Studies on calcium excretion in diabetic children

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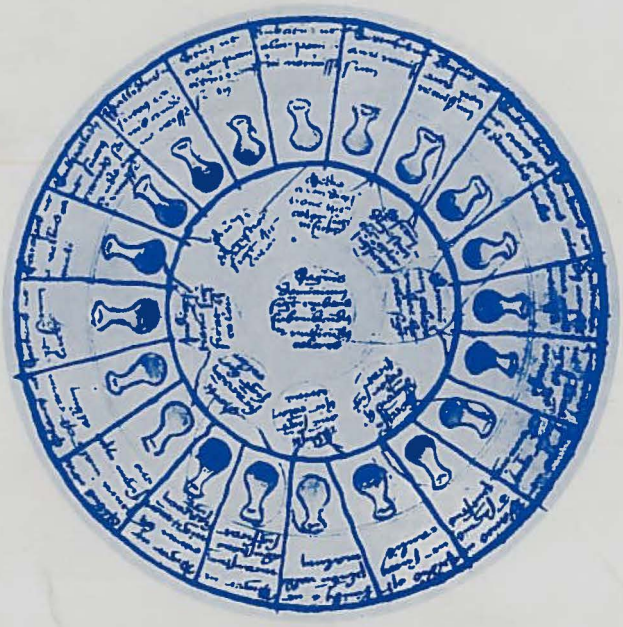
R. Van Damme - Lombaerts



Studies on urinary calcium excretion in diabetic children

The illustration on the cover shows a uroscopic table with twenty matulae, each having one of the distinctive colours described in the uroscopic manuals of the medieval physicians.

From a manuscript in the university library of Leipzig.
The illustration was kindly provided by Dr. A. Van Den Bon
In : Het achthonderd jaar oud sint-Janshospitaal van de stad Brugge, Dr. A. Van Den Bon, Brugge, 1974.

STELLINGEN

1. Het bepalen van de 24 uurs excretie van calcium in de urine dient opgenomen te worden in het nefrologisch exploratie programma van diabetische kinderen.

2. De bruikbaarheid van de orale calcium belastingstest in de differentiaal diagnose van hypercalciurie is vooral toe te schrijven aan de korte duur van de test.

   Pak et al., New Engl. J. Med. 292, 497.

3. Het advies inzake insuline spuiten uitgebracht door het Nationaal Centrum voor Kruiswerk aan de apothekers en apotheekhoudende artsen in Nederland is voor kinderen met diabetes mellitus onjuist.

4. Bij patienten met chronische proteinurie en verminderde nierfunctie is het aangewezen de diameter van de glomeruli te meten.

5. Pharmacologische sluiting van de ductus Botalli bij pasgeborenen verdient wel de aandacht, maar (nog) geen toepassing.

6. De veronderstelling dat akute post streptokokken glomerulonefritis een progressieve aandoening is, is onwaarschijnlijk.

7. Het nut van een groot aantal laboratoriumbepalingen bij een gezonde populatie is niet aangetoond.

8. Bij de kort segment Hirschsprung dient naast de dilatatie ook plaats te worden ingeruimd voor de posterior myectomie.

9. Bij kinderen met pseudohypoaldosteronisme wijst de bestudering van de zoutreabsorptie onder hypotoon zoutinfuus op een proximaal tubulair defect.

10. Ouderparticipatie in de school betekent niet dat de ouders plaats nemen in de stoel van de leerkrachten.

11. De lofzang over huishoudelijk werk, als een eervolle taak voor de vrouw, is een hoffelijke manier van de man om dit soort werk aan haar over te laten.

Stellingen bij "Studies on urinary excretion of calcium in diabetic children",
R. Van Damme-Lombaerts, 15 juni 1977

RIJKSUNIVERSITEIT TE GRONINGEN

Studies on Urinary Calcium Excretion in Diabetic Children

PROEFSCHRIFT

ter verkrijging van het doctoraat in de Geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. M.J. Janssen
in het openbaar te verdedigen op woensdag 15 juni 1977
des namiddags te 3 uur precies.

door

Rita Melania Marcel Maria
Van Damme - Lombaerts
geboren te Ninove

Drukkerij: C. Govaerts N.V. - Deurne.

PROMOTOR : DR. N.M. DRAYER

COPROMOTOR : DR. J.A. TROELSTRA

Aan mijn ouders

Aan Bo, Tom, An en Philip

This investigation was performed in the Paediatric Department of the University Hospital of Groningen.
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The children, their parents, Sister I. Holwerda and the nursing staff, who co-operated in this study are gratefully acknowledged.
Dr. N.M. Drayer and Drs. C. Rouwé helped me during this study.
The samples of urine and blood were analysed in the Central Chemical Laboratory of the University Hospital, with the help of Dr. K. Deggeller,Dr. F.R. Hindriks, Dr. E.W. Kwarts, Dr. B. Wolters, Mr. E. Ligeon, Mr. J. van der Meulen and the staff of analysts, and of Dr. W.J. Sluiter from the Department of Internal Medicine of the University Hospital. Determinations in the Isotopic Laboratory were performed by Drs. J.J. Pratt and Miss M. Kwants. The estimation of the plasma levels of hormones was performed by Dr. W.H. Hackeng of the Bergwegziekenhuis of Rotterdam.
The diets were analysed by Miss W. Stevenson, Miss M. Kreumer, Miss N. Kooyman and Miss W. Euwe in the Department of Dietetics of the University Hospital.
Miss N. de Vries, Miss G. Geudens and Mrs. A. Van Rengen-Van den Panhuysen typed the manuscript. Miss J. Otten, Miss H. Jansen, Miss H. Marra and Miss M.Th. Snel helped in the preparative work.
Mr. G.M. Meschendorp drew the figures. The photographical work was performed in the Central Photographical Service of the University of Groningen.
The results were analysed with the help of Mr. L. Th. van der Weele and Mr. T. Wierstra of the Computer Centre of the University of Groningen.
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But what I believe I would try to do is to select one question, just one, out of the many questions. It would be a question about which I could easily collect quantitative data from my patients. I would collect the data, sort and analyze them, and then I would collect more data. I believe that after studious collection of data about a specific question for many years, I would surely known a little more, if ever so little more, that would advance knowledge about that single project. It seems to me that the discipline of constantly collecting, sorting and analyzing data would also sharpen my skills as a physician.

A. Kornberg.
New England Journal of Medicine
294, 1212, 1976.

ABBREVIATIONS

cAMP	cyclic 3', 5'-adenosine monophosphate (μMoles/g of creatinine)
$\frac{C_{PO4}}{C_{Creat}}$	phosphate to creatinine clearance rate (no dimension)
DF	degrees of freedom
e	natural logarithm
FFA	free fatty acids (μMoles/l)
GFR	glomerular filtration rate (ml/min)
hCT	human calcitonin (ng/ml)
hGH	human growth hormone (ng/ml)
hr	hour(s)
24 hr	24 hour(s)
I_{Ca}	calcium intake (mg/kg/24 hr)
I_{CHO}	carbohydrate intake (g/kg/24 hr)
I_{Fat}	fat intake (g/kg/24 hr)
I_{P}	phosphate intake (mg/kg/24 hr)
I_{Prot}	protein intake (g/kg/24 hr)
iPTH	immunoreactive parathyroid hormone (ng equivalent bovine PTH/ml)
kg	kilogram body weight
n, N°	number
n.s.	not significant
P	phosphate (mg/dl)
PCA	percent cortical area
SD	standard deviation
SDS	standard deviation score
TRP	tubular reabsorption of phosphate (%)
TmP/GFR	tubular reabsorption maximum of phosphate per 100 ml glomerular filtrate (mg P/100 ml GFR)
U_{Ca}	urinary calcium (mg/kg/24 hr)
U_{Cl}	urinary chloride (mEq/kg/24 hr)
U_{Cr}	urinary creatinine (mg/kg/24 hr)

U_{Gluc}	urinary glucose (g/kg/24 hr)
U_{K}	urinary potassium (mEq/kg/24 hr)
U_{Mg}	urinary magnesium (mg/kg/24 hr)
U_{Na}	urinary sodium (mEq/kg/24 hr)
U_{P}	urinary phosphate (mg/kg/24 hr)
U_{Vol}	urinary volume (ml/kg/24 hr)
yr	year(s)

CONTENTS

ORIGIN AND PURPOSE OF THE STUDY

A study on the urinary calcium excretion in diabetic children was set up in 1972 after an incidental finding of unexplained hypercalciuria in a diabetic boy presenting with a history suggestive of a renal colic.

The purpose of this study was to establish the incidence of hypercalciuria in diabetic children and to elucidate the pathogenesis and clinical implications of the hypercalciuria.

CHAPTER 1

REVIEW OF THE LITERATURE

The physiological importance of the divalent cation, calcium, is well established (Nordin et al., 1967 ; Epstein, 1968 ; Manery, 1969 ; Walser, 1969).

In neonates and infants the physiology of calcium metabolism is quite different from that of adults (David and Anast, 1974 ; Tsang et al., 1976). When the child is about the age of one year the metabolism is similar to that of adults. As all our diabetic patients are more than two years old, the literature on the calcium metabolism in neonates and infants is not discussed.

After a short review of the regulation of the blood calcium concentration, some non-hormonal and some hormonal factors influencing the renal handling of calcium (Bacq, 1964 ; Walser, 1969 and 1973) are discussed.

1.1. REGULATION OF BLOOD CALCIUM CONCENTRATION

The blood calcium concentration is maintained within certain ranges, and fluctuations in the blood calcium level are kept to a minimum (Carruthers et al., 1964).

35 % to 40 % of calcium in the blood is bound to plasma proteins (McLean and Hastings, 1935) and only the unbound fraction can pass freely across the glomerular basement membrane (Toribara et al., 1957). Blood calcium levels are primarily determined by the movement of calcium from the bone and from the gut, and are regulated by the renal reabsorption of calcium (Nordin and Peacock, 1969 ; Nordin et al., 1972/a).

As DeLuca reviewed in 1975 and 1976, the parathyroid gland senses hypocalcaemia and parathormone is released in response. Parathyroid hormone acts directly on bone mobilising calcium, on the kidney causing phosphaturia and acts also in the kidney stimulating the synthesis of 1,25 dihydroxycholecalciferol (Garabedian et al., 1972). This hormonal form of vitamin D is responsible for calcium mobilisation from intestinal contents without the assistance of parathyroid hormone, and from bone together with parathyroid hormone.

A rise in blood calcium induces an inhibition of the parathyroid gland and stimulates the C-cells in the thyroid gland to produce calcitonin (Copp, 1967). The hypocalcaemic effect of calcitonin is the result of an inhibition of bone destruction (Milhaud et al. 1965) and an increased renal excretion rate of calcium in the kidney (Cochran et al., 1970).

1.2. RENAL HANDLING OF CALCIUM

In the human kidney a remarkably effective reabsorption of calcium by the renal tubules occurs and only 0.5 to 1 % of the filtered calcium appears in the urine.

Micropuncture and microperfusion studies on animals (Lassiter et al., 1963 ; Frick et al., 1965 ; Duarte and Watson, 1967 ; Brunette et al., 1969), provide evidence that calcium is actively reabsorbed in the proximal and distal tubule. The bulk of calcium reabsorption takes place in the proximal tubule (Lassiter et al. 1963 ; Duarte and Watson, 1967), 20 % to 25 % is reabsorbed in the loop of Henle, 10 % in the distal tubule and most of the remainder in the collecting ducts (Lassiter et al., 1963).
There is no evidence for secretion of calcium by the renal tubule, although transtubular flux of calcium can occur in dogs (Ginn et al., 1959 ; Bronner and Thompson, 1961) and rats (Frick et al., 1965). Also a maximum reabsorptive capacity for calcium has not been demonstrated (Massry et al., 1973).

Anions which bind calcium in urine are present in concentrations higher than that in serum. The influence of these anions on calcium binding is conditioned by variations in total ionic strength and pH of the urine (Schubert and Lindenbaum, 1952).
Different calcium complexes are formed which may be reabsorbed at different rates.

The normal range of urinary calcium excretion in humans varies greatly in different parts of the world. Evidence is mounting that the calcium excretion has risen during the past twenty years (Nordin et al., 1967 ; Nordin et al., 1972/b). In children the urinary calcium excretion is expressed as a function of body weight, and the reported values for normal children are discussed in Chapter 3.

1.2.1. SOME NON-HORMONAL FACTORS INFLUENCING URINARY EXCRETION OF CALCIUM

Extracellular volume expansion and interrelationship with sodium excretion

An increase in urinary calcium excretion in animals occurs if sodium is infused (Walser, 1961 ; Antoniou, 1969), even if the aorta is experimentally constricted (Levinsky and Lalone, 1963 ; Massry et al., 1967/b ; Blythe et al., 1968). These observations indicate that the volume expansion due to saline infusion causes a decrease in the renal tubular reabsorption of calcium, as well as of sodium. Micropuncture studies by Duarte and Watson (1967) demonstrate that indeed volume expansion produced by saline infusion decreases the proximal reabsorption of both calcium and sodium.

In animals the relationship between the clearances of filtrable calcium and sodium, which is near unity over a wide range of changes in sodium clearance (Walser, 1961 ; Massry et al., 1967/b ; Brickman et al., 1971), can be modified by the urinary excretion of large quantities of unreabsorbable anions, particularly those which complex with calcium (Walser, 1961). Also, when saline infusions are

administered with phosphate infusions (Coburn et al., 1971), or when saline infusions are administered to calcium depleted animals (Brickman et al., 1971), the renal tubule reabsorbs calcium in preference to sodium.

Davis and Murdaugh reported in 1970 that the infusion of hyperoncotic albumin administered to dogs, a procedure which decreases the sodium reabsorption by the proximal tubule, failed to increase the renal excretion of calcium. This provides strong evidence that it is the extracellular volume expansion produced by saline infusions that decreases the distal tubular reabsorption of calcium. That the distal segments of the nephron play an important role in determining calcium excretion was shown by an increase in calcium excretion, without inhibition of sodium reabsorption by the proximal tubule, following bradykinin and prostaglandin E_2 administration to dogs (Schneider et al., 1973)

Changes in glomerular filtration rate

In dogs an acute rise in glomerular filtration rate without extracellular volume expansion has little effect on calcium and sodium excretion (Massry and Kleeman, 1972), but a chronic rise may be associated with a significant loss of calcium (Massry et al.,1973).

Acute reductions in the glomerular filtration rate reduce the urinary calcium excretion in animals (Widrow and Levinsky, 1962 ; Blythe et al., 1968).

Chronic reduction in the glomerular filtration rate tends to decrease the calcium clearance (Hodgkinson and Pyrah, 1958) in the human kidney.

Hypercalcaemia

The filtered calcium load can be increased by augmenting the glomerular filtration rate, resulting in small changes in the urinary calcium excretion or by elevating the serum calcium level, causing a marked increase in calcium excretion.
When calcium infusions are administered to animals whereby hypercalcaemia is produced,the absolute amount of calcium reabsorbed increases, but the fractional reabsorption of the filtered calcium decreases (Massry et al., 1968/b ; Coburn et al., 1970/a). This effect could be due to hypercalcaemia itself or its suppression of the parathyroid gland. The concomitant increase in urinary sodium excretion with these calcium infusions, even if the glomerular filtration rate is reduced, suggests that the tubular reabsorption of sodium is inhibited (Coburn et al., 1970/a).

Micropuncture studies located the reduced sodium reabsorption during hypercalcaemia in the proximal tubule (Dibona, 1971) and in the loop of Henle (Suki et al., 1969). The inhibition of sodium reabsorption could be due to hypercalcaemia or competition between calcium and sodium for a common transport mechanism.

Acidosis

As early as 1899 Gerhardt and Schlesinger reported that in metabolic acidosis induced by uncontrolled diabetes, hypercalciuria usually occurs.

An acid-ash diet induces hypercalciuria (Nelson, 1928) in epileptic children, and oral administration of hydrochloric acid (Givens, 1918; Stehle and McCarty, 1921 ; Logan, 1935) does so in human beings and in animals. This hypercalciuria results from a decrease in tubular reabsorption as it has been observed when filtered calcium is decreased in man (Lemann et al., 1967). The hypercalciuric response to metabolic acidosis is not due to acidosis itself, as respiratory acidosis does not increase calcium excretion in man (Epstein, 1960) and in rats (Silberg et al., 1964).

During chronic acidosis there is a correlation between the urinary excretion of calcium and sodium plus ammonium ions in man (Lemann et al., 1967 ; Walser, 1971).
Acute metabolic acidosis inhibits the proximal tubular reabsorption of both calcium and sodium, but enhances the Na^+ - H^+ exchange in the distal nephron, causing the increase in clearance of calcium to be greater than that of sodium (Stein et al., 1968 ; Levine and Nash, 1971).

Diuretic agents

Osmotic diuresis induced by mannitol, sucrose, urea or saline causes a calciuresis and natriuresis (Walser, 1961 ; Better et al., 1966 ; Blythe et al., 1968). The greater inhibition of reabsorption of water in the proximal tubule, compared to sodium decreases the concentration of calcium in the tubular fluid and probably inhibits the tubular reabsorption of calcium (Massry et al., 1973).

In the dog those diuretics which affect the loop of Henle and distal nephron segments cause a greater increase in calcium excretion than those diuretics which primarily affect the proximal tubule (Eknoyan et al., 1970). Also in man, diuretic agents have variable effects on the urinary calcium excretion. The benzothiazide diuretics given over longer periods of time produce a decrease in the urinary excretion of calcium in man (Lamberg and Kuhlback, 1959 ; Walser and Trounce, 1961 ; Higgins et al., 1964 ; Brickman et al., 1972 ; Parfitt, 1972) and are used for treatment of patients with renal stones and idiopathic hypercalciuria (Yendt et al., 1970).

Ingestion of carbohydrate

The ingestion of glucose, galactose or fructose results in an increase in urinary calcium excretion in humans (Lindeman et al. 1964; Hodgkinson and Heaton, 1965 ; Lindeman et al. 1967 ; Lemann et al., 1970/a), with little or no augmentation in sodium excretion. After glucose ingestion the sodium reabsorption increases in the proximal tubule and decreases in the distal tubule. A fall in urinary pH and an increase in acid excretion is seen after glucose ingestion. The reduced tubular reabsorption of calcium due to acidosis and glucose ingestion is through separate mechanisms (Lennon and Piering, 1970).

Perfusion of the proximal tubules of the newt kidney with glucose-containing solutions produces a concentration related increase in the transtubular potential difference (lumen negative) and an increase of active sodium transport (Maruyama and Hoshi, 1972). Kokko (1973) showed that when isolated rabbit proximal tubules are perfused with solutions containing mixtures of glucose, alanine and bicarbonate the lumen potential which is negative near the glomerulus becomes positive later on in the nephron due to the transtubular chloride diffusion potential. The positive lumen potential difference facilitates the reabsorption of cations.

Using Kokko's model (Kokko, 1973), Lennon et al. (1974) proposed that in humans, with the increased glucose delivery to the proximal tubule, glucose absorption proceeds along a longer segment of the proximal tubule. The glucose absorption augments sodium absorption (Hoffman et al., 1969 ; Schloeder and Stinebaugh, 1970), and thereby delays the development of a positive potential difference later on in the nephron and inhibits the reabsorption of other cations, such as calcium.

Dietary intake of protein, fat, sodium, calcium and magnesium

A high protein diet increases the urinary calcium excretion (Margen and Calloway, 1967) in healthy subjects, but a diet rich in fat does not (Lindeman et al., 1967).

In humans the urinary calcium varies with alterations in sodium intake (Hills et al., 1959 ; Kleeman et al., 1964 ; Philips and Cooke, 1967 ; Epstein, 1968).

Some dependency of urinary calcium excretion on calcium intake has been recognized for many years (Bauer et al., 1929 ; Knapp, 1947). The efficiency with which calcium salts are absorbed from the intestine varies considerably from one individual to another (Bhandarkar et al., 1961), and depends also on the particular calcium containing compounds which are ingested.
Calcium chloride, lactate, gluconate and citrate are absorbed relatively rapidly, whereas calcium carbonate and phosphate are not (Bhandarkar et al., 1961). Peacock et al. (1967) calculated from the data of Knapp (1947) that in the majority of people the urinary excretion of calcium changes only slightly, even with rather large changes in dietary calcium. In normal adult subjects Nordin et al. (1972/b) calculated that the slope of the regression equation between urinary (mg/24 hr) (y) and dietary calcium (mg/24 hr) (x) was only 0.056 ($y = 0.056 x + 126$). Dietary calcium intake must therefore, be very high before it produces hypercalciuria.

A low magnesium intake decreases and a high magnesium intake increases the urinary calcium excretion (Cunningham and Cunningham, 1938 ; Heaton and Parsons, 1961 ; Alcock and McIntyre, 1964 ; Heaton, 1965 ; Clark, 1969/b).

Phosphate depletion and loading

During phosphate depletion a marked hypercalciuria occurs in humans (Lotz et al., 1968) and animals (Day and McCollum, 1939 ; Young et al., 1966 ; Coburn and Massry, 1970/b) caused by a reduction in tubular reabsorption of calcium, which is not due to changes in the tubular reabsorption of other ions (Coburn and Massry, 1970/b). Extrarenal mechanisms related to events occurring in bone or secondary hyperparathyroidism, may contribute to the hypercalciuria.

In contrast, the acute oral or intravenous administration of phosphate is associated with a significant reduction in urinary calcium (Albright et al., 1932 ; Bernstein and Newton, 1966 ; Hebert et al., 1966 ; Massry et al., 1968/c; Coburn et al., 1971), and mild hypocalcaemia can occur (Smith and Nordin, 1964).

Activity

The urinary excretion of calcium and sodium decreases after exercise, probably due to the decrease in the filtered load of these ions which is due to the slight fall in glomerular filtration rate during exercise (Kattus et al., 1949 ; Heaton and Hodgkinson, 1963 ; Loutit, 1965). Bed rest leads to an increase in urinary calcium excretion (Cuthbertson, 1929), which can not be prevented by a low calcium intake (Howard et al., 1945) and is due to a relative increase in rate of bone resorption.

Diurnal variation

Many investigators have reported a diurnal variation in the urinary excretion of calcium (Heaton and Hodgkinson, 1963 ; Wesson, 1964 ; Edwards and Hodgkinson, 1965/b ; Briscoe and Ragan, 1966). A rapid rise in the urinary calcium excretion to a maximum during the late morning which coincides with, or slightly precedes, the peak in urinary sodium excretion, is followed by a decrease in the afternoon and a rise during the evening, and again a decrease during the night (Heaton and Hodgkinson, 1963). The mid'day peak in the calcium excretion relates to the hours food is taken as this peak is eliminated during fasting (McIntosh et al., 1962; Heaton and Hodgkinson, 1963), or by giving food at regular intervals around the clock (Loutit, 1965).

Seasonal variation

Morgan et al. (1972) and Robertson et al. (1974) reported a seasonal variation in urinary calcium excretion, with a maximum excretion in July and August and a minimum in December and January. The difference between the mean values in summer and winter was 120-150 mg/24 hr. These seasonal variations in the daily urinary excretion of calcium can take place independently of changes in the dietary calcium intake (Transbol et al., 1975).
The seasonal changes in urinary calcium follow the monthly pattern of hours of sunshine, and changes in vitamin D levels were therefore, thought to be the cause. However, the peak in serum level of 25-hydroxycholecalciferol occurs in the late summer (McLaughlin, 1974 ; Stamp and Round, 1974).

