Parathyroid function in idiopathic hypercalciuria — response to oral calcium tolerance test.

Stanley Wong

This thesis is submitted in part fulfilment
of the requirements for the degree of

Master of Science

in the Department of Physiology in the Faculty of Science
at the University of Sydney, Australia.

August 1990

Acknowledgements

The majority of the work for this thesis was performed at the Metabolic Unit, Concord Hospital, Concord. I would like to acknowledge the support of the Department of Veterans' Affairs during that time. I am grateful to the director of the Metabolic Unit, Dr Richard A. Evans, for his support, encouragement and supervision. His enthusiasm and dedication to research lead me to undertake this project. I would also like to thank Dr Brian J. Morris, Department of Physiology, University of Sydney, for his guidance and supervision.

I am especially indebted to my co-workers in the Metabolic Unit for their many and varied assistance towards this study. Mary Evans, RN; Colin Dunstan, MSc; Ellen Hills, BSc (hons); Elizabeth Lancaster, BSc (hons); and Diane Ivers all made valuable contributions which are much appreciated.

I would like to give thanks the staff of the Department of Biochemistry, especially Steven Slater, BASc, and Sue Acland, BSc, for the analysis of majority of the test samples; the Department of Nutrition and Dietitics; the Department of Endocrinology, Royal North Shore Hospital, St Leonards, for the determination of serum vitamin D; and the Department of Clinical Chemistry, Prince of Wales Hospital, Randwick, for the determination of urine oxalate. I wish to also thank Micheal M. S. Sidholm, the director of the University Computer Service, for the allowing me the use of computer facilities during the writing of this thesis.

Special thanks go to those volunteers who submitted themselves to the demands and rigors of the oral calcium tolerance test. Their altruism and forbearance will not go unrecognized.

Abstract

Idiopathic hypercalciuria is a syndrome of calcium-containing renal stone formation characterized by normocalcaemia, hypophosphataemia and hypercalciuria. Several modalities have been proposed as the primary defect in this disease including intestinal hyperabsorption of calcium, renal "leak" of calcium, renal "leak" of phosphate, hyperparathyroidism, and disturbance of vitamin D metabolism. The oral calcium tolerance test has been used in previous studies to differentiate and investigate the different modalities. Previous reports have suggested that parathyroid hormone may have a role in the pathogenesis of idiopathic hypercalciuria. Recent availability of radioimmunoradiometric assay for intact parathyroid hormone molecule (the main biologically active metabolite) in serum allows the opportunity to further elucidate its involvement in idiopathic hypercalciuria.

Parathyroid hormone concentrations where found to be normal in kidney stone forming subjects and the response to calcium loading is normal. However, there is some evidence that a subtle defect in the parathyroid 'set-point' for calcium is present in stone forming patients. Reports in the literature also suggest there is a defect in the transport of calcium across cell membranes in stone forming subjects. The combination of both these defects would account for the findings in this study. Protein metabolism may have some role in nephrolithiasis and might be more important in hypercalciuric than normocalciuric stone forming patients.

Contents

Tables

Figures

Abbreviations

$1,25\text{-}(OH)_2$-vitamin D	1α,25-dihydroxycholecalciferol
ATP	adenosine triphosphate
BMI	body mass index
BSA	body surface area
cAMP	cyclic adenosine monophosphate
EDTA	ethylenediaminetetraacetic acid
GFR	glomerular filteration rate
HC	hypercalciuric
iPTH	immunoreactive parathyroid hormone
$iPTH_{1\text{-}84}$	intact parathyroid hormone (1-84 amino acid)
IRMA	immunoradiometric assay
NC	normocalciuric
NS	not significant
p	probability
PTH	parathyroid hormone
r	correlation co-efficient
RIA	radioimmunoassay
SF	stone former
t	t statistic
UV	ultraviolet
z	z statistic

INTRODUCTION

Calcium Nephrolithiasis

In the United Kingdom the annual incidence of new urinary stone formation is 5 per 10,000 population and the male to female ratio is 2:1 (Robertson, 1984). Throughout the world, between 50% to 80% of the sufferers of renal calculi have stones which contain calcium (Coe and Favus, 1981; Robertson, 1984). Approximately half of the these are classified as idiopathic hypercalciuria (Coe and Favus, 1981; Aurbach *et al.*, 1981). Renal stones can be caused by high urinary calcium concentrations in many other disorders, such as primary hyperparathyroidism, Cushing's disease, sarcoidosis, renal tubular acidosis, immobilization and vitamin D excess. When these diseases are excluded (although, sometimes it is difficult to differentiate from primary hyperparathyroidism) a diagnosis of idiopathic (unknown causes) hypercalciuria is established. A number of metabolic patterns have been observed within this large group of patients but the exact aetiology remains obscure.

Idiopathic Hypercalciuria

In a series of 35 patients admitted with calcium-containing renal stones, Flocks (1939) found that many had had an increased or high urinary excretion of calcium in comparison with his normal volunteers. These "idiopathic" high urinary calcium patients represent over half the cases with calcium stones (Flocks, 1940; Hodgkinson and Pyrah, 1958) whereas only a small proportion of the normal population have a 24 hour urine calcium excretion above 7.5 mmol/day (for men) and 6.25 mmol/day (for women) (Knapp, 1947; Hodgkinson and Pyrah, 1958; Robertson and Morgan, 1972).

Pathogenesis of Hypercalciuria

Albright and his colleagues (1953) applied the term "idiopathic hypercalciuria" to describe the syndrome of calcium-containing renal stone formation characterized by a normal serum calcium concentration, frequently a low serum phosphorus concentration and an increased urinary excretion of calcium. They initially suggested that the condition was primarily due to renal tubular damage, possibly from staphylococcal pyelonephritis (which they found was commonly present or past). Further investigation by the same group (Henneman *et al.*, 1958) suggested that the disease might reflect a metabolic defect (as indicated by the unexplained low faecal calcium in a small number of patients) rather than a peripheral renal defect. Harrison (1959) did not find any evidence of urinary infection in his patients and only a minority of patients had had any significantly low plasma phosphate concentration. With the results from limited metabolic balances, he proposed that a few of the patients may have had a primary disorder of an over-absorption

of calcium from the gut, though he admitted that it was not the case in the majority. Jackson and Doncaster (1959) suggested that the increased gastro-intestinal absorption they found in two patients were secondary to the primary loss of calcium in the urine.

Hodgkinson (1961) suggested that the disorder might not be homogeneous and that the primary abnormality could be renal in some and intestinal in others. Furthermore, Parfitt *et al.* (1964) made the comment that the various findings up until then were unlikely to be applicable to a single homogeneous disease, although the term "idiopathic hypercalciuria" should be retained, but without any nosological implications. He described the two (then) postulated mechanisms for the disease as:

i) renal hypercalciuria; the result of impaired renal tubular reabsorption of calcium. The patients might be therefore be in incipient negative calcium balance, so that the serum calcium would tend to fall, stimulating the parathyroid glands, lowering serum phosphorus and secondarily increasing intestinal calcium absorption.

ii) hypercalciuria from intestinal over-absorption. The patients would then need to excrete the excess absorbed calcium from the diet in order to remain in zero balance, raising the renal handling of calcium, possibly predisposing to stone formation, and the serum calcium would tend to be higher.

Parfitt *et al.* (1964) found, in a small series of patients, some degree of increased calcium absorption was present in almost every case. They considered that the stone formers with idiopathic hypercalciuria might be hyperabsorbers of calcium since with the administration of cellulose phosphate (which binds calcium in the gut) the faecal calcium increased, the urinary calcium excretion decreased and there was no change in the calcium balance in

these patients. Using a calcium radioisotope to measure the time course and amount of absorption, Caniggia *et al.* (1965) found a faster and greater intestinal absorption in stone formers than normal and suggested the principal abnormality was hyperabsorption. Dent and Watson (1965), in a long-term study of one idiopathic hypercalciuria patient, found excessive absorption of calcium and hypercalciuria which could be controlled by a strict low-calcium diet and the patient was in approximate zero balance, again suggesting that the primary defect was overabsorption of calcium by the gut.

Renal tubular reabsorption of calcium appears to be normal in idiopathic hypercalciuria (Peacock and Nordin, 1968) and, after an overnight fast, both the slightly increased serum and the increased urine calcium concentrations fall almost into the normal range (Peacock *et al.*, 1968) and an even longer deprivation of calcium reduces them to normal. On subsequent administration of calcium there was a significant increase in both serum and urine calcium in normals and hypercalciuric subjects, but the rise in the latter group was significantly greater. The total amount of calcium excreted was also greater than controls. It was thus claimed that the hypercalciuria of renal stone disease is absorptive in the majority of instances (Peacock *et al.*, 1968; Peacock and Nordin, 1969). The same Leeds group (Nordin *et al.*, 1972) also noted that reduced tubular resorption of calcium (in renal hypercalciuria) could not *per se* cause hypercalciuria because at equilibrium it would be expected to lower plasma calcium rather than raise the urinary calcium. Though, normally one could also expect some compensatory mechanism (such as increased PTH or gut absorption) would bring the plasma calcium back to normal. In a study utilizing fractional counting of calcium radioisotopes in the forearm (Pak *et al.*, 1972), patients with idiopathic hypercalciuria had an elevated fractional calcium

absorption in comparison with controls and renal stone patients with normal urinary calcium concentration. The authors argued against "renal leak" of calcium as a significant factor in idiopathic hypercalciuria and they found no evidence of abnormal parathyroid function. The increased fractional absorption of calcium by the gut appeared to wholly account for the hypercalciuria.

Edwards and Hodgkinson (1965a) found, in their patients, relatively large amounts of calcium in the urine during fasting and while taking low calcium diets. In addition, the excretion of calcium continued to be elevated during the administration of ethylenediaminetetraacetic acid (EDTA). However, they subsequently reported that the renal responses to drugs and diets were essentially normal and they suggested that an extra-renal factor, possibly hormonal, was primarily responsible (Edwards and Hodgkinson, 1965b). This was the hypothesis proposed by Liberman *et al.* (1968), whose patients were found to have a decreased calcium excretion in the urine while on low calcium diets (confirming earlier studies), an elevated true intestinal calcium absorption rate, an enlarged miscible calcium pool size and an increased calcium turnover rate. Interestingly, Adams *et al.* (1970) found, by using a provocative test of parathyroid activity, that a significant number of their so-called idiopathic hypercalciuria patients had, in fact, parathyroid adenomas and they suggested that the two disease populations may not be different. However, Davies *et al.* (1971), in correspondence, disagreed since the original diagnosis was based on a relatively wide control range of plasma calcium. The same question was also raised by a second correspondent (Keynes, 1971) though he also expressed the view that provocative testing might prove to be useful.

One study, using a carboxy-terminal specific parathyroid hormone assay (Coe *et al.*, 1973), found serum parathyroid hormone concentrations were elevated in 26 of 40 patients with idiopathic hypercalciuria and they fell to normal or near normal during periods of thiazide administration. It was noted that in primary hyperparathyroid disease the hormone plasma concentration does not fall when hypercalciuria is reversed. In patients with initially normal serum concentrations of parathyroid hormone, hypercalciuria was induced by furosemide and an increase in hormone serum concentration equivalent to the first group was found. Hyperabsorption could not be a factor in these patients, and the mechanism by which it produces hypercalciuria would not increase the secretion of parathyroid hormone. The authors concluded that idiopathic hypercalciuria is primarily due to a defect in the renal tubular reabsorption of calcium, leading to secondary hyperparathyroidism. However, Burckhardt and Jaeger (1981) were unable to find any evidence of secondary hyperparathyroidism in their patients, other than in one case, and suggested that secondary hyperparathyroidism in this disease is quite rare.

With the development of a radioligand assay for vitamin D in 1974 (see Maggio *et al.*, 1979) a number of studies were initiated to define the role of this metabolite in idiopathic hypercalciuria. Serum concentrations of 1α,25-dihydroxycholecalciferol (1,25-$(OH)_2$-vitamin D — the main active vitamin D metabolite) were found to be raised in some patients with idiopathic hypercalciuria in comparison with normal controls (Shen *et al.*, 1975). In a larger study, these findings were confirmed (Shen *et al.*, 1976) and the authors reported higher urine calcium, normocalcaemia, decreased parathyroid hormone, increased serum 1,25-$(OH)_2$-vitamin D and decreased serum phosphorus in the patients as compared with normal controls. The increase in serum 1,25-$(OH)_2$-vitamin D was found to correlate

significantly with the decrease in serum phosphorus. Since there is a direct relationship between phosphorus and parathyroid hormone and 1,25-$(OH)_2$-vitamin D increases calcium absorption, it was proposed that a renal phosphorus leak led to hypophosphataemia, increasing 1,25-$(OH)_2$-vitamin D and consequent hypercalciuria (Shen *et al.*, 1977ab). An increase in immunoreactive calcitonin led support to this theory (Ivey *et al.*, 1981). However, Tschöpe *et al.* (1980) doubted phosphate leak as a primary factor in absorptive hypercalciuria because of the circadian variation of the decreased plasma phosphate and because of their findings of increased fractional excretion of sodium and chloride in their patients which would suggest some alternative pathogenesis. Furthermore, Lau *et al.* (1982) found a defect in the proximal tubular reabsorption of sodium in idiopathic hypercalciuria patients which could account for the phosphate wasting. Subsequently, Colussi *et al.* (1983) found that the reduced proximal tubular resorption of sodium was not a 'defect' but rather a physiological adaption to a habitual high sodium intake. Muldowney *et al* (1982) reported significant reduction of urine calcium, sodium and phosphate excretion of SF subjects on low sodium diets. Broadus *et al.* (1984) considered that their results were not consistent with the phosphate leak theory for the pathogenesis of idiopathic hypercalciuria. They found no predictable relationship between a reduction in serum phosphate concentration and/or the renal tubular maximum phosphate threshold and plasma 1,25-$(OH)_2$-vitamin D. They postulated a proximal tubule abnormality causing both hypophosphataemia and an increase in 1,25-$(OH)_2$-vitamin D.

Kaplan *et al.* (1977) found raised 1,25-$(OH)_2$-vitamin D in one third of their patients with absorptive hypercalciuria and the normal serum concentrations were thought to be inappropriately high for the circulating concentration of parathyroid hormone. They

suggested that intestinal hyperabsorption of calcium may be vitamin D dependent but may not be the sole cause since, in some patients, the intestinal calcium absorption is inappropriately high for the plasma concentration of 1,25-$(OH)_2$-vitamin D. Zerwekh *et al.*, (1980) thought the calcium absorption in absorptive hypercalciuria was vitamin D independent. Most other workers have found elevated 1,25-$(OH)_2$-vitamin D levels in patients with high urinary calcium (Broadus *et al.*, 1980; Berlin *et al.*, 1982; Bataille *et al.*, 1987) but some have not (Coe *et al.*, 1982; Audran *et al.*, 1987). Duncombe *et al.* (1980) reported an increased permeability to calcium ions in the brush border of the jejunum in hypercalciuria patients. This could be the primary defect or the result of an increased level of or sensitivity to vitamin D compounds. Broadus *et al.* (1987) concludes that absorptive hypercalciuria is regularly a 1,25-$(OH)_2$-vitamin D-mediated syndrome and suggests that there is an unknown qualitative defect in the control of 1,25-$(OH)_2$-vitamin D production in patients with absorptive hypercalciuria. The authors also hint that a proximal tubular hypersensitivity to the effects of parathyroid hormone may explain their findings.

In primary hyperparathyroidism, after a calcium load, the urinary cyclic 3',5'-adenosine monophosphate (cyclic AMP) excretion did not change significantly, in contrast to the decrease found in normal controls. The calcium challenge does not adequately suppress parathyroid hormone secretion in this disease, so it was suggested that this test might identify primary hyperparathyroidism better than does the measurement of immunoreactive hormone. Measurement of simple urinary cyclic AMP (expressed as nanomoles per 100 ml glomerular filtrate) was found, in most instances, to be comparable to using nephrogenous cyclic AMP and better than immunoreactive parathyroid hormone for differentiating primary hyperparathyroid patients from normal controls (Broadus *et al.*,

1977). Caniggia *et al.* (1981) reported on the usefulness of 24 hour urinary cyclic AMP/creatinine ratios for assessing parathyroid function. Kraiem *et al.* (1983) found a spot sample or 2 hour test of urinary or nephrogenous cyclic AMP per 100 ml glomerular filtrate was quite satisfactory for separating primary hyperparathyroid patients and normal controls. In a study of primary hyperparathyroid patients, Broadus *et al.* (1980) found two subpopulations in this disease: one having hyperabsorption, hypercalciuria, elevated plasma 1,25-$(OH)_2$-vitamin D and a high incidence of renal stones; and another group having normocalciuria, normal or high normal plasma 1,25-$(OH)_2$-vitamin D concentration and a low incidence of stones. However, in absorptive hypercalciuria the calcium absorption may be vitamin D independent (Zerwekh *et al.*, 1980; Duncombe *et al.*, 1980). Furthermore, when Pak *et al.* (1981) divided their primary hyperparathyroid patients by the presence or absence of renal stones, they noted that the biochemical findings were similar in both groups.

Calcium Load Test

In an attempt to differentiate the various types of idiopathic hypercalciurias, Pak *et al.* (1974) introduced a four day study protocol involving a synthetic diet and treatments based on the expected responses of the different forms of hypercalciurias to a number of stimuli. They found that most of their idiopathic patients had absorptive hypercalciuria. The evidence was not conclusive but the authors considered that the renal hypercalciuria patients might have had secondary hyperparathyroidism, although it was noted that the patients shared the same features found in patients with "normocalcaemic" primary

hyperparathyroidism as described by Wills *et al.* (1969). In a later report (Pak *et al.*, 1975) the study protocol was refined into a six hour test which could be performed in a routine laboratory. This test involved the measurement of serum calcium, and of urinary calcium, creatinine and cyclic AMP. The theoretical basis for the test was: during a fast, urinary calcium would be raised if there was impaired renal tubular reabsorption or skeletal mobilization of calcium; after an oral load of calcium, urinary calcium may be elevated in the presence of intestinal hyperabsorption of calcium; and urinary cyclic AMP, obtained after an oral calcium load, may provide a reliable measure of parathyroid function.

The protocol required subjects to be on a low calcium diet for at least a week before the test. After an overnight fast (except for distilled water) the test begins at 7 am when the bladder is voided and 600 ml distilled water is drunk. A 2 hour urine collection is made (*ie*, at 9 am) for measurement of calcium, creatinine and cyclic AMP. At 9 am blood is drawn for measurement of calcium. An oral load of 1 gram of calcium is given at this time. Urine is then collected for 4 hours (until 1 pm) for measurements of calcium, creatinine and cyclic AMP. Four groups of nephrolithiasis patients were used to establish the effectiveness of the test: normocalciuric stone formers, absorptive, resorptive (primary hyperparathyroidism) and renal hypercalciuria patients. The previously defined absorptive hypercalciuria patients demonstrated in the test: normocalcaemia, fasting normocalciuria, exaggerated increase in urinary calcium after an oral load and a normal or low urinary cyclic AMP. In primary hyperparathyroid patients: the fasting plasma calcium and urinary calcium was high with high urinary calcium and cyclic AMP after load. Normocalciuric stone formers had: normal plasma and urinary calcium and cyclic AMP with some increase in urinary calcium after load. The renal hypercalciuria patients were found to

have: normocalcaemia and high fasting urinary calcium which increased after load while the fasting cyclic AMP was high to normal. There were only a small number of renal hypercalciuria patients and the authors were unable to make any definitive statements as to whether the disorder was the secondary hyperparathyroidism suggested by Coe *et al.* (1973).

Absorptive hypercalciuria was subsequently further subcategorized into 3 types (Pak *et al.*, 1980; Pak, 1984). Absorptive hypercalciuria Type I is represented by the classical presentation characterized by hypercalciuria on a calcium-sodium restricted diet, normal fasting urinary calcium with normocalcaemia, normal or low serum parathyroid hormone or urinary cyclic AMP and an high urinary calcium excretion during the 4 hours following an oral load of 1 g calcium. Type II absorptive hypercalciuria presentation is identical to Type II except for normal urinary calcium on the restricted diet. The patients with Type III have persistently low serum phosphorus and theorectical renal phosphate threshold in addition to the biochemical profiles found in Type I or Type II.

Muldowney *et al.* (1976) reported the usefulness of ionized calcium (*ie*, "active" calcium) and the parathyroid hormone levels in a group of idiopathic hypercalciuria patients. Although their total serum calcium were within 3 standard deviations above the normal mean, a third of the patients had raised ionized calcium and immunoreactive parathyroid hormone plasma concentration and subsequently 9 of these 10 patients were shown to have parathyroid adenomas. They concluded "*that in many cases, idiopathic hypercalciuria and so-called normocalcaemic hyperparathyroidism (albeit with high ionized concentrations) may be one and the same entity.*" In the remaining non-

hyperparathyroid idiopathic hypercalciuria patients, they considered that the disease is "renal" rather than "absorptive" in origin (Muldowney *et al.*, 1980). However, Bordier *et al.* (1977) was able to distinguish the 3 main types of idiopathic hypercalciuria patients (*ie*, renal, absorptive and resorptive). They also further subdivided the first 2 groups into 2 subtypes; one renal hypercalciuria subtype of which was considered to have secondary hyperparathyroidism which had eventually led to tertiary hyperparathyroidism. Other workers, using measurements of cyclic AMP and calcium tolerance tests, were able to distinctly differentiate renal from absorptive hypercalciuria (Broadus *et al.*, 1978; Pak and Galosy, 1979; Iguchi *et al.*, 1980; Kitamura *et al.*, 1988).