1.2.2. SOME HORMONAL FACTORS INFLUENCING URINARY EXCRETION OF CALCIUM

Parathyroid hormone

Many clinical and experimental conditions, such as parathyroidectomy in animals and in patients with parathyroid adenomas, provide evidence that parathyroid hormone increases tubular reabsorption of calcium for any level of the filtered load of calcium without changing the renal excretion of sodium (Talmage and Kraintz, 1954 ; Talmaga, 1956 ; Canary and Kyle, 1959 ; Kleeman et al., 1961 ; Walser, 1973).

After the administration of parathyroid extract the urinary calcium excretion may be reduced in humans as well as in experimental animals, (Kleeman et al., 1961 ; Widrow and Levinsky, 1962 ; Bernstein et al. , 1963 ; Edwards and Hodgkinson, 1965/b ; Eisenberg, 1965 ; Bethune et al., 1968) but most commonly no change (Buchanan, 1961), or an increase is seen (Albright et al., 1929 ; Pechet et al., 1961).

Stop-flow (Widrow and Levinsky, 1962), micropuncture (Agus et al., 1971) and clearance studies (Massry et al., 1968/b) in animals located the effect of parathyroid hormone on the calcium reabsorption primarily in the distal nephron.

Thyrocalcitonin

Thyrocalcitonin increases the urinary excretion of calcium and sodium in normal subjects and patients with hypoparathyroidism (Cochran et al., 1970 ; Paillard et al., 1972). As the increase of urinary sodium produced by calcitonin is due to decreased reabsorption in the proximal nephron (Paillard et al., 1972), it is likely that the increase in the excretion of calcium is due also to an inhibition of the reabsorption in the proximal tubule.

Growth hormone

Growth hormone increases the urinary excretion of calcium in man without changing the level of serum calcium (Ikkos et al., 1959 ; Beck et al., 1960 ; Henneman et al., 1960 ; Hanna et al., 1961 ; Karam et al., 1961), suggesting a decreased tubular reabsorption of calcium. Patients with acromegaly often show hypercalciuria and can develop renal stones (Bauer and Aub, 1941 ; Harrison et al., 1960 ; De Gennes et al., 1961).

Thyroid hormone

Hypercalciuria accompanies hyperthyroidism, and a reduction in urinary calcium excretion, hypothyroidism (Aub et al., 1929 ; Krane et al., 1956 ; Kleeman et al., 1958 ; Cook et al., 1959). The calcium balance is distinctly negative in hyperthyroidism (Cook et al., 1959), suggesting that the effects of thyroid hormone on the kidney are due to the effects of the hormone on bone metabolism.

Vitamin D

Vitamin D and its 25-hydroxylated derivative increase the urinary excretion of calcium in human as well as in animals (Hanna, 1961 ; Litvak et al., 1958 ; Edwards and Hodgkinson, 1965/a ; Puschett et al., 1972).

Following the identification of 1,25-dihydroxycholecalciferol (Holick et al., 1971 and 1972), DeLuca (1976) hypothesised that 1,25-dihydroxycholecalciferol directly stimulates the intestinal absorption of calcium in the absence of parathyroid hormone, but mobilises calcium from bone only in the presence of parathyroid hormone. Stanbury (1976) suggested that the action of 1,25-dihydroxycholecalciferol on the gut is permissive, and that other factors determine the quantitative extent of intestinal calcium absorption.

Adrenocortical steroids

In humans glucocorticoids cause osteoporosis and an increased urinary calcium excretion (Pechet et al., 1959 ; Edwards and Hodgkinson, 1965/a) due to the catabolic action of these hormones, and probably partly due to an increased glomerular filtration rate (Bethune, 1962).

The acute administration of mineralocorticoids has no influence on calcium excretion in man or dog (Massry et al., 1967/a ; Lemann et al., 1970/b), but the chronic administration increases the urinary calcium excretion (Massry et al., 1968/a). The sodium retention that occurs during prolonged mineralocorticoid administration leads to extracellular volume expansion, and this produces a decrease in the proximal tubular reabsorption of sodium and calcium. By promoting the sodium but not the calcium reabsorption in the distal segment of the nephron an increase in urinary calcium excretion is produced.

Antidiuretic hormone

When lysine vasopressin or Pitressin(R) was administered to human beings an inverse correlation between calcium excretion and urine flow was found by some investigators (Dicker and Eggleston, 1961 ; Nielsen, 1964), but not by others (Gardner et al., 1962 ; Leaf et al., 1963 ; Fisch et al., 1965).

Other hormones

Catecholamines (Morey and Kenney, 1964), glucagon (Charbon et al., 1963 ; Pullman et al., 1967) and angiotensin (Gantt and Carter, 1964), all increase the urinary excretion of calcium in animals.

Oestrogens reduce the urinary excretion of calcium in elderly women (Ackerman et al., 1954).

Androgens reduce the urinary calcium excretion or have no effect (Fischer and Hastrup, 1964).

1.3. CAUSES OF HYPERCALCIURIA

There are many factors influencing the urinary calcium excretion and many clinical diseases are associated with hypercalciuria (Fourman, 1965 ; Massry et al., 1973). The cause for the hypercalciuria such as excessive intake of calcium or vitamin D, hyperparathyroidism, other endocrinopathies, bone diseases, or diseases associated with renal tubular defects, can often not be established.
In these circumstances these patients are then classified as belonging to the group having "idiopathic hypercalciuria" (Flocks, 1940 ; Albright et al., 1953 ; Henneman et al., 1958 ; Parfitt et al., 1964 ; Edwards and Hodgkinson, 1965/b).

Two major causes are proposed for the hypercalciuria in idiopathic hypercalciuria.
The first is an enhanced intestinal absorption of calcium : absorptive hypercalciuria (Dent and Watson, 1965 ; Eisenberg, 1965 ; Nordin et al., 1967 and 1972/b ; Pak et al., 1974). With rigid restriction of

dietary calcium the urinary excretion of calcium falls (Nordin et al., 1967).
The second is a primary defect in the renal tubular reabsorption of calcium : renal hypercalciuria (Edwards and Hodgkinson, 1965/b ; Coe et al., 1973).

Coe et al. (1971) reported increased levels of circulating parathyroid hormone in many patients with idiopathic hypercalciuria. They suggested that it is highly unlikely that a normocalcaemic form of primary hyperparathyroidism (Wills et al., 1969/b) is the basis of idiopathic hypercalciuria, because the parathyroid hormone levels are not elevated in all patients and, when the parathyroid hormone levels are high they are lowered when the hypercalciuria is suppressed with thiazide. Coe et al. (1973) hypothesised that idiopathic hypercalciuria is often due to a primary renal defect of calcium handling that leads to secondary hyperparathyroidism.

CHAPTER 2

PATIENTS, HEALTHY CONTROL CHILDREN, PROCEDURES AND METHODS

2.1. PATIENTS

The study was started in 1972 and all children with diabetes mellitus attending the diabetic clinic participated in it. New patients were admitted to the study, provided they had received insulin for at least one month.
In total forty-seven diabetic children were studied, aged 2.9-16.5 years.
Fig. 2.1. illustrates the chronological age distribution and table 2.1. lists the age, duration of diabetes, height and weight of the diabetic boys and girls when first studied.

Table 2.1. : Age, duration, height and weight of the diabetic boys and girls at the onset of the study.

	Boys (n= 27)	Girls (n= 20)
	Mean ± 1 SD	Mean ± 1 SD
Age (yr)	11.02 ± 3.94	11.66 ± 3.32
Duration of diabetes (yr)	4.30 ± 3.23	4.23 ± 3.52
Height (SDS)	+ 0.50 ± 1.15	+ 0.25 ± 0.86
Weight (SDS)	+ 0.32 ± 1.12	+ 0.17 ± 0.85

All children received long-acting insulins, lente (Novo^R) and semi-lente (Novo^R) and followed a prescribed diet. The mean duration of the insulin administration to the children, when they were studied for the first time, was 4.3 years (range : 1 month - 12.5 years). The caloric content of the prescribed diet taken as three meals and three snacks, consisted of 45 % - 55 % carbohydrate, 35 % - 40 % fat and 15 % - 20 % protein. Sweets and food of a high carbohydrate content were omitted from the diet. Exchange lists were used. No extra vitamin D was prescribed.

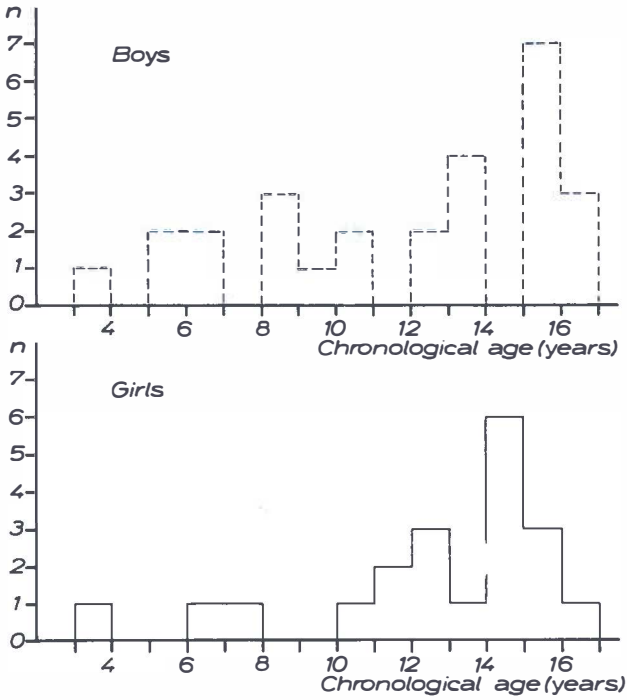


Fig. 2.1. Chronological age distribution of the diabetic boys and girls.

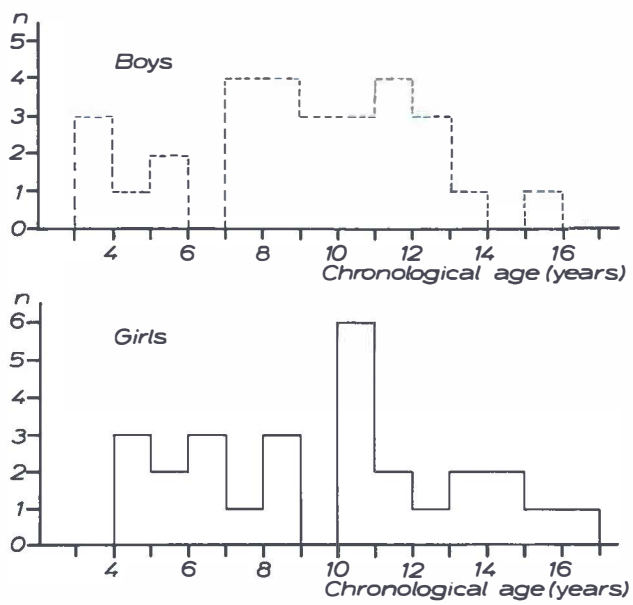


Fig. 2.2. Chronological age distribution of the healthy boys and girls.

2.2. HEALTHY CONTROL CHILDREN

The urinary excretion of several substances was studied in sixty healthy children, aged 3.3 - 16.3 years, in June 1975. These children did not receive any medication or extra vitamins. All of them were children of members of the medical or nursing staff or their friends. Only families where the parents were sure that accurate facts could be obtained cooperated with this study. The dietary habits of the children were not changed. The mean caloric intake consisted of 45 % - 49 % carbohydrate, 38 % - 43 % fat, and 11 % - 13 % protein.

Fig. 2.2. shows the chronological age distribution and table 2.2. lists the age, height and weight of the twenty-nine boys and the twenty-seven girls.

Table 2.2. : Age, height and weight of the healthy boys and girls.

	Boys (n= 29)	Girls (n = 27)
	Mean ± 1 SD	Mean ± 1 SD
Age (yr)	9.2 ± 3.11	10.08 ± 3.73
Height (SDS)	+ 0.33 ± 0.87	+ 0.51 ± 1.17
Weight (SDS)	- 0.20 ± 0.90	- 0.12 ± 0.75

The urinary cyclic AMP excretion was studied in a fasting state for 2 hours allowing 200 ml of water at the onset of the collection period, in seventeen healthy children, aged 3 - 15 years, while at home.

A fasting blood sample was taken for serum FFA, after informed consent was obtained from the parents, from thirty-seven children aged 4 - 17 years hospitalised for elective minor operations. In twelve of these children iPTH was also determined.

2.3. PROCEDURES

The estimation of the dietary intake in the diabetic children was based on analysis of the diet they received in the hospital, and also by asking what amounts of foods and fluid were ingested the previous day.

On an out-patient basis at regular intervals and during annual check-ups of the patients in the hospital, 24 hour urine samples were collected in bottles which did not contain any preservatives or acid. To minimise the precipitation of calcium salts the urine was kept at room temperature. The day the urine collection was terminated the urinary analyses were performed.

From each of the sixty healthy children one 24 hour urine sample was collected at home, in the same manner as that for the diabetic children. In these children the dietary habits were not changed during the urine collection period and the amounts of food and fluid, estimated by weighing and measuring, were recorded by the parents.

The completeness of each 24 hour urine collection was judged on the creatinine excretion. For the healthy control children and also for the diabetic children, the correlation between the body surface and the 24 hour urine creatinine excretion proved to be more reliable than similar equations with body weight, height or age.
The 24 hour creatinine excretion (mg) for the healthy children was :
1,001.18 x body surface (m^2) - 400.97
($r = 0.891$; $p < 0.01$; $n = 56$; estimated standard deviation of error : 139.44)
and for the diabetic children :
1,202.97 x body surface (m^2) - 550.42
($r = 0.862$; $p < 0.01$; $n = 47$; estimated standard deviation of error : 227.63).
The 24 hour urine collection of a child was excluded from the study if the creatinine excretion was outside the limits of confidence.

For the determination of the urinary hydroxyproline excretion the diabetic children were kept on a gelatin-free diet the day of the urine collection and for two days prior to it.

In the diabetic children urine was also collected from 7 a.m. - 12 noon, 12 noon - 5 p.m., 5 p.m. - 10 p.m., 10 p.m. - 7 a.m.

During the yearly one day in-patient check-up of the diabetic children, blood glucose was estimated at 11.30 a.m., 3 p.m., 5 p.m., 10 p.m., 4 a.m. and 8 a.m. After an overnight fast the urine was collected from 7 a.m. to 9 a.m. for studies on the renal handling of phosphate and creatinine clearance, and urinary acetone determination. At 8 a.m., while still fasting and not having yet received insulin, a blood sample was taken for the determination of calcium, phosphate, magnesium, creatinine, alkaline phosphatase, albumin and free fatty acids.
The day before the hospitalisation 2 - 8 extra units of insulin were administered to avoid high blood glucose levels.
While in hospital, one year oral calcium, and another year indomethacin was administered to the children who were kept fasting from 7 p.m. until 10 a.m. the next day.
Distilled water was given at specific times to ensure an adequate urine flow during the study : 250 ml at 8 p.m. and again at 10 p.m., 150 ml at 6 a.m., 7 a.m. and 9 a.m.. The daily insulin administration was delayed until completion of the test at 10 a.m. Urine was collected from 6 a.m. until 8 a.m. (control period) and from 8 a.m. until 10 a.m. (test period). Blood samples were taken at 8 a.m. and at 10 a.m. The oral calcium and indomethacin tests were explained to the parents and the children. Only when signed informed consent from the parents was obtained could their children participate in these investigations.

2.4. GENERAL METHODS

In the diabetic children the measurement of height was performed according to Tanner et al. (1966/a and 1966/b) using the Harperden stadiometer. The measurement of weight was performed with beam scales.
The healthy control children were measured and weighed by their parents at home.

The dietary intake of fat, protein, carbohydrate, calcium and phosphate was estimated using the Dutch food tables listing the contents of these substances (Nederlandse Voedingsmiddelentabel, 1973).

Urine pH was determinated by dip strip method (Merck) or with a pH meter (PHM G2 Standard pH meter, Radiometer, Copenhagen), the presence of acetone and of ketone bodies were determined by the acetest (Ames) and by ketostix (Ames), respectively. Later on in the study urinary acetone was measured gaschromatographically (Packard-Becker, Model 409, 2 m x 2 mm i.d. glascolumn packed with Chromosorb 102, 80-100 mesh, FID detector).

Quantitative glucose analysis in urine was performed with a Thorn automatic polarimeter (Thorn Automation Limited,Nottingham, England), which is an electronically controlled, self balancing photo-electric polarimeter.

In the laboratory the urine was shaken well, and after measuring the volume the aliquots taken for the determination of the calcium content were acidified to pH 4, by means of 25 % w/v hydrochloric acid.
All urinary assays, except magnesium, were performed by automated methods on continuous flow systems (Auto-analyser-I and SMA-6 from Technicon Instruments Corporation, Tarrytown, N.Y.). Calcium was determined according to the method of Gitelman (1967), based on the reaction with cresolphtalein complexone, including the use of 8-hydroxyquinoline to virtually eliminate the interference of magnesium. Inorganic phosphate was determined according to the method of Amador (1972), based on the formation of phosphomolybdic acid. The determination of magnesium (Bohnon, 1962), creatinine (Chasson et al., 1961) and chloride (Skeggs and Hochstrasser, 1964) was based on the reaction with xylidylblue, alkaline picrate and mercuric thiocyanate, respectively. Sodium and potassium were simultaneously determined using a two-channel flame photometer with lithium as internal standard. The precision of the method for determining the urinary concentration of the different substances was estimated by statistical analysis of fifteen determinations on the same batch and of determinations on the same specimen twenty consecutive days (table 2.3.). The coefficient of variation for these determinations was below 3 %, except for magnesium, where it was 5.6 % in the day to day variation and 4.2 % within the batch variation.
The accuracy was tested by regression analysis in a dilution curve. The intercept was always zero. The coefficient of variation was less than 2 % in all instances. The curve was strictly linear.

Calcium ion in tap water was determinated by a titration method with murexide as indicator.

Urinary cyclic AMP was measured by a competitive binding assay using a commercially available kit (Radio Chemical Centre, Amersham, Buckinghamshire, England).

Table 2.3. : Estimation of the precision of the methods for the determination of several urinary substances.

	Day to day variation (n = 20)		Within batch variation (n = 15)	
	Mean ± 1 SD	Coefficient of variation	Mean ± 1 SD	Coefficient of variation
sodium (mEq/l)	84 ± 1	1.2 %	84 ± 0.7	0.8 %
potassium (mEq/l)	27 ± 0.9	3.0 %	28 ± 0.6	2.0 %
chloride (mEq/l)	94 ± 1.6	1.7 %	95 ± 1.0	1.1 %
creatinine (mg/dl)	66 ± 1.5	2.3 %	66 ± 1.1	1.7 %
calcium (mg/l)	119 ± 1.7	1.4 %	120 ± 1.0	1.7 %
phosphate (mg/l)	410 ± 5.5	1.3 %	410 ± 3.4	0.8 %
magnesium (mg/l)	39 ± 2.2	5.6 %	40 ± 1.7	4.2 %

Urinary hydroxyproline analyses were performed with the Hypronosticon kit (Organon Technika, Oss, The Netherlands). This method is based on the Stegemann (1958) procedure modified by Goverde and Veenkamp (1972).

Serum calcium, phosphate, magnesium and creatinine determinations were performed with the same methods employed for the urinary determination of these substances.
Serum ionised calcium was determinated with an Orion SS-20 ionised calcium Analyser (Research Inc. 1975, U.S.A.). Serum alkaline phosphatase was determined according to the method of Morgenstern et al. (1965).
Blood glucose was measured by the glucose-oxidase method (Saifer and Gerstenfeld, 1958). The serum free fatty acids were estimated colorimetrically (Regouw et al., 1972).
Blood pH, actual bicarbonate and standard bicarbonate were performed on an I.L. 313 digital pH blood-gasanalyser (Instrumentation Laboratory Inc., Lexington, Massachusetts, U.S.A.).

Plasma PTH, hCT and hGH were measured by radio-immunoassay. Plasma iPTH was determined with a slightly modified method of Lequin et al., (1970). Values exceeding 0.25 ng equiv. b. PTH/ml were considered as elevated. Plasma hGH was determined according to the method of Touber (1964) after the modification of Schopman and Hackeng (1971) and plasma hCT according to the method of Hackeng et al. (1970).

Indomethacin was determined in serum according to the method of Holt and Hawkins (1965) after the modification of Hvidberg (1972).

X-rays of the left hand and wrist were taken according to Tanner and Whitehouse (1962). The length (L) of the second metacarpal bone was measured with a ruler, and the external diameter (D) and the internal diameter (d) of the cortex at the midpoint of the shaft were measured with a measuring lens (Flubacher & Co, Horgen, Switzerland) to the nearest tenth of a millimeter (fig. 2.3.). The measurements were taken again one month later by the same person.
The average of the two measurements was used for the calculations thus diminishing the variability of the measurements (Naor et al., 1972).

Formula

The standard deviation score (SDS) for height and weight was calculated according to Tanner et al., (1971) :

$$SDS : \frac{X - \bar{x}}{s_x}$$

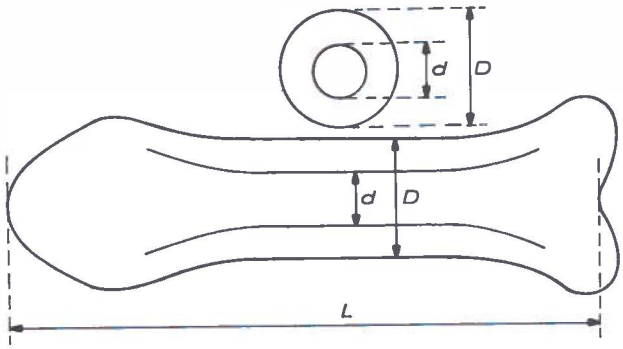
X is the measurement, $\bar{x}$ the mean at the relevant age and s_x the SD at that age.
The tables of van Wieringen (1972) for Dutch children were used for the mean height and weight, and the standard deviation at the relevant age.

The values of the tubular maximum reabsorption of phosphate per 100 ml glomerular filtration rate (TmP/GFR) were calculated using the formula of Bijvoet (1972).

When TRP $<$ 80 % : $TmP/GFR = TRP \times (P)$

When TRP $>$ 80 % : $TmP/GFR = \left[e^{10.3180x^2 - 5.1848x + 0.4022} \right] \times (P)$

$$x = \frac{C_{PO4}}{C_{Creat}}$$



$D - d$: cortical thickness

$\frac{D - d}{D}$ % : cortical index

$D^2 - d^2$: cortical area index

$\frac{D^2 - d^2}{D^2} \times 100$: percent cortical area

$\frac{D^2 - d^2}{DL}$: index of Exton-Smith

Fig. 2.3. Measurements taken from the X-ray of the second metacarpal bone, and the calculations based on these measurements.

On the basis of the measurements of the left second metacarpal bone (fig. 2.3.) the cortical thickness can be obtained by a simple subtraction D - d, for which Garn et al. (1967) published standards for Ohio whites, and Bonnard (1968) for Swiss children.
The ratio of the cortical thickness to the total diameter of bone is known as the cortical index $\frac{D - d}{D}$ % .