In contrast to the above findings, a number of reports have been unable to clearly distinguish such subgroups. Fuss *et al.* (1978) found that in 30% of their cases the calcium loading test was not diagnostic. Sutton and Walker (1980) found no differences in immunoreactive parathyroid hormone concentrations or nephrogenous cyclic AMP excretion among renal leak, absorptive hypercalciuria and normal controls. Coe *et al.* (1982) found a uniform elevation of intestinal calcium absorption and a variable defect of renal calcium reabsorption in idiopathic hypercalciuria patients which "*suggest that renal and absorptive hypercalciuria may not be distinct entities but rather the two extremes of a continuum of behaviour.*" Holdaway *et al.* (1982) were unable to distinguish different types of hypercalciuria patients within their non-primary hyperparathyroid subjects when they used a slightly modified calcium loading test of Pak *et al.* (1975). In addition, Lien and Keane (1983ab) considered that the responses to oral calcium loading did not serve a useful role in the identification of pathogenic subgroups. They found only limited separation of hypercalciuria stone formers from normal individuals using the post-calcium

load response and did not find any evidence of "renal leak" in their hypercalciuria patients. Drexler (1982) proposed that there is a single pathology which would encompass the divergent findings: hypersecretion of parathyroid hormone. Evans *et al.* (1984) could not identify different groups of hypercalciuric patients in their study and suggest that parathyroid hyperfunction is the the primary dysfunction in this disease. Hess and Binswanger (1986) did not consider classification of subjects into "absorptive" and "renal hypercalciuria" was justified while the primary metabolic defect remained unidentified. Nunziatta *et al.* (1987) considered that the disorder is the result of the presence of both (gut hyperabsorption and renal leak) and a profound alteration of the body calcium turnover. The division of idiopathic hypercalciuria patients into absorptive and renal hypercalciuria may have some clinical relevance but it does not suggest a basis for the pathogenesis (Vosburgh and Peters, 1987).

The delineation of several apparent cause(s) of hypercalciuria suggested that the various subgroups might require varying treatments (Pak, 1977, 1979; Pak *et al.*, 1981, 1982; Lemann and Gray, 1989). The treatment of hypercalciuric stone formers with primary hyperparathyroidism involves the surgical removal of the glands, which usually abolishes any further stone disease (see Coe and Favus, 1980). Other types of hypercalciuric stone formers require long-term therapy (Pak and Nicar, 1981). These may involve the use of thiazides (Yendt and Cohanim, 1978; Sutton and Walker, 1980; Scholz *et al.*, 1982; Coe *et al.*, 1988), allopurinol (Coe, 1977), phosphate (Ettinger and Kolb, 1973; Van der Berg *et al.*, 1980) and low calcium diets (Pak *et al.*, 1981; Coe and Favus, 1981) or any combination thereof (Kocián, 1987). Although, Ettinger (1979) found that the stone passage rates were markedly similar during therapy in placebo, phosphate and

low-calcium diet groups. He suggests "*a proposed treatment that does not bring nearly 100 per cent remission over five years in stone forming patients should be regarded as possibly ineffective.*"

Martinez-Maldonado (1979) discussed some of the difficulties associated with attempting to understand the pathophysiology of hypercalciuria. In particular, (besides trying to define hypercalciuria itself) he mentions the problems in the interpretation of data from immunoreactive parathyroid hormone and cyclic AMP assays as a measure of parathyroid function. In a review of primary hyperparathyroidism and idiopathic hypercalciuria, Halabé and Sutton (1987) noted the difficult distinction between subtle or mild primary hyperparathyroidism and idiopathic hypercalciuria and suggested that further investigation of the role of abnormal parathyroid function in idiopathic hypercalciuria is required.

The proposed study is an attempt to overcome some of the difficulties previous workers encountered in this area. It is intended to examine the physiological responses of parathyroid hormone in normal controls and hypercalciuric stone formers to the calcium load test. Levels of parathyroid hormone will be estimated with a immunoradiometric assay for intact (1-84 amino acid chain) parathyroid hormone. Developments in assay technology have enabled a 2 to 3 order of magnitude improvement in sensitivity and resolution for hormone detection, as well as greater specificity for the major circulating biologically active form of the hormone (intact parathyroid hormone). In addition, present technology allows rapid, convenient and precise measurements of calcium and other metabolites in biological fluids.

MATERIALS AND METHODS

Subjects

Sixty patients were studied who were referred to the Metabolic Unit for investigation of recurrent calcium-containing renal calculi over a period of 9 months. There were 51 men, aged 47 ± 14 (mean ± standard deviation) years, range 18 to 72 years and 9 women, aged 46 ± 13 years, range 25 to 62 years. The patients did not have any known causes of calculi such as primary hyperparathyroidism, sarcoidosis, vitamin D intoxication, or renal tubular acidosis. All the patients had normal renal function (as determined by the GFR as estimated from the creatinine clearance). The plasma creatinine in the patients was less than 0.12 mmol/l, excepting for one patient with plasma creatinine of 0.13 mmol/l, and there was no evidence of urinary tract infection at the time of the study.

Nineteen patients had a 24 hour urinary calcium excretion greater than 0.1 mmol/kg body weight on a normal diet and were thus categorized as the hypercalciuric (HC) group of stone formers (SF) (Broadus *et al.*, 1978; Lien and Keane, 1983a; Bianchi *et al.*, 1988; Lemann and Gray, 1989). The remaining 41 patients were considered to be normocalciuric (NC) stone formers. Knapp (1947) in a survey of 606 normal men, women and children concluded that urinary excretion of calcium in normal individuals is

dependent on the intake of calcium per unit body weight and on an endogenous factor or factors characteristic of the individual. Later Hodgkinson and Pyrah (1958) defined the normal range of 24 hour urinary calcium excretion as 2.5-7.5 mmol/day for men and 2.5-6.25 mmol/day for women. In more recent studies, HC subjects have also been defined as those patients with a 24 hour calcium excretion greater than 0.1 mmol/day/kg and, in most instances, the criteria are equivalent. In this report, the latter single criterion was chosen to avoid ambiguity.

Thirty-one healthy volunteers with no history of nephrolithiasis or bone disease were used as controls. There were 20 men, aged 46 ± 13 years, range 27 to 75 years and 11 women, aged 39 ± 11, range 21 to 61 years recruited from the hospital and general population by Ms E. Lancaster. All had plasma calcium and phosphorus levels within normal limits. Two of the controls had a 24 hour urinary calcium excretion greater than 0.1 mmol/kg body weight, but this was accepted as part of the expected (asymmetrical) distribution in normal subjects (Knapp, 1947; Hodgkinson and Pyrah, 1958) and hence they remained in the study.

Study Protocol

Twenty-four hour urine specimens were collected while subjects were on a normal diet before any treatment had begun. All subjects were cautioned about accuracy of the collections and were asked to record their collection times. Two collections, at least one week apart, were usually obtained from each patient to evaluate consistency. In addition, no significant outliers were found when the body mass to 24 hour urinary creatinine

excretion ratio was examined. Results from the collection immediately prior to the test period are reported in this study. The urine was collected without preservative except storage at 0-4°C. The 24 hour urine volume was measured and the urine was aliquoted and acidified, where necessary, in the metabolic laboratory within 24 hours of collection. Samples were then kept frozen at −15°C until analysis for sodium, urea, creatinine, calcium, magnesium, phosphate, urate, oxalate and citrate.

The study was conducted on an out-patient basis in an air-conditioned room adjacent to the metabolic laboratory. The subjects had a diet restricted in calcium (10 mmol/day) and sodium (100 mmol/day) for one week prior to the calcium loading test. No medication to control calculi was taken during this period, but other medications were permitted. On the evening prior to the test, the subjects fasted from 2100 hours, though water was taken as desired. The importance of fasting was emphasised and fasting status was confirmed by the subjects on the day of the test.

On the morning of the test, at 0800 hours subjects voided their bladders and the urine discarded. Subsequently, 3 urine collections were taken from the periods 0800-1000, 1000-1200 and 1200-1400 hours and 3 blood samples were taken at 0900, 1100 and 1300 hours. To maintain hydration, 600 ml of distilled water was taken in the first half hour, and then 150 ml every half hour until 1300 hours. At 1000 hours 1000 mg of calcium was given orally (*Sandocal 1000*, 5.23 g calcium lactate gluconate, 0.8 g calcium carbonate and 2 g citric acid; Sandoz Ltd, Basle, Switzerland) in 150 ml of distilled water. The subjects remained supine for 30 minutes before each blood sample was taken, and activity was restricted throughout the remainder of the test. The tests were usually conducted

under the supervision of RN M. Evans and the blood specimens were obtained by Dr R. Evans.

Thirty ml of venous blood was taken at each collection time anaerobically into a plastic syringe. Ten ml of blood was aliquoted for measurement of serum intact parathyroid hormone ($iPTH_{1-84}$), 1,25-$(OH)_2$-vitamin D and lipids; 5 ml for the measurement of plasma cyclic AMP (treated with a final concentration of 5 mmol/l EDTA); and 2.5 ml for the measurement of blood glucose. Heparin, titrated to the normal ionized calcium level of 1.25 mmol/l (*Radiometer S4500 Heparin* for ionized calcium analyses, 875 IU/ml sodium heparin, 3 nmol/ml calcium chloride, 155 nmol/ml sodium chloride; Radiometer A/S, Copenhagen NV, Denmark), was then added to the remaining blood in the syringe, 0.02 ml heparin per 3 ml blood, and then the syringe was inverted 3 times. Air was then expelled from the syringe and the whole blood ionized calcium was measured within 15 minutes. After the analysis, the remaining blood was then dispensed into an ammonium heparin tube for plasma biochemistry. Aliquots of separated plasma or serum were stored frozen at –20°C until analysis.

The blood samples from the 3 periods were analyzed for sodium, potassium, chloride, bicarbonate, urea, creatinine, albumin, alkaline phosphatase, total calcium, phosphate, ionized calcium, parathyroid hormone ($iPTH_{1-84}$) and cyclic adenosine monophosphate (cAMP) (for the estimation of nephrogenous cAMP). The 0900 hour blood sample was also examined for glucose, urate, cholesterol and triglycerides. The volume of the urine samples from the three periods were measured and the urine analyzed for pH, sodium, potassium, urea, creatinine, calcium, phosphate and cAMP (for the estimation of

nephrogenous cAMP). In addition, each subject's height, weight and skinfold thickness (the sum of the triceps, subscapular and suprailiac skinfolds for the estimation of body fat) were measured on the day of the test by the duty nutritionist.

Analytical Methods

Plasma sodium, potassium, chloride, bicarbonate, urea, creatinine, albumin (Doumas *et al.*, 1971), alkaline phosphatase, total calcium (Gitelman, 1967) and phosphate were measured on a random access *Hitachi 705* automatic analyzer (Hitachi Ltd, Tokyo, Japan). Serum cholesterol and triglyceride was also measured on the automatic analyzer. The calcium, phosphate and albumin levels were measured twice: once during the course of the study; and again at the end of the study when all the samples were measured in one assay (to avoid interassay variation). During the nine-months duration of the study, low and high biological standards were measured daily and the coefficient of variation for calcium was 1.8%, phosphate 1.7% and albumin 1.8%. The intra-assay variation for calcium was 0.7%, phosphate 1.8% and albumin 0.9%. There was some variation between the two measurements (correlation coefficient of 0.62 for fasting samples, $t = 7.51$, $p < 0.001$) so the values obtained from the single assay were used in subsequent analyses. For this study, plasma calcium is corrected to a plasma albumin level of 38 g/l (see appendix A). These analyses were performed by the Department of Biochemistry.

Ionized calcium in the whole blood was measured using a *Nova 2* ionized calcium analyzer (Nova Biomedical, Newton, Massachusetts, USA) (Urban *et al.*, 1985). No adequate biological standard is yet available for these analyzers. Aqueous standards and

the slope of the concentration response curve was checked before unknowns were measured. I measured each sample in triplicate and also measured an aqueous standard between each unknown sample. The maximum acceptable error in the 1.50 mmol/l standard was ± 2% and the coefficient of variation over the period of the study was 1.0%.

Serum levels of intact 84 amino acid chain of PTH (the major biologically active form (Goltzman *et al.*, 1986)) were measured using a two-site immunoradiometric assay (IRMA) (Nussbaum *et al.*, 1987) available in kit form as the *Allégro* Intact PTH Immunoassay System (Nichols Institute Diagnostics, San Juan Capistrano, California, USA). The assay uses two different goat polyclonal antibodies to human PTH; one antibody is prepared to bind only to the mid-region and C-terminal PTH 39-84 and this antibody is immobilized onto plastic beads; the other antibody is prepared to bind only to the N-terminal PTH 1-34 and this antibody is radiolabeled for detection. Only the intact PTH 1-84 present in the sample is bound by both the immobilized and labeled antibodies to form the 'sandwich' complex necessary for detection. The kit was evaluated for suitability both by myself in the Metabolic Unit Laboratory and by Ms S. Acland in the Department of Biochemistry. The study samples were measured in duplicate in one assay by Ms S. Acland and Mr S. Slater from the Department of Biochemistry. Apart from the dilutions, the procedures of the assay were automated using a *Hybritech photon Era* automated immunoassay analyzer (Hybritech Inc, San Diego, California, USA). The sensitivity obtained was 2 pg/ml and the intra-assay variation was 10.6% (at 16 pg/ml), 4.9% (at 55 pg/ml) and 6.2% (at 95 pg/ml) which were comparable with the assay kit manufacturer's performance specifications.

The cAMP in plasma and urine was measured by radioimmunoassay (RIA) using the *INCSTAR* cAMP RIA kit (Incstar Corporation, Stillwater, Minnesota, USA). The plasma and urine were diluted sufficiently and acetylated so that both samples could be measured in the same assay. The overnight option was used for the assay to improve sensitivity. In my hands, the sensitivity achieved was 0.02 nmol/l. The intra-assay coefficient of variation was 7% (at 0.2 nmol/l) and 5% (at 0.5 nmol/l) for both plasma and urine. The inter-assay coefficient of variation was 9% for plasma and 11% for urine. The slight loss of plasma cAMP from phosphodiesterase activity even after treatment with EDTA and storage at −20°C (Holmegaard, 1982) was not apparent in the samples in this study. Measurements were made in 20 controls (12 men and 8 women) and 25 patients (18 men and 7 women). The urinary and nephrogenous cAMP levels were calculated and expressed per 100 ml glomerular filtrate (GF) (Broadus *et al.*, 1977; Broadus, 1979).

Fasting serum levels of 1,25-$(OH)_2$-vitamin D were measured according to the method of Reinhardt *et al.* (1984) with modifications as outlined in the paper by Seshadri *et al.* (1985). Measurements were made in 30 control subjects (19 men and 11 women) and in 27 SF subjects (20 men and 7 women) by Ms D. Lissner and Ms S. Taliaferro at the Department of Endocrinology, RNSH. The detection limit was 2.2 fmol/tube. The inter-assay coefficients of variation were 24% (at 15.8 fmol/tube) and 5.6% (at 32.2 fmol/tube) and the intra-assay coefficients of variation were 13.7% (at 24.7 fmol/tube) and 13.5% (at 44.6 fmol/tube).

Urinary sodium, potassium, urea, creatinine and phosphate were measured on a random access Hitachi 705 automatic analyzer in the Department of Biochemistry. The

coefficient of variation during the period of the study for creatinine and phosphate was 1.3% and 2.0%, respectively. Urinary calcium and magnesium was measured in duplicate using a *Varian SpectAA 10* atomic absorption spectroscope (Varian Techtron P/L, Mulgrave, Victoria, Australia), also in the Department of Biochemistry, and the coefficent of variation for calcium was 2.8% and for magnesium was 2.6%.

Urine levels of citrate were estimated by the adaption of a kit (*Boehringer Mannheim* citric acid UV method; Boehringer Mannheim GmbH, Mannheim, West Germany) based on the use of citrate lyase (EC 4.1.3.6) (Warty *et al.*, 1984). Samples were measured in duplicate by Mr C. Dunstan in the Metabolic Unit Laboratory. The results were read using a *Pye Unicam SP6-350* visible spectrophotometer (Pye Unicam Ltd, Cambridge, England, UK) interfaced with a *Hewlett-Packard HP9815A* desktop calculator (Hewlett-Packard, Loveland, Colorado, USA). The intra-assay variation was 5.5% (at 0.18 mmol/l) and 3.1% (at 3.1 mmol/l) and the inter-assay variation was 8.4% (at 0.22 mmol/l) and 3.2% (at 3.1 mmol/l). Urine levels of oxalate were estimated by a modified oxalate oxidase method (Stauss *et al.*, 1987) by the Department of Clinical Chemistry, POWH. The reported inter-assay variation was 3.8% (at 0.82 mmol/l)

Statistical Analysis

The results are presented as the group mean ± standard deviation (mean ± SD) and comparisons between groups were made by Student's t-test and by one way and multiple analysis of variance. When these analysis models were inappropriate (*eg*, due to lack of normality and/or unequal variances) then transformations or non-parametric tests (*ie*,

Mann-Witney and Kruskal-Wallis) were utilized (pp 615-635, Neter *et al.*, 1985). The level of significance reported is 0.05. A lower level of probability was not considered necessary within the limits of the study design (p 205, Armitage and Berry, 1987). The association between stone formation and laboratory measurements were studied using correlation coefficents and linear regression models. Multiple linear regression was used to evaluate associations of groups of variables.

The analyses were carried out on (variously) a PDP 11/23+, a MicroVAX 3500 and a MIPS 2000 minicomputers using Minitab (Minitab Inc., State College, Pennesylvania, USA), SPSS-X (SPSS International, Gorinchem, The Netherlands), and New S (AT&T Bell Laboratories, Greensboro, North Carolina, USA) statistical programs.

RESULTS

Somatometric data

The controls matched the patients for age and body size (Table 1). The only significant differences observed were between the men and women in height, BSA and percentage body fat. As expected from general observations, the men were taller, had a greater body surface area and lower percentage body fat than the women.

The number of women in the SF subject sample population is low (9 out of 60 or 15%) reflecting the trend that stone formation occurs predominantly in men. There was only one woman who could be classified as hypercalciuric by the selected criteria.

Subject type (*ie*, control, NC or HC) correlated with subject percentage body fat ($r = 0.22$, $p < 0.05$). Multiple linear regression analysis showed that, in addition to BMI, sex and age, subject status (*ie*, control or SF) was a significant contributor to percentage body fat ($r = 0.89$, $p < 0.001$; BMI: $t = 11.00$, $p < 0.001$; sex: $t = -11.13$, $p < 0.001$; age: $t = 4.55$, $p < 0.001$; status: $t = -2.15$, $p < 0.05$). Furthermore, subject type remains a significant correlate after the contributions of BMI, sex and age have been removed ($r = 0.90$, $p < 0.001$; BMI: $t = 11.41$, $p < 0.001$; sex: $t = -10.97$, $p < 0.001$; age: $t = 4.69$, $p < 0.001$; type: $t = -3.01$, $p < 0.005$). In men, a similar relationship was found with body fat

TABLE 1 *Subjects' age and somatometric data (mean ± standard deviation).*

Group (*n*)	Control (31) men (20) women (11)	Normocalciuric (41) men (33) women (8)	Hypercalciuric (19) men (18) women (1)	Stone Formers (60) men (51) women (9)
age (years)	43.4 ± 13.0 45.7 ± 13.5 39.3 ± 11.6	46.0 ± 14.5 45.9 ± 14.8 46.9 ± 14.2	47.5 ± 12.1 47.9 ± 12.3 40.0	46.5 ± 13.7 46.6 ± 13.9 45.8 ± 13.4
height (m)	1.70 ± 0.08 1.74 ± 0.06 1.64 ± 0.06[d]	1.72 ± 0.10 1.75 ± 1.72 1.59 ± 0.06[d]	1.72 ± 0.12 1.72 ± 0.12 1.60	1.72 ± 0.10 1.74 ± 0.10 1.59 ± 0.06[d]
weight (kg)	73.4 ± 8.8 75.7 ± 8.0 69.3 ± 9.5	75.6 ± 12.4 77.7 ± 10.8 67.1 ± 15.9	78.7 ± 18.1 78.2 ± 18.5 86.9	76.6 ± 14.4 77.9 ± 13.8 69.3 ± 16.2
BMI (kg/m^2)	25.3 ± 3.2 25.0 ± 3.0 25.9 ± 3.8	25.6 ± 4.2 25.4 ± 3.5 26.5 ± 6.6	26.4 ± 4.5 26.0 ± 4.3 33.9	25.9 ± 4.2 25.6 ± 3.7 27.3 ± 6.6
BSA (m^2)	1.86 ± 0.13 1.90 ± 0.11 1.76 ± 0.12[b]	1.89 ± 0.18 1.93 ± 0.15 1.71 ± 0.19[a]	1.92 ± 0.27 1.92 ± 0.28 1.94	1.90 ± 0.21 1.93 ± 0.20 1.73 ± 0.19[a]
body fat (%)	29 ± 8 24 ± 6 36 ± 6[d]	26 ± 7 25 ± 6 35 ± 6[d]	24 ± 7 23 ± 6 41	26 ± 7 24 ± 6 35 ± 6[d]

[a, b, c, d] significantly different, women from men; $p < 0.05, 0.01, 0.005, 0.001$, respectively.

significantly correlated with BMI, age and subject type ($r = 0.82$, $p < 0.001$; BMI: $t = 9.53$, $p < 0.001$; age: $t = 3.39$, $p < 0.005$; type: $t = -2.56$, $p < 0.05$). However, in women, their body fat correlated with BMI, age and subject status (rather than subject type as there was only one HC woman) ($r = 0.89$, $p < 0.001$; BMI: $t = 7.04$, $p < 0.001$; age: $t = 3.54$, $p < 0.005$; status: $t = -2.29$, $p < 0.05$).