This index is constant in young adults (Barnett and Nordin, 1961). However, in children a defect in the bone cortex may be accompanied by a defect in the growth of the bone in width, bringing the index back to normal in spite of an abnormality such as osteoporosis. Exton-Smith et al. (1969/a) introduced a cortical area index : D^2-d^2 and for a uniform cylindrical bone it would be directly related to the ash content per unit length.
To make a valid comparison between the left second metacarpal bones of individuals, variations in skeletal size need to be taken into

account. This was done by introducing the product of metacarpal length (L) and diameter (D) into the calculation of the index $\frac{D^2 - d^2}{DL}$, yielding a dimensionless ratio of cortical area to surface area (Exton-Smith et al., 1969/b).
Gryfe et al. (1971) published values for the index of the cortical area $D^2 - d^2$ and the ratio $\frac{D^2 - d^2}{DL}$ in children.
Garn et al. (1971) introduced the percent cortical area (PCA) as $\frac{D^2 - d^2}{D^2} \times 100$. This measurement is directly related to bone density.

2.5. STATISTICAL METHODS

The results obtained in the diabetic children and in the control subjects are given as mean $\pm$ 1 standard deviation (Mean $\pm$ 1 SD).

The statistical analyses were performed using a CDC Cyber 74-16 computer. Linear and multiple regression equations were calculated (Croxton, 1953; Siegel, 1956; Snedecor and Cochran, 1967).

For comparison of independent values the Student "t" test was used. The statistical analyses of the paired control and test values (Chapter 5) were performed making use of the Student "t" test for paired samples.
Two-sided values of 0.05 or less were considered to be statistically significant in the Student "t" test as well as in the Student "t" test for paired samples.

When the chi-square test was applied it was calculated with Yates correction (Croxton, 1953). In instances where the numbers were rather small (Snedecor and Cochran, 1967), the probability of combinations of non-parametric data was also tested by a one-sided Fisher exact test (Fisher, 1958), logarithmically transformed and programmed on a HP 25 pocket calculator.

CHAPTER 3

REFERENCE VALUES IN HEALTHY CONTROL CHILDREN

The concept "reference values" was introduced in 1969 by Gräsbeck and Saris as being the scientific basis for clinical interpretation of laboratory data.

3.1. URINARY EXCRETION OF CALCIUM, INORGANIC PHOSPHATE, MAGNESIUM AND CREATININE

3.1.1. INTRODUCTION

In this study on the calcium metabolism of diabetic children it became necessary to know the urinary excretion of calcium, phosphate, magnesium and creatinine in healthy children living at home, and on their regular diet. Available data on the 24 hour excretion of these substances have been obtained only from hospitalized children and to our knowledge have not been reported for healthy Dutch children.

3.1.2 PATIENTS

Observations were made on sixty children, aged 3.3 to 16.3 years (Chapter 2.2.).
One 24 hour urine sample was collected at home from each child. During the day of collection the intake of food and fluid were estimated by weighing or measuring and were recorded by the parents.

3.1.3. RESULTS

The 24 hour urine collections of four of the sixty children were not included in the statistical analysis of the data. In two children the creatinine excretion was more than four standard deviations below the mean and, therefore, these two children were not included in the study.
In two other children the urinary calcium excretion was more than four standard deviations above the mean calcium excretion as calculated on the remaining fifty-six children. The discrepancies were not due to inadequate urine collection, for the urine creatinine excretion rates fell within the normal range. The results from these two children have been regarded as outliers, and have not been included in subsequent calculations. By an oversight one urinary magnesium determination was not carried out, and the data on urinary magnesium therefore concern fifty-five samples.

In table 2.2. are listed the height and weight, expressed in standard deviation score, and the age for boys and girls separately.

The 24 hour urinary excretion of calcium, phosphate and magnesium correlates with body weight (kg) : $r = 0.304$ ($p < 0.05$) ; $r = 0.712$ ($p < 0.01$) ; $r = 0.306$ ($p < 0.05$), respectively. The urinary excretion of these substances is therefore, expressed in terms of body weight unless otherwise mentioned.
There is no correlation between the urinary excretion of these substances expressed as mg/kg/24 hr and age or sex.

In table 3.1. are listed the mean and standard deviation of the dietary intake of calcium, phosphate, carbohydrate, fat and protein, as well as the urinary excretion of creatinine, calcium, phosphate, magnesium, sodium, potassium and chloride.

Table 3.1.: Dietary intake and urinary excretion of several substances in fifty-six healthy children.

	Mean	Standard deviation
Intake		
calcium (mg/kg/24 hr)	29.30	11.90
phosphate (mg/kg/24 hr)	39.70	12.80
carbohydrate (g/kg/24 hr)	8.50	2.70
fat (g/kg/24 hr)	3.20	1.10
protein (g/kg/24 hr)	2.20	0.60
Urinary excretion		
calcium (mg/kg/24 hr)	2.40	1.40
phosphate (mg/kg/24 hr)	18.80	5.15
magnesium (mg/kg/24 hr)	1.70	0.80
sodium (mEq/kg/24 hr)	3.40	1.20
potassium (mEq/kg/24 hr)	1.40	0.40
chloride (mEq/kg/24 hr)	3.50	1.10
creatinine (mg/kg/24 hr)	22.20	4.03
volume (ml/kg/24 hr)	24.08	8.60

* n = 55

The mean caloric intake of the boys is 2,114 cal/24 hr, with 12.8 % protein, 41 % fat and 46.2 % carbohydrate. The girls have a mean caloric intake of 2,081 cal/24 hr, with 12.3 % protein, 40.1 % fat and 47.6 % carbohydrate.
The positively skewed distribution of the urinary excretion (mg/kg/24 hr) of calcium and magnesium per child is shown in fig. 3.1.

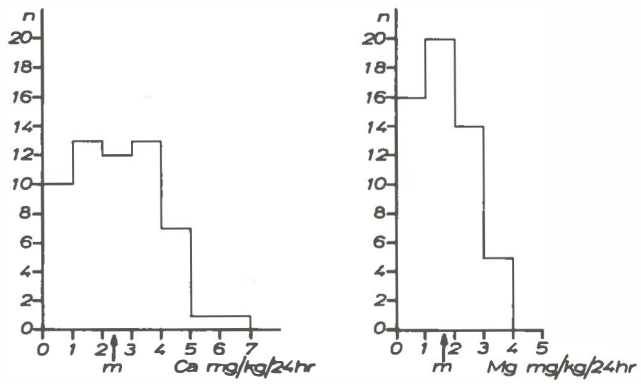


Fig. 3.1. Frequency distribution of urinary calcium and magnesium (mg/kg/24 hr) in the healthy children.

The one-sided 5 %, 2.5 % and 1 % upper limits of confidence for the urine calcium excretion are 4.70 mg/kg/24 hr, 5.20 mg/kg/24 hr and 6 mg/kg/24 hr.

Table 3.2. shows the correlation between the analysed urinary and dietary substances expressed in terms of body weight for the fifty-six children.
Urinary calcium excretion does not correlate with any analysed dietary substance, but does correlate with urinary creatinine ($r = 0.316$; $p < 0.01$), sodium ($r = 0.416$; $p < 0.01$) and phosphate ($r = 0.335$; $p < 0.05$).
There is also a significant correlation between urinary phosphate and dietary calcium ($r = 0.501$; $p < 0.01$), phosphate ($r = 0.422$; $p < 0.01$), protein ($r = 0.519$; $p < 0.01$), urinary sodium ($r = 0.454$; $p < 0.01$) and creatinine ($r = 0.377$; $p < 0.01$).
The urinary magnesium does not correlate with the calcium intake, but correlates significantly with the intake of fat ($r = 0.397$; $p < 0.01$) and protein ($r = 0.341$; $p < 0.05$).

In another approach to study possible multifactorial influences, the urinary excretion of calcium, phosphate and magnesium was calculated according to multiple linear regression equations, using the formula :

$$X_o = \beta_o + \beta_1 X_1 + \beta_2 X_2 \ldots\ldots\ldots \beta_p X_p$$

The constant factor (β_o) and the estimated regression coefficients ($\beta_1 \ldots\ldots \beta_p$) for the different factors ($X_1 \ldots\ldots X_p$) in the regression equation for the urinary calcium excretion (mg/kg/24 hr) are listed in table 3.3. The variance of error for this equation is 1.2154, the value of the F statistic is 3.9703 (13 - 42 DF; $p < 0.001$) and the multiple correlation coefficient is 0.74.
In a similar manner multiple regression equations for the urinary phosphate and magnesium have been calculated.

Table 3.2. : Correlation between dietary and urinary substances in the healthy children.

	I_{Ca}	I_P	I_{Prot}	I_{Fat}	I_{CHO}	U_{Ca}	U_P	U_{Mg}	U_K	U_{Na}	U_{Cl}	U_{Cr}
Intake												
calcium (mg/kg/24 hr)	1											
phosphate (mg/kg/24 hr)	0.903**	1										
protein (g/kg/24 hr)	0.826**	0.938**	1									
fat (g/kg/24 hr)	0.425**	0.642**	0.755**	1								
carbohydrate (g/kg/24 hr)	0.578**	0.744**	0.754**	0.670**	1							
Urine												
calcium (mg/kg/24 hr)	- 0.002	- 0.038	0.038	0.072	0.099	1						
phosphate (mg/kg/24 hr)	0.501**	0.490**	0.519**	0.141	0.422**	0.335*	1					
magnesium (mg/kg/24 hr)	0.214	0.193	0.341*	0.397**	0.199	0.157	0.200	1				
potassium (mEq/kg/24 hr)	0.471**	0.440**	0.432**	0.130	0.258*	- 0.023	0.507**	0.415**	1			
sodium (mEq/kg/24 hr)	0.364**	0.454**	0.507**	0.426**	0.470**	0.416**	0.454**	0.231	0.341*	1		
chloride (mEq/kg/24 hr)	0.296*	0.387**	0.420**	0.379**	0.382**	0.500**	0.408**	0.328*	0.372**	0.908**	1	
creatinine (mg/kg/24 hr)	0.111	- 0.140	0.057	- 0.159	- 0.018	0.316*	0.377**	0.169	0.076	0.247	0.208	1

* $p < 0.05$; ** $p < 0.01$

Table 3.3. : Multiple regression equation for urinary calcium (mg/kg/24 hr) in the healthy children.

	Estimated regression coefficient	Standard deviation	Value of T statistic (42 DF)
constant term	0.0003	1.2155	0.0002
Urine			
phosphate (mg/kg/24 hr)	0.1161	0.0461	2.5162
creatinine (mg/kg/24 hr)	0.0291	0.0505	0.5759
potassium (mEq/kg/24 hr)	-0.8182	0.5077	-1.6115
sodium (mEq/kg/24 hr)	-0.4829	0.3381	-1.4282
chloride (mEq/kg/24 hr)	1.1816	0.3632	3.2537
volume (ml/kg/24 hr)	-0.0364	0.0320	-1.1368
Intake			
calcium (mg/kg/24 hr)	0.0861	0.0407	2.1153
phosphate (mg/kg/24 hr)	-0.1308	0.0523	-2.5007
calories (cal/kg/24 hr)	1.7840	1.1512	1.5497
protein (g/kg/24 hr)	-7.1738	4.8030	-1.4936
fat (g/kg/24 hr)	-15.7586	10.3696	-1.5197
carbohydrate (g/kg/24 hr)	-7.0362	4.6054	-1.5278
fluid (ml/kg/24 hr)	0.0055	0.0217	0.2557

To simplify these equations the value of the T statistic was used, and then a combination of T statistic and correlation coefficient.
Table 3.4. lists the most useful equations with the value of the F statistic, the multiple correlation coefficient and the estimated standard deviation of error.

Table 3.4. : Multiple regression equations for urinary calcium, phosphate and magnesium in the healthy children.

$U_{Ca} = 1.5954 + 0.6232\ U_{Na} - 0.0320\ I_P$

$U_P = -2.9210 + 0.5803\ U_{Cr} + 0.2223\ I_P$

$U_{Mg} = -0.2007 + 0.2166\ I_{Fat} + 0.0521\ U_{Vol}$

	F statistic	Multiple correlation coefficient	Estimated standard deviation of error
U_{Ca}	8.2825* : $p < 0.005$	0.4880	1.6355
U_P	21.0414* : $p < 0.005$	0.6653	3.9196
U_{Mg}	17.4312**: $p < 0.005$	0.6335	0.6936

* 5-53 DF; ** 2-52 DF;

The mean and standard deviation of the urinary concentration ratios of calcium to creatinine, magnesium to creatinine and calcium to magnesium are listed in table 3.5. The use of these concentration ratios is valid as the correlation between urinary calcium excretion (mg/24 hr) and urinary creatinine excretion (mg/24 hr) is 0.425 ($p < 0.01$; $n = 56$), and between urinary creatinine (mg/24 hr) and urinary magnesium (mg/24 hr) is 0.403 ($p < 0.01$; $n = 55$).

Table 3.5. : 24 hour urinary concentration ratios in the healthy children.

	n	Mean	SD
$\frac{\text{calcium (mg)}}{\text{creatinine (mg)}}$	56	0.111	0.063
$\frac{\text{magnesium (mg)}}{\text{creatinine (mg)}}$	55	0.079	0.040
$\frac{\text{calcium (mg)}}{\text{magnesium (mg)}}$	55	1.822	1.333

The urinary calcium/creatinine concentration ratio correlated negatively with the phosphate intake (mg/24 hr) and positively with the urinary sodium excretion (mg/24 hr) : $r = -0.348$ ($p < 0.05$), and $r = 0.362$ ($p < 0.01$), respectively.

3.1.4. SUMMARY

Reference values are presented for the urinary calcium, magnesium, inorganic phosphate and creatinine excretions in healthy Dutch children who were living at home, with no dietary restrictions.

The 24 hour excretion of these urinary substances correlates with body weight.

The mean urinary calcium excretion in healthy Dutch children is 2.40 mg/kg/24 hr $\pm$ 1.40(SD). As the distribution of the urinary calcium excretion is positively skewed (fig. 3.1.) the one-sided upper 5 % confidence limit of 4.70 mg/kg/24 hr is considered to be a reference value.
The mean urinary calcium to creatinine concentration ratio is 0.111 $\pm$ 0.063 (SD), and the upper limit of this ratio is 0.234.

In this study on healthy children the urinary calcium excretion does not correlate with the calcium intake. The calcium excretion correlates negatively with the phosphate intake only when other factors are taken into account (table 3.4.). The sodium intake could not be estimated, but it was found that the urinary calcium excretion correlated with the sodium excretion.

The urinary phosphate excretion correlated strongly with the creatinine excretion and the phosphate intake.

The mean magnesium excretion in the healthy children is 1.70 mg/kg/24 hr $\pm$ 0.80(SD). The urinary magnesium to creatinine ratio is 0.079 $\pm$ 0.040(SD).Similarly to the urinary calcium excretion the urinary magnesium excretion correlates significantly with the urinary sodium excretion.

No correlation could be shown between urinary magnesium and urinary calcium excretion. The calcium intake did nog significantly influence the urinary magnesium extretion.

To conclude, in the evaluation of the urinary calcium extretion the sodium excretion and phosphate intake should be considered.

3.2 URINARY CYCLIC AMP EXCRETION

In seventeen healthy control children, a mean excretion of 5.80 μM cAMP/g creatinine $\pm$ 2.43 (SD) was found in a 2 hour urine specimen obtained after an overnight fast.
The 2 hour urinary excretion of cAMP was found to decrease with age ($r = -0.732$; $p < 0.01$; $n = 17$).

3.3. PLASMA iPTH AND SERUM FFA CONCENTRATIONS

A fasting blood sample was taken for serum FFA in thirty-seven children, hospitalised for elective minor operations. In twelve of these children plasma iPTH was also determined.

The mean plasma iPTH level was 0.181 ng equiv.b. PTH/ml $\pm$ 0.091 (SD).
Three children had values above 0.25 ng equiv.b. PTH/ml.

The mean fasting serum FFA level of the healthy control children was 562 μM/l $\pm$ 294 (SD).
The fasting serum FFA levels of the healthy control children were compared with those from the diabetic children. To distinguish good from poor diabetic control the value of 800 μM FFA/l was used. For this discriminatory value, the test gave negative results in 89 % of all subjects tested who had good diabetic control (specificity of the test), and gave positive results in 65 % of the patients who had poor control (sensitivity of the test) (Holland and Whitehead, 1974).

CHAPTER 4

STATISTICAL STUDIES ON THE URINARY EXCRETION OF CALCIUM IN DIABETIC CHILDREN

4.1. INTRODUCTION

In the first part of this Chapter the statistical validity of the incidence of hypercalciuria in diabetic children is demonstrated. The constancy of the hypercalciuria on follow-up is also shown.

As calciuria is governed by hormonal as well as by non-hormonal factors, as discussed in Chapter 1, the contribution of some of these factors to the urinary calcium excretion in the diabetic children was analysed statistically. The investigated non-hormonal factors were the urinary glucose excretion, the metabolic acidosis and the dietary intake of several substances. The hormonal factors studied were parathyroid hormone, calcitonin and growth hormone.

Operational definition of hypercalciuria.

If one uses the 5 % one-sided upper limit of confidence, a urinary excretion of calcium exceeding 4.70 mg/kg/24 hr should be considered as hypercalciuria, as shown in Chapter 3.1.3. In the course of the follow-up of the diabetic patients it became clear that children with a urinary excretion of calcium between 4.70 mg/kg/24 hr and 5.20 mg/kg/24 hr did not show constantly high urinary calcium excretion values. To avoid this,the 2.5 % one-sided upper limit of confidence for the urinary excretion of calcium,i.e. 5.20 mg/kg/24 hr was used.

4.2. INCIDENCE AND FOLLOW-UP OF HYPERCALCIURIA

The first twenty-four hour urine specimen collected from forty-seven diabetic children showed that the urinary excretion of calcium in the diabetic children had a bimodal frequency distribution as illustrated in fig. 4.1. Thirty-two diabetic children were normocalciuric with a mean urinary excretion of calcium of 2.41 mg/kg/24 hr $\pm$ 1.02 (SD). Fifteen diabetic children were hypercalciuric with a mean calcium excretion rate of 7.54 mg/kg/24 hr $\pm$ 1.79 (SD).

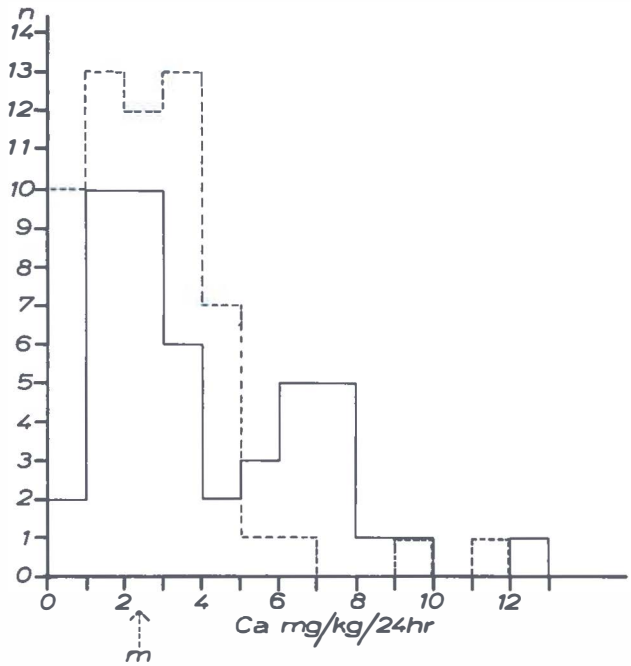


Fig. 4.1. Frequency distribution of urinary calcium excretion (mg/kg/24 hr) in forty-seven diabetic (——) and in fifty-eight healthy children (---).

Four of the fifty-eight control children, in contrast to fifteen of the forty-seven diabetic children, had a urinary calcium excretion rate exceeding 5.20 mg/kg/24 hr. The occurence of hypercalciuria was significantly higher in the diabetic group than in the control group.
The chi-square test = 9.19 ($p < 0.01$).

In the follow-up period, four diabetic children who at their first examination were normocalciuric became hypercalciuric.

In the next paragraph (4.4.1.) it is shown that this group of nineteen hypercalciuric children comprised thirteen children with a glucose-independent type of hypercalciuria, and six children with a glucose-dependent type of hypercalciuria.

Table 4.1. lists the occurrence of the established hypercalciuria during the follow-up period, in both hypercalciuric diabetic groups. For the four children who became hypercalciuric during the follow-up period, only the determinations of the urinary excretion of calcium from the onset of the hypercalciuria were included.
In the glucose-dependent hypercalciuric group, the hypercalciuria occurred often but not constantly. The glucose-independent hypercalciuria, on the other hand, was not an incidental finding. The child who had this characteristic kept it on follow-up. Of the patients with a glucose-independent hypercalciuria, only patient number 2 had once a normal urinary excretion of calcium.

Table 4.1. : Occurrence of urinary calcium excretion exceeding 5.2 mg/kg/24 hr in the diabetic children

	Patient	N° of determinations $U_{Ca} > 5.2$ (mg/kg/24hr) / Total N° of determinations
Glucose-independent hypercalciuria	1	4/4
	2	3/4
	3	7/7
	4	4/4
	5	9/9
	6	6/6
	7	5/5
	8	3/3
	9	4/4
	10	4/4
	11	3/3
	12	2/2
	13	4/4
Glucose dependent hypercalciuria	1	5/7
	2	1/1
	3	4/5
	4	3/7
	5	4/4
	6	4/5

4.3. COMPARISON OF THE URINARY EXCRETION OF SEVERAL SUBSTANCES BY NORMOCALCIURIC AND HYPERCALCIURIC DIABETIC CHILDREN AND BY CONTROL CHILDREN

As demonstrated in tables 2.1. and 2.2., the group of diabetic children was anthropometrically comparable with te group of healthy children. The age, height and weight were not statistically different.

Table 4.2. lists the mean age, height and weight, and the duration of diabetes in the hypercalciuric and the normocalciuric children at the beginning of the study, separately for boys and girls.

Table 4.2. : Age, duration of diabetes, height and weight in the normocalciuric and hypercalciuric diabetic boys and girls at the onset of the study.

	$U_{Ca} \leq 5.2$(mg/kg/24hr)		$U_{Ca} > 5.2$(mg/kg/24hr)	
	Boys (n= 19)	Girls (n= 13)	Boys (n= 8)	Girls (n= 7)
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
Age (yr)	11.75 ± 3.81	12.92 ± 1.70	9.29 ± 3.92	9.33 ± 4.39
Duration of diabetes (yr)	5.02 ± 2.85	5.56 ± 3.31	2.58 ± 3.61	1.75 ± 2.53
Height (SDS)	+0.45 ± 1.20	+0.19 ± 0.70	+0.61 ± 1.09	+0.35 ± 1.17
Weight (SDS)	+0.49 ± 1.21	+0.14 ± 0.81	-0.07 ± 0.80	+0.22 ± 0.98

There was no difference in height and weight between the hypercalciuric and normocalciuric boys and girls (Student "t" test : n.s.). In the diabetic boys there was no significant difference in age and duration of diabetes between hypercalciuric and normocalciuric boys. The hypercalciuric girls were significantly younger and their diabetes was of a shorter duration than that of the normocalciuric girls (Student "t" test : $p < 0.02$ in both cases).

In table 4.3. are listed the means and standard deviations of several substances excreted in the first 24 hour urine specimen collected from thirty-two normocalciuric and fifteen hypercalciuric diabetic children. In the same table the dietary intake of these children is also listed. The dietary intake is discussed later in Chapter 4.4.3.

Table 4.4. lists the 24 hour urinary concentration ratios of several urinary substances in the hypercalciuric and normocalciuric diabetic children.

As the diabetic group comprises two types of children only the normocalciuric diabetic children were compared with the healthy children (table 4.3.).
There was no significant difference in the excretion rate of calcium and sodium between these two groups (table 4.3.).
The urinary calcium to creatinine concentration ratio was also not different between normocalciuric diabetic and the control children (table 4.4.).
There was a significant difference between the urinary calcium to magnesium, and magnesium to creatinine concentration ratios of these two groups of children (table 4.4.).

In tables 4.3. and 4.4. are also compared the hypercalciuric and normocalciuric children. The urinary excretion of phosphate, magnesium, potassium and glucose were higher in the hypercalciuric diabetic group compared to that of the normocalciuric group, but the urinary excretion of sodium, chloride and creatinine was not different.
The 24 hour urinary concentration ratios of calcium to creatinine and calcium to magnesium were also significantly different between the hypercalciuric and the normocalciuric diabetic group (table 4.4.).
The hypercalciuric diabetic group is not homogenous, and comprises several "glucose-dependent" and "glucose-independent" hypercalciuric children, this is discussed in Chapter 4.4.1., and therefore, these conclusions may be open to criticism.

Table 4.3. : Dietary intake and urinary excretion of several substances in the normocalciuric and hypercalciuric diabetic children.

	$U_{Ca} \leq 5.2$ (mg/kg/24 hr) (n = 32) Mean ± 1 SD	Student "t" test compared to healthy children*	$U_{Ca} > 5.2$ (mg/kg/24 hr) (n = 15) Mean ± 1 SD	Student "t" test compared to normocalciuric diabetic children*
Intake				
calcium (mg/kg/24 hr)	35.33 ± 16.53	n.s.	52.03 ± 10.29	0.001
phosphate (mg/kg/24 hr)	43.31 ± 12.11	n.s.	56.82 ± 13.40	0.005
carbohydrate (g/kg/24 hr)	5.81 ± 1.79	0.001	6.78 ± 1.11	n.s.
fat (g/kg/24 hr)	2.45 ± 1.51	0.01	3.78 ± 2.23	0.005
protein (g/kg/24 hr)	2.21 ± 0.61	n.s.	2.67 ± 0.47	0.01
Urinary excretion				
calcium (mg/kg/24 hr)	2.41 ± 1.02	n.s.	7.54 ± 1.79	0.001
phosphate (mg/kg/24 hr)	22.40 ± 8.72	0.02	29.49 ± 7.07	0.002
magnesium (mg/kg/24 hr)	3.83 ± 1.94	0.001	4.56 ± 1.42	0.05
sodium (mEq/kg/24 hr)	3.07 ± 1.31	n.s.	4.08 ± 1.55	n.s.
potassium (mEq/kg/24 hr)	1.64 ± 0.59	0.02	2.30 ± 0.55	0.005
chloride (mEq/kg/24 hr)	2.23 ± 0.89	0.001	2.95 ± 1.23	n.s.
creatinine (mg/kg/24 hr)	24.77 ± 5.06	0.01	26.09 ± 6.19	n.s.
volume (ml/kg/24 hr)	38.39 ± 15.96	0.001	49.73 ± 18.71	0.05
glucose (g/kg/24 hr)	1.17 ± 0.88	-	1.96 ± 1.59	0.05

* : nearest value greater than p.

Table 4.4. : 24 hour urinary concentration ratios
in the normocalciuric and hypercalciuric diabetic children.

	$U_{Ca} \leq 5.2$ (mg/kg/24 hr) (n = 32)	Student "t" test compared to the healthy children *	$U_{Ca} > 5.2$ (mg/kg/24hr) (n = 15)	Student "t" test compared to the normocalciuric diabetic children*
	Mean ± 1 SD		Mean ± 1 SD	
$\frac{\text{calcium (mg)}}{\text{creatinine (mg)}}$	0.10 ± 0.03	n.s.	0.31 ± 0.15	0.001
$\frac{\text{magnesium (mg)}}{\text{creatinine (mg)}}$	0.16 ± 0.07	0.001	0.19 ± 0.07	n.s.
$\frac{\text{calcium (mg)}}{\text{magnesium (mg)}}$	0.88 ± 0.95	0.001	1.73 ± 0.72	0.005

* : nearest value greater than p

Table 4.5. shows that in the hypercalciuric diabetic group no correlation could be established between the urinary excretion of calcium and the urinary phosphate, magnesium, creatinine, glucose, and 24 hour urinary volume. However, in the normocalciuric diabetic group, the urinary excretion of calcium correlates with the urinary creatinine and glucose excretion. The relationships between urinary calcium and glucose excretion, and between the urinary calcium and sodium excretion, is discussed in Chapter 4.4.1. and 4.4.3., respectively.

Table 4.5. : Correlation coefficients between urinary calcium and urinary phosphate, magnesium, creatinine and glucose in the normocalciuric and hypercalciuric diabetic children.

	$U_{Ca} \leq$ 5.2 (mg/kg/24 hr) (n = 32)	$U_{Ca} >$ 5.2 (mg/kg/24hr) (n = 15)
U_P (mg/kg/24 hr)	0.106	0.153
U_{Mg} (mg/kg/24 hr)	0.287	0.213
U_{Cr} (mg/kg/24 hr)	0.545**	-0.134
U_{Gluc} (g/kg/24 hr)	0.664**	0.374
U_{Vol} (ml/kg/24 hr)	0.055	0.059

** : $p < 0.01$

During the follow-up of the normocalciuric and the hypercalciuric diabetic children, the correlation data were similar to those obtained from the first 24 hour urine collections.

No significant correlation could be obtained when multiple regression equations, in which dietary substances were also included, were calculated.

4.4. STUDIES ON SOME NON-HORMONAL FACTORS INFLUENCING URINARY EXCRETION OF CALCIUM

4.4.1. RELATIONSHIP BETWEEN THE URINARY CALCIUM AND GLUCOSE EXCRETION

The relationship between the urinary excretion of calcium and glucose in the normocalciuric and hypercalciuric diabetic children on the first urine examination is illustrated in fig. 4.2.

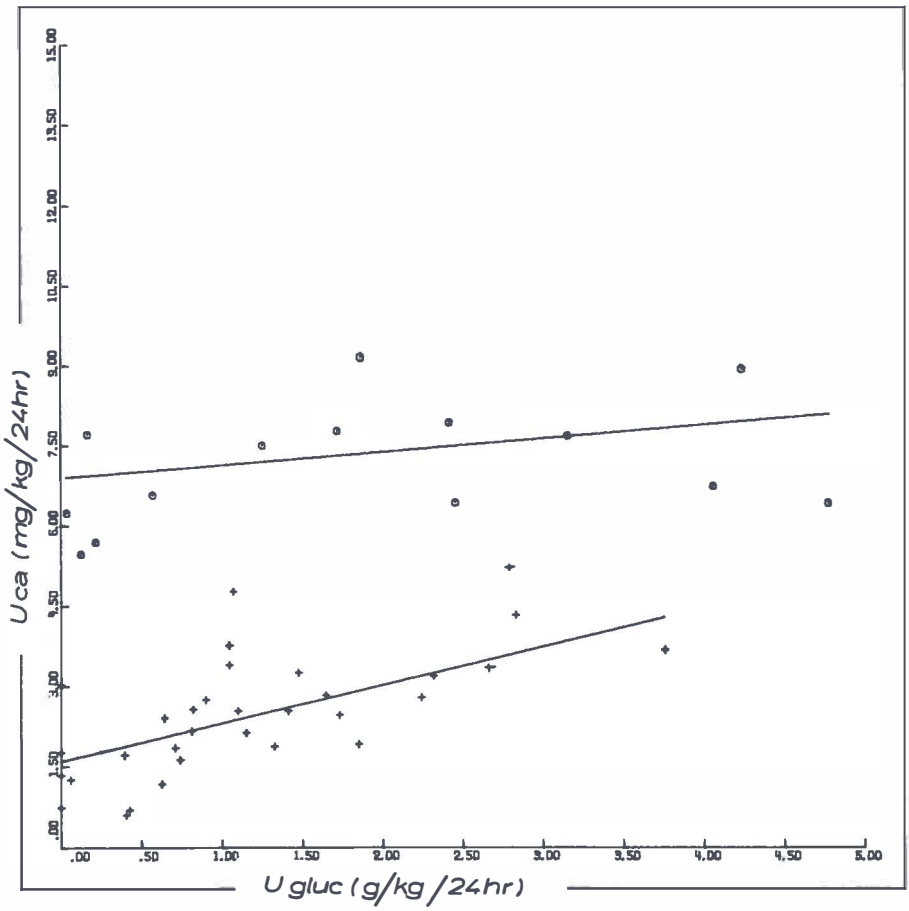


Fig. 4.2. Relationship between urinary calcium and glucose excretion in the normocalciuric(+) and hypercalciuric(⊙) diabetic children.

The regression equation for the urinary excretion of calcium (mg/kg/24 hr) in the normocalciuric group is 1.52 + 0.76 x urinary excretion of glucose (g/kg/24 hr); (estimated standard deviation of error : 0.8189; $r = 0.664$; $p < 0.01$; $n = 32$).
This correlation between the urinary excretion of calcium and glucose in the normocalciuric children was confirmed in the follow-up period, and similar regression equations and correlation coefficients were found.

For the hypercalciuric group no significant correlation between the urinary excretion of calcium and glucose could be established ($r = 0.374$; $p > 0.05$; $n = 15$).

When analysing separately for each hypercalciuric child the urinary excretion of calcium and glucose during the follow-up period, five children showed a hypercalciuria related to the degree of glucosuria and ten children a hypercalciuria that was independent of the urinary excretion of glucose.
An example of such a glucose-dependent hypercalciuria and of a glucose-independent hypercalciuria is shown in fig. 4.3.

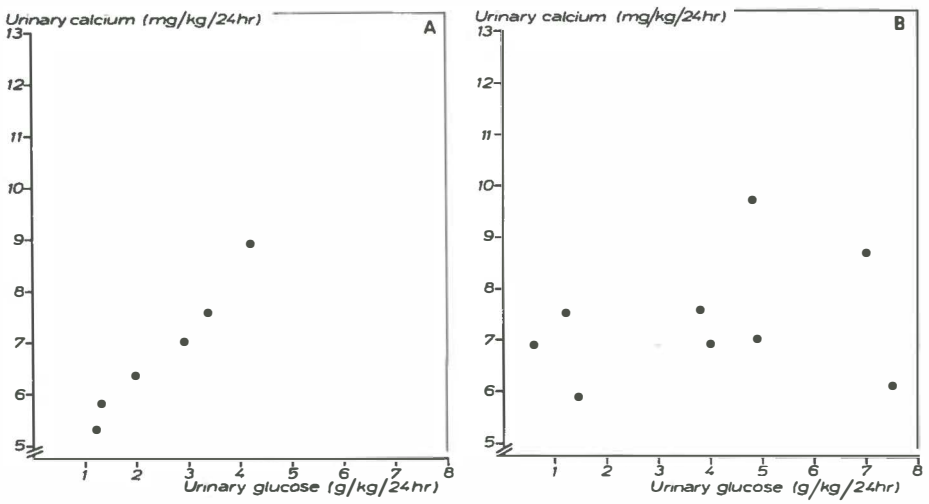


Fig. 4.3. Example of a glucose-dependent (A) and a glucose-independent (B) hypercalciuric child. (A: r = 0.968; $p < 0.01$) (B : r = 0.263; $p > 0.05$).

Of the four children who became hypercalciuric in the course of the follow-up period, one showed a glucose-dependent and three a glucose-independent type of hypercalciuria.

Urine was collected at intervals throughout the 24 hours as described in Chapter 2.3. six times over a period of four years, and analysed to demonstrate a possible difference in the diurnal pattern of the urinary excretion of calcium between the hypercalciuric and the normocalciuric children, and also to illustrate a possible relationship between the diurnal patterns of the urinary excretion of calcium and glucose. The number of normocalciuric children participating in each series of urine collections ranged from twelve to twenty-four, and the number of hypercalciuric children from six to thirteen.
To equate the levels of the urinary excretion of calcium and glucose between the two groups of diabetic children, the urinary excretion of calcium (and glucose) in each urine sample was expressed as excretion per hour as a percentage of the mean calcium (and glucose) excretion per hour of the pooled 24 hour period samples. These values are plotted at the midpoint of each collection period in fig. 4.4. and 4.5. which illustrate the diurnal pattern of the urinary excretion of calcium and glucose, respectively, in the six series of normocalciuric and glucose-independent hypercalciuric diabetic children.
There is no difference in the calcium excretion pattern between the glucose-independent hypercalciuric and normocalciuric diabetic children (Student "t" test : n.s.). In the hypercalciuric children

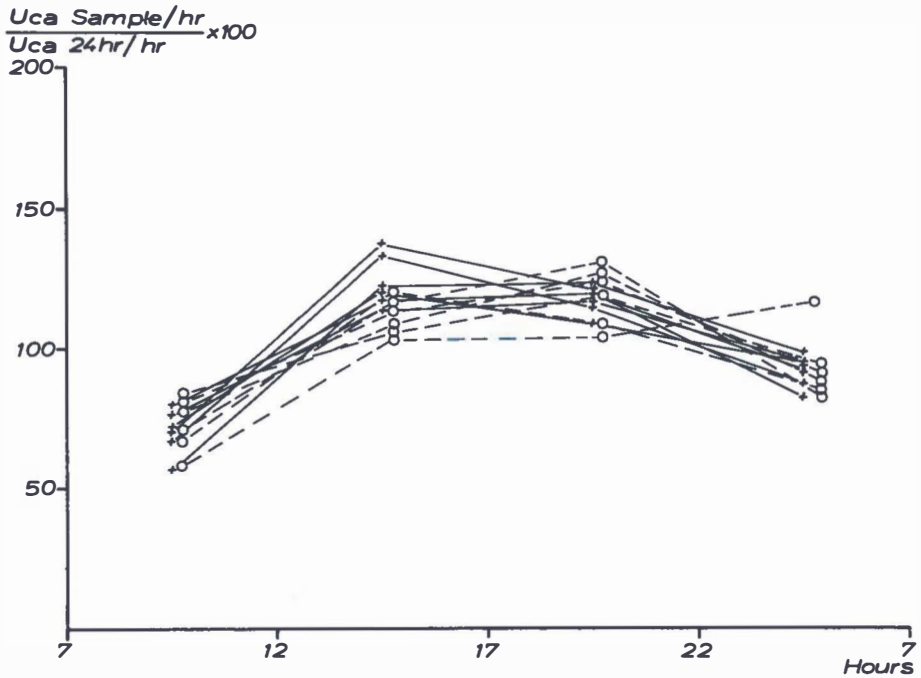


Fig. 4.4. Mean diurnal pattern of the urinary calcium excretion in each of the six series of the normocalciuric(+) and glucose-independent hypercalciuric(o) diabetic children.

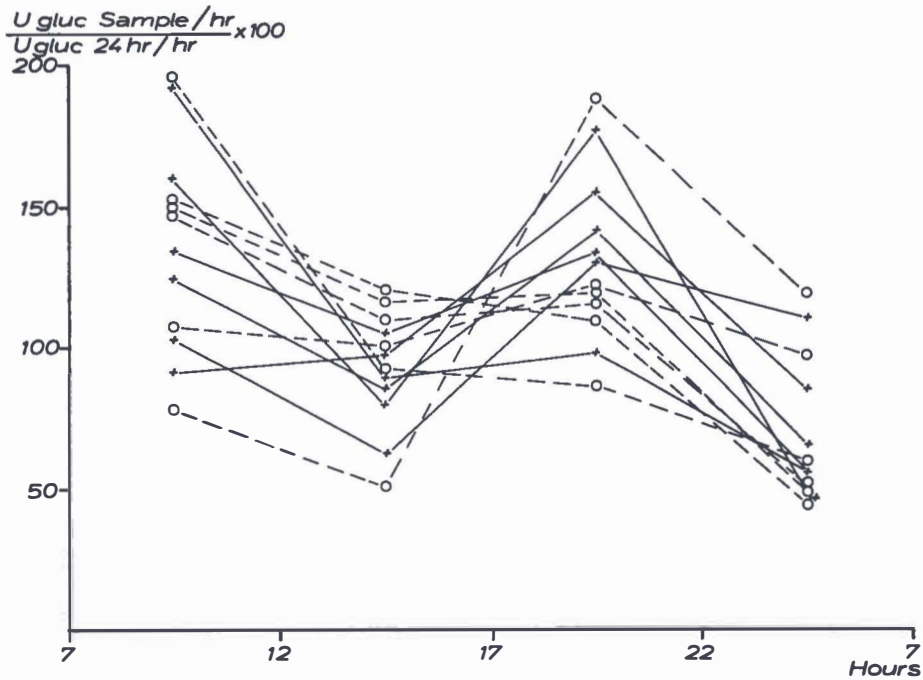


Fig. 4.5. Mean diurnal pattern of the urinary glucose excretion in each of the six series of the normocalciuric(+) and glucose-independent

the lowest value of the urinary excretion of calcium was observed in the morning samples. A nocturnal minimum was never observed in the normocalciuric, or the hypercalciuric diabetic patients.

The diurnal pattern of the urinary excretion of calcium did not coincide with that of glucose in the six series of normocalciuric nor hypercalciuric diabetic children.

4.4.2. RELATIONSHIP BETWEEN THE URINARY EXCRETION OF CALCIUM AND METABOLIC ACIDOSIS

All urine samples were tested with urine pH dip stix, ketostix and acetest. The acetest and ketostix were constantly negative, and the pH (dip stix) was 6 or more in the urine samples from the patients who were glucose-independent hypercalciuric. More accurate data were obtained by measuring the pH and bicarbonate levels in venous blood, the urine pH by pH meter and the urine acetone by gaschromatography. These results are dealt with in detail in Chapter 5. The estimation of titrable acidity and of urinary ammonium excretion was not performed.

Good diabetic control has been defined by White (1965) as less than 25 g of urinary glucose excretion in 24 hours.
As shown in table 4.6. good diabetic control was found in six of the twenty-eight normocalciuric children, and in six of the thirteen glucose-independent children. In the latter group, only one child had poor control.

Table 4.6. : 24 hour urinary excretion of glucose (g/24 hr) in the normocalciuric and hypercalciuric diabetic children.

	0-25	25-50	50-100	100
Normocalciuric children	6	9	9	4
Hypercalciuric children :				
glucose-dependent			5	1
glucose-independent	6	3	3	1

In the six children with a glucose-dependent hypercalciuria, the glucosuria was more than 50 g/24 hr in all cases.
A urinary glucose excretion pattern, similar to that listed in table 4.6., was obtained for the different groups of diabetic children during follow-up.

Fig. 4.6. illustrates the mean blood glucose levels, determined at specific times over the 24 hour period as described in Chapter 2.3. during three annual check-ups in the hospital. The number of normocalciuric children participating was sixteen, seventeen and seventeen and the number of hypercalciuric children nine, ten and ten. There was no difference in the blood glucose levels between glucose-independent hypercalciuric and normocalciuric diabetic children (Student "t" test : n.s.).

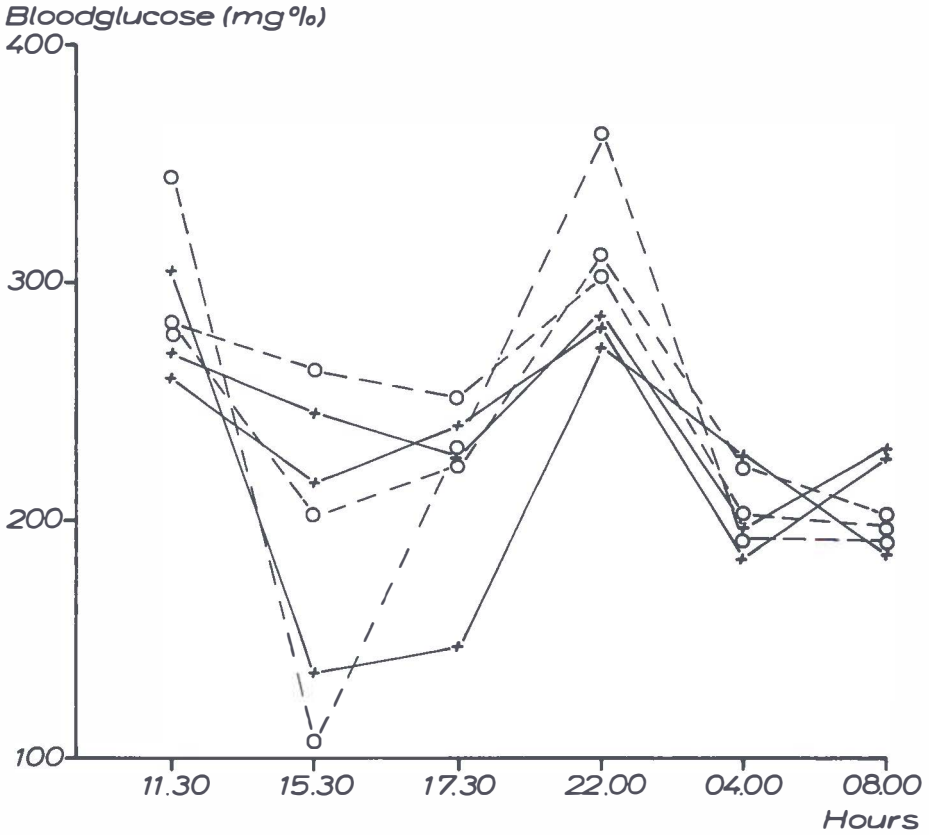


Fig. 4.6. Mean fluctuations of blood glucose throughout 24 hours in each of the three series of the normocalciuric (+) and glucose-independent hypercalciuric (o) diabetic children.

In the course of this study, fasting serum free fatty acids (FFA) levels and fasting blood glucose levels were determined (table 4.10).
The values of blood glucose and serum FFA are lower in the glucose-independent hypercalciuric group when compared with the normocalciuric diabetic group, but the differences are not statistically significant.

4.4.3. RELATIONSHIP BETWEEN THE URINARY EXCRETION OF CALCIUM AND THE DIETARY INTAKE OF SEVERAL SUBSTANCES

The dietary intake of calcium, phosphate, carbohydrate, protein and fat are listed in table 4.3. for the normocalciuric and hypercalciuric diabetic children, and in table 3.1. for the healthy control children.
The healthy children had a higher intake of carbohydrate and fat compared to the normocalciuric diabetic children, but the intake of calcium, phosphate and protein was not different (table 4.3.).
The hypercalciuric diabetic group had a significantly different intake of calcium, phosphate, fat and protein compared to normocalciuric diabetic children (table 4.3.).

However, the statistical analysis of the dietary intake of the control children, the normocalciuric diabetic children and the hypercalciuric diabetic children lends itself to criticism, as prepuberal and puberal children were not studied separately, and the hypercalciuric diabetic group is not homogenous as it contained "glucose-dependent" and "glucose-independent" hypercalciuric children.

The mean caloric intake of all the diabetic boys was 2,248 cal/day with 18 % protein, 33 % fat and 49 % carbohydrate. The diabetic girls had a mean caloric intake of 1,862 cal/day with 17 % protein, 34.5 % fat and 48.5 % carbohydrate.
The contribution of carbohydrate to the total caloric intake in diabetic children was in accordance with values determined in control children (Chapter 3.1.3.). The contribution of protein to the total caloric intake in diabetic children was higher than in the healthy control children.
No difference in caloric intake, nor in the contribution of protein, fat and carbohydrate to the caloric intake, could be established between the hypercalciuric and normocalciuric diabetic children. The latter group had a mean caloric intake of 2,195 cal/day with 18% protein, 34 % fat and 48 % carbohydrate. The hypercalciuric diabetic children had a mean caloric intake of 2,057 cal/day with 17 % protein, 34 % fat and 49 % carbohydrate.