24 hour urine analysis

The results of the 24 hour urine analysis are shown in Table 2. Although patients were segregated using the amount of urinary calcium excretion per day per kilogram body mass,

TABLE 2 *Results of analysis of 24 hour urine samples (mean ± SD) on a normal diet.*

Group (*n*)	Control (31) men (20) women (11)	Normocalciuric (41) men (33) women (8)	Hypercalciuric (19) men (18) women (1)	Stone Formers (60) men (51) women (9)
volume (ml)	1709 ± 689 1655 ± 514 1807 ± 951	1733 ± 726 1717 ± 769 1799 ± 552	1867 ± 825 1882 ± 846 1585	1775 ± 754 1775 ± 792 1775 ± 521
sodium (mmol/day)	148 ± 52 157 ± 54 131 ± 47	150 ± 75 158 ± 80 114 ± 34[a]	176 ± 69 178 ± 70 128	158 ± 73 165 ± 76 116 ± 32[c]
urea (mmol/day)	298 ± 136 430 ± 148 341 ± 90[a]	395 ± 149 415 ± 153 312 ± 99[a]	441 ± 102 440 ± 105 466	410 ± 137 424 ± 138 329 ± 106[d]
creatinine (mmol/day)	14.4 ± 3.7 16.0 ± 3.5 11.3 ± 1.2[d]	14.8 ± 4.3 16.0 ± 3.7 9.9 ± 2.7[d]	16.7 ± 4.6 16.7 ± 4.7 16.0	15.4 ± 4.4 16.3 ± 4.1 10.6 ± 3.2[d]
calcium (mmol/day)	4.44 ± 2.64 4.91 ± 2.74 3.58 ± 2.31	4.43 ± 1.94 4.68 ± 1.86 3.40 ± 2.03	9.42 ± 2.21[4] 9.34 ± 2.25[4] 10.8	6.01 ± 3.08[1] 6.32 ± 3.00 4.22 ± 3.11
magnesium (mmol/day)	4.68 ± 1.69 5.14 ± 1.55 3.83 ± 1.68[a]	4.20 ± 1.68 4.48 ± 1.75 3.04 ± 0.44[d]	5.32 ± 1.53 5.22 ± 1.51 7.10	4.55 ± 1.70 4.74 ± 1.69 3.49 ± 1.41[a]
phosphate (mmol/day)	29.5 ± 8.7 31.6 ± 8.8 25.8 ± 7.7	29.4 ± 10.6 31.8 ± 10.3 19.5 ± 4.7[d]	32.8 ± 9.9 32.8 ± 10.2 32.3	30.4 ± 10.4 32.1 ± 10.2 20.9 ± 6.1[d]
urate (mmol/day)	4.16 ± 1.22 4.40 ± 1.45 3.73 ± 0.42	3.69 ± 1.88 3.92 ± 1.99 2.72 ± 0.90[a]	3.85 ± 1.39 3.82 ± 1.42 4.60	3.74 ± 1.73 3.89 ± 1.79 2.93 ± 1.05[a]
oxalate (µmol/day)	274 ± 110 317 ± 92 196 ± 98[c]	316 ± 186 341 ± 190 217 ± 137	352 ± 144 353 ± 148 330	326 ± 174 345 ± 176 230 ± 133[a]
citrate (mmol/day)	2.86 ± 0.98 2.71 ± 0.80 3.14 ± 1.24	2.27 ± 1.33[1] 2.40 ± 1.30 1.62 ± 1.36[1]	2.66 ± 1.30 2.49 ± 1.12 5.51	2.39 ± 1.32 2.43 ± 1.23 2.11 ± 1.86

[1, 2, 3, 4] significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
[a, b, c, d] significantly different, women from men; $p < 0.05, 0.01, 0.005, 0.001$, respectively.

the results in the table are expressed as urinary excretion per day since the preponderence of previous studies report excretion per day and during the analysis, no relationships are being assumed between excretion and body mass.

There were no significant differences between control and SF subjects for their respective 24 hour urine volume, sodium, urea, creatinine, magnesium, phosphate, urate and oxalate excretion. The 24 hour urinary citrate was lower in NC subjects than in the controls (NC, 2.3 ± 1.3 mmol/24 hour; control, 2.9 ± 1.0, t = 2.16, $p < 0.05$) (mean ± SD) but the reduced mean citrate was mainly due to the NC women who had a much lower urine citrate than control women (NC women, 1.6 ± 1.4; control women, 3.1 ± 1.2, t = 2.39, $p < 0.05$) whereas the difference between the mean values in men was negligible (NC men, 2.4 ± 1.3; control men, 2.7 ± 0.8, t = 1.05, NS). The single HC woman in the study had a high to normal amount of urine citrate.

The 24 hour urinary calcium was higher in SF subjects than controls. When the SF subjects are separated into NC and HC groups then the 24 hour urinary calcium excretion in the HC group was twice that of the NC and control groups (HC, 9.4 ± 2.2; NC, 4.4 ± 1.9, t = –8.45, $p < 0.001$; control, 4.4 ± 2.6, t = –7.17, $p < 0.001$). In both men and women separately, HC subjects had greater 24 hour calcium than the controls or NC subjects.

Women were found to have lower 24 hour excretion of urea, creatinine, magnesium and oxalate than men in both control and SF subjects. In SFs alone, the SF women also had lower 24 hour excretion of sodium, phosphate and urate than SF men. These differences were not significant in the control subjects.

The 24 hour calcium excretion in control subjects correlated with 24 hour sodium (r = 0.37, $p < 0.05$), urea (r = 0.41, $p < 0.05$), magnesium (r = 0.60, $p < 0.001$) and phosphate (r = 0.52, $p < 0.005$). However, with multiple linear regression analysis of the 24 hour urine results, magnesium was found to be the only variable significantly correlated with calcium.

In control men, only the 24 hour magnesium correlated with 24 hour calcium ($r = 0.48$, $p < 0.05$). In control women, calcium correlated with magnesium ($r = 0.76$, $p < 0.01$) and phosphate ($r = 0.68$, $p < 0.05$) though phosphate was not a significant correlate when magnesium was included in the multiple linear correlation analysis.

The 24 hour calcium excretion in SF subjects correlated with 24 hour sodium ($r = 0.38$, $p < 0.005$), urea ($r = 0.40$, $p < 0.005$), creatinine ($r = 0.56$, $p < 0.001$), magnesium ($r = 0.47$, $p < 0.001$) and phosphate ($r = 0.41$, $p < 0.005$). In contrast to the control subjects, creatinine appears to be the only significant correlate with calcium after multiple linear regression analysis in SF subjects. Similarly in the SF men alone, only 24 hour creatinine excretion correlates with calcium with multiple linear regression analysis ($r = 0.49$, $p < 0.001$). In SF women, it was 24 hour phosphate which was significantly correlated with calcium ($r = 0.82$, $p < 0.01$).

The 24 hour urinary calcium excretion in all subjects combined significantly correlated with 24 hour creatinine and magnesium ($r = 0.56$, $p < 0.001$; creatinine: $t = 3.14$, $p < 0.005$; magnesium: $t = 2.98$, $p < 0.005$). In men alone, only 24 hour creatinine correlates with 24 hour calcium ($r = 0.41$, $p < 0.001$). However, in women alone, both 24 hour magnesium and 24 hour creatinine correlated with 24 hour calcium ($r = 0.79$, $p < 0.001$; magnesium: $t = 3.43$, $p < 0.005$; creatinine: $t = 2.21$, $p < 0.05$). The 24 hour creatinine correlated with BSA ($r = 0.65$, $p < 0.001$). When somatometric data was included in the multiple linear regression analysis the 24 hour calcium correlated with 24 hour magnesium and body weight ($r = 0.57$, $p < 0.001$; magnesium: $t = 3.97$, $p < 0.001$; weight: $t = 3.32$, $p < 0.005$).

Plasma electrolytes

In Table 3 are the fasting plasma (or serum) concentration of electrolytes and metabolites. No significant differences were found for fasting plasma sodium, potassium, chloride, bicarbonate, albumin, alkaline phosphatase, urate, cholesterol and triglycerides between the control and SF groups. The SF fasting glucose was slightly higher in comparison with controls (SF, 5.1 ± 0.8 mmol/l; control, 4.8 ± 0.4, t = 2.56, $p < 0.05$) but only the NC group of SF's was significantly higher (NC, 5.2 ± 0.9; control, 4.8 ± 0.4, t = 2.28, $p < 0.05$) whereas in the HC group, it was not significant (HC, 5.0 ± 0.4; control, 4.8 ± 0.4, t = 1.85, NS).

The fasting plasma creatinine was slightly raised in the patients than controls (SF, 0.09 ± 0.02 mmol/l; control, 0.08 ± 0.02, t = 2.69, $p < 0.01$) but this represents the difference of the ratio of men and women in the sample populations. The fasting plasma creatinine was lower in women than in men in all groups (women, 0.07 ± 0.01; men, 0.09 ± 0.01, t = 7.72, $p < 0.001$) as it is essentially proportional to muscle mass. The fasting plasma urate in control women was lower than in control men (women, 0.24 ± 0.09 mmol/l; men, 0.38 ± 0.08, t = 4.37, $p < 0.001$). Similarly, the SF women fasting plasma urate was lower than in SF men (SF women, 0.31 ± 0.07; SF men, 0.38 ± 0.12, t = 2.61, $p < 0.05$). The plasma alkaline phosphatase concentration was lower in women than in men (women, 58 ± 15 U/l; men, 71 ± 15, t = 3.22, $p < 0.005$). These differences between the men and the women are related to the differences in body composition.

The women patients had lower fasting plasma potassium than in the SF men (SF women, 3.6 ± 0.1 mmol/l; SF men, 4.0 ± 0.4, t = 4.76, $p < 0.001$) but in normal subjects,

TABLE 3 *Fasting plasma (or serum) concentrations of electrolytes and metabolites (mean ± SD).*

Group (*n*)	Control (31) men (20) women (11)	Normocalciuric (41) men (33) women (8)	Hypercalciuric (19) men (18) women (1)	Stone Formers (60) men (51) women (9)
sodium (mmol/l)	138 ± 2 139 ± 2 138 ± 3	139 ± 2 139 ± 2 138 ± 2	139 ± 2 139 ± 2 141	139 ± 2 139 ± 2 139 ± 2
potassium (mmol/l)	3.9 ± 0.4 3.9 ± 0.4 3.8 ± 0.2	3.9 ± 0.4 4.0 ± 0.4 3.6 ± 0.2[d]	4.0 ± 0.2 4.0 ± 0.2 3.7	3.9 ± 0.4 4.0 ± 0.4 3.6 ± 3.6[d]
chloride (mmol/l)	104 ± 3 104 ± 3 104 ± 4	104 ± 3 104 ± 2 105 ± 2	105 ± 2 104 ± 2 107	104 ± 2 104 ± 2 105 ± 2
bicarbonate (mmol/l)	25 ± 3 26 ± 3 24 ± 2[a]	26 ± 3 26 ± 3 25 ± 2	26 ± 3 27 ± 3 24	26 ± 3 26 ± 3 25 ± 2
albumin (mmol/l)	38 ± 2 38 ± 2 38 ± 2	39 ± 3 39 ± 3 37 ± 2	38 ± 2 38 ± 2 41	39 ± 2 39 ± 2 38 ± 2
alkaline phosphatase (U/l)	67 ± 18 71 ± 16 59 ± 20	69 ± 15 72 ± 15 59 ± 9[b]	68 ± 16 69 ± 16 49	67 ± 15 71 ± 15 58 ± 9[b]
glucose (mmol/l)	4.8 ± 0.4 5.0 ± 0.3 4.6 ± 0.4[a]	5.2 ± 0.9[1] 5.2 ± 0.9 5.2 ± 1.0	5.0 ± 0.4 5.1 ± 0.4 4.9	5.1 ± 0.8[1] 5.1 ± 0.8 5.2 ± 1.0
urate (mmol/l)	0.33 ± 0.11 0.38 ± 0.08 0.24 ± 0.09[d]	0.36 ± 0.10 0.37 ± 0.10 0.32 ± 0.07	0.39 ± 0.14 0.40 ± 0.14 0.23	0.37 ± 0.11 0.38 ± 0.12 0.31 ± 0.07[a]
cholesterol (mmol/l)	5.2 ± 1.0 5.2 ± 0.9 5.0 ± 1.2	5.4 ± 1.1 5.3 ± 1.1 5.8 ± 1.2	5.5 ± 1.0 5.5 ± 1.1 5.7	5.4 ± 1.1 5.4 ± 1.1 5.7 ± 1.0
triglycerides (mmol/l)	1.1 ± 0.6 1.3 ± 0.6 0.9 ± 0.5	1.6 ± 1.3 1,6 ± 1.2 1.5 ± 1.6	1.6 ± 1.9 1,6 ± 1.9 0.9	1.6 ± 1.5[1] 1.6 ± 1.5 1.4 ± 1.5
creatinine (mmol/l)	0.08 ± 0.02 0.09 ± 0.02 0.07 ± 0.01[d]	0.09 ± 0.02[1] 0.09 ± 0.01 0.07 ± 0.01[d]	0.09 ± 0.01[1] 0.09 ± 0.01 0.07	0.09 ± 0.02 0.09 ± 0.01 0.07 ± 0.01[d]
1,25-$(OH)_2$-D* (pmol/l)	86 ± 21 84 ± 23 90 ± 16	96 ± 29 98 ± 31 89 ± 20	107 ± 27[1] 107 ± 27[1] –	99 ± 28[1] 101 ± 30[1] 89 ± 20

[1, 2, 3, 4] significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.

[a, b, c, d] significantly different, women from men; $p < 0.05, 0.01, 0.005, 0.001$, respectively.

* 1,25-$(OH)_2$-vitamin D group *n*'s were 30 (11/19), 27 (7/20), 10 (0/10), and 37 (7/30), respectively (women/men).

women did not have a significantly lower plasma potassium (control women, 3.8 ± 0.2 mmol/l; control men, 3.9 ± 0.4, t = 1.48, NS).

Fasting serum 1,25-$(OH)_2$-vitamin D concentrations in HC subjects were significantly higher than in control subjects (HC, 107 ± 27 pmol/l; control, 86 ± 21, t = –2.10, $p < 0.05$) and concentrations in SF men were raised in comparison with control men (SF men, 101 ± 30; control men, 84 ± 23, t = 2.28, $p < 0.05$). However, only 4 SF subjects had 1,25-$(OH)_2$-vitamin D concentrations greater than the upper limit of the 95% confidence interval of the control subjects and only 1 of which was a HC subject. Only 2 HC subjects had concentrations below the mean of the normal values.

The fasting 1,25-$(OH)_2$-vitamin D concentration correlated with fasting serum triglyceride and urine excretion of creatinine (r = 0.42, $p < 0.005$; triglyceride: t = 2.74, $p < 0.01$; urine creatinine: t = 2.58, $p < 0.05$) which was similar in men separately (r = 0.46, $p < 0.005$; triglyceride: t = 2.60, $p < 0.05$; urine creatinine: t = 2.56, $p < 0.05$). The correlation was not significant in women subjects though the 1,25-$(OH)_2$-vitamin D did correlate with fasting urine phosphate excretion (r = 0.56, $p < 0.05$). In control subjects separately, fasting plasma urea correlated with 1,25-$(OH)_2$-vitamin D (r = 0.45, $p < 0.05$) with an additional correlate, urine potassium excretion (r = 0.56, $p < 0.01$; plasma urea: t = –2.58, $p < 0.05$; urine potassium: t = –2.13, $p < 0.05$) which was not observed in SF subjects. Male control subjects 1,25-$(OH)_2$-vitamin D concentrations correlated with corrected fasting plasma calcium (r = 0.50, $p < 0.05$) and was not observed in other subject groups.

Response to calcium load

The response in plasma to calcium loading for all subjects is shown in Table 4 and the response in urine is shown in Table 5. The response in men is shown separately in Table 6 (plasma) and Table 7 (urine) and the response in women is shown in Table 8 (plasma) and Table 9 (urine). The changes between fasting and post-load plasma and urine concentrations are also shown in Figures 1 and 2, respectively.

The fasting and post-load plasma calcium was higher in SF than normal controls (fasting: SF, 2.30 ± 0.06 mmol/l; controls, 2.23 ± 0.07, t = –4.85, $p < 0.001$; post-load: SF, 2.38 ± 0.07; controls, 2.33 ± 0.06, t = –3.66, $p < 0.001$; second post-load: SF, 2.40 ± 0.09; controls, 2.33 ± 0.07, t = –4.37, $p < 0.001$). There was no apparent difference between the NC subjects and HC subjects. The post-load plasma calcium increased by similar amounts in controls and SF (Figure 1). The uncorrected plasma calcium concentrations were similarly raised in SF (fasting control, 2.23 ± 0.08 mmol/l; fasting SF, 2.31 ± 0.08, t = 4.35, $p < 0.001$) though they were within the standard laboratory normal range (2.10-2.60) and the plasma albumin did not vary between groups (Table 3). The fasting plasma calcium in normal subjects on a free diet is 2.24 ± 0.08 (CR Dunstan, personal communication) which is similar to the control subjects.

The whole blood ionized calcium was not significantly different between controls and SF (fasting control, 1.23 ± 0.05 mmol/l; fasting SF, 1.25 ± 0.05, t = –1.94, NS). The post-load ionized calcium increased to a similar extent in all groups. There was a good correlation between corrected plasma calcium and ionized calcium (r = 0.54, r = 0.52, r = 0.49, $p < 0.001$; for fasting, first and second post-load periods, respectively). There were

TABLE 4 *Response in plasma to calcium fasting and loading (mean ± SD).*

Group (n)	Period*	Control (31)	Normocalciuric (41)	Hypercalciuric (19)	Stone Formers (60)
plasma calcium (corrected) (mmol/l)	1	2.23 ± 0.07	2.30 ± 0.06^{4}	2.29 ± 0.06^{4}	2.29 ± 0.06^{4}
	2	$2.33 \pm 0.06^{\delta}$	$2.38 \pm 0.06^{\delta,3}$	$2.39 \pm 0.08^{\delta,1}$	$2.38 \pm 0.07^{\delta,4}$
	3	$2.33 \pm 0.07^{\delta}$	$2.40 \pm 0.10^{\delta,4}$	$2.41 \pm 0.08^{\delta,3}$	$2.40 \pm 0.09^{\delta,4}$
ionized calcium (mmol/l)	1	1.23 ± 0.05	1.25 ± 0.05	1.25 ± 0.04	1.25 ± 0.05
	2	$1.27 \pm 0.05^{\delta}$	$1.29 \pm 0.06^{\delta}$	$1.30 \pm 0.05^{\delta}$	$1.29 \pm 0.06^{\delta}$
	3	$1.28 \pm 0.05^{\delta}$	$1.29 \pm 0.05^{\delta}$	$1.30 \pm 0.03^{\delta}$	$1.29 \pm 0.05^{\delta}$
plasma phosphate (mmol/l)	1	1.24 ± 0.25	1.03 ± 0.22^{4}	1.05 ± 0.20^{3}	1.03 ± 0.21^{4}
	2	$1.60 \pm 0.39^{\delta}$	$1.42 \pm 0.26^{\delta,1}$	$1.34 \pm 0.22^{\delta,3}$	$1.39 \pm 0.24^{\delta,2}$
	3	$1.51 \pm 0.23^{\delta}$	$1.36 \pm 0.24^{\delta,2}$	$1.44 \pm 0.29^{\delta}$	$1.38 \pm 0.26^{\delta,1}$
plasma urea (mmol/l)	1	4.3 ± 1.1	5.6 ± 1.6^{4}	5.5 ± 1.4^{3}	5.6 ± 1.5^{4}
	2	4.1 ± 1.2	$5.2 \pm 1.5^{\delta,3}$	$5.1 \pm 1.5^{\delta,1}$	$5.2 \pm 1.4^{\delta,4}$
	3	$3.7 \pm 1.0^{\delta}$	$4.9 \pm 1.4^{\delta,4}$	$4.9 \pm 1.3^{\delta,3}$	$4.9 \pm 1.3^{\delta,4}$
parathyroid hormone (pg/ml)	1	25 ± 11	23 ± 12	23 ± 10	23 ± 11
	2	$14 \pm 4^{\delta}$	$13 \pm 8^{\delta}$	$12 \pm 6^{\delta}$	$13 \pm 7^{\delta}$
	3	$16 \pm 10^{\gamma}$	$17 \pm 9^{\delta}$	$16 \pm 7^{\delta}$	$17 \pm 8^{\delta}$
nephrogenous cyclic AMP† (nmol/100ml GF)	1	1.9 ± 1.4	1.9 ± 1.6	1.5 ± 1.1	1.8 ± 1.4
	2	1.7 ± 1.1	2.1 ± 1.2	1.6 ± 1.5	2.0 ± 1.3
	3	1.5 ± 1.0	1.4 ± 0.8	2.1 ± 1.6	1.6 ± 1.1

$\alpha, \beta, \gamma, \delta$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$1, 2, 3, 4$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.
† nephrogenous cAMP group n's were 20, 18, 7, and 25, respectively.

no differences between men and women for blood calcium in any group.

Fasting urinary calcium was higher in HC subjects than controls ($p < 0.001$), measured either by excretion (Table 5) or clearance (Table 10). The NC subjects urinary calcium was raised though it was not significant (fasting NC, 2.2 ± 1.7 μmol/min; fasting control, 1.6 ± 1.1, t = −1.87, NS). The NC women had a lower urinary calcium excretion compared with NC men (fasting NC women, 1.4 ± 1.0; fasting NC men, 2.4 ± 1.7, t = 2.19, $p < 0.05$).