As shown in table 4.7., no correlation could be found between the urinary excretion of calcium and the dietary intake of phosphate, protein, fat and carbohydrate in either the hypercalciuric or the normocalciuric diabetic group.

As the diabetic children were not studied under metabolic ward conditions the sodium intake was not calculated.
The urinary excretions of calcium and sodium when determined for the first time in the glucose-independent hypercalciuric group, were not related ($r = 0.280$; $p > 0.05$; $n = 13$). Nor could a correlation be found in any of the subsequently examined series of urine samples.
In the normocalciuric group it occured more often than not that significant correlations were obtained between the urinary excretion of calcium and sodium in the different series of urine collections.

Table 4.7. : Correlation coefficients between urinary calcium and dietary substances in the normocalciuric and hypercalciuric diabetic children.

	$U_{Ca} \leq 5.2$ (mg/kg/24 hr) (n = 32)	$U_{Ca} > 5.2$ (mg/kg/24 hr) (n = 15)
I_P (mg/kg/24 hr)	- 0.090	0.270
I_{Prot} (g/kg/24 hr)	- 0.125	- 0.041
I_{Fat} (g/kg/24 hr)	0.027	0.507
I_{CHO} (/kg/24 hr)	- 0.140	0.253

Although a significant correlation between the urinary excretion of calcium and sodium was found in the control group (Chapter 3.1.3.), multiple regression analysis did not reveal a significant relationship between the urinary excretion of calcium and sodium in the normocalciuric diabetic children.

Table 4.8. shows that there is a difference in the calcium intake between the glucose-independent hypercalciuric and the normocalciuric diabetic children (Student "t" test : $p < 0.02$).

Table 4.8. : Dietary calcium intake (mg/kg/24 hr) in the normocalciuric and glucose-independent hypercalciuric diabetic children.

	$U_{Ca} \leq 5.2$ (mg/kg/24 hr)		$U_{Ca} > 5.2$ (mg/kg/24 hr)	
	n	Mean $\pm$ 1 SD	n	Mean $\pm$ 1 SD
Prepuberal	7	47.00 $\pm$ 8.40	8	52.10 $\pm$ 17.20
Puberal	21	31.60 $\pm$ 8.15	5	39.00 $\pm$ 13.40
Total	28	35.60 $\pm$ 10.60	13	47.20 $\pm$ 16.80

However, the calcium intake per kg body weight of the diabetic children, and also of the control children, decreases with age ($r = - 0.788$; $p < 0.01$; $n = 47$ and $r = - 0.613$; $p < 0.01$; $n = 56$ respectively). As the hypercalciuric diabetic group contains a comparatively large number of young children, a high mean calcium intake compared to the normocalciuric group could possibly be explained by the difference in ages between the two groups. Subdivision of the hypercalciuric and normocalciuric diabetic children into two groups, prepuberal and puberal (table 4.8.), did indeed fail to show a significant difference between the calcium intake (Student "t" test : n.s.).

In the four children who became hypercalciuric during follow-up the calcium intake had not changed in the course of the follow-up.

In healthy control children no relationship between the urinary and dietary calcium could be established (Chapter 3.1.3.). Fig.4.7.

illustrates that no relationship could be found in the diabetic children either. In the normocalciuric group the correlation coefficient between the urinary excretion of calcium and the calcium intake was -0.059 ($p > 0.05$; $n = 32$) and in the hypercalciuric group it was 0.431 ($p > 0.05$; $n = 15$).

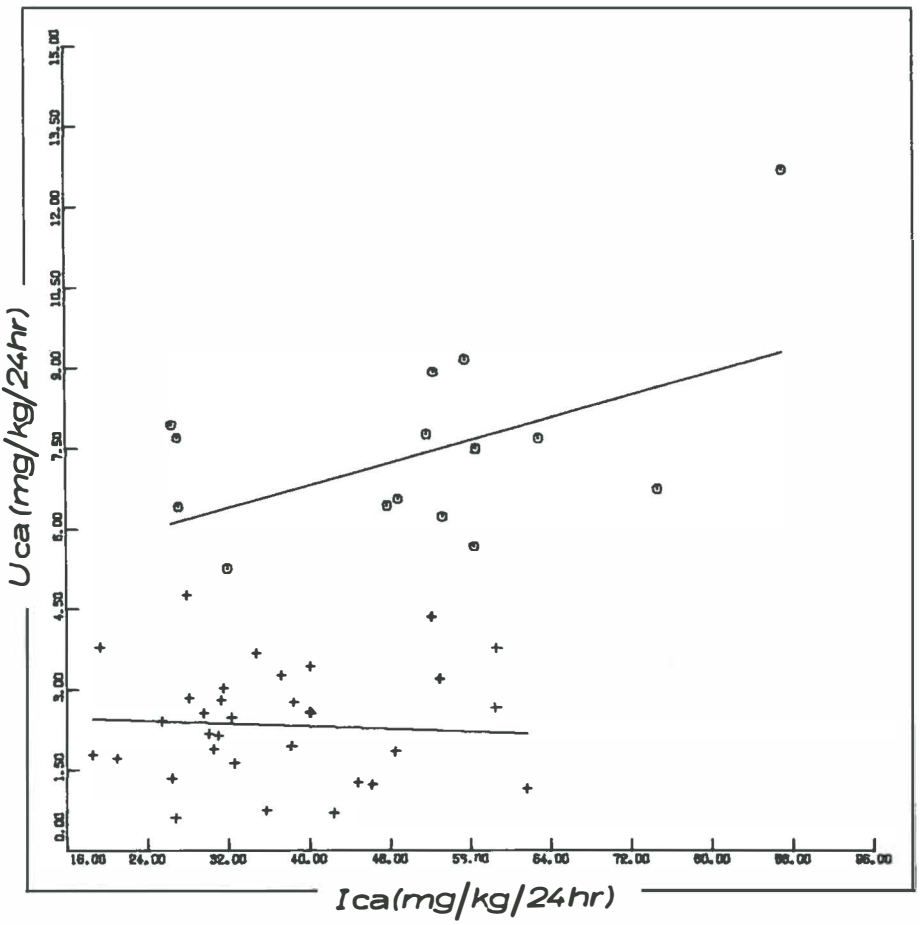


Fig. 4.7. Relationship between urinary calcium excretion and dietary calcium intake in the normocalciuric(+) and hypercalciuric(⊙) diabetic children.

Between the hypercalciuric and the normocalciuric diabetic children no difference exists in the regression coefficient of the correlation between the dietary intake and urinary excretion of calcium, as demonstrated by an analysis of co-variance (F statistic = 2.5 ; 1-41 DF ; $p > 0.1$).
There is, however, a significant difference between the intercepts of these correlations ($F = 68.8$; 1-42 DF; $p < 0.01$), indicating that for the same calcium intake the children in the hypercalciuric group have

a higher urinary excretion of calcium. A similar lack of correlation between the urinary calcium and dietary calcium was observed during the follow-up period.
On statistical grounds it can be concluded that in the diabetic children the calcium intake exerts no influence on the urinary excretion of calcium.

The calcium intake of the diabetic children compared to that of the control children was high, and this could be the cause of the high incidence of hypercalciuria in the diabetic children.
Instead of lowering the calcium intake of the diabetic children, the calcium intake of twenty-one healthy children was increased to levels similar to that of the diabetic children.
By taking more milk and cheese for a period of four days, the calcium intake (mg/kg/24 hr) of ten healthy prepuberal children was 51.40 $\pm$ 0.32 (SD) and of eleven healthy puberal children 34.40 $\pm$ 0.42 (SD). From these children 24 hour urine samples were collected on the fourth day. The mean urinary calcium excretion (mg/kg/24 hr) in the prepuberal children was 3.14 $\pm$ 1.23 (SD) and in the puberal children 2.91 $\pm$ 1.19 (SD). The upper limit of a normal urinary calcium excretion was exceeded by only one child, whose urinary calcium excretion was of 5.70 mg/kg/24 hr.
The incidence of hypercalciuria in the diabetic children remains significantly different from that in healthy control children (Fisher exact test : $p = 0.026$) even after increasing the dietary calcium intake.

Drinking water.

To evaluate the contribution of calcium from drinking water to the occurrence of hypercalciuria in the diabetic children a geographic study was set up in 1974.
Seventeen hypercalciuric, and nineteen normocalciuric diabetic children, who lived in the three northern provinces of the Netherlands participated in this study. The only water used in the homes of these children was that supplied by the local waterworks. The water is regularly analysed for the calcium-ion content by the waterworks'staff. There were large differences in the calcium content of the drinking water from the different waterworks (table 4.9.).

There was no connection between the occurence of hypercalciuria in the diabetic children and the calcium content of the drinking water. The correlation between the urinary excretion of calcium (mg/kg/24 hr) and the calcium content of drinking water (mg/l) was not significant ($r = 0.198$; $p > 0.05$).

4.5. STUDIES ON SOME HORMONAL FACTORS INFLUENCING URINARY EXCRETION OF CALCIUM

Table 4.10. lists the values of several blood constituents in the first examined fasting blood samples of twenty-four normocalciuric and thirteen glucose-independent hypercalciuric diabetic children. (Calci-

Table 4.9. : The number of normocalciuric and hypercalciuric diabetic children and the calcium-ion content of the drinking water.

Waterworks in the province of	Drinking water Ca^{++} (mg/l) content	Number of diabetic children		
		$U_{Ca} \leqslant 5.2$ (mg/kg/24hr)	$U_{Ca} > 5.2$ (mg/kg/24 hr)	
			Glucose-dependent	Glucose-independent
Groningen				
Glimmen	50.1	1	2	4
Onnen	64.5	4	1	1
De Groeve	51.0	3	1	1
Sellingen	32.2	2	1	1
Nietap	70.6	2	-	3
Drenthe				
Norg	27.5	-	-	1
Zuidlaren	23.1	1	-	-
Emmen Noord-largeres	40.0	1	-	1
Friesland				
Terwischa	37.8	1	-	-
Oldeholtpade	76.9	1	-	-
's Gravesande	93.0	3	-	-

tonin determinations were performed further on in the study and are discussed in Chapter 5). There is no difference between the two diabetic groups (Student "t" test = n.s.) for any listed values.

In the diabetic group five children, two of which were hypercalciuric, showed an iPTH plasma level exceeding 0.25 ng equiv. b. PTH/ml. In the control group (Chapter 3.3.) however, three children were also found to have an iPTH plasma level of more than 0.25 ng equiv. b. PTH/ml. As described in Chapter 2.4. a plasma level of more than 0.25 ng equiv.b. PTH/ml is to be considered as abnormal. The plasma iPTH of the seventeen control children (Chapter 3.3.) does not differ from that of the normocalciuric and glucose-independent hypercalciuric diabetic children.

Table 4.10. : The concentration of several bloodconstituents at 8 a.m. (fasting) in the normocalciuric and glucose-independent hypercalciuric diabetic children

	$U_{Ca} \leq 5.2$ (mg/kg/24 hr) (n = 24)	$U_{Ca} > 5.2$ (mg/kg/24 hr) (n = 13)
	Mean ± 1 SD	Mean ± 1 SD
Serum		
calcium (mg/dl)	9.33 ± 0.20	9.38 ± 0.21
phosphate (mg/dl)	4.48 ± 0.39	4.20 ± 0.55
alk.phosph. (IE)	20.07 ± 5.92	20.70 ± 7.26
magnesium (mg/dl)	1.83 ± 0.15	1.90 ± 0.17
albumin (mg/dl)	4.39 ± 0.28	4.43 ± 0.14
FFA (μM/dl)	790 ± 281*	596 ± 186**
iPTH (ng equiv.b. PTH/ml)	0.152± 0.125	0.153± 0.085
Blood		
glucose (mg/dl)	230.80 ± 92.80	188.60 ± 85.90

* n = 15

** n = 8

4.6. SUMMARY

From forty-seven diabetic children fifteen children showed a hypercalciuria when first examined. On follow-up the hypercalciuric group consisted of thirteen children with a glucose-independent type, and six children with a glucose-dependent type, of hypercalciuria. The glucose-independent hypercalciuria is not an incidental finding, the child who has this characteristic keeps it on follow-up. In contrast, normocalciuric diabetic children may become hypercalciuric later on, this did occur in four children.
The glucose-independent hypercalciuria could not be due to metabolic acidosis, or be explained by excessive glucosuria or natriuresis. It was not possible on statistical grounds to consider factors such as age, sex, duration or control of the diabetic process as being implicated in the occurrence of hypercalciuria. Neither the dietary calcium intake nor the calcium content of drinking water were the cause of glucose-independent hypercalciuria.

Hypercalciuria occurs significantly more frequently in diabetic children than in healthy children on a diet with a calcium content equal to that of the diabetic children.

CHAPTER 5

INVESTIGATIONS ON HYPERCALCIURIA IN DIABETIC CHILDREN

5.1. INTRODUCTION

From Chapter 4 it can be concluded that hypercalciuria in the presence of a normal serum calcium occurs with abnormal high frequency in diabetic children. This hypercalciuria is either dependent or independent of the degree of glucose excretion. Causes such as a high calcium intake and primary hyperparathyroidism were unlikely.

The hypercalciuria can be associated with abnormalities in bone metabolism. Therefore, the growth of the diabetic children and the amount of bone in the skeleton were analysed.
Measurements of cortical thickness in the second metacarpal bone are used for the detection of loss of bone substance (Barnett and Nordin, 1961; Juliani et al., 1966; Garn et al., 1967). The 24 hour urinary excretion of hydroxyproline shows a high positive correlation with growth rates (Smiley and Ziff, 1964) and is an index of collagen metabolism (Udendriend, 1966).

In general the cause of hypercalciuria may be an enhanced release of minerals from bone, a decrease in renal tubular reabsorption, or an increase in gastrointestinal absorption. To determine the cause of hypercalciuria in the diabetic children, the following studies were undertaken.
An oral calcium load was given to differentiate whether the hypercalciuria in the patients was of primary intestinal origin (absorptive hypercalciuria) or of primary renal origin (renal hypercalciuria).
Indomethacin was administered to study the possible contribution by prostaglandins to the occurrence of hypercalciuria in the diabetic patients.

5.2. RELATIONSHIP BETWEEN HYPERCALCIURIA AND BONE METABOLISM

5.2.1. GROWTH

There is no statistical significant difference in height and weight between the diabetic and healthy control children (table 2.1. and 2.2.), nor between the hypercalciuric and normocalciuric diabetic children (table 4.2.).
The height of twenty-two normocalciuric children, and of thirteen glucose-independent hypercalciuric children, was followed-up for one to four years. Also on follow-up there was no difference in height between normocalciuric and glucose-independent hypercalciuric children (table 5.1.) (Student "t" test : n.s.). The number of hypercalciuric children who could be followed-up is, however, very small.

5.2.2. STUDIES OF THE CORTICAL THICKNESS OF THE LEFT SECOND METACARPAL BONE

5.2.2.1. patients

X-rays were taken of the left hand of twenty-six diabetic children (thirteen normocalciuric and thirteen hypercalciuric) in 1975 and of thirty diabetic children (sixteen normocalciuric and fourteen hypercalciuric) in 1976. Eleven of the normocalciuric children and eleven of the hypercalciuric children had X-rays of the left hand in 1975 and also in 1976. In the hypercalciuric group, the glucose-dependent and glucose-independent children were studied separately.

5.2.2.2. results

In tables 5.2. and 5.3. are listed the ages of the children at the time the first X-ray films were taken and the values of the external diameter (D) and internal diameter (d) and also the lenght (L) of the second metacarpal bone. Boys and girls, normocalciuric and hypercalciuric children are listed separately.

Figs. 5.1., 5.2. and 5.3. illustrate the most important parameters in the patients in comparison with those published by Bonnard (1968), Garn et al. (1971) and Gryfe et al. (1971). These parameters have not been published for healthy Dutch children.
The index of Exton-Smith, the cortical thickness, and the percent cortical area did not separate the hypercalciuric diabetic children

Table 5.1. : Height (SDS) in the normocalciuric and glucose-independent hypercalciuric diabetic children on follow-up.

	Onset of study	Follow-up			
		1 yr	2 yr	3 yr	4 yr
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
Normocalciuric children	0.41 ± 1.02 (n = 31)	0.64 ± 1.03 (n = 21)	0.51 ± 1 (n = 15)	0.48 ± 1.09 (n = 13)	0.34 ± 1.30 (n = 11)
Glucose-independent hypercalciuric children	0.50 ± 1.05 (n = 13)	0.46 ± 1.23 (n = 13)	0.15 ± 1.37 (n = 7)	0.21 ± 1.67 (n = 6)	0.60 ± 1.85 (n = 3)

Table 5.2. : External diameter (D), internal diameter (d) and lenght (L) of the left second metacarpal bone in 1975 (1) and 1976 (2) and the age of the normocalciuric diabetic children in 1975.

Patient N°	Age (yr)	D1	D2	d1	d2	L1	L2
BOYS							
1	15	8.65	9.00	4.85	5.50	6.50	6.80
2	16	9.00	8.95	5.50	5.55	7.10	7.20
3	16	8.95	9.05	4.30	4.30	7.00	7.10
4	16	8.85	9.25	4.55	4.60	7.40	7.70
5	9	6.25	6.50	3.75	3.65	5.00	5.20
6	14	8.70	8.65	5.10	4.50	7.55	7.80
7	8	6.50	6.75	4.40	4.45	4.45	4.60
8	10	7.15	7.55	4.15	4.40	4.90	5.00
9	12		7.80		4.40		6.40
10	13		8.90		6.10		5.65
11	9		5.90		3.80		4.20
GIRLS							
1	13	9.25		5.00		6.70	
2	13	7.00	7.40	4.10	4.35	5.80	6.20
3	17	7.95		4.25		6.10	
4	15	8.10	8.30	4.90	5.40	6.40	6.50
5	11	6.40	7.00	4.05	4.55	5.70	5.90
6	17		7.60		3.90		6.50
7	15		8.20		4.20		6.90

Table 5.3. : External diameter (D), internal diameter (d) and lenght (L) of the left second metacarpal bone in 1975 (1) and 1976 (2) and the age of the hypercalciuric diabetic children in 1975.

Patient N°	Age (yr)	D1	D2	d1	d2	L1	L2
Glucose-independent : BOYS							
1	11	6.55	6.75	3.95	4.35	4.80	5.00
2	9	6.95	7.15	4.70	5.10	5.20	5.40
3	5	6.65	6.90	5.20	5.45	4.00	4.25
4	8	6.60	6.65	4.05	3.85	5.10	5.20
5	12	7.80		4.55		5.85	
6	15	8.20		4.35		5.95	
7	13		7.15		4.15		5.75
		GIRLS					
1	13		8.50		4.60		6.60
2	14	8.10	8.10	4.45	4.20	6.90	6.90
3	11	6.10	6.35	3.00	3.10	5.30	5.50
4	15		6.75		2.85		6.00
Glucose-dependent : BOYS							
1	15	8.30	8.50	4.40	4.55	6.40	6.50
2	16	8.10	8.50	4.20	3.70	6.75	6.80
		GIRLS					
1	7	6.00	6.20	3.65	3.75	4.05	4.20
2	10	6.65	7.00	3.55	3.75	4.50	4.70
3	5	5.00	5.15	3.15	3.20	3.90	4.20

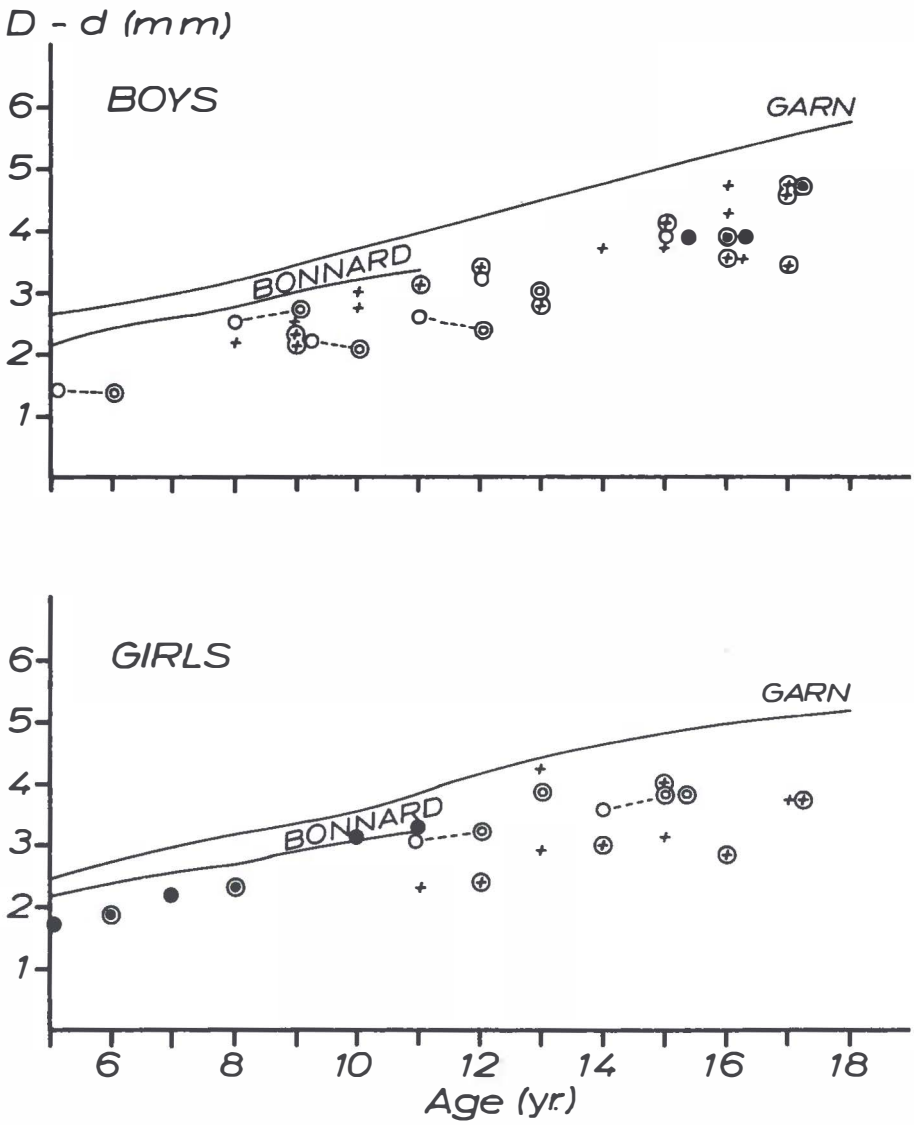


Fig. 5.1. Cortical thickness of the left second metacarpal bone in the normocalciuric (+ in 1975; ⊕ in 1976), glucose-dependent (● in 1975; ◉ in 1976) and glucose-independent (o in 1975; ⊙ in 1976) hypercalciuric diabetic children : lines connect the two measurements in the glucose-independent hypercalciuric children.

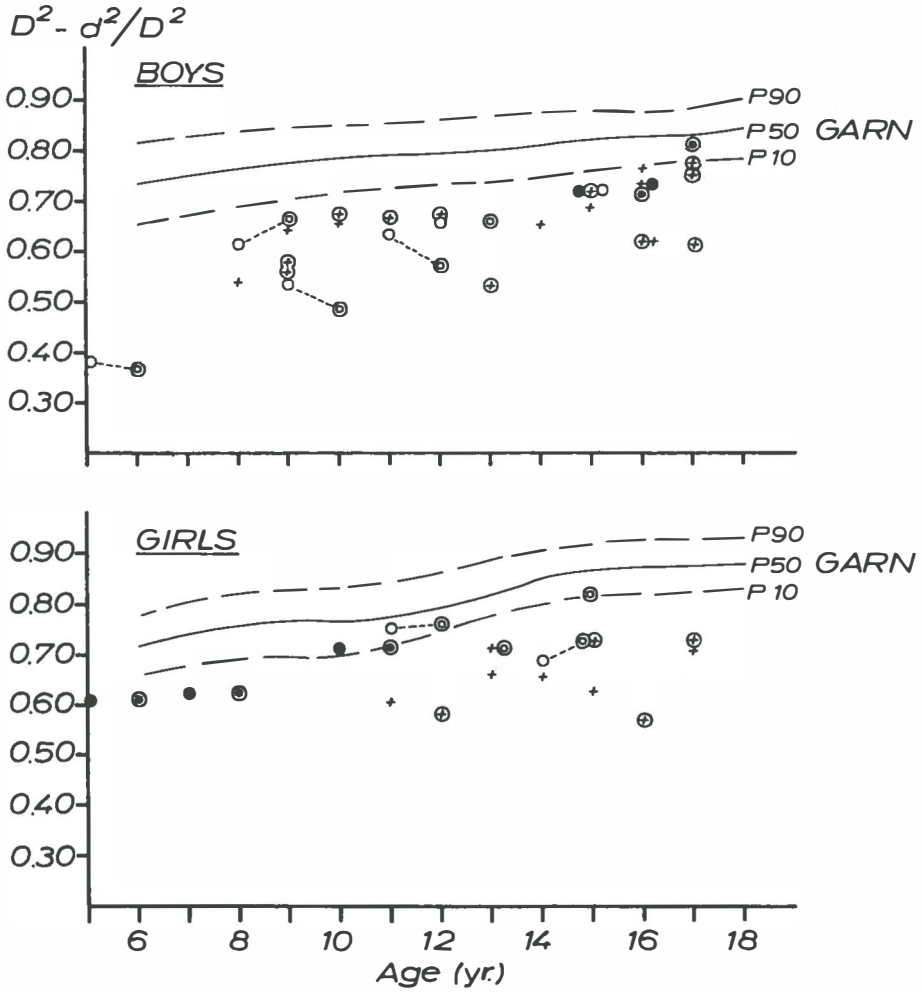


Fig. 5.2. Percent cortical area of the left second metacarpal bone in the normocalciuric (+ in 1975; ⊕ in 1976), glucose-dependent (● in 1975; ◉ in 1976) and glucose-independent (o in 1975; ⊙ in 1976), hypercalciuric diabetic children : lines connect the two measurements in the glucose-independent hypercalciuric children.

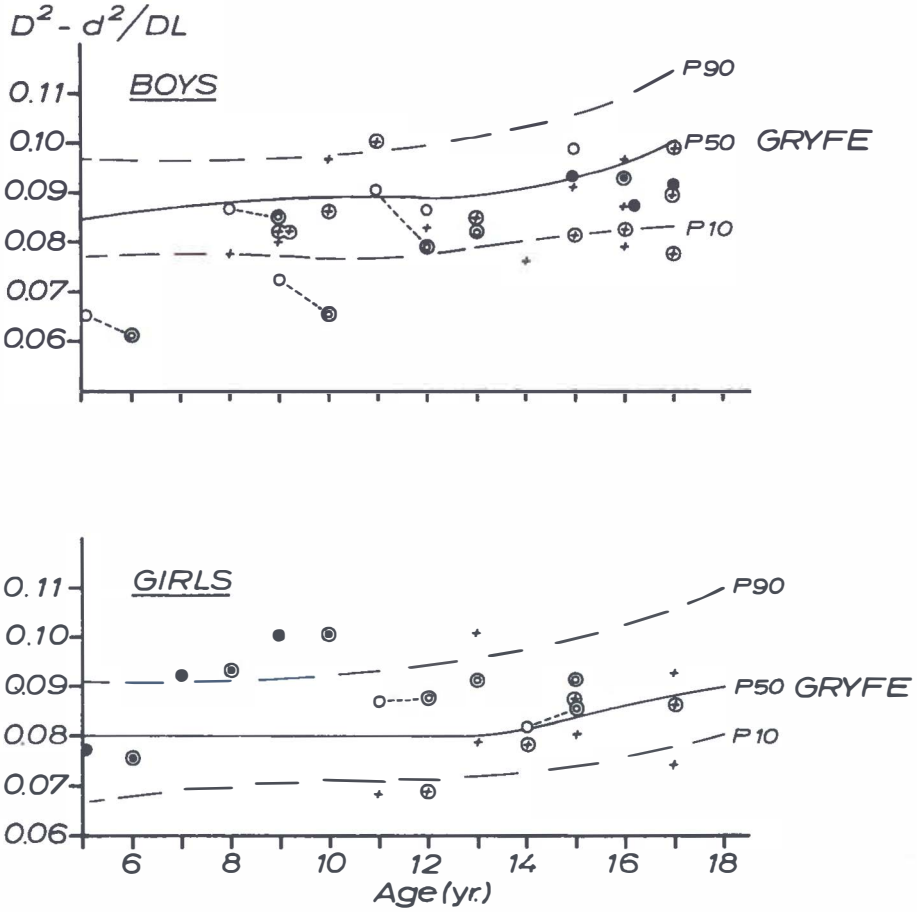


Fig. 5.3. Index of Exton-Smith on the left second metacarpal bone in the normocalciuric (+ in 1975; ⊕ in 1976), glucose-dependent (● in 1975; ◉ in 1976) and glucose-independent (o in 1975; ○ in 1976) hypercalciuric diabetic children : lines connect the two measurements in the glucose-independent hypercalciuric children.

from the normocalciuric ones.
In three out of four diabetic boys with glucose-independent hypercalciuria, the PCA and the index of Exton-Smith decreased after one year, while in six out of eight normocalciuric boys, these parameters increased. In the hypercalciuric girls no change occurred.
All diabetic children have values of cortical thickness below those published by Bonnard (1968) and Garn et al. (1971). No significant differences between boys and girls are noted. The PCA in diabetic children is also lower than that published by Garn et al. (1971) and again, no differences were found between boys and girls. In the diabetic boys and girls only the index of Exton-Smith is within the normal limits published by Gryfe et al. (1971).

5.2.3. URINARY EXCRETION OF HYDROXYPROLINE

5.2.3.1. patients

In 1976, the 24 hour urinary excretion of creatinine and hydroxyproline was determined in fourteen normocalciuric, nine glucose-independent hypercalciuric and five glucose-dependent hypercalciuric diabetic children while on a gelatin-free diet.

5.2.3.2. results

Fig. 5.4. shows the urinary hydroxyproline excretion (mg/24 hr/ m2 of body surface) in normocalciuric, glucose-independent and glucose-dependent hypercalciuric children. The values reported by Williams (1974) were used as reference values.
There was no change in the mean hydroxyproline excretion in the three age groups of the diabetic patients in contrast to the data of Williams (1974). The 24 hour urine creatinine excretion was not abnormally low and, therefore, incomplete urine collections could not account for the low excretory rate of hydroxyproline in the eight children in the age groups one to fourteen years. In the age group above fourteen years where many of the diabetic children were not post-puberal eight children had an abnormally high urine hydroxy-proline excretion.
No significant difference between hypercalciuric and normocalciuric diabetic children could be shown.

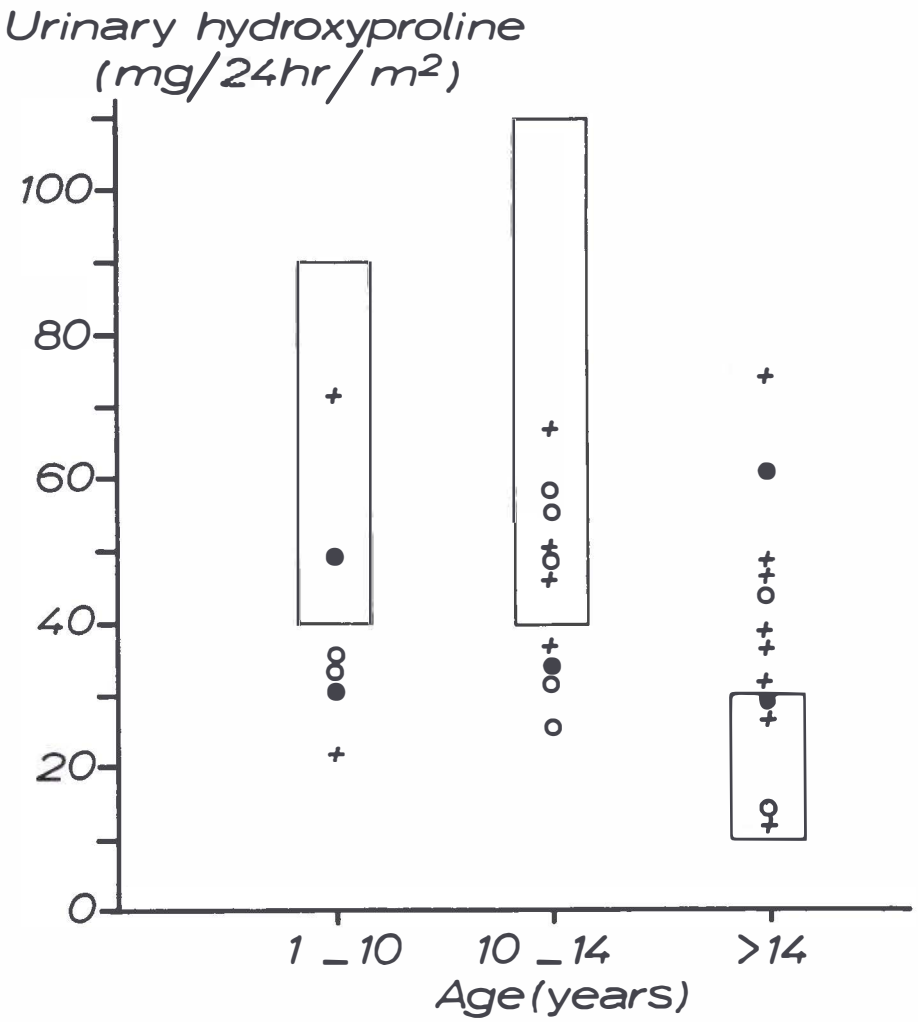


Fig. 5.4. Urinary hydroxyproline excretion in the normocalciuric (+), glucose-dependent (●) and glucose-independent (o) hypercalciuric diabetic children.

5.3. EFFECT OF AN ORAL CALCIUM LOAD

5.3.1. INTRODUCTION

Pak et al. (1975) developed a test to facilitate the differentiation between absorptive and renal hypercalciuria. After an oral calcium load, the urinary excretion of calcium is high in the presence of intestinal hyperabsorption of calcium, and Pak et al. (1975) suggested that under these conditions the urinary cyclic AMP excretion provides a reliable measure of parathyroid response. The test was found to be as reliable when fasting urine collection periods of two hours instead of four hours were used.

5.3.2. PATIENTS

In fifteen normocalciuric (group 1) and ten glucose-independent hypercalciuric (group 2) diabetic children the test was carried out as described in Chapter 2.3. while the children were fasting. After collecting urine during a two hour fasting period (control period), 1 g of ionised calcium (2 tablets of calcium Sandoz) was administered to each child, and urine was collected again during the two following hours (test period).
Table 5.4. lists the chronological age, number of years of insulin administration, heigt and weight of the diabetic children of each group. The age of the children ranged from 5.5 to 16.5 years.

Table 5.4. : Age, duration of diabetes, height and weight of the normocalciuric and glucose-independent hypercalciuric diabetic children before the calcium load.

	Group 1 (n = 15)	Group 2 (n = 10)
	$U_{Ca} \leq 5.2$ (mg/kg/24 hr)	$U_{Ca} > 5.2$ (mg/kg/24 hr)
	Mean $\pm$ 1 SD	Mean $\pm$ 1 SD
Age (yr)	13.7 $\pm$ 2.6	11.2 $\pm$ 2.9
Duration of diabetes (yr)	7.5 $\pm$ 3.3	4.2 $\pm$ 3.5
Height (SDS)	+ 0.63 $\pm$ 0.88	+ 0.51 $\pm$ 1.34
Weight (SDS)	+ 0.41 $\pm$ 0.66	- 0.07 $\pm$ 0.69

5.3.3. RESULTS

Table 5.5. lists the concentrations of blood glucose, serum FFA and urinary glucose during control and test periods. Urinary acetone was measured only during the control period.
No difference in urinary glucose, acetone, blood glucose and serum FFA could be established between the two groups, although these values are somewhat lower in group 2 (Student "t" test : n.s.).
The urinary excretion of glucose remained unchanged in group 2 after the calcium load, whereas in group 1 it rose. This change in group 1 is however, not significantly different from that measured in group 2.

Table 5.6. lists the concentrations of serum calcium, inorganic phosphate, sodium, potassium, alkaline phosphatase and placma iPTH, hCT and hGH at 8 a.m., before the calcium load, and at 10 a.m.,during

Table 5.5. : Urinary glucose and acetone, blood glucose and serum FFA before (control period), and after (test period) the calcium load.

	Group 1 (n = 15) $U_{Ca} \leq 5.2$ (mg/kg/24 hr)		Group 2 (n = 10) $U_{Ca} > 5.2$ (mg/kg/24 hr)	
	Period :		Period :	
	Control	Test	Control	Test
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
Urinary glucose (g/kg/24 hr)	0.13 ± 0.14	0.19 ± 0.20	0.13 ± 0.17	0.13 ± 0.19
Urinary acetone (mg/dl)	9.80 ± 15.07		3.20 ± 4.5	
Blood glucose (mg/dl)	228.00 ± 105.30	242.30 ± 96.90	199.30 ± 121.70	207.70 ± 109.00
Serum FFA (μ M/l)	765 ± 335	809 ± 391	687 ± 321	545 ± 237

Table 5.6. : The concentration of several blood constituents before (control period) and after (test period) the calcium load.

	Group 1 (n = 15) $U_{Ca} \leq 5.2$ (mg/kg/24 hr)		Group 2 (n = 10) $U_{Ca} > 5.2$ (mg/kg/24 hr)	
	Period :		Period :	
	Control	Test	Control	Test
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
Serum				
calcium (mg/dl)	9.72 ± 0.48	10.36 ± 0.46	9.75 ± 0.59	10.35 ± 0.46
phosphate (mg/dl)	4.25 ± 0.35	4.17 ± 0.40	4.43 ± 0.48	4.50 ± 0.37
sodium (mEq/l)	136.08 ± 3.35	137.15 ± 2.97	137.14 ± 1.95	136.50 ± 1.60
potassium (mEq/l)	4.43 ± 0.44	4.47 ± 0.35	4.41 ± 0.30	4.35 ± 0.32
alk. phosph. (IE)	20.98 ± 9.91	21.02 ± 10.42	25.27 ± 5.47	26.06 ± 5.57
Plasma				
iPTH (ng equiv.b.PTH/ml)	0.13 ± 0.08	0.15 ± 0.10	0.10 ± 0.07	0.10 ± 0.07
hCT (ng/ml)	0.31 ± 0.14	0.31 ± 0.12	0.24 ± 0.11	0.25 ± 0.12
hGH (ng/ml)	4.45 ± 7.30	5.09 ± 7.43	3.29 ± 4.55	2.91 ± 5.27

the test period, after the calcium load.
The serum level of calcium, inorganic phosphate, sodium, potassium, alkaline phosphatase and the plasma iPTH, hCT and hGH concentrations were not different in group 2 when compared to group 1 (Student "t" test : n.s.).
The serum calcium level rose significantly after the oral calcium load in each of the two groups (paired "t" statistic : $p < 0.01$).
The calcium increment was not significantly different between the two groups. After the calcium load no change was measured in the serum inorganic phosphate, sodium, potassium, alkaline phosphatase, plasma iPTH, hCT and hGH concentrations (paired "t" statistic : n.s.).
There is a wide spread of hGH levels in both diabetic groups.

The urinary excretion of several substances during the control and test periods of each group are listed in table 5.7. The urinary excretion of the substances is expressed either as per kg of body weight or as per mg and g of creatinine excreted in the urine.

Noteworthy is the high fasting calcium/creatinine ratio of 0.21 $\pm$ 0.11 (SD) during the control period in group 2 as compared to group 1 (Student "t" test : $p < 0.001$).
The rise in the calcium/creatinine ratio in group 1 from 0.08 $\pm$ 0.06 (SD) to 0.13 $\pm$ 0.09 (SD) after the calcium load is significant in group 1 (paired "t" statistic : $p < 0.01$), but not so in group 2. The increment in the calcium/creatinine ratio after the calcium load in group 1 is, however, not significantly different from that in group 2.

The urinary cyclic AMP concentration per unit creatinine during the control period was lower in diabetic children than in the seventeen healthy children, where a mean of 5.80 μ Moles/g of creatinine $\pm$ 2.43 (SD) was found (Student "t" test : $p < 0.005$) decreasing with age ($r = -0.732$; $p < 0.01$). The urinary cAMP excretion in diabetic children also decreased with age ($r = 0.418$; $p < 0.05$; $n = 25$).
The urinary cAMP concentration was somewhat higher in group 2 when compared to group 1, but was not statistically significant.
The urinary cyclic AMP excretion did fall significantly in group 2 from 3.98 $\pm$ 1.02 (SD) to 3.16 $\pm$ 0.69 (SD) after the calcium load (paired "t" statistic : $p < 0.05$), but did not decrease significantly in group 1. However, the decrement of urinary cyclic AMP excretion after the calcium load in group 2 was not significantly different from that in group 1.
There was no difference between the two groups in the urinary excretion of sodium which rose after the calcium load in both groups, but not significantly.
There was no correlation between the urinary excretions of calcium and sodium in the hypercalciuric children during the test period ($r = 0.234$; $p > 0.05$; $n = 10$).

The urinary excretion of potassium in the control period was higher in group 2 (Student "t" test : $p < 0.05$), but did not change significantly after the calcium load.

There was no significant difference between the volumes of the urines of group 1 when compared to group 2 during the control and test periods.

The creatinine clearance was high in both groups, but there was no significant difference.

The urinary inorganic phosphate excretion showed no difference between the two groups, but decreased significantly after the calcium load only in group 2 (paired "t" statistic : $p < 0.05$).
The values of the tubular reabsorption of phosphate (TRP) and of the tubular maximum reabsorption of phosphate per 100 ml of glomerular filtrate (TmP/GFR) were not significantly different in group 2 when

Table 5.7. : The renal excretion and handling of several substances before (control period) and after (test period) the calcium load.

	Group 1 (n = 15) $U_{Ca} \leq 5.2$ (mg/kg/24 hr)		Group 2 (n = 10) $U_{Ca} > 5.2$ (mg/kg/24 hr)	
	Period :		Period :	
	Control	Test	Control	Test
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
calcium (mg/mg creat)	0.08 ± 0.06	0.13 ± 0.09	0.21 ± 0.11	0.24 ± 0.10
sodium (mEq/kg/2 hr)	0.33 ± 0.24	0.37 ± 0.21	0.29 ± 0.20	0.43 ± 0.26
potassium (mEq/kg/2 hr)	0.13 ± 0.07	0.17 ± 0.09	0.21 ± 0.10	0.19 ± 0.13
phosphate (mg/kg/2 hr)	1.65 ± 1.15	1.29 ± 0.81	2.10 ± 1.36	1.40 ± 0.85
TRP (%)	89.00 ± 6.50	89.70 ± 6.30	88.90 ± 4.90	92.50 ± 4.00
TmP/GFR (mgP/100 ml GFR)	4.30 ± 1.02	4.31 ± 1.00	4.35 ± 0.76	4.97 ± 0.95
cAMP (μM/g creat)	3.43 ± 0.88	3.07 ± 0.93	3.98 ± 1.02	3.16 ± 0.69
creatine clearance (ml/min/1.73 m^2)	181.20 ± 71.00	152.20 ± 41.50	173.20 ± 42.70	172.50 ± 39.90
volume (ml/2 hr)	207.64 ± 93.03	212.00 ± 81.90	206.00 ±102.27	203.89 ±112.88

compared to those of group 1.
These parameters were the same before and after the calcium load in group 1. However, a significant increase in TRP (%) from 88.92 ± 4.90 (SD) to 92.50 ± 3.97 (SD) and in TmP/GFR from 4.35 ± 0.76 (SD) to 4.97 ± 0.95 (SD), respectively, (paired "t" statistic : $p < 0.01$) was observed in group 2 after the calcium load (fig. 5.5.). This increment in TRP and TmP/GFR in group 2 was also significantly different from that noted in group 1 (Student "t" test : $p < 0.02$).

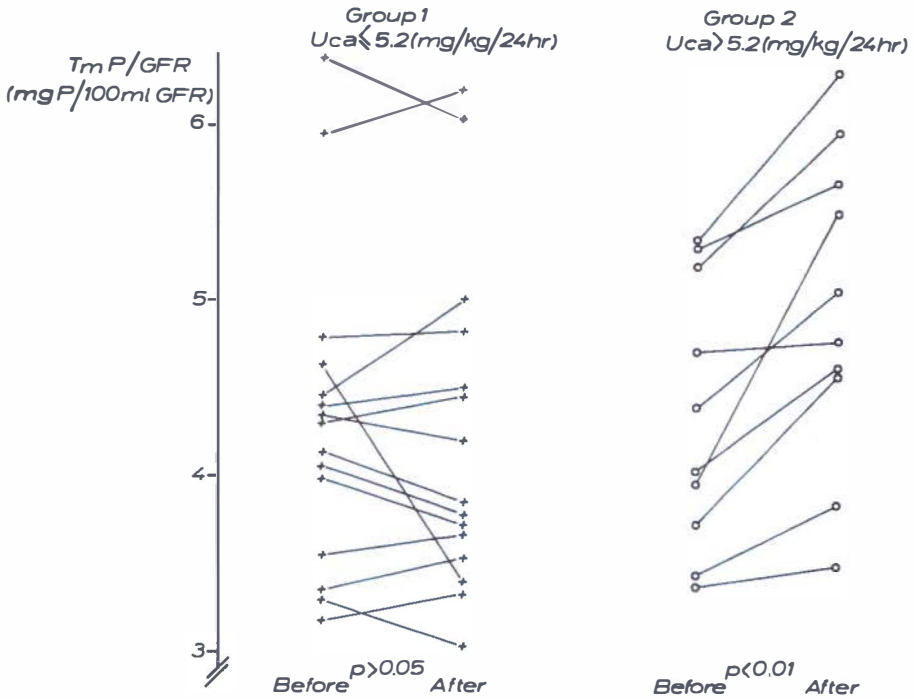


Fig. 5.5. TmP/GFR in the normocalciuric (+) (group 1) and glucose-independent (o) (group 2) hypercalciuric diabetic children before and after the oral calcium load.

5.4. EFFECT OF INDOMETHACIN

5.4.1. INTRODUCTION

It was suggested in the previous Chapter that parathyroid hormone or substances related to PTH, could not be the factors responsible for the hypercalciuria found in the diabetic children. Most of the hypercalciuric children did not have elevated immunoreactive parathyroid hormone plasma levels.

The results of the oral calcium load test suggested that the hypercalciuria could be of primary renal origin. Therefore, the possible influence of prostaglandins on the hypercalciuria in the diabetic children was evaluated by inhibiting prostaglandin synthesis with indomethacin.

The suggestion that prostaglandins may be of importance derives from the following observations.