TABLE 5 *Response in urine to calcium fasting and loading (mean ± SD).*

Group (*n*)	Period*	Control (31)	Normocalciuric (41)	Hypercalciuric (19)	Stone Formers (60)
urine cyclic AMP† (nmol/100ml GF)	1	3.4 ± 1.6	3.5 ± 1.7	3.3 ± 1.8	3.4 ± 1.7
	2	2.9 ± 1.2	3.5 ± 1.3	3.1 ± 1.6	3.4 ± 1.4
	3	2.8 ± 1.3	2.8 ± 0.9	3.7 ± 2.6	3.0 ± 1.6
urine calcium (μmol/min)	1	1.6 ± 1.1	1.3 ± 1.7	3.0 ± 1.2^{4}	2.4 ± 1.6^{3}
	2	$2.5 \pm 2.0^{\delta}$	$2.9 \pm 1.8^{\delta}$	$4.9 \pm 2.0^{\delta,4}$	$3.5 \pm 2.1^{\delta,1}$
	3	$3.9 \pm 2.5^{\delta}$	$4.0 \pm 2.3^{\delta}$	$6.0 \pm 2.3^{\delta,3}$	$4.6 \pm 2.4^{\delta}$
urine phosphate (μmol/min)	1	11 ± 7	11 ± 6	13 ± 5	12 ± 6
	2	12 ± 5	$13 \pm 6^{\gamma}$	$14 \pm 5^{\delta}$	$13 \pm 6^{\gamma}$
	3	13 ± 5	12 ± 5	$16 \pm 7^{\delta}$	$13 \pm 6^{\alpha}$
urine urea (μmol/min)	1	290 ± 75	370 ± 106^{4}	366 ± 95^{2}	369 ± 102^{4}
	2	279 ± 63	$339 \pm 82^{\alpha,4}$	349 ± 99^{1}	$342 \pm 87^{\alpha,4}$
	3	290 ± 119	$309 \pm 76^{\delta}$	321 ± 129	$313 \pm 94^{\delta}$
urine creatinine (μmol/min)	1	9.8 ± 2.4	11.8 ± 7.9	14.6 ± 8.1^{1}	12.7 ± 8.0^{1}
	2	9.2 ± 2.4	$9.3 \pm 1.9^{\alpha}$	$10.3 \pm 3.1^{\alpha}$	$9.6 \pm 2.4^{\beta}$
	3	9.3 ± 3.1	$8.9 \pm 2.4^{\alpha}$	$10.2 \pm 3.1^{\alpha}$	$9.3 \pm 2.7^{\delta}$
urine sodium (μmol/min)	1	82 ± 50	109 ± 59^{1}	85 ± 40	102 ± 55
	2	97 ± 77	$121 \pm 60^{\alpha}$	$108 \pm 55^{\alpha}$	$117 \pm 58^{\gamma}$
	3	$139 \pm 98^{\delta}$	$138 \pm 65^{\delta}$	$119 \pm 56^{\beta}$	$132 \pm 62^{\delta}$
urine potassium (μmol/min)	1	83 ± 37	77 ± 30	98 ± 44	84 ± 36
	2	$65 \pm 33^{\delta}$	$56 \pm 24^{\delta}$	$80 \pm 43^{\alpha}$	$64 \pm 33^{\delta}$
	3	$56 \pm 32^{\delta}$	$47 \pm 19^{\delta}$	$71 \pm 39^{\alpha}$	$55 \pm 29^{\delta}$
urine pH	1	6.7 ± 0.6	6.5 ± 0.6	6.8 ± 0.5	6.6 ± 0.6
	2	6.7 ± 0.5	6.6 ± 0.5	6.7 ± 0.6	6.6 ± 0.5
	3	6.7 ± 0.5	6.6 ± 0.5	6.7 ± 0.5	6.6 ± 0.5

$^{\alpha, \beta, \gamma, \delta}$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{1, 2, 3, 4}$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.
† urine cAMP group *n*'s were 20, 18, 7, and 25, respectively.

There was a weak correlation between the calcium in the urine and calcium in the blood for all time periods and for both corrected total and ionized calcium (corrected plasma calcium *vs* urine calcium: r = 0.24, $p < 0.05$; r = 0.30, $p < 0.005$; r = 0.16, $p < 0.001$; ionized calcium *vs* urine calcium: r = 0.26, $p < 0.05$; r = 0.23, $p < 0.05$; r = 0.33, $p < 0.005$; for fasting, first and second post-load periods, respectively). All groups had

TABLE 6 *Response in plasma to calcium fasting and loading in men (mean ± SD).*

Group (n)	Period*	Control (20)	Normocalciuric (33)	Hypercalciuric (18)	Stone Formers (51)
plasma calcium (corrected) (mmol/l)	1	2.22 ± 0.05	2.31 ± 0.06^{4}	2.29 ± 0.06^{4}	2.30 ± 0.06^{4}
	2	$2.32 \pm 0.06^{\delta}$	$2.38 \pm 0.06^{\delta,3}$	$2.39 \pm 0.08^{\delta,2}$	$2.38 \pm 0.07^{\delta,4}$
	3	$2.32 \pm 0.06^{\delta}$	$2.41 \pm 0.10^{\delta,4}$	$2.41 \pm 0.08^{\delta,3}$	$2.41 \pm 0.09^{\delta,4}$
ionized calcium (mmol/l)	1	1.24 ± 0.04	1.25 ± 0.05	1.25 ± 0.04	1.25 ± 0.05
	2	$1.27 \pm 0.04^{\delta}$	$1.29 \pm 0.06^{\delta}$	$1.30 \pm 0.05^{\delta}$	$1.29 \pm 0.06^{\delta}$
	3	$1.28 \pm 0.04^{\delta}$	$1.29 \pm 0.04^{\delta}$	$1.30 \pm 0.04^{\delta}$	$1.29 \pm 0.04^{\delta}$
plasma phosphate (mmol/l)	1	1.24 ± 0.28	1.02 ± 0.23^{2}	1.03 ± 0.19^{1}	1.02 ± 0.22^{2}
	2	$1.59 \pm 0.44^{\gamma}$	$1.42 \pm 0.24^{\delta}$	$1.33 \pm 0.22^{\delta}$	$1.39 \pm 0.24^{\delta}$
	3	$1.50 \pm 0.24^{\gamma}$	$1.36 \pm 0.26^{\delta}$	$1.40 \pm 0.25^{\delta}$	$1.37 \pm 0.26^{\delta}$
plasma urea (mmol/l)	1	4.6 ± 1.0	5.7 ± 1.7^{2}	5.6 ± 1.4^{1}	5.7 ± 1.6^{3}
	2	4.5 ± 1.2	$5.3 \pm 1.6^{\delta}$	$5.2 \pm 1.4^{\delta}$	$5.3 \pm 1.5^{\delta,1}$
	3	$4.1 \pm 1.0^{\delta}$	$5.0 \pm 1.5^{\delta,3}$	$5.0 \pm 1.3^{\delta,1}$	$5.0 \pm 1.4^{\delta,3}$
parathyroid hormone (pg/ml)	1	26 ± 12	23 ± 11	23 ± 10	23 ± 11
	2	$13 \pm 5^{\delta}$	$12 \pm 6^{\delta}$	$12 \pm 6^{\delta}$	$12 \pm 6^{\delta}$
	3	$17 \pm 10^{\beta}$	$17 \pm 8^{\delta}$	$16 \pm 8^{\delta}$	$17 \pm 8^{\delta}$
nephrogenous cyclic AMP† (nmol/100ml GF)	1	1.8 ± 1.3	2.2 ± 1.8	1.5 ± 1.1	1.9 ± 1.6
	2	1.5 ± 1.2	2.4 ± 1.3	1.6 ± 1.5	2.1 ± 1.4
	3	1.4 ± 1.6	1.5 ± 0.8	2.1 ± 1.6	1.8 ± 1.2

$\alpha, \beta, \gamma, \delta$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$1, 2, 3, 4$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.
† nephrogenous cAMP group n's were 20, 18, 7, and 25, respectively.

significant increases in urinary calcium after load. In all groups combined, the increase in urinary calcium after load correlated with the increase in plasma calcium ($r = 0.24$, $p < 0.05$; $r = 0.31$, $p < 0.005$; for the first and second post-load periods, respectively). The HC subjects post-load increase in urinary calcium excretion was significantly greater than the increase in control subjects (HC, 1.92 ± 1.95 μmol/min; control, 0.90 ± 1.27, $z = -2.34$, $p < 0.02$).

Plasma phosphate was lower in the SF than in controls during all time periods and the post-load increment increase of plasma phosphate was similar in all groups (Table 4 and

TABLE 7 *Response in urine to calcium fasting and loading in men (mean ± SD).*

Group (*n*)	Period*	Control (20)	Normocalciuric (33)	Hypercalciuric (18)	Stone Formers (51)
urine cyclic AMP† (nmol/100ml GF)	1	3.2 ± 1.5	4.0 ± 2.0	3.3 ± 1.8	3.7 ± 1.9
	2	2.6 ± 1.3	3.7 ± 1.1^{1}	3.1 ± 1.6	3.4 ± 1.3
	3	2.9 ± 1.4	3.0 ± 1.1	3.7 ± 2.6	3.2 ± 1.8
urine calcium (μmol/min)	1	1.7 ± 1.0	2.4 ± 1.7	3.0 ± 1.2^{4}	2.6 ± 1.6^{2}
	2	2.5 ± 2.2$^{\alpha}$	3.0 ± 2.0$^{\beta}$	4.8 ± 2.1$^{\delta,3}$	3.7 ± 2.2$^{\delta}$
	3	3.7 ± 2.1$^{\delta}$	4.1 ± 2.4$^{\delta}$	6.0 ± 2.3$^{\delta,3}$	3.8 ± 1.6$^{\delta}$
urine phosphate (μmol/min)	1	10 ± 6	11 ± 6	13 ± 5	12 ± 6
	2	11 ± 5	13 ± 6$^{\beta}$	14 ± 5$^{\gamma}$	14 ± 6$^{\gamma}$
	3	14 ± 6	13 ± 4	16 ± 7$^{\alpha}$	14 ± 5$^{\alpha}$
urine urea (μmol/min)	1	239 ± 77	381 ± 105^{3}	370 ± 96	377 ± 101
	2	291 ± 64	342 ± 83$^{\alpha,1}$	348 ± 102$^{\beta}$	344 ± 89$^{\beta,2}$
	3	314 ± 111	319 ± 75$^{\gamma}$	323 ± 132	321 ± 98$^{\delta}$
urine creatinine (μmol/min)	1	10.5 ± 2.5	13.2 ± 8.2	15.0 ± 8.1^{1}	13.8 ± 8.1^{1}
	2	10.1 ± 2.5	9.8 ± 1.6$^{\alpha}$	10.3 ± 3.2$^{\gamma}$	10.0 ± 2.3$^{\gamma}$
	3	10.7 ± 2.7	9.6 ± 2.2$^{\alpha}$	10.2 ± 3.2$^{\delta}$	9.9 ± 2.6$^{\delta}$
urine sodium (μmol/min)	1	77 ± 45	111 ± 59^{1}	84 ± 42	102 ± 55
	2	87 ± 73	120 ± 64	101 ± 48$^{\alpha}$	113 ± 59$^{\alpha}$
	3	124 ± 71$^{\gamma}$	135 ± 65$^{\gamma}$	113 ± 52$^{\delta}$	127 ± 61$^{\delta}$
urine potassium (μmol/min)	1	79 ± 34	82 ± 29	98 ± 45	88 ± 36
	2	69 ± 33	59 ± 24$^{\delta}$	81 ± 44$^{\delta}$	66 ± 34$^{\delta}$
	3	66 ± 32	48 ± 20$^{\delta,1}$	73 ± 40$^{\delta}$	57 ± 30$^{\delta}$
urine pH	1	6.7 ± 0.6	6.5 ± 0.6	6.8 ± 0.5	6.6 ± 0.6
	2	6.7 ± 0.5	6.5 ± 0.5	6.7 ± 0.6	6.6 ± 0.5
	3	6.7 ± 0.6	6.5 ± 0.5	6.7 ± 0.5	6.6 ± 0.5

$^{\alpha, \beta, \gamma, \delta}$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{1, 2, 3, 4}$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.
† urine cAMP group *n*'s were 12, 11, 7, and 18, respectively.

Figure 1). There was no difference between the NC and HC groups. Urinary phosphate clearance was higher in the HC group than the control subjects for all time periods (fasting HC, 0.105 ± 0.052 mmol phosphate/mmol creatinine; fasting control, 0.075 ± 0.046, t = −2.10, $p < 0.05$) though the total phosphate excretion rates were similar (fasting HC, 12.7 ± 5.4 μmol/min; fasting control, 11.0 ± 6.8, t = −0.98, NS). In SF subjects, the phosphate

TABLE 8 *Response in plasma to calcium fasting and loading in women (mean ± SD).*

Group (*n*)	Period*	Control (11)	Normocalciuric (8)	Hypercalciuric (1)	Stone Formers (9)
plasma calcium (corrected) (mmol/l)	1	2.24 ± 0.09	2.27 ± 0.07	2.19	2.27 ± 0.07
	2	$2.35 \pm 0.06^{\delta}$	$2.39 \pm 0.07^{\delta}$	2.33	$2.38 \pm 0.07^{\delta}$
	3	$2.34 \pm 0.08^{\beta}$	$2.36 \pm 0.08^{\alpha}$	2.47	$2.38 \pm 0.08^{\beta}$
ionized calcium (mmol/l)	1	1.22 ± 0.06	1.24 ± 0.07	1.21	1.24 ± 0.07
	2	$1.27 \pm 0.06^{\delta}$	$1.29 \pm 0.06^{\alpha}$	1.25	$1.28 \pm 0.06^{\beta}$
	3	$1.28 \pm 0.07^{\delta}$	$1.30 \pm 0.07^{\gamma}$	1.27	$1.30 \pm 0.07^{\delta}$
plasma phosphate (mmol/l)	1	1.26 ± 0.18	1.06 ± 0.19^{1}	1.30	1.09 ± 0.20
	2	$1.62 \pm 0.29^{\beta}$	$1.38 \pm 0.31^{\gamma}$	1.52	$1.40 \pm 0.29^{\delta}$
	3	$1.53 \pm 0.22^{\delta}$	$1.33 \pm 0.10^{\alpha}$	2.13	$1.42 \pm 0.28^{\beta}$
plasma urea (mmol/l)	1	3.7 ± 0.9^{a}	5.2 ± 0.7^{4}	3.7	5.0 ± 0.8^{3}
	2	3.4 ± 0.9^{b}	$4.7 \pm 0.8^{\delta,2}$	3.3	$4.6 \pm 0.9^{\delta,1}$
	3	$3.1 \pm 0.8^{\alpha,b}$	$4.2 \pm 0.7^{\delta,3,a}$	3.1	$4.1 \pm 0.7^{\delta,2,b}$
parathyroid hormone (pg/ml)	1	21 ± 8	25 ± 14	21	25 ± 13
	2	$14 \pm 4^{\beta}$	$16 \pm 13^{\delta}$	11	$15 \pm 12^{\delta}$
	3	16 ± 10	$18 \pm 14^{\alpha}$	10	$17 \pm 13^{\alpha}$
nephrogenous cyclic AMP† (nmol/100ml GF)	1	2.1 ± 1.5	1.4 ± 0.9	-	1.4 ± 0.9
	2	1.9 ± 0.9	1.7 ± 1.1	-	1.7 ± 1.0
	3	1.7 ± 1.0	1.3 ± 0.6	-	1.3 ± 0.6

$\alpha, \beta, \gamma, \delta$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
1, 2, 3, 4 significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
a, b, c, d significantly different, women from men; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.
† nephrogenous cAMP group *n*'s were 20, 18, 7, and 25, respectively.

excretion increased after load though, in control subjects, the change was not significant. In all subjects, there was little change to fasting phosphate clearance with calcium load. The theorectical renal phosphate threshold (Tm_{PO_4}/GFR) is lower in SF than in controls for all periods (Table 10, 11 and 12). A similar increment in Tm_{PO_4}/GFR was observed within all subject groups after the calcium challenge. There was no difference between men and women for plasma phosphate, urine phosphate excretion, clearance and Tm_{PO_4}/GFR. The Tm_{PO_4}/GFR was greater in the only HC woman in the study than in control subjects in all time periods.

TABLE 9 *Response in urine to calcium fasting and loading in women (mean ± SD).*

Group (n)	Period*	Control (11)	Normocalciuric (8)	Hypercalciuric (1)	Stone Formers (9)
urine cyclic AMP† (nmol/100ml GF)	1	3.7 ± 1.6	2.8 ± 0.6	-	2.8 ± 0.6
	2	3.2 ± 1.0	3.2 ± 1.6	-	3.2 ± 1.6
	3	2.7 ± 1.1	2.5 ± 0.6	-	2.5 ± 0.6
urine calcium (μmol/min)	1	1.3 ± 1.1	1.4 ± 1.0^{a}	1.7	1.4 ± 0.9^{c}
	2	2.3 ± 1.6$^{\gamma}$	2.3 ± 0.9$^{\alpha}$	6.1	2.7 ± 1.5$^{\alpha}$
	3	4.2 ± 3.2$^{\beta}$	3.5 ± 1.3$^{\gamma}$	6.6	3.8 ± 1.6$^{\gamma}$
urine phosphate (μmol/min)	1	12 ± 8	9 ± 5	6	9 ± 4
	2	12 ± 4	10 ± 3	10	10 ± 3^{a}
	3	10 ± 4	8 ± 5	4	8 ± 4^{b}
urine urea (μmol/min)	1	285 ± 73	327 ± 102	300	324 ± 96
	2	258 ± 56$^{\alpha}$	327 ± 78	375	332 ± 75^{1}
	3	247 ± 126	267 ± 63$^{\alpha}$	283	268 ± 60$^{\alpha,1}$
urine creatinine (μmol/min)	1	8.4 ± 1.6^{b}	6.2 ± 2.2^{1,d}	7.5	6.3 ± 2.1^{1,d}
	2	7.4 ± 1.0^{d}	7.1 ± 1.5^{d}	10.0	7.4 ± 1.7^{c}
	3	6.8 ± 1.9^{d}	6.4 ± 1.1^{d}	9.2	6.7 ± 1.4^{d}
urine sodium (μmol/min)	1	91 ± 61	101 ± 61	98	101 ± 57
	2	114 ± 86	125 ± 47	227	137 ± 55
	3	166 ± 133$^{\alpha}$	150 ± 67	220	158 ± 67$^{\alpha}$
urine potassium (μmol/min)	1	90 ± 42	53 ± 20^{1,c}	104	59 ± 25^{a}
	2	59 ± 34^{d}	44 ± 19	68	47 ± 19$^{\alpha,a}$
	3	41 ± 25$^{\gamma,a}$	40 ± 14$^{\alpha}$	38	39 ± 13$^{\alpha,b}$
urine pH	1	6.7 ± 0.4	6.6 ± 0.5	7.0	6.7 ± 0.4
	2	6.8 ± 0.4	6.7 ± 0.3	7.0	6.8 ± 0.3
	3	6.9 ± 0.4	6.9 ± 0.2$^{\alpha,b}$	6.8	6.9 ± 0.2^{b}

$^{\alpha, \beta, \gamma, \delta}$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{1, 2, 3, 4}$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{a, b, c, d}$ significantly different, women from men; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.
† urine cAMP group n's were 8, 7, 0, and 7, respectively.

Fasting plasma phosphate did not correlate with fasting urinary excretion of phosphate. Despite the presence of hypophosphataemia and raised 1,25-$(OH)_2$-vitamin D in SF subjects, plasma phosphate and/or Tm_{PO_4}/GFR were not significantly correlated with serum 1,25-$(OH)_2$-vitamin D. There were no correlations observed in SF subjects alone, control subjects alone or all subjects combined.

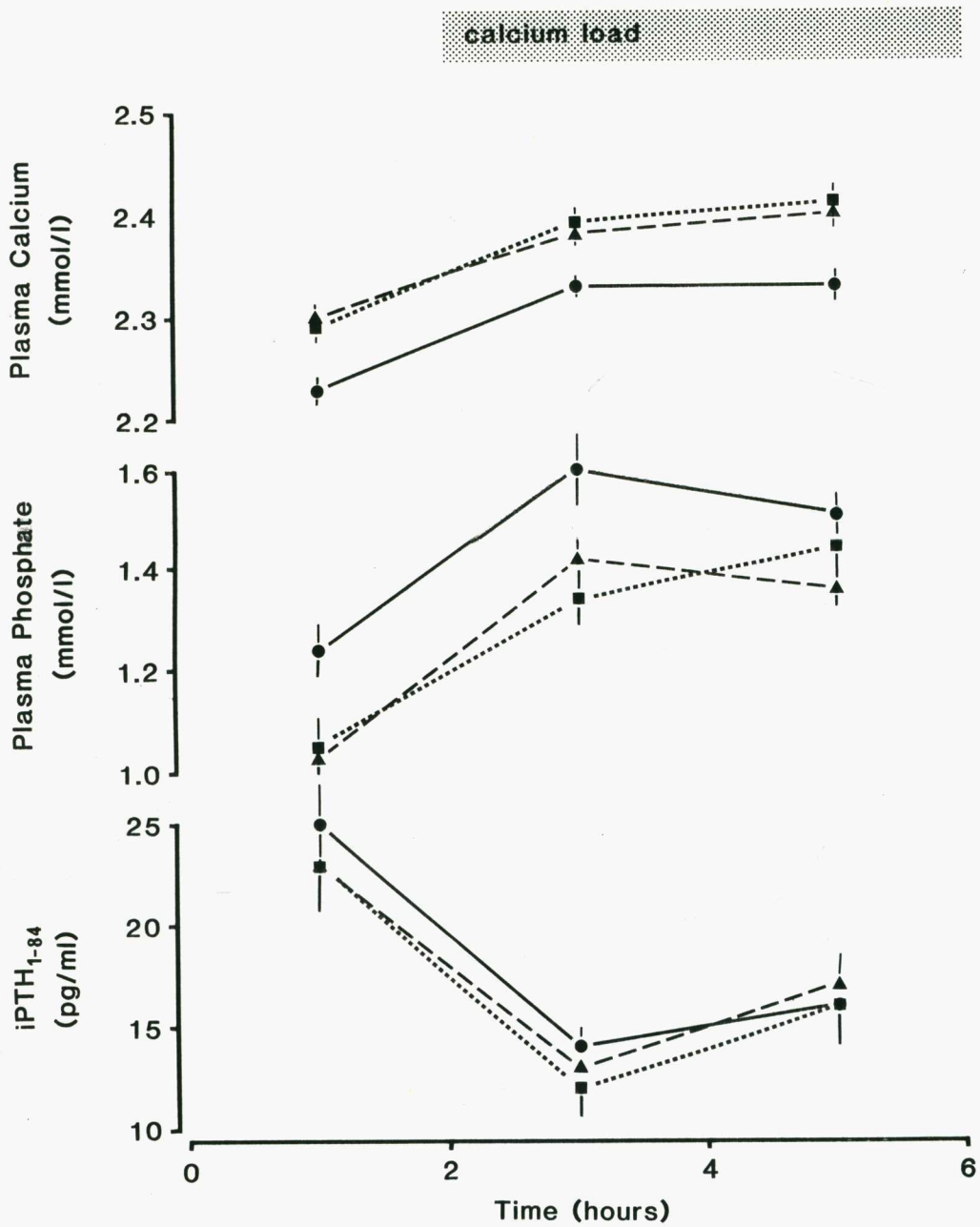

FIGURE 1. Mean plasma calcium, phosphate and intact PTH_{1-84} concentrations before and after oral calcium load. The closed circles and solid lines represent the control subjects, the closed triangles and dashed lines represent the NC subjects and the closed squares and dotted lines represent the mean of the HC subjects. The vertical bars represent 1 standard error from the mean.