Prostaglandins of the E series are potent stimulators of bone resorption in vitro (Klein and Raisz, 1970), and altered bone metabolism in diabetic patients was described by Levin et al.(1976), De Leeuw (1976), and in this study in Chapter 5.2.2.
It has been proposed that prostaglandins may be the humoral agents responsible for the hypercalcaemia seen in animals and humans with solid tumors (Tashjian et al., 1972 and 1974; Brereton et al., 1974; Blum, 1975; Seyberth et al., 1975; Voelkel et al. (1975).
Indomethacin and aspirin, potent inhibitors of the prostaglandin synthesis are used clinically to correct hypercalcaemia associated with solid tumours (Vane, 1971; Smith and Willis, 1971).
In the syndrome of Bartter, the high urinary excretion of sodium, potassium (Verberckmoes et al. 1976) and calcium (Donker et al. 1976/b) decreases when the patients are treated with indomethacin..
The ubiquitous quasi-hormones, the prostaglandins, have been credited with numerous biological functions (Ramwell and Shaw, 1970), such as the activation of adenyl cyclase in a wide variety of tissues and cell systems, and involvement in cation transport in the cell membrane (Paton and Daniel, 1967; Strong and Bohr, 1967).

5.4.2. PATIENTS

Eleven diabetic children with a normal urinary calcium excretion (group 1), and ten diabetic children with glucose-independent hypercalciuria (group 2), were given 0.5 mg of indomethacin per kg body weight orally at 8 a.m. after an overnight fast. Four other normocalciuric diabetic children did not receive indomethacin. Urine was collected from 6 a.m. until 8 a.m. (control period) and from 8 a.m. until 10 a.m. (test period) (see Chapter 2.3.).
The chronological age, height and weight of the children and the duration of insulin administration to the children of both groups are listed in table 5.8.

Table 5.8. : Age, duration of diabetes, height and weight of the normocalciuric and glucose-independent hypercalciuric diabetic children before the indomethacin administration.

	Group 1 (n = 11)	Group 2 (n = 10)
	$U_{Ca} \leqslant 5.2$ (mg/kg/24 hr)	$U_{Ca} > 5.2$ (mg/kg/24 hr)
	Mean $\pm$ 1 SD	Mean $\pm$ 1 SD
Age (yr)	13.00 $\pm$ 2.65	11.80 $\pm$ 2.57
Duration of diabetes (yr)	5.73 $\pm$ 2.22	4.59 $\pm$ 4.13
Height (SDS)	+ 0.83 $\pm$ 0.92	+ 0.24 $\pm$ 1.44
Weight (SDS)	+ 0.39 $\pm$ 0.58	- 0.40 $\pm$ 0.78

The children of group 2 (glucose-independent hypercalciuric) when compared to those of group 1(normocalciuric) were younger, lighter in weight and their diabetes was of a shorter duration, but these differences were of no significance, except for the weight (Student "t" test : $p < 0.05$).

5.4.3. RESULTS

Two hours after the indomethacin administration the indomethacin serum levels were 0.98 μg/ml $\pm$ 0.38 (SD) (n = 7) in group 1 and 1.50 μg/ml $\pm$ 0.69 (SD) (n = 8) in group 2 (Student "t" test : n.s.).

Table 5.9. shows that between the two groups of diabetic children the blood glucose, pH, and bicarbonate levels did not differ significantly nor show any significant change after indomethacin administration.
The urinary acetone and serum FFA increased significantly during the test period. However, only in group 1 and not in group 2, was this increment of urinary acetone and serum FFA significant (paired "t" statistic : $p < 0.05$; $p < 0.005$, respectively). Between the two groups the increment of the urinary acetone and serum FFA was not significantly different.

The urinary pH was significantly higher in group 2 compared to that in group 1 (Student "t" test : $p < 0.05$ during control period ; $p < 0.005$ during test period). Urinary pH and urinary glucose did not significantly change after indomethacin administration.

Table 5.10. shows that there was no difference in serum calcium, ionised calcium, phosphate, alkaline phosphatase, sodium and potassium between the two diabetic groups.
Serum calcium, ionised calcium and alkaline phosphatase showed no significant alterations after indomethacin. Serum phosphate decreased in group 1 (paired "t" statistic : $p < 0.02$) and not in group 2, but the decrement of the serum phosphate in group 1 was not different from group 2.
Serum sodium decreased in both diabetic groups after indomethacin administration (paired "t" statistic : $p < 0.005$ in group 1; $p < 0.01$ in group 2). The decrement was significantly different in group 1 when compared to that of group 2 (Student "t" test : $p < 0.05$). Serum potassium did not increase significantly after indomethacin administration in either groups.

As shown in table 5.11., the urinary calcium to creatinine ratio was high during the control period in group 2 (0.32) compared to that in group 1 (0.07) (Student "t" test : $p < 0.001$) and decreased in both groups (paired "t" statistic : $p < 0.02$). The decrement of the calcium/creatinine ratio was different between group 1 and group 2 (Student "t" test : $p < 0.02$) : in the hypercalciuric group (group 2) the calcium/creatinine ratio decreased more than in the normocalciuric group (group 1) after indomethacin administration. The urinary sodium excretion was not significantly different between the two groups and did not change significantly during the test period. Figs. 5.6. and 5.7. illustrate the urinary calcium to creatinine ratio and sodium excretion for each patient in the two groups during control and test period.

The urinary excretion of sodium and the calcium to creatinine ratio (table 5.11.) correlates significantly in the normocalciuric group during the control period ($r = 0.900$; $p < 0.01$) and during the

Table 5.9. : Urinary glucose, acetone, pH, blood glucose, serum FFA, blood pH, standard and actual bicarbonate before (control period) and after (test period) indomethacin administration.

	Group 1 (n = 15) $U_{Ca} \leq 5.2$ (mg/kg/24 hr)		Group 2 (n = 10) $U_{Ca} > 5.2$ (mg/kg/24 hr)	
	Period :		Period :	
	Control	Test	Control	Test
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
Urinary				
glucose (g/kg/2 hr)	0.13 ± 0.18	0.25 ± 0.23	0.10 ± 0.16	0.10 ± 0.14
acetone (mg/dl)	2.13 ± 2.58	5.73 ± 6.61	2.12 ± 3.26	3.41 ± 4.46
pH	5.57 ± 0.40	5.57 ± 0.34	6.27 ± 0.74	6.37 ± 0.62
Blood				
glucose (mg/dl)	231 ± 100.3	247 ± 86.3	214 ± 111.9	216 ± 86
pH	7.37 ± 0.09	7.38 ± 0.10	7.37 ± 0.05	7.35 ± 0.07
st HCO_3	23.38 ± 4.19	21.8 ± 4.05*	24 ± 2.54	20.7 ± 3.8**
ac HCO_3	25.38 ± 4.16	22.57 ± 4.49*	25.2 ± 2.62	21.4 ± 4.27**
Serum				
FFA (μM/l)	652 ± 460	1,224 ± 563	580 ± 310	1,070 ± 732

* n = 9 ** n = 8

Table 5.10. : The concentration of several blood constituents before (control period) and after (test period) indomethacin administration.

	Group 1 (n = 11) $U_{Ca} \leq 5.2$ (mg/kg/24 hr)		Group 2 (n = 10) $U_{Ca} > 5.2$ (mg/kg/24 hr)	
	Period :		Period :	
	Control	Test	Control	Test
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
Serum				
calcium (mg %)	9.4 ± 0.17	9.37 ± 0.31	9.42 ± 0.19	9.44 ± 0.32
ionised calcium (mEq/l)	1.9 ± 0.09	1.91 ± 0.08	1.92 ± 0.06	1.94 ± 0.08
phosphate (mg %)	4.45 ± 0.39	4.17 ± 0.44	4.16 ± 0.60	4.02 ± 0.48
potassium (mEq/l)	4.39 ± 0.34	4.44 ± 0.41	4.15 ± 0.40	4.24 ± 0.40
sodium (mEq/l)	137.5 ± 2.94	134.7 ± 3.38	137.3 ± 3.65	136.5 ± 2.83
alk.phosph. (IE/ml)	22.4 ± 5.29	23.18 ± 6.58	21.9 ± 7.28	21.33 ± 6.65

Table 5.11. : The renal excretion and handling of several substances before (control period) and after (test period) indomethacin administration.

	Group 1 (n = 11) $U_{Ca} \leq 5.2$ (mg/kg/24 hr)		Group 2 (n = 10) $U_{Ca} > 5.2$ (mg/kg/24 hr)	
	Period :		Period :	
	Control	Test	Control	Test
	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD	Mean ± 1 SD
calcium (mg/mg/creat)	0.07 ± 0.04	0.04 ± 0.03	0.32 ± 0.18	0.17 ± 0.15
sodium (mEq/kg/2 hr)	0.30 ± 0.26	0.21 ± 0.22	0.44 ± 0.24	0.36 ± 0.23
potassium(mEq/kg/2 hr)	0.14 ± 0.08	0.18 ± 0.08	0.17 ± 0.09	0.22 ± 0.11
phosphate(mg/kg/2 hr)	1.6 ± 0.72	1.63 ± 0.66	1.76 ± 0.99	1.64 ± 0.69
TRP (%)	89.4 ± 5.36	87.5 ± 5.47	86.27 ± 8.47	86.86 ± 9.89
TmP/GFR(mgP/100 ml GFR)	4.30 ± 0.89	3.93 ± 0.55	3.9 ± 0.91	4.01 ± 0.79
cAMP (μg/g creat	3.64 ± 1.58	3.82 ± 1.07	4.85 ± 1.54	4.27 ± 1.39
creatinine clearance (ml/min/1.73 m^2)	161.4 ± 36.2	155.4 ± 49.1	132.6 ± 35.3	142.5 ± 42.6
volume (ml/2 hr)	216 ± 102	185 ± 106	256 ± 167	231 ± 120

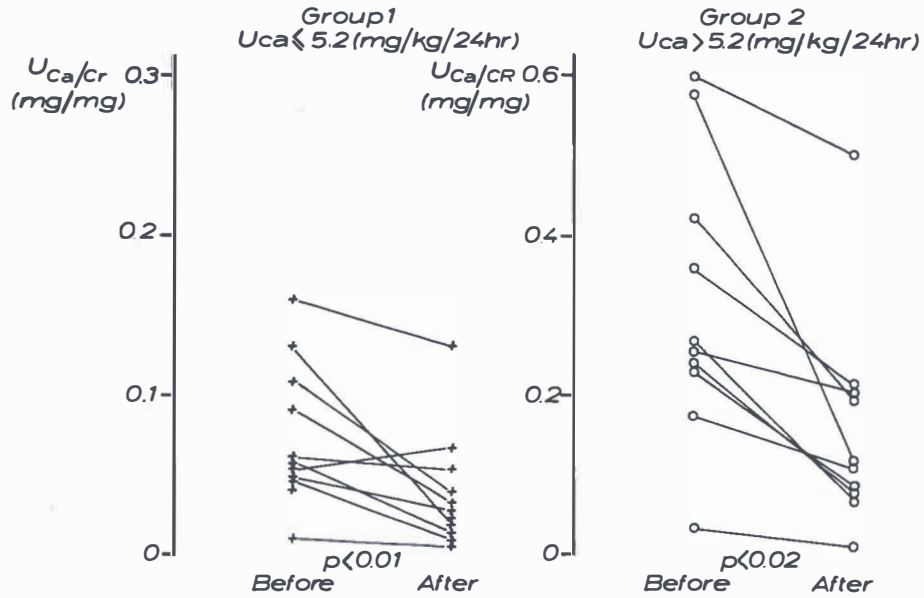


Fig. 5.6. Urinary calcium to creatinine concentration ratio in the normocalciuric (+) (group 1) and glucose-independent (o) (group 2) hypercalciuric diabetic children before and after indomethacin administration.

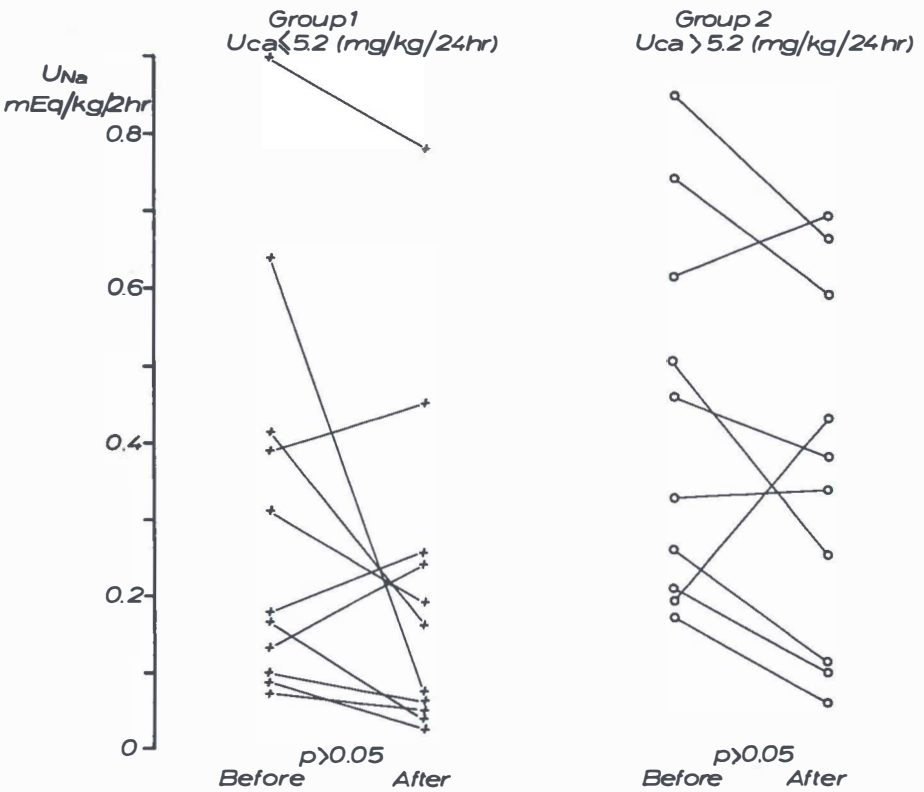


Fig. 5.7. Urinary sodium excretion in the normocalciuric (+) (group 1) and glucose-independent (o) (group 2) hypercalciuric diabetic children before and after indomethacin

test period ($r = 0.940$; $p < 0.01$).
In the hypercalciuric group the correlation between the excretion of sodium and the calcium to creatinine ratio was not significant during the control period ($r = 0.420$; $p > 0.05$), but was significant after the indomethacin administration ($r = 0.634$; $p < 0.05$).

The TRP, TmP/GFR, creatinine clearance, and the urinary excretion of potassium, inorganic phosphate and cAMP and the urinary volume (table 5.11.) showed no significant difference between the two groups of children during the control period, and also during the test period.
The slightly higher excretion rate of potassium and cAMP in the hypercalciuric group during the control period is noteworthy.

The four children who had not received indomethacin did not show alterations in the calcium to creatinine ratios, nor in the urinary sodium excretion. The sodium excreted (mEq/kg/2 hr) and the calcium to creatinine ratio (mg/mg) determined in the 6 to 8 a.m. urine sample was 0.35 ± 0.29 (SD) and 0.11 ± 0.05 (SD) respectively, and in the 8 to 10 a.m. urine sample 0.44 ± 0.36 (SD) and 0.11 ± 0.06 (SD) respectively.

5.5. SUMMARY

During the four year follow-up period the growth in height of the hypercalciuric diabetic children was not different from that of the normocalciuric children.
In the diabetic patients the cortical thickness and the percent cortical area, but not the index of Exton-Smith were below the values reported for normal children. These parameters did not significantly differ between the hypercalciuric and normocalciuric diabetic children. Between the hypercalciuric and normocalciuric children there was no significant difference in the urinary hydroxyproline excretion.

Compared with the normocalciuric diabetic group, a significantly higher urinary calcium to creatinine concentration ratio and a significantly higher urinary pH were noted during fasting in the glucose-independent hypercalciuric group. The two groups did not differ in blood pH and bicarbonate levels. The urinary potassium excretion during fasting was higher in the glucose-independent hypercalciuric group than in the normocalciuric diabetic group, but only once out of the two determinations significantly. The urinary cAMP excretion in the diabetic children was significantly lower compared to that in the healthy children.

In the glucose-independent hypercalciuric group the oral calcium load decreased the urinary cAMP and increased the TmP/GFR and TRP significantly. The levels of iPTH were not different between the hypercalciuric and normocalciuric diabetic patients.

After indomethacin administration, the decrement of the urinary calcium to creatinine concentration ratio in the hypercalciuric children was significantly different from the normocalciuric children, with no alterations in the urinary excretion of sodium.

CHAPTER 6

DISCUSSION

The purpose of this study was to investigate the urinary excretion of calcium in diabetic children. The study originated from the finding of abnormally high urinary calcium excretion levels in some of the diabetic children of the Paediatric Department of the University Hospital of Groningen. The hypercalciuria was not accompanied by signs or symptoms of primary hyperparathyroidism, or renal stone formation, and on plain X-ray films of the abdomen no abnormal calcifications could be found.

Two methods of investigation were employed in this study, statistical and experimental. The statistical analyses were carried out on the 24 hour urine calcium excretion and on the dietary calcium intake, of the diabetic children and also of a group of healthy children, to ascertain whether the hypercalciuria found in the diabetic children did indeed occur with an abnormal frequency. By analysing statistically other urinary and dietary substances also, a possible contribution of these substances on the occurrence of hypercalciuria in the diabetic children could be sought.

Possible abnormalities in calcium absorption from the gut, in bone metabolism and in renal function were investigated by employing a calcium load test and a prostaglandin inhibiting test, and by comparing the two groups of diabetic children, normocalciuric and hypercalciuric, under these test conditions.

6.1. REFERENCE VALUES

The statistical analyses on the urinary excretion of the several substances, and on the dietary intake of the groups of children, were performed on groups of children in which the children were not matched. However, the groups are comparable in age, height and weight.

The dietary intake of calories and the proportion of the contribution of carbohydrate, fat and protein to the diet of the fifty-six healthy children were in accordance with those reported for 7 - 10 year old Dutch children (van der Haar et al., 1973 ;de Wijn,1976).

The urinary calcium excretions of the diabetic and healthy control children were determined employing the same methods. To make a valid comparison the healthy children were living in the same region as the diabetic children (Nordin et al., 1967; Rose and Harrison,1974).

Table 6.1. shows that the urinary calcium excretion in the group of fifty-six healthy control children compared well with that reported by Ghazali and Barratt (1974), but not with that reported by Royer (1961) and by Paunier et al. (1970). The observed differences in the

mean excretion of calcium could be due to differences in methods, in the selection of children, in the dietary intake of calcium and vitamins, and in exposure to sunlight (Parry and Lister, 1975).

Table 6.1. : Reported urinary calcium and magnesium in healthy children.

	Age(yr)	U_{Ca} (mg/kg/24 hr)			U_{Mg} (mg/kg/24 hr)		
		n	Mean	± 1 SD	n	Mean	± 1 SD
France							
Royer, P.(1961)	0-18	74					
		65 < 4					
		1 > 6					
Switzerland							
Paunier et al. (1970)	0-14	38	3.6	2.4	38	2.8	1.1
U.K.							
Ghazali and Barratt (1974)	1-15	52	2.38	0.66	23	2.82	0.79
The Netherlands							
Van Damme et al. (1976)	3.3-16.3	56	2.4	1.4	55	1.7	0.8

Ghazali and Barratt (1974) collected urine in bottles containing thiomersalyl, Paunier et al. (1970) did not use a preservative but acidified the urine after completion of the collection, as we did. Paunier et al. (1970) and Ghazali and Barratt (1974), used atomic absorption in the determination of the calcium and magnesium content of the urine, in our study chemical methods were employed. The children in the other studies were hospitalised and Royer (1961) and also Paunier et al. (1974) included many children under one year of age.

The positively skewed distribution of the urinary calcium excretion in our control group is in accordance with earlier observations (Knapp, 1947; Hodgkinson and Pyrah, 1958; Bulusu et al., 1970; Nordin et al., 1972/b).

The urinary calcium to creatinine concentration ratio of 0.111 ± 0.063 (SD) in this study is in agreement with the results of the study of Ghazali and Barratt (1974). The upper value of this ratio of 0.23 is somewhat less than that of 0.28 reported in normal adults by Nordin (1959).

The urinary magnesium excretion determined in our study differed from that reported by Ghazali and Barratt (1974), as did the urinary magnesium to creatinine ratio. However, the magnesium to creatinine concentration ratio of 0.079 is nearly the same as the ratio of 0.076 reported in adults (Hodgkinson, 1974).

In this study a good correlation was found between the urinary excretion of sodium and calcium, a not too surprising finding as it is known that the calcium excretion in the urine depends on the sodium intake (Modlin, 1966; Epstein, 1968; Antoniou et al., 1969).

In the control children the urinary excretion of calcium did not correlate with the calcium intake. A similar finding in stone forming subjects was reported by Peacock et al. (1967), and also when extreme amounts of calcium (2 - 3 g/day) were given to adults (Nordin et al. 1972/b).

The influence of the phosphate intake on the calciuria, which has been reported in adults (Bernstein and Newton, 1966; Coburn et al., 1971), was found when the urinary sodium excretion was also taken into account.

In contrast to other studies in humans (Clarkson et al., 1967; Paunier et al., 1970) and animals (Clark, 1969/a) the calcium intake did not significantly influence the urinary magnesium excretion. As in adult humans (Duarte and Watson, 1967) the urinary magnesium excretion correlated with the urinary sodium excretion, but not with the urinary calcium excretion (Heaton and Parsons, 1961).

6.2. HYPERCALCIURIA IN DIABETIC CHILDREN

The urinary excretion of creatinine was higher in the diabetic children than in the healthy control children. This cannot be attributed to the presence of ketones in the urine (Watkins, 1967). Not only was ketonuria not detectable by ketostix tests in the urine of the diabetic children but also the method employed for the determination of creatinine is not interfered if acetone is added. The low plasma values of creatinine, also reported by Aviram et al., (1966) in diabetic patients, together with the high level of urinary creatinine excretion implies that the creatinine clearance values were elevated. The creatinine clearances in the diabetic children were determined on non-catheterised urine specimens and over short periods of time (2 hours), leaving room for marginal errors. It is known that in juvenile diabetic patients there is an increased glomerular filtration rate (Farber et al., 1951; Robertson and Gray, 1953 ; Mogensen, 1972 ; Mogensen and Andersen, 1973).

The large urine flow of the diabetic patients can be explained by osmotic diuresis due to high glucose levels in the glomerular filtrate.

An increased excretion of phosphate in the presence of a normal serum phosphate which was found in the diabetic children, was also reported in non-acidotic diabetic patients without manifestations of renal impairment (Astrug, 1966) . The saturation of the tubular reabsorptive capacity for glucose impairs the phosphate reabsorption (Martini, 1964), and can account for this finding.

A high filtered load of calcium due to a high GFR, does not explain the hypercalciuria. The creatinine clearance rates of the glucose-independent hypercalciuric diabetic patients did not differ from that of the normocalciuric diabetic children.

Oral glucose loads increase the urinary excretion of calcium in normal adults (Lindeman et al., 1964; Lemann et al., 1969 and 1970/a; Lennon et al., 1974). A good correlation was obtained between the calciuria and the glucosuria in the diabetic children with a normal

urinary calcium excretion, and also in six of the nineteen children with hypercalciuria. In the thirteen other hypercalciuric children the hypercalciuria remained high, irrespective of the glucosuria.

In comparison with the group of healthy control children, the group of diabetic children was found to have an abnormally high incidence of hypercalciuria. Three of the thirthy-two diabetic children who originally had normal urinary calcium excretions, showed a hypercalciuria independent of glucosuria, during the four year follow-up.