The plasma urea was higher in SF than in controls in all time periods (fasting SF, 5.6 ± 1.6 mmol/l; fasting controls, 4.3 ± 1.1, t = –4.75, $p < 0.001$) as was the urinary urea (fasting SF, 369 ± 102 μmol/min; fasting controls, 290 ± 75, t = –4.21, $p < 0.001$). In the control subjects, the plasma urea was only reduced in the third period (fasting, 4.3 ± 1.1 mmol/l; second post-load, 3.7 ± 1.0, t = 5.64, $p < 0.001$) whereas in SF subjects, the reduction was in the second period (fasting, 5.6 ± 1.5; post-load, 5.2 ± 1.5, t = 12.11, $p < 0.001$). Control women had lower plasma urea than the men though the urinary urea was not significantly different. The changes in urinary urea excretion with load in control subjects was not significant except in control women where the urea was reduced (fasting, 285 ± 73; post-load, 258 ± 56, t = 2.39, $p < 0.05$). In SF subjects, urinary urea was significantly lower after the calcium load.

The urinary excretion of urea correlated with plasma urea in all time periods (fasting, r = 0.54, $p < 0.001$; first post-load, r = 0.54, $p < 0.001$; second post-load, r = 0.43, $p < 0.001$). It was found with multiple linear regression analysis that plasma creatinine correlated with plasma urea in addition to urinary urea excretion (fasting, r = 0.69, $p < 0.001$; plasma creatinine: t = 5.64, $p < 0.001$; urine urea: t = 5.39, $p < 0.001$). The urinary urea excretion with multiple linear regression analysis was found to be correlated with plasma urea, urinary calcium and phosphate excretion (fasting, r = 0.69, $p < 0.001$; plasma urea: t = 6.73, $p < 0.001$; urine calcium: t = 4.23, $p < 0.001$; urine phosphate: t = 2.76, $p < 0.01$). In control subjects alone, only plasma urea and urine phosphate correlated with urine urea (fasting, r = 0.62, $p < 0.001$; plasma urea: t = 3.80, $p < 0.001$; urine phosphate: t = 3.52, $p < 0.005$) whereas in SF subjects alone, only plasma urea and urine calcium correlated with urine urea (fasting, r = 0.62, $p < 0.001$; plasma urea: t = 4.66, $p < 0.001$;

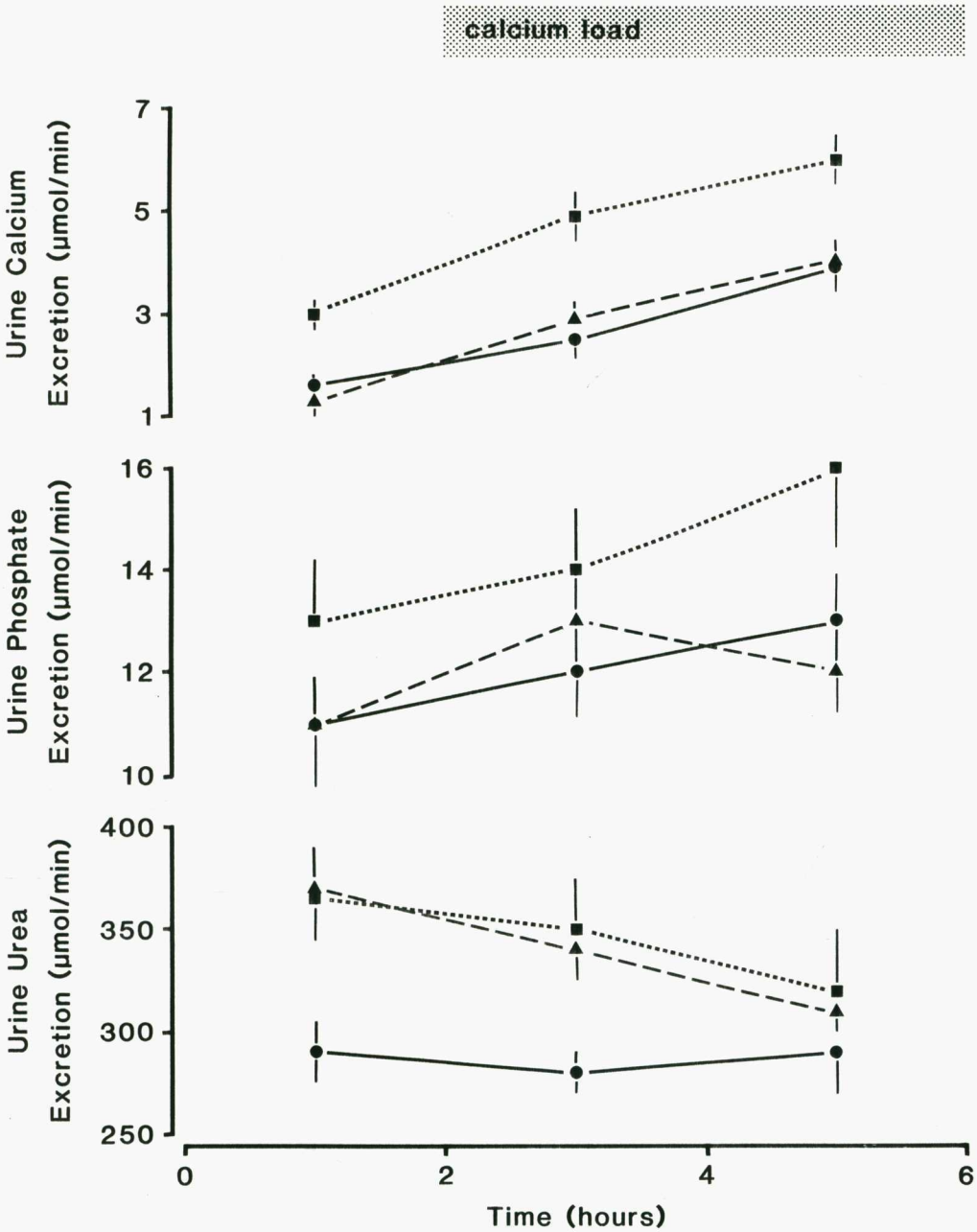

FIGURE 2. Mean urine calcium, phosphate and urea excretion rates before and after oral calcium load. The closed circles and solid lines represent the control subjects, the closed triangles and dashed lines represent the NC subjects and the closed squares and dotted lines represent the HC subjects. The vertical bars represent 1 standard error from the mean.

TABLE 10 *Renal clearance and Tm_{PO_4}/GFR in response to calcium fasting and loading (mean ± SD).*

Group (*n*)	Period*	Control (31)	Normocalciuric (41)	Hypercalciuric (19)	Stone Formers (60)
C_{Ca}/C_{creat}	1	0.006 ± 0.004	0.008 ± 0.004	0.011 ± 0.004^{4}	0.009 ± 0.005^{2}
	2	0.010 ± 0.010$^{\gamma}$	0.011 ± 0.006$^{\delta}$	0.018 ± 0.006$^{\delta,4}$	0.013 ± 0.007$^{\delta}$
	3	0.015 ± 0.011$^{\delta}$	0.016 ± 0.007$^{\delta}$	0.022 ± 0.007$^{\delta,3}$	0.018 ± 0.008$^{\delta}$
C_{PO_4}/C_{creat}	1	0.075 ± 0.046	0.107 ± 0.086^{1}	0.105 ± 0.052^{1}	0.106 ± 0.076^{1}
	2	0.080 ± 0.070	0.100 ± 0.093	0.099 ± 0.040^{1}	0.100 ± 0.080
	3	0.069 ± 0.026	0.094 ± 0.063^{1}	0.104 ± 0.045^{1}	0.097 ± 0.058^{1}
Tm_{PO_4}/GFR (mmol/l)	1	1.38 ± 0.36	1.06 ± 0.30^{4}	1.07 ± 0.27^{3}	1.06 ± 0.28^{4}
	2	1.78 ± 0.64$^{\beta}$	1.49 ± 0.41$^{\delta,1}$	1.37 ± 0.33$^{\delta,2}$	1.45 ± 0.38$^{\beta}$
	3	1.68 ± 0.34	1.43 ± 0.34$^{\delta,3}$	1.47 ± 0.48$^{\delta}$	1.44 ± 0.39^{3}
C_{creat} (GFR) (ml/min)		120 ± 25	117 ± 39	126 ± 32	120 ± 37

$^{\alpha, \beta, \gamma, \delta}$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{1, 2, 3, 4}$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.

urine calcium: $t = 4.44$, $p < 0.001$).

The serum parathyroid hormone ($iPTH_{1-84}$) concentrations were the same in all subject groups (Table 4 and Figure 1). There was no difference between men and women in serum $iPTH_{1-84}$ concentrations (Table 6 and 8). In all subjects, there was a decrease in $iPTH_{1-84}$ after the calcium load (fasting subjects, 23.7 ± 10.9 pg/ml; post-load, 12.9 ± 6.4, $t = -12.48$, $p < 0.001$; second post-load, 16.7 ± 8.8, $t = -7.19$, $p < 0.001$).

Fasting blood calcium (corrected total or ionized) did not correlate with $iPTH_{1-84}$ when all subjects were combined or separately (controls and SF). However, the second post-load total calcium concentrations correlated with second post-load $iPTH_{1-84}$ for all subjects ($r = -0.42$, $p < 0.001$), controls alone ($r = -0.51$, $p < 0.005$) and SF subjects alone ($r = -0.45$, $p < 0.01$). The decrease in $iPTH_{1-84}$ after calcium load correlated with the increase in corrected total plasma calcium (Figure 3) (post-load, $r = -0.41$, $p < 0.001$;

TABLE 11 *Renal clearance and Tm_{PO_4}/GFR of men in response to calcium fasting and loading (mean ± SD).*

Group (*n*)	Period*	Control (20)	Normocalciuric (33)	Hypercalciuric (18)	Stone Formers (51)
C_{Ca}/C_{creat}	1	0.007 ± 0.005	0.008 ± 0.005	0.011 ± 0.004^{3}	0.009 ± 0.005
	2	0.010 ± 0.012	$0.011 \pm 0.006^{\delta}$	$0.018 \pm 0.007^{\delta,1}$	$0.014 \pm 0.007^{\delta}$
	3	$0.013 \pm 0.009^{\gamma}$	$0.016 \pm 0.008^{\delta}$	$0.023 \pm 0.007^{\delta,4}$	$0.018 \pm 0.009^{\delta,1}$
C_{PO_4}/C_{creat}	1	0.071 ± 0.041	0.109 ± 0.092	0.109 ± 0.050^{1}	0.109 ± 0.079^{1}
	2	0.082 ± 0.083	0.106 ± 0.101	0.102 ± 0.040^{1}	0.104 ± 0.084
	3	0.071 ± 0.024	0.101 ± 0.067^{1}	0.109 ± 0.041^{3}	0.104 ± 0.059^{3}
Tm_{PO_4}/GFR (mmol/l)	1	1.39 ± 0.38	1.04 ± 0.31^{3}	1.04 ± 0.24^{3}	1.04 ± 0.28^{3}
	2	$1.78 \pm 0.75^{\alpha}$	$1.48 \pm 0.41^{\delta}$	$1.35 \pm 0.32^{\delta,1}$	$1.44 \pm 0.39^{\delta}$
	3	1.65 ± 0.35	$1.41 \pm 0.37^{\delta,1}$	$1.39 \pm 0.34^{\delta,1}$	$1.40 \pm 0.36^{\delta,2}$
C_{creat} (GFR) (ml/min)		126 ± 25	123 ± 40	127 ± 33	124 ± 37

$^{\alpha, \beta, \gamma, \delta}$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{1, 2, 3, 4}$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.

second post-load, $r = -0.32$, $p < 0.005$). Increased ionized calcium in control subjects in the second post-load period correlated with the decrease in $iPTH_{1-84}$ ($r = -0.37$, $p < 0.05$). There was no significant correlation between change of ionized calcium and $iPTH_{1-84}$ in SF subjects. In controls, the post-load urine calcium excretion correlated with post-load $iPTH_{1-84}$ ($r = -0.39$, $p < 0.05$). In SF subjects, both the first and second post-load urine calcium correlated with $iPTH_{1-84}$ (first post-load: $r = -0.37$, $p < 0.005$; second post-load: $r = -0.35$, $p < 0.01$). The change in urine calcium after load did not correlate with the change in $iPTH_{1-84}$ concentration.

Fasting plasma alkaline phosphatase correlated with fasting $iPTH_{1-84}$ ($r = 0.27$, $p < 0.05$) but not after the calcium load. This correlation was not observed when control subjects were examined separately. Fasting plasma urate was found, with multiple linear regression analysis, to be an additional correlate with alkaline phosphatase to $iPTH_{1-84}$ (r

TABLE 12 *Renal clearance and Tm_{PO_4}/GFR of women in response to calcium fasting and loading (mean ± SD).*

Group (n)	Period*	Control (11)	Normocalciuric (8)	Hypercalciuric (1)	Stone Formers (9)
C_{Ca}/C_{creat}	1	0.005 ± 0.003	0.006 ± 0.004	0.007	0.006 ± 0.003
	2	$0.009 \pm 0.006^{\delta}$	$0.009 \pm 0.003^{\gamma}$	0.021	$0.010 \pm 0.005^{\alpha}$
	3	$0.017 \pm 0.013^{\gamma}$	$0.015 \pm 0.004^{\delta}$	0.021	$0.016 \pm 0.004^{\delta}$
C_{PO_4}/C_{creat}	1	0.082 ± 0.055	0.098 ± 0.057	0.038	0.091 ± 0.057
	2	0.076 ± 0.038	0.079 ± 0.045	0.053	0.076 ± 0.043
	3	0.066 ± 0.029	0.062 ± 0.032^{a}	0.016	$0.057 \pm 0.034^{\alpha,c}$
Tm_{PO_4}/GFR (mmol/l)	1	1.36 ± 0.33	1.10 ± 0.26	1.62	1.16 ± 0.30
	2	$1.78 \pm 0.40^{\beta}$	$1.51 \pm 0.41^{\beta}$	1.78	$1.54 \pm 0.39^{\beta}$
	3	1.73 ± 0.38	$1.52 \pm 0.18^{\beta}$	2.93	$1.68 \pm 0.50^{\beta}$
C_{creat} (GFR) (ml/min)		109 ± 22	94 ± 22	121	97 ± 22

$^{\alpha, \beta, \gamma, \delta}$ significantly different from fasting level; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{1, 2, 3, 4}$ significantly different from control group; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
$^{a, b, c, d}$ significantly different, women from men; $p < 0.05, 0.01, 0.005, 0.001$, respectively.
* Periods 1, 2 and 3 refer to 2 h fasting, first 2 h post-calcium load, and second 2 h post-calcium load periods, respectively.

= 0.34, $p < 0.01$; alkaline phosphatase: t = 2.29, $p < 0.05$; urate: t = 2.03, $p < 0.05$).

Nephrogenous and urinary cAMP were similar in all subjects and did not differ between men and women. In contrast to $iPTH_{1\text{-}84}$, there was no significant decrease in cAMP with calcium load. Fasting nephrogenous cAMP in control subjects correlated weakly with fasting $iPTH_{1\text{-}84}$ ($r = -0.45$, $p < 0.05$). After calcium load, there was no correlation between nephrogenous cAMP and $iPTH_{1\text{-}84}$. However, the change of nephrogenous cAMP after calcium load correlated with the change in $iPTH_{1\text{-}84}$ ($r = -0.75$, $p < 0.001$) and with the change in plasma calcium ($r = -0.62$, $p < 0.01$). In SF subjects alone, fasting nephrogenous cAMP correlated with fasting plasma calcium ($r = 0.46$, $p < 0.05$) and in NC subjects alone, the correlation between the change in nephrogenous cAMP and the change in plasma calcium was significant ($r = 0.53$, $p < 0.05$).

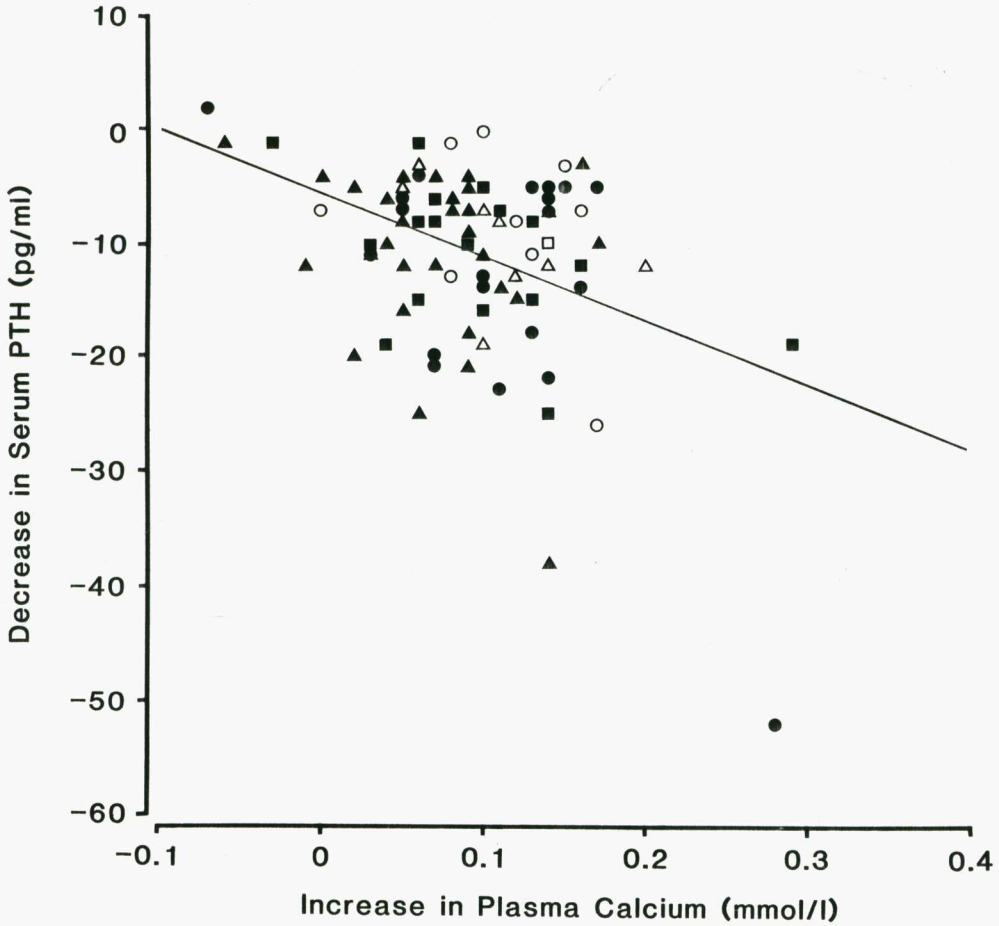

FIGURE 3. Decrease of serum intact PTH_{1-84} with increase of plasma calcium from fasting concentrations 1 hour after oral calcium load. The closed and open circle represent control men and women, repectively; the closed and open triangles represent NC men and women, respectively; and the closed and open squares represent HC men and women, repectively. There is a significant linear correlation between the fall in $iPTH_{1-84}$ and rise in plasma calcium ($r = -0.41, p < 0.001$).

Mean urinary creatinine excretion of control subjects did not change with calcium load (Table 5). Control women had a lower urinary excretion of creatinine than control men (Table 7 and 9). In male SF subjects, there was a significant reduction of urine creatinine after calcium load (fasting, 13.8 ± 8.1 μmol/min; post-load, 10.0 ± 2.3, t = −3.47, $p <$ 0.001) and male HC subjects had a significantly higher fasting creatinine excretion than male controls (fasting HC, 15.0 ± 8.1 μmol/min; fasting control, 10.5 ± 2.5, t = −2.24, $p <$ 0.05).

Urinary sodium excretion increased after load, in the second calcium post-load period in controls, and in the first period in SF subjects (Table 5). The NC subjects had a greater fasting urine sodium excretion than controls (NC, 109 ± 59 μmol/min; control, 82 ± 50, t = 2.12, $p < 0.05$) which was not significant in the NC women. Sodium excretion correlates with calcium excretion (fasting, r = 0.27, $p < 0.01$) when all subjects are combined but not when controls and SF's are examined separately. Multiple linear regression of fasting urine calcium excretion found urea, sodium and creatinine excretion to be significant correlates (r = 0.48, $p < 0.001$; urea: t = 2.51, $p < 0.05$; sodium: t = 2.80, $p < 0.01$; creatinine: t = 2.63, $p < 0.01$).

There was no change in urinary potassium excretion in control men after calcium load (Table 7) but it decreased after load in control women (Table 9). Fasting excretion were not significantly different between control men and women. Urine potassium in SF subjects decreased after calcium load (fasting, 84 ± 36 μmol/min; post-load, 64 ± 33, t = −5.33, $p < 0.001$; second post-load, 55 ± 29, t = −6.41, $p < 0.001$) and fasting excretion in NC women were lower than NC men (NC women, 53 ± 20; NC men, 83 ± 29, t = 3.35, $p <$

0.005). Fasting urine potassium correlated weakly with fasting urine calcium ($r = 0.21$, $p < 0.05$).

Urine pH was not significantly different between controls and SF subjects (Table 5) or between men and women (Table 7 and 9). There was no significant change in urine pH after calcium load except in NC women in the second post-load period where the pH was raised slightly (fasting pH, 6.6 ± 0.5; second post-load, 6.9 ± 0.2, $t = 2.52$, $p < 0.05$).

The results from the multiple linear regression show that there is a strong association between subject status (control or SF) and plasma calcium, Tm_{PO_2}/GFR and plasma urea (fasting, $r = 0.66$, $p < 0.001$; plasma calcium: $t = 4.86$, $p < 0.001$; Tm_{PO_2}/GFR: $t = -3.69$, $p < 0.001$; plasma urea: $t = 3.35$, $p < 0.005$). In the absence of Tm_{PO_2}/GFR from the analysis, plasma phosphate was a significant correlate (fasting, $r = 0.66$, $p < 0.001$; plasma calcium: $t = 4.66$, $p < 0.001$; plasma urea: $t = 3.84$, $p < 0.001$; plasma phosphate: $t = -3.55$, $p < 0.001$). In SF subjects alone, subject type (NC or HC) did not correlate with other variables apart from with 24 hour urine calcium excretion (since this was the determinate) and with an additional contribution from the 24 hour urine creatinine excretion ($r = 0.82$, $p < 0.001$; 24h urine calcium: $t = 10.40$, $p < 0.001$; 24 h urine creatinine: $t = -3.66$, $p < 0.001$). The 24 hour urine creatinine was not significant alone.

DISCUSSION

Several modalities have been proposed as the primary defect in idiopathic hypercalciuria (see Vosburgh and Peters, 1987). They include intestinal hyperabsorption of calcium, renal "leak" of calcium, primary hyperparathyroidism, renal "leak" of phosphate, and disturbance of vitamin D metabolism. In order to differentiate these disorders, investigators have used provocative testing with a calcium load and measurement of hormones such as 1,25-$(OH)_2$-vitamin D and parathyroid hormone. The tests have been used to identify subgroups of hypercalciuric subjects and separate them from normal individuals.

The findings from this study are in partial agreement with an earlier study from this laboratory (Evans *et al.*, 1984). Fasting and post-load plasma calcium concentrations are higher in SF subjects than in control subjects but there was no separation between NC and HC subjects. Plasma phosphate and renal tubular resorption of phosphate is lower in SF subjects than in control subjects. Urine calcium excretion in HC subjects is higher than control subjects but NC subjects are not different to controls. The patients did not easily fall into the classical groups of "absorptive", "renal", or "resorptive" hypercalciuria as defined by Pak (1984). It was suggested that patients with idiopathic hypercalciuria

suffered from parathyroid hyperfunction. The following Discussion will explore the different proposed pathologies in relation to the findings in this study.

Sex dependent factors

Nephrolithiasis is more common in men than in women (Rose and Harrison, 1974; Robertson, 1984). The incidence in this study is representative of this trend. There were some differences between the men and women SF subjects but they were also observed in normal subjects. Adult women, on average have a lower urinary calcium excretion than men (see Peacock, 1988; this study, Table 2). This apparently reflects a lower dietary calcium intake. Although oestrogen regulates both the rate of bone resorption and the secretion threshold for PTH and calcitriol, the five-fold changes in plasma oestrogen concentration during the menstrual cycle are not associated with changes in calcium excretion. After menopause, on the other hand, the oestrogen deficiency causes a rise in calcium excretion. Treatment with replacement oestrogen in postmenopausal women reduces calcium excretion to lower levels (see Peacock, 1988). It is probable that oestrogen's inhibition of bone resorption reduces, to some extent, the calcium excretion in women. Weitbrecht *et al.* (1987), in a study of the 24 hour urine samples from patients with recurrent idiopathic calcium urolithiasis, found that men have a higher excretion rates of lithogenic substances (calcium, uric acid and phosphate) and of inhibitory agent magnesium than women. This was found in both patients and controls and is consistent with the findings from this study. They considered that differences in diet and body weight perhaps has a greater influence on the the risk of stone formation than any action of

sex hormones on urinary constitution.

Parks and Coe (1986) reported significantly lower urinary citrate excretion in SF women relative to normal women. The urine citrate concentration can influence the likelihood of calcium oxalate crystallization in at least 2 ways. It can bind to calcium ions (reducing the supersaturation of calcium oxalate) and it reduces the growth rate of preformed calcium oxalate monohydrate seed crystals. Hypocitraturia can be successfully treated with potassium citrate (Pak and Fuller, 1986). In this study, urinary citrate excretion was low in NC women but it was high-normal in the single HC woman. In contrast, Weitbrecht *et al.* (1987) found SF women had greater urinary citrate excretion than SF men (and not different to normal women) when excretion was expressed per unit body weight. The lower incidence of SF women and the lower incidence of risk factors generally associated with stone formation in SF men together with lower body mass in women may accentuate finding of hypocitraturia in some SF women. Although it may be an important risk factor in certain individuals, hypocitraturia does not appear to be a common feature in all SF subjects.

Griffith *et al.* (1986a) examined the dietary intake of SF and normal controls in Ireland and found women SF had many nutrient intake differences from normal women which put them more at risk of stone formation, whereas SF men had fewer differences from normal men and both were apparently at some risk of stone formation. It is more than likely that some combination of diet, body mass and, to a limited extent, hormones reduces the risk of stone formation in women. Further investigation of these factors would seem warranted.

Hyperabsorption of calcium by the intestine

Many investigators have concluded that intestinal hyperabsorption of calcium is the commonest cause of idiopathic hypercalciuria (Pak *et al.*, 1974; Broadus *et al.*, 1978; Fuss *et al.*, 1978). Ingested calcium is absorbed from the intestine at the duodenum, where it is rapidly transported actively against an electrochemical gradient, and the jejunum, where the bulk of calcium absorption takes place (see Lyles and Drezner, 1981). The transport of calcium in the jejenum is by facilitated diffusion, which is enhanced by 1,25-$(OH)_2$-vitamin D. In idiopathic hypercalciuria, *in vitro* studies have shown that the jejenum uptake of calcium is higher than in normal controls (Duncombe *et al.*, 1980). It has been postulated that in idiopathic hypercalciuria, the primary increase in calcium absorption results in elevated circulating calcium (albeit usually within the normal range), causing hypercalciuria by the rise in the filtered load of calcium. Furthermore, the concurrent parathyroid suppression inhibits the renal tubular reabsorption of calcium. Following an oral calcium load the urinary calcium is high in absorptive hypercalciuria, in the face of normal serum calcium and fasting urinary calcium. The results from this study do not support this hypothesis as the primary defect in idiopathic hypercalciuria. Although the mean fasting serum 1,25-$(OH)_2$-vitamin D concentration in HC subjects is significantly greater than control subjects, only a single HC patient had a 1,25-$(OH)_2$-vitamin D concentration outside the normal range. If the primary lesion was absorptive then the post-load calcium increases would be expected to be greater than in controls or NC. Fasting plasma calcium concentrations were significantly higher than the control concentrations but the increase after calcium load was parallel to normal subjects. Moreover, the increases in urinary calcium after load were also parallel in NC and HC

patients to the control subjects. Also, the $iPTH_{1-84}$ concentrations in SF subjects were not suppressed by the higher circulating calcium concentration.

Renal tubule leak of calcium

Patients with renal hypercalciuria are postulated to have impaired renal tubular reabsorption ("renal leak") of calcium (see Pak, 1981; Vosburgh and Peters, 1987). The resulting reduction in serum calcium, though typically within the normal range, stimulates parathyroid activity. This, in turn, raises intestinal calcium absorption consequent to the PTH-dependent stimulated increase of 1,25-$(OH)_2$-vitamin D. Fasting urinary calcium is invariably increased with normocalcaemia. The patients from this study do not exhibit such characteristics. The serum calcium is higher than the controls and the PTH is not significantly different from the controls.

A variation of this hypothesis is the renal "leak" of phosphate leading to reduced calcium reabsorption. Lowered serum calcium results eventually in secondary hyperparathyroidism with raised PTH and 1,25-$(OH)_2$-vitamin D concentrations. Alternatively, renal phosphate leak leads to low plasma phosphate. This stimulates production of 1,25-$(OH)_2$-vitamin D which increases the absorption of calcium from the gut. The increased plasma calcium places an increased renal filtered load resulting in excess calcium in the urine. In this scenario, PTH would be suppressed slightly by the increase in plasma calcium concentration. In this study, there were no subjects who meet these criteria. Although plasma phosphate concentrations were significantly lower in SF subjects and fasting serum concentration of 1,25-$(OH)_2$-vitamin D were significantly

higher in HC subjects, the plasma calcium was slightly elevated and SF subjects had normal serum concentrations of PTH. After the calcium load the increase in plasma calcium and urinary calcium excretion was no greater in HC subjects than in NC subjects or controls. In addition, the association of stone forming status and plasma phosphate (negative) secondary to plasma calcium (positive) found in this study would further suggest a primarily calcium metabolic disorder which is not resulting in lower blood calcium caused by renal leak of phosphate.

Resorptive hypercalciuria

Sutton and Walker (1986) reported higher urinary hydroxyproline in SF patients and suggested that increased bone turnover may contribute to the hypercalciuria. This is in concordance with findings earlier by Anderson *et al.* (1967) who found evidence of increased turnover of bone calcium and differences suggestive of hyperparathyroidism. Bordier *et al.* (1977) reported increased osteoclast surface in their SF patients with increased carboxy-terminal immunoreactive PTH concentrations. Though, Malluche *et al.* (1980) found osteoid surface and volume from bone biopsies of patients with idiopathic hypercalciuria were increased which suggests a slowdown of the secondary mineralization of the osteoid seams. There was no evidence of increase osteoclast activity (as measured by resorptive lacunae and morphological identification) which would be expected with increased turnover. In contrast, Zechner *et al.* (1979) demonstrated reduction of basal serum calcium from statistically high to normal and hydroxyproline excretion in hypercalciuria with calcitonin infusions which reflect the direct contribution of increased

calcium mobilization from the bone. Filipponi *et al.* (1988) used injections of calcitonin to experimentally reduce urine calcium excretion in fasting hypercalciuric SF subjects and found a linear correlation between percentage urine calcium decrease and percentage urine hydroxyproline decrease. Since calcitonin is an inhibitor of osteoclast activity, the correlation suggests bone resorption may be involved. In addition, reduced bone mass in hypercalciuric subjects have been reported (Lawoyin *et al.*, 1979; Barkin *et al.*, 1985; Fuss *et al.*, 1990). Serum PTH from SF subjects was within normal limits in this study though serum 1,25-$(OH)_2$-vitamin D was significantly raised in HC subjects, and so might promote bone resorption and urinary calcium excretion. However, the increased amount of calcium excreted in the urine cannot solely be from the skeleton, since hypercalciuria can persist for many years and, at the levels of excretion found, would rapid deplete the bones of calcium. The gradual decline in skeletal calcium would be a response to the continuing hypercalciuria rather than a cause.

Parathyroid hyperfunction

Some investigators (by using RIA for carboxy-terminal or amino-terminal fragments of the parathyroid hormone, or estimation of urinary or nephrogenous cyclic AMP to assess parathyroid function) have reported increased fasting concentrations in renal hypercalciuria and normal or reduced parathyroid hormone in absorptive hypercalciuria (Bordier *et al.*, 1977; see Pak, 1979). More recently, von Lilienfeld-Toal *et al.* (1982) found carboxyl-terminal immunoreactive PTH in patients with idiopathic hypercalciuria was no higher than in normal controls. Burckhardt (1986) noted that hypercalciuria is

rarely the indirect result of excess PTH. Kitamura *et al.* (1987) reported small, but significantly, elevated carboxy-terminal $iPTH_{46-84}$ concentrations in SF patients. They speculate that there is a renal calcium leak with mild secondary parathyroid hyperfunction. The results from this present study show that the serum concentration of intact $iPTH_{1-84}$ is identical in SF and control subjects and the decrease in response to calcium loading is similarly no different between SFs and controls. Furthermore, urinary and nephrogenous cyclic AMP was the same in patients and controls. These results are similar to those found by Halabé *et al.* (1990) who also measured $iPTH_{1-84}$. The differences from earlier studies may be the result of the use previously of poorer resolution RIA (for both PTH and cyclic AMP) which frequently had detection limits near normal serum concentrations (Broadus *et al.*, 1977). In addition, subjects with impaired renal function can have increased accumulation of carboxy-terminal fragments of PTH which may give falsely elevated results (Bill, *et al.*, 1984). Though this problem is not as evident with amino-terminal assays. The subjects in this study did not have impaired renal function and assay of intact $iPTH_{1-84}$ was used. There was no *direct* evidence for increased PTH activity.

Ionized calcium is the principle physiologic regulator of PTH secretion (see Rosenblatt, 1982). High calcium concentrations suppress PTH secretion and low concentrations simulate secretion. The response is linear over a narrow range; as calcium concentrations fall, PTH secretion increases rapidly to a maximal rate; at high calcium concentrations, suppression of PTH is incomplete (Figure 4). There is a very narrow range of 'set-point' for parathyroid cells (0.97 ± 0.04 mmol/l calcium ion) from normal individuals at which PTH release is half maximally inhibited. In adenoma, primary, and secondary hyperplasia, the set point was found to be increased to 1.26 ± 0.13, 1.09 ± 0.15,

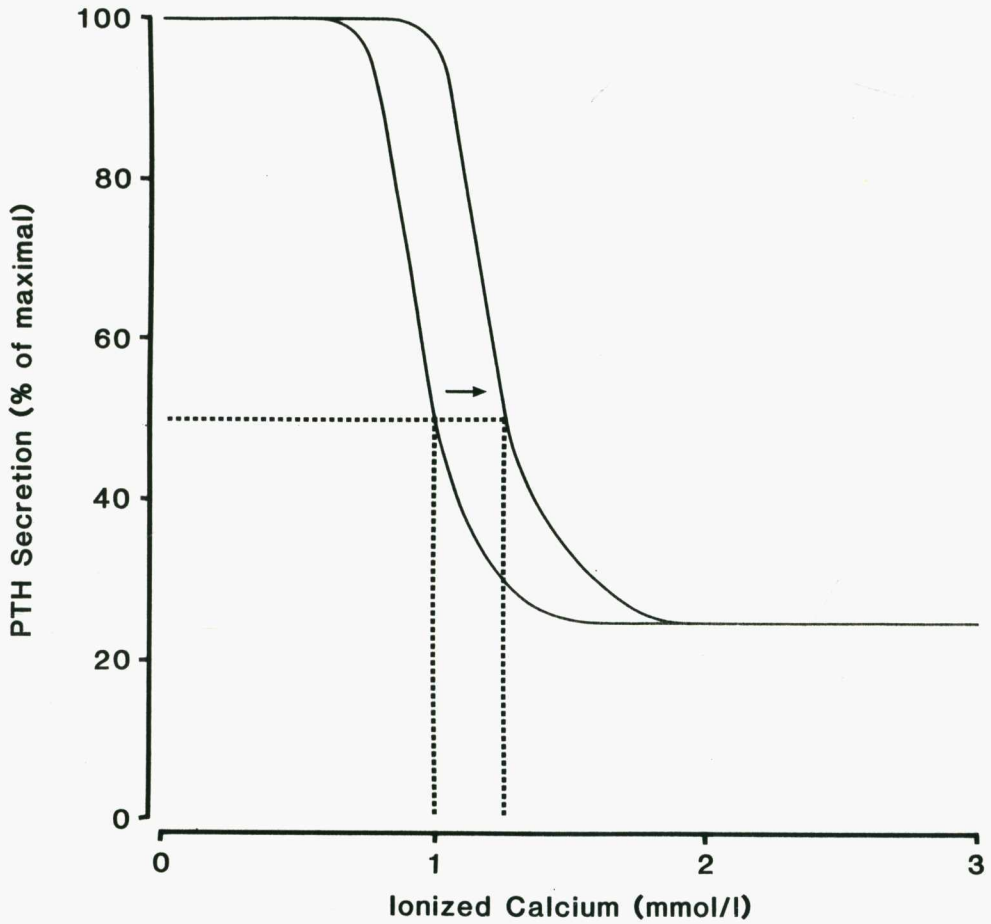

FIGURE 4. Relationship between PTH secretion as a function of calcium concentration. In normal individuals, the maximal PTH secretion is decreased 50% at an ionized calcium concentration of 0.97 mmol/l (Brown, 1983). This is the set-point for parathyroid gland activity. In adenoma parathyroid tissue, the set-point is raised but the maximal and minimal PTH secretion and the response (*ie*, the slope) does not change.

and 1.17 ± 0.19 mmol/l calcium ion, respectively (Brown, 1983). The degree of responsiveness of hyperplastic parathyroid tissue across the sensitive range is also affected. The elevated PTH concentrations in hyperparathyroidism can result from increased parathyroid tissue mass, increase in the set point for calcium to inhibit PTH secretion, and a change in the degree of suppression be calcium throughout the calcium sensitive range (*ie*, slope of the suppression line) (Slatopolsky *et al.*, 1984). In the SF subjects from this study, the concentration of PTH before and after oral calcium load is the same as that found in normal subjects but the total plasma calcium concentration is raised and the plasma phosphate concentration reduced (Figure 1). The increase in plasma calcium concentration after load in SF patients is parallel to control subjects and there is a linear correlation between the increase and the fall in $iPTH_{1-84}$ (Figure 3). This suggests that the set point in SF subjects may be higher than in normal subjects but there is no change in the response (*ie*, slope) or maximal PTH secretion. This is similar to the findings *in vitro* with normal parathyroid gland extracts from patients with adenoma (Brown, 1983), albeit the *in vivo* ionized calcium concentration were only marginally and not significantly raised in SF subjects. The plasma albumin concentrations were not different in control and SF subjects which would indicate that a greater proportion of the plasma calcium is complexed to other ions in SF than in control subjects. These findings have been reported previously (Evans *et al.*, 1984) where the clinical value of the measurement of ionized calcium with the available technology was questioned despite its superior discrimination of hypercalcaemia over total calcium measurement. Recent reports using latter technology have found significantly increased serum/plasma ionized calcium concentrations in SF patients in comparison to controls (Halabé *et al.*, 1990;

Thode, 1990). Thus it appears likely that a subtle alteration in the calcium set point for parathyroid hormone secretion occurs in SF subjects.

A number of studies have reported no differences in plasma or serum calcium between normal control and idiopathic hypercalciuria patients and/or SF subjects. Other studies report differently. Coe *et al.* (1973) and Bordier *et al.* (1977) found idiopathic hypercalciuria patients with elevated serum PTH had significantly raised serum calcium. Weinberger *et al.* (1977) found significantly higher serum calcium in hypercalciuric SF patients compared to normocalciuric SF subjects. Fuss *et al*, (1990) found significantly raised serum calcium in their SF subjects who were maintained on a free diet. Previously in this laboratory, plasma calcium was found to be significantly raised in both normocalciuric and hypercalciuric SF patients (Evans *et al.*, 1984). The results from this study were similar (*ie*, plasma calcium in SF subjects were significantly higher than in control subjects) (Table 4) and plasma calcium was found to be the primary variable associated with stone forming status. In the studies from this laboratory, plasma calcium concentration was measured in the fasting state after 7 days of a calcium and sodium restricted diet. The variance of plasma calcium is greater on a free diet than when subjects are on a low-calcium diet (Coe *et al.*, 1982). Thus, the differences between normal controls and SF patients may be obscured unless such a regimen is not imposed before measurement of plasma calcium. However, although similar protocols have been followed in earlier studies (Broadus *et al.*, 1978; Fuss *et al.*, 1978) as well as more recently (Kitamura *et al.*, 1987), the absence of statistical hypercalcaemia may be due the use of routine methodology in the earlier studies or the smaller sample of normal controls in the latter study. Interestingly, although Halabé *et al.* (1990) found significantly increased

serum ionized calcium in their SF patients, they did not find statistically significant increased serum total calcium compared to control subjects. Thode (1990), on the other hand, found that the albumin corrected plasma total calcium concentration was significantly increased in SF patients in comparison to his controls.