The relationship between the hypercalciuria and the diabetic process is not clear. Neither poor control of the diabetes nor metabolic acidosis can explain the glucose-independent type of hypercalciuria. The pH and the standard bicarbonate levels of venous blood, blood and urine glucose levels, serum FFA concentrations and urinary excretions of acetone in the glucose-independent hypercalciuric group were not different from those found in the normocalciuric group. The urinary pH of fasting samples was in fact lower in the latter group. The duration and the quality of control of the diabetic process were not found to be relevant to the occurrence of hypercalciuria.

Walsh et al. (1975) reported a high incidence of hyperparathyroidism in diabetic patients, and Schneider et al. (1974) reported elevated levels of parathormone in alloxane induced diabetes in rats. In the group of glucose-independent hypercalciuric patients the serum calcium, ionised calcium, phosphate and the plasma iPTH were all within normal limits, and did not differ from the values found in the normocalciuric patients.
The limitations of the radioimmunoassay for the determination of intact human PTH are well known (Silverman and Yalow, 1973; Arnaud et al., 1974 ; Reiss and Canterbury, 1974; Bouillon, 1977).

In conclusion, in the diabetic children with the glucose-independent type of hypercalciuria, the hypercalciuria could not be associated with manifestations of the diabetic process, such as glucosuria, increased GFR, high blood glucose, low blood pH and standard bicarbonate. Neither could the hypercalciuria be associated with elevated iPTH plasma levels.

6.3. ROLE OF THE DIET

The relative contribution of protein, but not of carbohydrate, to the total caloric intake was higher in the diabetic children compared to that in the group of fifty-six healthy control children. 20 % of the caloric intake of the diet was in the form of proteins, as is usually prescribed for diabetic patients (Kaufmann et al.,1975; McArthur et al., 1976). There was no difference between the protein intake of the normocalciuric and hypercalciuric diabetic children. In the diabetic children no correlation could be established between the relative or absolute amounts of protein or carbohydrate in the diet, and the observed level of calciuria.

Modlin (1966) and others (Kleeman et al., 1964; Epstein, 1968) described a correlation between the urinary excretion of calcium and sodium in 24 hour urine samples of healthy subjects. Only Wills et al. (1969/a) were unable to find a consistent correlation between the sodium and calcium excretion.

In the control group of healthy children a significant correlation between the calcium and sodium excretion was demonstrated. The diabetic children with a normal calcium excretion showed a correlation between the calcium and sodium excretion that was occasionally significant, but not so when multiple regression equations were calculated. There was no correlation between the urinary output of calcium and sodium in the hypercalciuric diabetic patients. The average urinary sodium excretion of the normocalciuric group was not different from that of the hypercalciuric group, and therefore, it is unlikely that the sodium intake is of any importance in the pathogenesis of the observed hypercalciuria.

The total group of hypercalciuric diabetic children showed a higher mean calcium intake than the normocalciuric diabetic children. Compared to the normocalciuric children many prepuberal children were in the hypercalciuric group. Prepuberal children had a relatively higher calcium intake than puberal children. When the normocalciuric and hypercalciuric groups were divided into prepuberal and puberal subgroups no difference could be established between the calcium intake of the normocalciuric and hypercalciuric patients. Analysing a possible contribution of calcium intake to the hypercalciuria, the correlation between the calcium intake and the calcium excretion in the normocalciuric, as well as in the hypercalciuric children was found not to be significant. Moreover, analysis of co-variance did not reveal a difference in the regression coefficients of the equations between the calcium intake and output in both groups, while a significant difference was demonstrated between the intercepts, indicating that for the same calcium intake the children of the hypercalciuric group still exhibited a higher calcium excretion.

An analysis of the contribution of the calcium-ion content of drinking water was performed, although the calcium content of tap water plays only a minor role in the total amount of calcium ingested. No relationship between the occurrence of hypercalciuria and the calcium-ion content of the drinking water was found.

The calcium intake in the groups of prepuberal and puberal diabetic children was higher than in the group of fifty-six healthy control children, but the same as in the group of twenty-one healthy control children. The incidence of hypercalciuria in the diabetic children was significantly higher than that in the control group of fifty-eight healthy children, who had a significantly lower calcium intake, and also higher than that in the control group of twenty-one healthy children, who had the same calcium intake.

No statistical evidence was found to incriminate the calcium intake in the pathogenesis of the hypercalciuria in the diabetic patients. This corresponds with the findings of Knapp (1947) that the relationship between the calcium intake and the urinary calcium is never close, and that only 2 - 7 % of the increment of calcium intake is excreted in the urine (Nicholaysen et al., 1953; McIntosh et al., 1962; Carruthers et al., 1964; Nordin et al., 1967; Peacock et al., 1967).

The hypercalciuria in these diabetic children was in all probability due to the fact that these children had diabetes mellitus, but the patho-physiological basis for this relationship was not demonstrated.

6.4. BONE STRUCTURE AND METABOLISM

The next question that arose was : could the hypercalciuria in the diabetic children be due to an abnormality in bone metabolism ?

Bone structure and metabolism were shown to be aberrant in diabetic patients (De Leeuw, 1976; Levin et al., 1976) and decreased bone mineral density was noted in patients with urolithiasis (Alhava et al., 1976).

Elevated levels of growth hormone in diabetic patients (Luft and Guillemin, 1974) can influence the growth and mineralisation of bone. The hypercalciuric children resemble acromegalic patients, inasmuch as they have an increased calcium excretion in the urine (Bauer and Aub, 1941; Harrison et al., 1960; De Gennes et al., 1961).
In the diabetic children the determination of growth hormone in the serum showed very high fasting levels in some children in both the normocalciuric and hypercalciuric groups, and no significant difference between the two groups was found.
No difference in height between the diabetic and healthy children, nor between the normocalciuric and hypercalciuric diabetic children was found. Although the groups of diabetic children were small, and the follow-up period was short, it was concluded that growth in height was within normal limits and that excess growth hormone did not explain the hypercalciuria.

The cortical thickness, and the PCA of the left second metacarpal bone of the diabetic children, when compared with those reported for healthy children, (Bonnard, 1968; Garn et al., 1971) were reduced. However, the hypercalciuric diabetic children did not differ from the normocalciuric diabetic children.

Not only in bone, but changes in the metabolism of all collagenous tissues are reflected in the amount of urinary hydroxyproline excreted (Williams, 1974).
Compared with the control values of healthy individuals (Williams, 1974), some of the prepuberal diabetic patients excreted low levels of hydroxyproline and some of the older children high levels. The diabetic children over fourteen years of age comprise the group of 14 - 16.5 years old patients and, therefore, are not comparable to an age group "over fourteen years", as it is known that in many diabetic children puberty is delayed (Rayner and Jivani, 1973).
No difference in hydroxyproline excretion between the normocalciuric and hypercalciuric children was found.

To recapitulate, the diabetic children showed a normal height but an aberrant bone structure. The hypercalciuric and normocalciuric diabetic children did not differ in this aberrant bone structure nor in the amount of urinary hydroxyproline excreted.

6.5. THE ORAL CALCIUM LOAD

To investigate whether the observed hypercalciuria in the diabetic children was of intestinal (Parfitt et al., 1964; Dent and Watson, 1965; Pak et al., 1972; Nordin et al., 1972/b) or renal (Edwards and Hodgkinson, 1965/a and 1965/b; Coe et al., 1973) origin an oral calcium load test as described by Pak et al. (1975) but modified for the diabetic children, was performed.

In contrast to Pak et al. (1975) the children were not kept on a low calcium diet preceding the test and a standard breakfast was not given with the calcium on the day of the test. Feeding the diabetic children without giving insulin would have resulted in glucosuria, and this would have interfered with the calcium reabsorption (Lindeman et al., 1964; Lemann et al., 1969 and 1970/a, Lennon et al., 1974).Fasting can decrease calciuria (Massry et al., 1973) but not substantially alter it in the studied diabetic children, as the calciuria in these patients is already at its lowest point in the morning.
Pak showed no difference in the reliability of the test if the duration of the fasting period was reduced from four to two hours. In contrast to Pak et al. (1975) the length of time after the calcium load in this study was reduced from four to two hours.
The accuracy of the collection of urine was ensured by giving the children large amounts of distilled water to drink, and thereby increasing the urine flow.

The pH of all urine samples was 5 or more (dip stix method) before and after the calcium load. The output of glucose and sodium in the urine did not change throughout the duration of the test. No correlation could be found between the urinary excretion of sodium and calcium, in either the hypercalciuric or the normocalciuric group of patients, before or after the calcium load.
Fasting is known to induce varying degrees of volume depletion and can change the tubular reabsorption of calcium (Massry et al., 1973). However, no evidence could be found that the hypercalciuric patients conserved sodium less adequately than the normocalciuric ones.

The same diabetic children with an abnormally high 24 hour urinary calcium excretion, showed abnormal high fasting calcium to creatinine concentration ratios, when compared to diabetic children with a normal 24 hour urinary calcium excretion.
The high fasting urinary calcium to creatinine concentration ratio is unlikely to be seen in absorptive hypercalciuria but is observed in primary hyperparathyroidism or in renal hypercalciuria (Pak et al., 1975).

In healthy control children as well as in diabetic children the 2 hour urinary excretion of cAMP decreases with age ($r = -0.732$; $p < 0.01$; $n = 17$ for the healthy control children and $r = -0.418$; $p < 0.05$; $n = 30$ for the diabetic children). A decrease in the 24 hour urinary cAMP excretion with age was also reported by Vitek and Lang (1976) in healthy subjects.
The elevated urinary excretion of cAMP decreases to normal levels in diabetic adult subjects, when they receive insulin (Tucci et al.,1973). Murad and Pak (1972) reported that normal volunteers excrete higher levels of cAMP than hospitalised patients. The healthy control children in our study had also a higher output of cAMP than the

hospitalised diabetic patients.

There is no statistically significant difference between the urinary output of cAMP during the two hour fast, before the calcium load, or before the indomethacin administration, in the normocalciuric and hypercalciuric diabetic children.
After the calcium load no significant difference in the urinary cAMP levels between the two groups was found, nor in the decrement of the urinary cAMP between the two groups. When tested with the "t" test for paired samples (Snedecor and Cochran, 1967), the oral calcium load test was found to reduce significantly the urinary cAMP excretion of the hypercalciuric children but not that of the normocalciuric children.
After an oral calcium load, a decrease of the high fasting urinary cAMP excretion accompanied by an increase of the high fasting urinary calcium to creatinine concentration ratio is indicative of renal hypercalciuria, and an increase of the low fasting urinary calcium to creatinine concentration ratio accompanied by a decrease of the low fasting urinary cAMP excretion is indicative of absorptive hypercalciuria (Pak et al., 1975).
Although the fasting values of the urinary cAMP excretion in our patients are low, intestinal hyperabsorption of calcium in diabetic patients seems unlikely since a blocked vitamin D metabolism was demonstrated in diabetes mellitus (Schneider, 1975) resulting in a decreased intestinal absorption of calcium (Schneider and Schedl,1972).

The values of TmP/GFR during the fasting period are as those reported by Corvillain and Abramov (1972) in normal children.
Most interesting is the finding that in the hypercalciuric group the TmP/GFR significantly rose after the calcium load and that in the normocalciuric group the TmP/GFR did not change significantly, despite an identical rise in the serum calcium and the same absolute serum calcium level. The increase in TmP/GFR after the oral calcium load in the hypercalciuric diabetic group suggests a suppression of the parathyroid function, but in our patients the iPTH levels did not change after the calcium load. The altered renal response to the calcium load is due to other factors, other than changes in iPTH levels.

Giving calcium did not change the plasma hGH and hCT levels in the normocalciuric or hypercalciuric diabetic children.

In conclusion, the glucose-independent hypercalciuric diabetic children show definite differences compared to the normocalciuric diabetic children : a high fasting urinary calcium to creatinine concentration ratio and after a calcium load, a fall in the urinary cAMP excretion and an increase in TmP/GFR.

As a cause for the hypercalciuria in the diabetic children a renal defect seems likely, as our findings share many similarities with the results of Pak et al. (1975). He reported the characteristic features of renal hypercalciuria as follows : "normocalcaemia, high fasting urinary calcium to creatinine concentration ratio, high fasting cAMP, and a normal cAMP after the calcium load with an increase of the calcium to creatinine concentration ratio."

In this study on diabetic children the hypercalciuria was not associated with elevated levels of iPTH, which occur frequently in idiopathic hypercalciuria (Coe et al., 1973).

The plasma hGH and hCT after the calcium load did not change in this study. There is the possibility that one or more unknown factors contribute to the hypercalciuria in the diabetic children.

6.6. EFFECT OF INHIBITION OF PROSTAGLANDIN SYNTHESIS ON THE HYPERCALCIURIA

The results of the oral calcium load showed that the hypercalciuria in the diabetic children had many similarities to renal hypercalciuria. In the absence of noticeable changes in plasma iPTH, hGH and hCT the role of the prostaglandins was investigated by inhibiting the prostaglandin synthesis with indomethacin.

Indomethacin was chosen as its pharmacological activities are well known, and its effectiveness in suppressing prostaglandin synthesis has been established (Vane, 1971). Administration of oral indomethacin induces a peak concentration in the serum after one hour (Haarman et al., 1976), which makes it suitable for observations over short periods. Two hours after administering 0.5 mg/kg indomethacin orally, the serum concentrations, at the end of the test, were about 1 μg/ml, sufficient for complete inhibition of the prostaglandin synthesis in man (Haarman et al., 1976).

After administration of indomethacin to the normocalciuric and hypercalciuric diabetic children, the urinary excretion of acetone and the serum levels of FFA were higher than before indomethacin was given. This could be expected in diabetic children who had received the last insulin injection the day before the test.
The urinary acetone excretion and serum FFA levels did not differ between the hypercalciuric and normocalciuric groups, and poor control of the diabetic process could not explain the observed differences in the urinary calcium excretion.
Also the urinary pH did not change after indomethacin administration.

The absence of a change in the urinary cAMP excretion after indomethacin administration as reported by Gross and Bartter (1973), was also observed in this study with diabetic children.

After indomethacin administration, the decrement in the urinary calcium to creatinine concentration ratio differed significantly between the glucose-independent hypercalciuric and the normocalciuric diabetic group. Neither the creatinine clearance nor the level of ionised calcium changed in either group of diabetic children after indomethacin administration, and there is therefore, no evidence for a decreased filtered calcium load during the test.
In the four children who did not receive indomethacin, the urinary calcium to creatinine concentration ratio did not change between 6 a.m. and 10 a.m.
A decreased calcium to creatinine concentration ratio was reported after indomethacin was given to patients with Bartter's syndrome (Donker et al., 1976/b). The urinary calcium to creatinine concentration ratio decreased significantly more in the hypercalciuric than in the normocalciuric children, which indicated an important role of the prostaglandins in the renal handling of calcium in the hypercalciuric children.

The reported natriuretic effect of the prostaglandins (Johnston et al., 1968; Martinez-Maldonado et al.,1972; Strandhoy et al.,1974) is probably caused by inhibition of sodium reabsorption at the level of the proximal tubular cells (Carr, 1970; Fichman et al., 1972). The calcium reabsorption is linked to the reabsorption of sodium, and it could be supposed that the observed decrease in the calcium to creatinine concentration ratio in the diabetic patients is due to a

decreased sodium excretion, occurring as a result of the inhibition of the prostaglandin effect. This supposition for the observed decrease in the calcium to creatinine concentration ratio is not valid, since there was no decrease in the sodium excretion after indomethacin administration in either group, and between the two groups no differences were noted in the sodium excretion.
The sodium excretion did not change probably because the children were not sodium restricted, nor was their blood volume depleted by administration of diuretics. This would have stimulated the renin-angiotensin system and by changing the filtration fraction contribute to the prostaglandin effect on natriuresis become discernable (Astoin et al., 1974;Itskovitz and McGiff, 1974; Arisz et al. 1976). Also the diabetic children received supplements of water which would suppress the renal renin-angiotensin system, and therefore, reduce the prostaglandin effect on the proximal tubules.

Another unexpected finding was noted. After administration of indomethacin the correlation between the urinary calcium and sodium, which was nonexistent in the hypercalciuric children before indomethacin administration, approached that found in the normocalciuric diabetic children before indomethacin administration. The effects of prostaglandins may not be restricted to the proximal tubules since effects on the collecting ducts were also described (Strandhoy et al., 1974) and suppression of the distal tubular reabsorption of sodium in animals was reported by Gill et al. (1975). In most of these studies on the effect of the prostaglandins on the sodium excretion, pharmacological doses of prostaglandins were administered through the renal artery which is not the physiologically normal way through which the prostaglandins reach the renal cortex. Prostaglandins are synthetised by the interstitial cells in the renal medulla (Larsson and Änggärd, 1973), from where they are transported to the cortex via the ascending limb of the loop of Henle, and therefore, the proximal tubule may play only a secondary role in the physiological effects of the prostaglandins.
The observed hypercalciuria in the diabetic patients may be due to prostaglandin effects distal to the proximal tubule. In the hypercalciuric children after indomethacin administration the normal relationship between urinary calcium and sodium was restored.
We hypothesise that the extra amount of calcium excreted in the urine by the hypercalciuric diabetic children is due to inhibition of reabsorption of calcium in the distal segments of the nephron, and that this inhibition is mediated by prostaglandins. In other words the hypercalciuric diabetic children have at that location an excess of prostaglandins, or are more sensitive to normal levels of prostaglandins.

Another surprising finding was the urinary pH (measured with a pH meter), which was significantly higher in the glucose-independent hypercalciuric children than in the normocalciuric diabetic children, before and also after indomethacin administration.
The normal blood pH and serum bicarbonate levels, the slightly but not significantly low serum potassium and the higher urinary potassium excretion in the hypercalciuric group compared to the normocalciuric group, suggest similarities with the syndrome of "incomplete renal tubular acidosis" (Wrong and Davies, 1959; Morris et al., 1972). A defect in the ability to acidify the urine after administration of ammoniumchloride is described in this syndrome, with other features such as potassium depletion, idiopathic hypercalciuria, nephrocalcinosis, nephrolithiasis and osteomalacia irregularly present (Buchalew et al., 1968; Malek and Kelalis, 1975; D'Angelo et al.,1976). An acid load test was not performed in the diabetic children and the final proof for the presence of renal tubular acidosis is lacking. The conflicting evidence as to the origin of hypercalciuria in the diabetic

children was also found in patients with incomplete tubular acidosis when these patients were tested with the oral calcium load test described by Pak et al. (1975) (Buchalew et al., 1976). Some results from these patients were compatible with hyperabsorption of calcium from the gut, and other results with renal defects.
The mechanism of incomplete distal tubular acidosis is probably related to an abnormality in the generation, or maintenance, of the distal tubular potential difference (Buchalew et al., 1968). This would fit perfectly with our hypothesis developed on the basis of the investigations with indomethacin, that the hypercalciuria in the diabetic children is due to a defect in the distal tubules, caused by abnormally elevated levels of prostaglandins, or an increased sensitivity to normal levels of prostaglandins.

In conclusion, hypercalciuria occurred approximatively in one in four children with diabetes mellitus. This hypercalciuria did not depend on the presence of glucose in the urine and could not be ascribed to known causes for hypercalciuria. Neither the duration nor the quality of control of the diabetic process could be related to the occurrence of the hypercalciuria.

From the investigations on the diabetic children, the loading with calcium, and the suppression of the prostaglandin synthesis we hypothesise that the hypercalciuria is due to a defect in the distal tubule of the kidney, caused by abnormally elevated levels of prostaglandins, or an increased sensitivity to normal levels of prostaglandins.

SUMMARY

Forty-seven diabetic children were examined systematically for the presence of hypercalciuria, and this was detected in fifteen of the children.

This finding was the origin of an investigation into the calcium excretion of healthy children. In this group of children the calcium excretion in the urine correlated with the urinary sodium excretion, and with the phosphate intake in the diet but not with the calcium intake in the diet.

Compared with the control group, in which four children showed hypercalciuria, the group of diabetic children had an abnormally high incidence of hypercalciuria ($X^2 = 9.90$; $p < 0.01$). The same conclusion was reached when the diabetic children were compared with a group of twenty-one healthy children who received a diet with a calcium content similar to that of the diabetic children.

During the follow-up period of four years four new diabetic patients developed hypercalciuria. In the total group of nineteen hypercalciuric children, the urinary calcium excretion was dependent on the excretion of glucose in six of the children, but was independent of the glucose excretion in thirteen of the children.

Metabolic acidosis could not be shown to be the cause of the hypercalciuria. No correlation was found between the hypercalciuria and the calcium intake in the diet.

The growth in height of the diabetic children with hypercalciuria was not different from that in the children with a normal calcium excretion in the urine. All diabetic children had a decreased cortical thickness of the second metacarpal bone when compared with normal values from the literature, there was however, no difference between the hypercalciuric and the normocalciuric group.

An oral calcium load test provided evidence for a renal rather than an intestinal origin of the hypercalciuria. Oral administration of indomethacin reduced significantly the calcium excretion, without altering the sodium excretion.

We hypothesise that the hypercalciuria found in the diabetic children is due to a renal tubular defect, caused by abnormally elevated levels of prostaglandins or an increased sensitivity to normal levels of prostaglandins.

SAMENVATTING

Tijdens systematisch onderzoek van zevenenveertig diabetische kinderen werd bij vijftien kinderen een hypercalciurie ontdekt.

Naar aanleiding hiervan werd een onderzoek ingesteld naar de calciumuitscheiding in de urine bij gezonde kinderen. In die groep bleek de calciumuitscheiding in de urine te correleren met de zoutexcretie en het phosphaatgehalte in de voeding, maar niet met de hoeveelheid calcium in de voeding.

Vergeleken met de controlegroep, waar vier van de achtenvijftig kinderen een hypercalciurie hadden, bleek dat hypercalciurie bij diabetische kinderen abnormaal frequent voorkomt. ($X^2 = 9.90$; $p < 0.01$). Dezelfde conclusie was geldig wanneer de diabetische kinderen vergeleken werden met een tweede controlegroep van eenentwintig gezonde kinderen, bij wie het calciumgehalte in de voeding hetzelfde was als bij de diabetische kinderen.

Gedurende de follow-up periode van vier jaar ontwikkelde zich bij vier diabetische kinderen een hypercalciurie. In de totale groep van negentien hypercalciurische kinderen was bij zes de calciumuitscheiding afhankelijk van de glucosurie maar bij dertien niet.

Metabole acidose was niet de oorzaak van de hypercalciurie. Er werd geen verband gevonden tussen de hypercalciurie en het calciumgehalte in de voeding.

De lengtegroei van de diabetische patienten met hypercalciurie was niet verschillend van die van de normocalciurische diabetische kinderen. In de totale groep diabetische kinderen was de corticalisdikte van het linker tweede metacarpaal bot in vergelijking met literatuur gegevens verminderd, maar ook hier was er geen verschil tussen hypercalciurische en normocalciurische diabetische kinderen.

Een orale calciumbelastingstest maakte waarschijnlijk dat de hypercalciurie een renale en geen intestinale oorzaak had. Na orale indometacine toediening trad een significante daling op van de calcium/creatinine ratio, bepaald in nuchtere urine. Deze daling ging niet gepaard met een vermindering in de natrium excretie.

We vermoeden dat een renaal tubulair defect de oorzaak is van de hypercalciurie bij deze diabetische kinderen.
Het is mogelijk dat een stoornis in de synthese en/of het metabolisme van prostaglandines daarvoor mede verantwoordelijk is.

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