There is some evidence suggesting that there is a defect in membrane calcium transport in idiopathic hypercalciuria. The jejunal brush border membrane permeability to calcium ions is increased in patients with idiopathic hypercalciuria (Duncombe *et al.*, 1980). SF subjects have lower proximal tubular maximum resorptive and secretory functions, diminished urinary concentrating mechanism and reduced urinary net acid excretion following an oral calcium load (Pabico *et al.*, 1987). Patients with hypercalciuria have increased erythrocyte-membrane calcium-magnesium-ATPase activity (Vezzoli *et al.*, 1987; Bianchi *et al.*, 1988) and increased sodium-potassium pump activity in comparison with control subjects (Bianchi *et al.*, 1988). Nunziatta *et al.* (1987) found that the total body clearance of infused calcium in "absorptive" and "renal" hypercalciurics was 1.5 and 2.0 times that of controls, respectively. They considered that idiopathic hypercalciuria was the result of the presence of both epithelial gut hyperabsorption and tubular renal leak. Between these there is a alteration of body calcium turnover which acts as a reservoir of calcium. And the reservoir is more or less consistently involved depending whether the defect in calcium handling is more or less severe as in renal or absorptive hypercalciuria, respectively.

Drexler (1982) hypothesised that idiopathic hypercalciuria is the result of hypersecretion of PTH which is active at the gut and bone target site but partially defective

at the renal site of action. The results from this study do not indicate that there is increased PTH secretion *per se* but there is a subtle defect in parathyroid gland calcium set point. Parathyroid hyperfunction (Evans *et al.*, 1984), either from a defect in parathyroid cell negative-feedback or adenoma, would increase intestinal calcium absorption (perhaps mediated by the PTH-induced increase in 1,25-$(OH)_2$-vitamin D) and/or bone calcium resorption. Plasma calcium would rise, increasing the renal filtered load. Renal tubule phosphate resorption would be reduced because of the presence of inappropriately high concentrations of PTH. Normally, PTH acts on the renal distal tubule to increase the theorectical calcium resorption threshold (Cirillo *et al.*, 1984). In primary hyperparathyroidism, calcium resorption increases by 20% whereas in idiopathic hypercalciuria the calcium resorption is the same as control subjects but the percentage renal resorption of filtered calcium load in idiopathic hypercalciuria is significantly reduced (Klöti and Binswanger, 1974). Thus in this disorder, the diffuse defect of cell membrane calcium channels could enhance the absorption of calcium from the gut and also hinder the reabsorption of calcium from the renal distal tubules. An increase in parathyroid set point in SF subjects is necessary as the enhance absorption of calcium from the intestine does not appear to suppress PTH secretion. Therefore both conditions must be present to some extent which would account for the present findings.

Dietary factors

Burckhardt (1986) noted that hypercalciuria is rarely the indirect result of excess PTH and suggested the mostly likely causes are increased dietary ingestion of sodium, meat,

calcium and possibly carbohydrates. Muldowney *et al.* (1982) found a marked reduction in hypercalciuria when dietary sodium was restricted and cautioned against studying of calcium stone forming subjects without regard to dietary sodium. High sodium diets induces calciuria which is compensated for in normal individuals by an increased 1,25-$(OH)_2$-vitamin D synthesis and intestinal calcium absorption (Zerwekh and Pak, 1982). The 24 hour urine excretion of sodium is a very good indication of the subject's dietary intake. The 24 hour urine sodium in HC subjects in this study were not statistically significantly elevated in comparison to the control and NC subjects. The differences were not large enough to account for the high 24 hour urine calcium excretion found in the HC subjects. During the calcium load test, the NC subjects had significantly higher urinary excretion of sodium than control and HC subjects but their calcium excretion rates were equivalent to the control subjects. In this study the dietary sodium does not appear to greatly contribute to the hypercalciuria as the intake levels are not significantly different.

Dietary protein is an important risk factor in hypercalciuria. Increase in dietary protein results in a concomitant rise in urinary calcium excretion (Margen *et al.*, 1974; Chu *et al.*, 1975; Allen *et al.*, 1979a; Linkswiler *et al.*, 1981). The protein-induced hypercalciuria is only minimally attributable to an increase in calcium absorption. The major calcium regulatory hormones are not generally affected by the variation in dietary protein. Protein metabolism appears to directly affect renal function in two ways (Zemel, 1988). Firstly, the glomerular filteration rate (GFR) increases 10-15% after a two- to threefold increase in dietary protein without significantly altering the plasma ultrafilterable calcium. Thus the increase in filtered load of calcium without a comparable increase in tubular reabsorption raises the urinary calcium. Secondly, raised dietary protein effects a 1% reduction in the

fractional renal tubular resorption of calcium (Allen *et al.*, 1979b). The protein-induced calciuresis is associated with an increase in urinary sulfate excretion (resulting from the catabolism of the sulphur amino acids in protein). The renal tubular reabsorption of calcium is reduced because the sulphate anion is poorly reabsorbed (thus increasing the electronegativity of the tubular lumen or increasing the complexed fraction of the filtered calcium load). Variation in the sulphur amino content of proteins determine their calcuretic potential. However, protein in the diet is accompanied by phosphorus and increase in phosphorus increases renal tubular reabsorption of calcium which offsets, in part, the hypercalciuretic effect of protein. Thus vegetable proteins appear to have a hypocalciuric effect (Zemel, 1988). Breslau *et al.* (1988) found vegetable protein diets do not increase urinary calcium excretion but they do increase the urinary oxalate excretion. Obversely, the diet rich in animal protein, in addition to hypercalciuria, is associated with increased urinary uric acid excretion (because of the high purine content). This poses a risk of uric acid stones but not for calcium stones. They concluded that because of the contradictory factors with vegetable and animal protein diets that another cause for the incidence of calcium stones in SF subjects should be sought.

Griffith *et al.* (1986ab) examined the dietary intake of stone forming patients and normal controls. The differences appeared in the purine content of their diets and the ingestion of vitamin C supplements. The higher purine diet indicated greater ingestion of organ meats, as the total protein and animal protein intake were not significantly different. Higher purine intake increases the risk of stone formation as it increases oxalate excretion (probably by increasing endogenous synthesis of oxalates). Foods high in purine often have low pH ash content and therefore reduce urine pH which facilitates the precipitation

of uric acid crystals. In addition, uric acid decreases the effect of glycosaminoglycans, inhibitors of calcium oxalate crystal growth. Normally 40% of dietary vitamin C is converted to oxalate thus the greater ingestion of vitamin C supplements by SF subjects places them at a greater risk of stone formation. The excretion of oxalate in the SF subjects from this study were not significantly raised, nor was urine urate (which correlates with uric acid, (see Coe and Parks, 1981)) or pH different from control values. This is consistent with findings of Conte *et al.* (1989) who found no significant differences in oxalate excretion in 24 hour urine collections from control and calcium oxalate stone forming subjects. It was also difficult to distinguish between stone formers and controls on the basis of urine uric acid excretion. They attributed the different relationship between SF and control subjects were due to low glycosaminoglycans. Unfortunately, it was outside the scope of this study to measure urine glycosaminoglycans.

The patients in this study did not feature unusually high protein diets as reported to the consultant dietitian. Plasma and urine urea reflects levels of protein metabolism and is proportional to dietary intake. The 24 hour urine excretion of urea in SF subjects were not statistically significantly different from the control subjects. Men had greater urine urea excretion than women. On their normal diets, the SF and control subjects appear to have similar dietary protein intake. Tschöpe *et al.* (1986) reported similar findings. However, in the fasting state the SF subjects had significantly raised plasma and urine urea. The fasting plasma urea concentration and urine urea excretion in SF subjects were 20-25% greater than in control subjects and after calcium load the urine urea was reduced in the second-post load period approaching near the control excretion rates. In control subjects, the urine urea excretion remains the same during the calcium loading. The plasma urea in SF

subjects reduces slightly with calcium load, parallel with the reduction in control subjects, but remains elevated. The GFR is normal (as estimated from creatinine clearance) but in HC subjects, the fasting urine creatinine excretion is significantly higher. Thus, in SF subjects there appears to be increased protein metabolism and it is perhaps greater in HC subjects. The raised (fasting) protein metabolism might reduce the fractional renal tubular resorption of calcium.

Conclusions

Serum PTH concentration was normal in SF subjects and PTH secretion responds normally to a calcium challenge. However, the total plasma calcium concentrations are significantly higher in SF subjects, albeit within the normal range. It is suggested that a subtle defect in the parathyroid set point for calcium is present in SF subjects. There is evidence in the literature that the transport of calcium ions across cell membranes is defective in SF subjects. Increased absorption of calcium from the gastrointestinal tract and decreased renal tubule resorption of calcium have been reported. Both conditions (raised set point and impaired membrane transport) are necessary to achieve the equilibrium observed in the subjects in this study. Sex and dietary factors did not appear to explain the results found in this study. Increased protein metabolism seems to have some role in nephrolithiasis and may be more important in HC subjects than in NC subjects.

REFERENCES

1. Adams P, Chalmers TM, Hill LF, Truscott BMcN. Idiopathic hypercalciuria and hyperparathyroidism. *British Medical Journal*, 1970; **4**: 582-585.

2. Albright F, Henneman P, Benidict PH, Forbes AP. Idiopathic hypercalciuria. A preliminary report. *Proceedings of the Royal Society of Medicine*, 1953; **46**: 1077-1081.

3. Allen LH, Bartlett RS, Block GD. Reduction of renal calcium reabsorption in man by consumption of dietary protein. *Journal of Nutrition*, 1979; **109**: 1345-1350. (b)

4. Allen LH, Oddoye EA, Margen S. Protein-induced hypercalciuria: a longer term study. *The American Journal of Clinical Nutrition*, 1979; **32**: 741-749. (a)

5. Anderson J, Lee HA, Tomlinson RWS. Some metabolic aspects of idiopathic hypercalciuria. *Nephron*, 1967; **4**: 129-138.

6. Armitage P, Berry G. Statistical methods in medical research. 2nd ed. Oxford: Blackwell Scientific, 1987; 559pp.

7. Audran M, Renier JC, Jallet P, Bidet M, Basle MF, Seret P. Étude des concentrations sériques en 1,25-dihydoxyvitamine D dans des cas d'ostéomalacie, de dysfonctionnement parathyroïdien et d'hypercalciurie idiopathique. *Revue du Rhumatisme et des Maladies Ostéo-articulair*, 1987; **54**: 163-169.

8. Aurbach GD, Marx SJ, Spiegel AM. Parathyroid hormone, calcitonin, and the calciferols. **In:**

William RH, ed. *Textbook of Endocrinology*. 6th ed. Philadelphia: W B Saunders Co, 1981; 922-1031.

9. Barkin J, Wilson DR, Manual MA, Bayley A, Murray T, Harrison J. Bone mineral content in idiopathic calcium nephrolithiasis. *Mineral and Electrolyte Metabolism*, 1985; **11**: 19-24.

10. Bataille P, Bouillon R, Fournier A, Renaud H, Gueris J, Idrissi A. Increased plasma concentrations of total and free 1,25-$(OH)_2D_3$ in calcium stone formers with idiopathic hypercalciuria. *Contributions to Nephrology*, 1987; **58**: 137-142.

11. Berlin T, Bjorkhem I, Collste L, Holmberg I, Wijkstrom H. Relation between hypercalciuria and vitamin D_3-status in patients with urolithiasis. *Scandanavian Journal of Urology and Nephrology*, 1982; **16**: 269-273.

12. Berry EM, Gupta MM, Turner SJ, Burns RR. Variation in plasma calcium with induces changes in plasma specific gravity, total protein, and albumin. *British Medical Journal*, 1973; **4**: 640-643.

13. Bianchi G, Vezzoli G, Cusi D, Cova T, Elli A, Soldati L, Tripodi G, Surian M, Ottaviano E, Rigatti P, Ortolani S. Abnormal red-cell calcium pump in patients with idiopathic hypercalciuria. *The New England Journal of Medicine*, 1988; **319**: 897-901.

14. Bill G, Jacquet A-F, Burckhardt P. Nephrogenous cyclic AMP and plasma parathyroid hormone in hypercalcaemia: the influence of renal function. *European Journal of Clinical Investigation*, 1984; **14**: 227-232.

15. Bordier P, Ryckewart A, Gueris J, Rasmussen H. On the pathogenesis of so-called idiopathic hypercalciuria. *The American Journal of Medicine*, 1977; **63**: 389-409.

16. Breslau NA, Brinkley L, Hill KD, Pak CYC. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. *Journal of Clinical Endocrinology and Metabolism*, 1988; **66**: 140-146.

17. Broadus AE. Nephrogenous cyclic AMP as a parathyroid function test. *Nephron*, 1979; **23**: 136-141.

18. Broadus AE, Burtis WJ, Oren DA, Sartori L, Gay L, Ellison A, Isogna KL. Concerning the pathogenesis of idiopathic hypercalciuria. *Contributions to Nephrology*, 1987; **58**: 127-136.

19. Broadus AE, Dominguez M, Bartter FC. Pathophysiological studies in idiopathic hypercalciuria: use of an oral calcium tolerance test to characterize distinctive hypercalciuric subgroups. *Journal of Clinical Endocrinology and Metabolism*, 1978; **47**: 751-760.

20. Broadus AE, Horst RL, Lang R, Littledike ET, Rasmussen H. The importance of circulating 1,25-dihydroxyvitamin D in the pathogenesis of hypercalciuria and renal-stone formation in primary hyperparathyroidism. *The New England Journal of Medicine*, 1980; **302**: 421-426.

21. Broadus AE, Insogna KL, Lang R, Mallette LE, Oren DA, Gertner JM, Kliger AS, Ellison A. A consideration of the hormonal basis and phosphate leak hypothesis of absorptive hypercalciuria. *Journal of Clinical Endocrinology and Metabolism*, 1984:**58**: 161-169.

22. Broadus AE, Mahaffey JE, Bartter FC, Neer RM. Nephrogenous cyclic adenosine monophosphate as a parathyroid function test. *The Journal of Clinical Investigation*, 1977; **60**: 771-783.

23. Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *Journal of Clinical Endocrinology and Metabolism*, 1983; **56**: 572-581.

24. Burckhardt P. Lithiase rénale dans le cadre de l'hypercalciurie idiopathique et de l'hyperparathyroïdie primitive. *Annales de Medecine Interne*, 1986; **137**: 216-219.

25. Burckhardt P, Jaeger P. Secondary hyperparathyroidism in idiopathic renal hypercalciuria: fact or theory? *Journal of Clinical Endocrinology*, 1981; **53**: 550-555.

26. Caniggia A, Gennari C, Cesari L. Intestinal absorption of ^{45}Ca in stone-forming patients. *British Medical Journal*, 1965; **1**: 427-429.

27. Caniggia A, Nuti R, Galli M. The 24-hour urinary cyclic adenosine 3'-5'-monophosphate/creatinine ratio: a useful approach to the diagnosis of parathyroid disorders and function. *The Journal of*

Endocrinological Investigation, 1981; **4**: 281-287.

28. Chu J-Y, Margen S, Costa FM. Studies in calcium metabolism. II. Effects of low calcium and variable protein intake on human calcium metabolism. *The American Journal of Clinical Nutrition*, 1975; **28**: 1028-1035.

29. Cirillo M, Strazzullo P, Mattioli PL, Siani A, Cioffi G. Basal parathyroid activity and renal calcium handling during and intravenous calcium load. *Nephron*, 1984; **38**: 167-169.

30. Coe FL. Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria, or no metabolic disorder. *Annals of Internal Medicine*, 1977; **87**: 404-410.

31. Coe FL, Canterbury JM, Firpo JJ, Reiss E. Evidence for secondary hyperparathyroidism in idiopathic hypercalciuria. *The Journal of Clinical Investigation*, 1973; **52**: 134-142.

32. Coe FL, Favus MJ. Idiopathic hypercalciuria in calcium nephrolithiasis. *Disease-a-Month*, 1980; **26**: 1-36.

33. Coe FL, Favus MJ. Hypercalciuric states. *Mineral and Electrolyte Metabolism*, 1981; **5**: 183-200.

34. Coe FL, Favus MJ, Crokett T, Strauss AL, Parks JL, Porat A, Gantt CL, Sherwood LM. Effects of low-calcium diet on urine calcium excretion, parathyroid function and serum $1,25(OH)_2D_3$ levels in patients with idiopathic hypercalciuria and in normal subjects. *The American Journal of Medicine*, 1982; **72**: 25-32.

35. Coe FL, Parks JH. Hyperuricosuria and calcium nephrolithiasis. *Urologic Clinics of North America*, 1981; **8**: 227-244.

36. Coe FL, Parks JH, Bushinsky DA, Langman CB, Favus MJ. Chlorthalidone promotes mineral retention in patients with idiopathic hypercalciuria. *Kidney International*, 1988; **33**: 1140-1146.

37. Colussi G, Surian M, De Ferrari ME, Pontoriero G, Rombolà G, Brando B, Malberti F, Cosci P, Aroldi A, Castelnovo C, Minetti L. Relationship between sodium intake, proximal tubular function and

calcium excretion in normal subjects and in idiopathic hypercalciuria. *Proceedings of the European Dialysis and Transplant Association*, 1983; **20**: 455-459.

38. Conte A, Roca P, Genestar C, Grases F. Uric acid and its relationship with glycosaminoglycans in normal and stone-former subjects. *Nephron*, 1989; **52**: 162-165.

39. Davies DR, Dent CE, Watson L. Idiopathic hypercalciuria and hyperparathyroidism [letter]. *British Medical Journal*, 1971; **1**: 108.

40. Dent CE, Watson L. Metabolic studies in a patient with idiopathic hypercalciuria. *British Medical Journal*, 1965; **2**: 449-452.

41. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*, 1971; **31**: 87-96.

42. Drexler J. The pathogenesis of idiopathic hypercalciuria: an alternate hypothesis. *Medical Hypotheses*, 1982; **9**: 353-360.

43. Duncombe VM, Watts RWE, Peters TJ. In-vitro calcium uptake by jejunal biopsy specimens from patients with idiopathic hypercalciuria. *The Lancet*, 1980; **ii**: 1334-1336.

44. Edwards NA, Hodgkinson A. Metabolic studies in patients with idiopathic hypercalciuria. *Clinical Sciences*, 1965; **29**: 143-157. (a)

45. Edwards NA, Hodgkinson A. Studies of renal function in patients with idiopathic hypercalciuria. *Clinical Sciences*, 1965; **29**: 327-338. (b)

46. Ettinger B. Recurrence of nephrolithiasis. A six-year prospective study. *The American Journal of Medicine*, 1979; **67**: 245-248.

47. Ettinger B, Kolb FO. Inorganic phosphate treatment of nephrolithiasis. *The American Journal of Medicine*, 1973; **55**: 32-37.

48. Evans RA, Hills E, Wong SYP, Wyndham LE, Eade Y, Dunstan CR. The pathogenesis of idiopathic hypercalciuria: evidence for parathyroid hyperfunction. *Quarterly Journal of Medicine*, 1984; **209**:

41-53.

49. Filipponi P, Mannarelli C, Gubbiotti G, Blass A, Moretti I, Tini S, Giuseppetti N, Ballanti S, Morucci P. The effects of calcitonin on idiopathic nephrolithiasis. Evidence of bone involvement in fasting hypercalciuria. *Journal of Endocrinological Investigation*, 1988; **11**: 509-513.

50. Flocks RH. Calcium and phosphorus excretion in the urine. *The Journal of the American Medical Association*, 1939; **114**: 1466-1471.

51. Flocks RH. Prophylaxis and medical management of calcium urolithiasis: the rôle of the quantity and precipitability of the urinary calcium. *Journal of Urology*, 1940; **44**: 183-190.

52. Fuss M, Verbeelen D, Geurts J, Simon J, Bergans A, de Backer M, Six R, Corvilain J. The use of a test for the differential diagnosis of hypercalciuria. *European Urology*, 1978; **4**: 324-329.

53. Fuss M, Pepersack T, Van Geel J, Corvilain J, Vandewalle J-C, Bergmann P, Simon J. Involvement of low-calcium diet in reduced bone mineral content of idiopathic renal stone formers. *Calcified Tissue International*, 1990; **46**: 9-13.

54. Gehan EA, George SL. Estimation of human body surface area from height and weight. *Cancer Chemotherapy Reports*, 1970; **54**: 225-235.

55. Gitelman HJ. An improved automated procedure for the determination of calcium in biological specimens. *Analytical Biochemistry*, 1967; **18**: 521-531.

56. Goltzman D, Bennet HPG, Koutsilieris M, Mitchell J, Rabbani SA, Rouleau MF. Studies of the multiple molecular forms of bioactive parathyroid hormone and parathyroid hormone-like substances. *Recent Progress in Hormone Research*, 1986; **42**: 665-703.

57. Griffith HM, O'Shea B, Keogh B, Kevany JP. A case-control study of dietary intake of renal stone patients. I. Preliminary Analysis. *Urological Research*, 1986; **14**: 67-74. (a)

58. Griffith HM, O'Shea B, Maguire M, Keogh B, Kevany JP. A case-control study of dietary intake of renal stone patients. II. Urine biochemistry and stone analysis. *Urological Research*, 1986; **14**: 75-82.

(b)

59. Halabé A, Sutton RAL. Primary hyperparathyroidism and idiopathic hypercalciuria. *Mineral and Electrolyte Metabolism*, 1987; **13**: 235-241.

60. Halabé A, Wong NLM, Wong EFC, Sutton RAL. Atrial natriuretic factor levels in renal stone patients with idiopathic hypercalciuria and in healthy controls: the effect of an oral calcium load. *Metabolism. Clinical and Experimental*, 1990; **39**: 209-212.

61. Harrison AR. Some results of metabolic investigations in cases of renal stone. *British Journal of Urology*, 1959; **31**: 398-403.

62. Henneman PH, Benedict PH, Forbes AP, Dudley HR. Idiopathic hypercalciuria. *The New England Journal of Medicine*, 1958; **259**: 802-807.

63. Hess B, Binswanger U. Idiopathische Hypercalciurie – neure pathogenetische und diagnostische aspekte. *Schweizerische medizinische Wochenschrift. Journal suisse de medecine*, 1986; **116**: 1569-1575.

64. Hodgkinson A. Idiopathic hypercalciuria. *Proceedings of the Association for Clinical Biochemistry*, 1961; **1**: 52-56.

65. Hodgkinson A, Pyrah LN. The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin. *The British Journal of Surgery*, 1958; **46**: 10-18.

66. Holdaway IM, Evans MC, Frengley PA, Ibbertson HK. Investigation and treatment of renal calculi associated with hypercalciuria. *Journal of Endocrinological Investigation*, 1982; **5**: 361-365.

67. Holmegaard SN. Measurement of cyclic AMP in clinical investigations. *Acta Endocrinologica*, 1982; **101**(S249): 1-47.

68. Iguchi M, Kohri K, Akiyama T, Yachiku S, Kurita T. Serum-ionized calcium as a diagnostic tool in hypercalciuria. *Urology International*, 1980; **35**: 36-41.

69. Ivey JL, Roos BA, Shen FH, Baylink DJ. Increased immunoreactive calcitonin in idiopathic hypercalciuria. *Metabolic Bone Disease and Related Research*, 1981; **3**: 39-42.

70. Jackson WPU, Doncaster C. A consideration of the hypercalciuria in sarcoidosis, idiopathic hypercalciuria, and that produced by vitamin D. A new suggestion regarding calcium metabolism. *The Journal of Clinical Endocrinology and Metabolism*, 1959; **19**: 658-680.

71. Kaplan RA, Haussler MR, Deftos LJ, Bone H, Pak CYC. The role of 1α,25-dihydroxyvitamin D in the mediation of intestinal hyperabsorption of calcium in primary hyperparathyroidsim and absorptive hypercalciuria. *The Journal of Clinical Investigation*, 1977; **59**: 756-760.

72. Keynes M. Idiopathic hypercalciuria and hyperparathyroidism [letter]. *British Medical Journal*, 1971; **5**: 108-109.

73. Kitamura T, Hirano Y, Ueda D, Aso Y. Biochemical changes before and during calcium tolerance test in calcium stone formers. *Urological Research*, 1988; **16**: 149-155.

74. Kitamura T, Hirano Y, Ueda D, Niijima T. Possibility of elevated parathyroid function in patients with calcium-containing nephrolithiasis as compared with normal controls. *European Urology*, 1987; **13**: 90-99.

75. Klöti J, Binswanger U. Die renale calciumausscheidung bie primärem hyperparathreoidismus und idiopathischer hypercalciurie. *Klinishe Wochenschrift*, 1975; **53**: 307-310.

76. Knapp EL. Factors influencing the urinary excretion of calcium. I. In normal humans. *The Journal of Clinical Investigation*, 1947; **26**: 182-202.

77. Kocián J. Idiopathic hypercalciuria. Diagnosis and treatment. *Acta Universitatis Carolinae Medica*, 1987; **33**: 609-664.

78. Kraiem Z, Glaser B, Rosenzweig A, Sheinfeld M, Ben-Ari J. A spot sample test for the estimation of urinary or nephrogenous cAMP in the evaluation of parathyroid function. *Nephron*, 1983; **35**: 264-266.

79. Lau YK, Wasserstein A, Westby GR, Bosanac P, Grabie M, Mitnick P, Slatopolsky E, Goldfarb S, Agus ZS. Proximal tubular defects in idiopathic hypercalciuria: resistance to phosphate administration. *Mineral and Electrolyte Metabolism*, 1982; **7**: 237-249.

80. Lawoyin S, Sismilich S, Browne R, Pak CYC. Bone mineral content in patients with calcium urolithiasis. *Metabolism. Clinical and Experimental*, 1979; **28**: 1250-1254.

81. Lien J, Keane P. Urinary cAMP and calcium excretion in the fasting state and their response to oral calcium loading in patients with calcium urolithiasis. *The Journal of Urology*, 1983; **129**: 401-404. (a)

82. Lien JW, Keane PM. Limitations of the oral calcium loading test in the management of recurrent calcareous renal stone former. *American Journal of Kidney Diseases*, 1983; **3**: 76-79. (b)

83. Lemann JJr, Gray RW. Idiopathic hypercalciuria. *The Journal of Urology*, 1989; **141**: 715-718.

84. Liberman UA, Sperling O, Atosmon A, Frank M, Modan M, de Vries A. Metabolic and calcium kinetic studies in idiopathic hypercalciuria. *The Journal of Clinical Investigation*, 1968; **47**: 2580-2589.

85. Linkswiler HM, Zemel MB, Hegsted M, Schuette S. Protein-induced hypercalciuria. *Federation Proceedings*, 1981; **40**: 2429-2433.

86. Lyles KW, Drezner MK. An overview of calcium homeostasis in humans. *Urologic Clinics in North America*, 1981; **8**: 209-226.

87. Maggio AJ, Coburn JW, Brosman SA, Das S. The role of vitamin D in idiopathic hypercalciuria. *The Journal of Urology*, 1979; **122**: 147-151.

88. Malluche HH, Tschoepe W, Ritz E, Meyer-Sabellek W, Massry SG. Abnormal bone histology in idiopathic hypercalciuria. *Journal of Clinical Endocrinology and Metabolism*, 1980; **50**: 654-658.

89. Margen S, Chu J-Y, Kaufmann NA, Calloway DH. Studies in calcium metabolism. I. The calciuretic effect of dietary protein. *The American Journal of Clinical Nutrition*, 1974; **27**: 584-589.

90. Martinez-Maldonado M. Continuing challenges to the understanding of the definition and pathophysiology of hypercalciuria. *Nephron*, 1979; **24**: 209-211.

91. Muldowney FP, Freaney R, McMullin JP, Towers RP, Spillane A, O'Connor P, O'Donohoe P, Moloney M. Serum ionized calcium and parathyroid hormone in renal stone disease. *Quarterly Journal of Medicine*, 1976; **45**: 75-86.

92. Muldowney FP, Freaney R, Moloney MF. Importance of dietary sodium in the hypercalciuria syndrome. *Kidney International*, 1982; **22**: 292-296.

93. Muldowney FP, Freaney R, Ryan JG. The pathogenesis of idiopathic hypercalciuria: evidence for renal tubular calcium leak. *Quarterly Journal of Medicine*, 1980; **49**: 87-94.

94. Neter J, Wasserman W, Kutner MH. Applied linear statistical models: regression, analysis of variance, and experimental designs. 2nd ed. Homewood: Richard D Irwin, Inc., 1985. 1127 pp.

95. Nordin BEC, Peacock M, Wilkinson R. Hypercalciuria and calcium disease. *Clinics in Endocrinology and Metabolism*, 1972; **1**: 169-183.

96. Nunziata V, Giannattasio R, di Giovanni G, Corrado MF, Riccio M. Altered calcium handling in idiopathic hypercalciuria. *Contributions to Nephrology*, 1987; **58**: 101-105.

97. Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LV, Keutmann HT, Wang C-A, Potts JTJr, Segre GV. A highly sensitive two-site immunoradiometric assay of parathyrin (PTH) and its' clinical utility in evaluating patients with hypercalcemia. *Clinical Chemistry*, 1987; **33**: 1364-1367.

98. Pabico RC, McKenna BA, Freeman RB. Renal tubular dysfunctions in patients with idiopathic calcium nephrolithiasis. *Mineral and Electrolyte Metabolism*, 1987; **13**: 462-468.

99. Pak CYC. Idiopathic hypercalciuria. *Advances in Experimental Medicine and Biology*, 1977; **81**: 309-17.

100. Pak CYC. Kidney stones: various forms and treatment. *Nephron*, 1979; **23**: 142-146.

101. Pak CYC. Pathogenesis of idiopathic hypercalciuria. **In**: Coe FL, ed. *Hypercalciuric States. Pathogenesis, Consequences, and Treatment.* Orlando: Grune & Stratton, Inc., 1984: 205-220.

102. Pak CYC, Britton F, Peterson R, Ward D, Northcutt C, Breslau NA, McGuire J, Sakhaee K, Bush S, Nicar M, Norman DA, Peters P. Ambulatory evaluation of nephrolithiasis. Classification, clinical presentation and diagnostic criteria. *The American Journal of Medicine*, 1980; **69**: 19-30.

103. Pak CYC, East DA, Sanzenbacher LJ, Delfa CS, Bartter FC. Gastrointestinal calcium absorption in nephrolithiasis. *The Journal of Clinical Endocrinology and Metabolism*, 1972; **35**: 261-270.

104. Pak CYC, Fuller C. Idiopathic hypocitraturic calcium-oxalate nephrolithiasis successfully treated with potassium citrate. *Annals of Internal Medicine*, 1986; **104**: 33-37.

105. Pak CYC, Galosy RA. Fasting urinary calcium and adenosine 3',5'-monophosphate: a discriminant analysis for the identification of renal and absorptive hypercalciurias. *The Journal of Clinical Endocrinology and Metabolism*, 1979; **48**: 260-265.

106. Pak CYC, Kaplan R, Bone H, Townsend J, Waters O. A simple test for the diagnosis of absorptive, resorptive and renal hypercalciurias. *The New England Journal of Medicine*, 1975; **292**: 497-500.

107. Pak CYC, Nicar MJ. Long-term persistence of metabolic abnormalities in absorptive or renal hypercalciuria. *The Lancet*, 1981; **i**: 356-359.

108. Pak CYC, Nicar M, Northcutt C. The definition of the mechanism of hypercalciuria *is* necessary for the treatment of recurrent stone formers. *Contributions to Nephrology*, 1982; **33**: 136-151.

109. Pak CYC, Ohata M, Lawrence EC, Snyder W. The hypercalciurias. Causes, parathyroid functions and diagnostic criteria. *The Journal of Clinical Investigation*, 1974; **54**: 387-400.

110. Pak CYC, Peters P, Hurt G, Kadesky M, Fine M, Reisman D, Splann F, Caramela C, Freeman A, Britton F, Sakhaee K, Breslau NA. Is selective therapy of recurrent nephrolithiasis possible? *The American Journal of Medicine*, 1981; **71**: 615-622.

111. Parfitt MM, Higgins BA, Nassim JR, Collins JA, Hilb A. Metabolic studies in patients with

hypercalciuria. *Clinical Sciences*, 1964; **27**: 463-482.

112. Parks JH, Coe FL. Urine citrate and calcium in calcium nephrolithiasis. *Advances in Experimental Medicine and Biology*, 1986; **208**: 445-449.

113. Peacock M. Renal excretion of calcium. **In**: Nordin BEC, ed. *Calcium in Human Biology*. London: Springer-Verlag, 1988: 125-169. (Macdonald I, ed. ILSI Human Nutrition Reviews)

114. Peacock M, Knowles F, Nordin BEC. Effect of calcium administration and deprivation on serum and urine calcium in stone-forming and control subjects. *British Medical Journal*, 1968; **2**: 729-731.

115. Peacock M, Nordin BEC. Tubular reabsorption of calcium in normal and hypercalciuric subjects. *The Journal of Clinical Pathology*, 1968; **21**: 353-358.

116. Peacock M, Nordin BEC. The hypercalciuria of renal stone disease. **In**: Hodgkinson A, Nordin BEC, eds. *Proceedings of the Renal Stone Research Symposium. Leeds, April 1968*. London: J & A Churchill Ltd., 1969: 253-261.

117. Reinhardt TA, Horst RL, Orf JW, Hollis BW. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromotography: application to clinical studies. *Journal of Clinical Endocrinolgy and Metabolism*, 1984; **58**: 91-98.

118. Robertson WG. Urinary tract calculi. **In**: Nordin BEC, ed. *Metabolic Bone and Stone Disease*. 2nd ed. Edinburgh: Churchill Livingstone, 1984: 271-326.

119. Robertson WG, Morgan DB. The distribution of urinary calcium excretions in normal persons, stone-formers. *Clinica Chimica Acta*, 1972; **37**: 503-508.

120. Rose GA, Harrison AR. The incidence, investigation, treatment of idiopathic hypercalciuria. *British Journal of Urology*, 1974; **46**: 261-274.

121. Rosenblatt M. Control of parathyroid hormone secretion. *Contributions to Nephrology*, 1982; **33**: 163-177.

122. Scholz D, Schwille PO, Sigel A. Double-blind study with thaizides in recurrent calcium lithiasis. *The Journal of Urology*, 1982; **128**: 903-907.

123. Seshadri MS, Frankel TL, Lissner D, Mason RS, Posen S. Bioactive parathyroid hormone in the rat: effects of calcium, calcitriol. *Endocrinology*, 1982; **117**: 2417-2423.

124. Shen F, Baylink D, Nielson R, Hughes M, Haussler M. Increased serum 1,25-dihydroxycalciferol (1,25-diOHD$_3$) in patients with idiopathic hypercalciuria (IH). *Clinical Research*, 1975; **23**: 423a.

125. Shen F, Baylink D, Nielsen R, Sherard D, Haussler M. A study of the pathogenesis of idiopathic hypercalciuria (IH). *Clinical Research*, 1976; **24**: 460a.

126. Shen FH, Baylink DJ, Nielsen RL, Sherrard DJ, Ivey JL, Haussler MR. Increased serum 1,25-dihydroxyvitamin D in idiopathic hypercalciuria. *The Journal of Laboratory and Clinical Medicine*, 1977; **90**: 955-962. (a)

127. Shen FH, Baylink DJ, Sherrard DJ, Haussler MR. Idiopathic hypercalciuria (IH): the "phosphate leak" hypothesis and the effects of oral phosphate supplement. *Clinical Research*, 1977; **25**: 448a. (b)

128. Slatopolsky E, Martin KJ, Morressey JJ, Hruska KA. Secretion, metabolism, and peripheral actions of parathyroid hormone. **In**: Coe FL, ed. *Hypercalciuric States. Pathogenesis, Consequences, and Treatment.* Orlando: Grune & Stratton, Inc., 1984: 101-141.

129. Strauss MM, Moshides J. Convenient urine oxalate assay with oxalate oxidase reagent and a centrifugal analyzer. *Clinical Chemistry*, 1987; **33**: 2301.

130. Sutton RAL, Walker VR. Responses to hydrochorothiazide and acetozolamide in patients with calcium stones. Evidence suggesting a defect in renal tubular function. *The New England Journal of Medicine*, 1980; **302**: 709-713.

131. Sutton RAL, Walker VR. Bone resorption and hypercalciuria in calcium stoneformers. *Metabolism: Clinical and Experimental*, 1986; **35**: 485-488.

132. Thode J. Ionized calcium and cyclic AMP in plasma and urine. *The Scandinavian Journal of Clinical*

and Laboratory Investigation, 1990; **50** S197: 1-45.

133. Transbøl I, Hahnemann S, Hornum I. Ionized, ultrafiltrable and total calcium in serum in hyperparathyroidism. *Acta Endocrinologica*, 1970; **65**: 385-400.

134. Tscöpe W, Ritz E, Schmidt-Gayk H. Is there a renal phosphorus leak in recurrent renal stone formers with absorptive hypercalciuria? *The European Journal of Clinical Investigation*, 1980; **10**: 381-386.

135. Tschöpe W, Horsch R, Ritz E. Therapeutic modalities for the prevention of nephrolithiasis. *Advances in Experimental Medicine and Biology*, 1986; **208**: 459-465.

136. Urban P, Buchmann B, Scheidegger D. Facilitated determination of ionized calcium. *Clinical Chemistry*, 1985; **31**: 264-266.

137. Van Der Berg CJ, Kumar R, Wilson DM, Heath H III, Smith LH. Orthophosphate therapy decreases urinary calcium excretion and serum 1,25-dihydroxyvitamin D concentrations in idiopathic hypercalciuria. *The Journal of Clinical Endocrinology and Metabolism*, 1980; **51**: 998-1001.

138. Vezzoli G, Elli A, Palazzi P, Cusi D, Cova T, Menghetti D, Scabini M, Ortolani S, Soldati L, Surian M, Quarto di Palo F, Bianchi G. Plasma membrane Ca-ATPase in idiopathic hypercalciuria. *Contributions to Nephrology*, 1987; **58**: 148-151.

139. von Lilienfeld-Toal H, Bach D, Hesse A, Franck H, Issa S. Parathyroid hormone is normal in renal stone patients with idiopathic hypercalciuria and high fasting urinary calcium. *Urological Research*, 1982; **10**: 205-207.

140. Vosburgh E, Peters TJ. Pathogenesis of idiopathic hypercalciuria: a review. *Journal of the Royal Society of Medicine*, 1987; **80**: 34-37.

141. Walton RJ, Bijvoet OLM. Nomogram for derivation of renal threshold phosphate concentration. *The Lancet*, 1975; **ii**: 309-310.

142. Warty VS, Busch RP, Virji MA. A kit for citrate in foodstuffs adapted for assay of serum and urine. *Clinical Chemistry*, 1984; **30**: 1231-1233.

143. Watson PE, Watson ID, Batt RD. Obesity indices [letter]. *The American Journal of Clinical Nutrition*, 1979; **32**: 736-737.

144. Weitbrecht M, Hegemann M, Pfab R, Wieser T. Geschlechtsspezifische faktoren bei der rezidivierenden idiopathischen kalzium-urolithiasis. *Urologe A*, 1987; **26**: 129-132.

145. Weinberger A, Sperling O, Schechter J, Liberman UA, Pinkhas J, de Vries A. Urolithiasis associated with hypercalciuria. *International Urology and Nephrology*, 1977; **9**: 213-216.

146. Wills MR, Pak CYC, Hammond WG, Bartter FC. Normocalcemic primary hyperparathyroidism. *The American Journal of Medicine*, 1969; **13**: 384-391.

147. Yendt ER, Cohanim M. Prevention of calcium stones with thiazides. *Kidney International*, 1978; **13**: 397-409.

148. Zechner O, Penner E, Willvonseder R. Calcitonin load in absorptive hypercalciuria type I. *European Urology*, 1979; **5**: 199-204.

149. Zemel MB. Calcium utilization: effect of varying level and source of dietary protein. *The American Journal of Clinical Nutrition*, 1988; **48**: 880-883.

150. Zerwekh JE, Pak CYC. Mechanisms of hypercalciuria. *Pathobiology Annual*, 1982; **12**: 185-201.

151. Zerwekh JE, Pak CYC, Kaplan RA, McGuire JL, Upchurch K, Breslau N, Johnson RJr. Pathogenetic role of 1α,25-Dihydroxyvitamin D in sarcoidosis and absorptive hypercalciuria: different response to prenisolone therapy. *The Journal of Clinical Endocrinology and Metabolism*, 1980; **51**: 381-386.

APPENDIX A

Formulae

Body Mass Index

The Body Mass Index (BMI) or Quetlet Index was calculated from:

$$BMI = \frac{W}{H^2}$$

where W is body mass in kilograms and H is height in metres (Watson *et al.*, 1979).

Body Surface Area

The Body Surface Area (BSA) was calculated by the formula:

$$BSA = 0.01545 \times H^{0.54468} \times W^{0.46336}$$

where H is height in centimetres and W is body mass in kilograms (Gehan and George, 1970).

Calcium Clearance

The renal clearance of calcium (and other substances) is expressed as the fraction of the clearance of endogenous creatinine:

$$\mathbf{C}_{Ca} = \frac{[U_{Ca}] \times [P_{creat}]}{[U_{creat}] \times [P_{Ca}]}$$

where $[U_{Ca}]$ is urine calcium concentration, $[P_{creat}]$ is plasma creatinine concentration, $[U_{creat}]$ is urine creatinine concentration and $[P_{Ca}]$ in plasma calcium concentration (Peacock, 1988).

Calcium Excretion

The renal excretion of calcium (and other substances) is expressed as the amount of calcium per unit time:

$$\mathbf{E}_{Ca} = \frac{[U_{Ca}] \times V}{t}$$

where $[U_{Ca}]$ is urine concentration of calcium, V is volume of urine and t is time over which it was collected.

Corrected Plasma Calcium

The plasma calcium was corrected for plasma albumin using the formula:

$$[cCa] = [P_{Ca}] + 0.02 \times (38 - [P_{alb}])$$

where $[P_{Ca}]$ is plasma calcium concentration in mmol/l and $[P_{alb}]$ is the plasma albumin level in g/l (Berry *et al.*, 1973).

Glomerular Filteration Rate

The glomerular filteration rate (GFR) was estimated from the creatinine clearance. The clearance is calculated from:

$$GFR = \frac{[U_{cr}] \times V}{[P_{cr}] \times t}$$

where $[U_{cr}]$ is the urine creatinine concentration in mmol/l, $[P_{cr}]$ is the plasma creatinine concentration in mmol/l, V is the volume of urine and t is time over which it was collected.

Nephrogenous cyclic AMP

The nephrogenous cAMP was calculated and expressed as the amount of cAMP (nmol) produced in the nephron per 100 ml glomerular filterate:

$$NcAMP = \frac{[U_{cAMP}] \times [P_{creat}]}{([U_{creat}] - [P_{cAMP}]) \times 10}$$

where $[U_{cAMP}]$ is urine cAMP concentration in nmol/l, $[P_{creat}]$ is plasma creatinine concentration in mmol/l, $[U_{creat}]$ is urine creatinine concentration in mmol/l and $[P_{cAMP}]$ is plasma cAMP concentration in nmol/l (Broadus *et al.*, 1977).

Urinary cyclic AMP

The urinary cyclic AMP is expressed as the amount of cAMP (nmol) excreted in the urine per 100 ml glomerular filterate:

$$UcAMP = \frac{[U_{cAMP}] \times [P_{creat}]}{[U_{creat}] \times 100}$$

where $[U_{cAMP}]$ is urine cAMP concentration in nmol/l, $[P_{creat}]$ is plasma creatinine

concentration, $[U_{creat}]$ is urine creatinine concentration.

Theorectical Renal Phosphate Threshold

The theorectical renal phosphate theshold (ratio of the maximum rate of renal tubular reabsorption of phosphate to GFR — Tm_{PO_4}/GFR) was estimated from the nomogram devised by Walton and Bijvoet (1975).