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Urinary albumin excretion rate and puberty in non-diabetic children and adolescents

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Slightly elevated urinary albumin excretion rate (microalbuminuria) is a marker of early diabetic nephropathy, but it is unclear if the established definition of microalbuminuria (20-200 μg/min) is correct for children and adolescents. We investigated the albumin excretion rate, albumin/creatinine ratio and urinary albumin concentration in 150 healthy schoolchildren and adolescents to (a) obtain a reference value for albumin excretion rate, (b) relate albumin excretion to pubertal stages and (c) evaluate albumin/creatinine ratio and morning albumin concentration as screening methods for elevated albumin excretion rate. Albumin concentration was measured by immunoturbidimetry in timed overnight urine samples. The albumin excretion showed a skewed distribution (geometric mean 3.2 μg/min, 95 percentile 15.1 μg/min). In girls, a peak in the albumin excretion rate was found at the pubertal stage 4 (Tanner) and in boys at stage 5. Albumin/creatinine ratio of 2.5 mg/mmol as a screening level for elevated albumin excretion (15 μg/min) showed a high positive (0.88) and negative (0.99) predictive value. □ *Microalbuminuria, non-diabetics, puberty, reference value, screening*

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Elevated urinary albumin excretion rate (AER) has been shown to be a predictor of overt nephropathy in insulin-dependent diabetes mellitus (1-3) and is now regarded as a marker of early nephropathy (4-8). As yet, there is no generally accepted normal range for urinary albumin excretion rate in non-diabetic children and adolescents. Previous studies in diabetic patients (9-12) and non-diabetic subjects (9, 12-14) have related urinary albumin excretion to age and gender, but not specifically to pubertal development.

Timed overnight urine samples are probably the most precise way to measure AER. The method is rather cumbersome and a more simple screening procedure is required (15-17).

The main purpose of this study was to investigate a possible relationship between urinary albumin excretion and graded pubertal development in healthy non-diabetic children and adolescents. A second objective was to obtain an age-related normal range for urinary albumin excretion and to evaluate the quality of screening methods (albumin concentration and albumin/creatinine ratio) for elevated AER in this age group.

Patients and methods

Subjects

One hundred and fifty children and adolescents (76 girls and 74 boys, mean age 13.5 years, range 10-18.5 years) from six different schools agreed to deliver timed urine samples from two consecutive nights. The children had no known renal or systemic disease. Informed consent was obtained from the children and their parents.

Samples

Each child received a careful written instruction to ensure accurate collection time. Girls with ongoing menstruation were not asked to deliver urine samples. No restrictions were given concerning physical activity the day preceding the collection of overnight urine. The first of the two samples was kept in the refrigerator at home for 24 h before the two samples were returned via the school. The urine samples were stored at 4 C and analysed within three weeks (18). Eight samples contained traces (1+) of either nitrite or leucocytes (not both). These urine samples were not cultivated for bacteria and were included in the statistical analysis. Both samples from 7 children (in 5 subjects because of sampling errors, 1 had proteinuria and 1 was newly treated for eosinophilic granuloma) and single samples from 3 patients (sampling errors) were rejected.

Methods

Urinary albumin concentration was measured by immunoturbidimetry. The inter-assay coefficient of variation was 4.7% in the range of 10-50 mg/l.

Nephur-Test plus Leuco (Boehringer Mannheim GmbH, Mannheim, FRG) was used for the determination of leucocytes, nitrite, glucose, ketones and albumin.

Urinary creatinine was analysed essentially according to Jaffe, without protein precipitation (19), and by recording colour development at 550 nm using an automated analyser (Hitachi 737). The samples were diluted 1 in 9 with distilled water.

Pubertal development was assessed by Tanner's staging of pubic hair. The scoring was performed by the local school nurses trained in using this method.

Body mass index (BMI) was calculated from: weight(kg)/[height(m)]^2.

Statistical methods

For the statistical analysis we used the results from one urine sample per patient. The first of two samples was used in 140 patients. In 3 patients, only a single sample could be obtained.

Variables with a continuous distribution are expressed as mean values with SD, median values, 5th and 95th percentiles and total ranges. Urinary AER, albumin concentration and albumin/creatinine ratio were found to have significantly skewed distributions and were logarithmically transformed before statistical analysis: 95% confidence intervals for the logarithmically transformed means are constructed using the Student's procedure (20). Comparisons between groups were carried out using the Student's *t*-test. Bonferroni corrections were used in single variable analysis (21). Methods were compared using a linear regression model with Fisher tests of the coefficients. Multiple linear regression was used to study the simultaneous effect of various variables on AER. All tests were carried out two-tailed and a significance level of 5% was used.

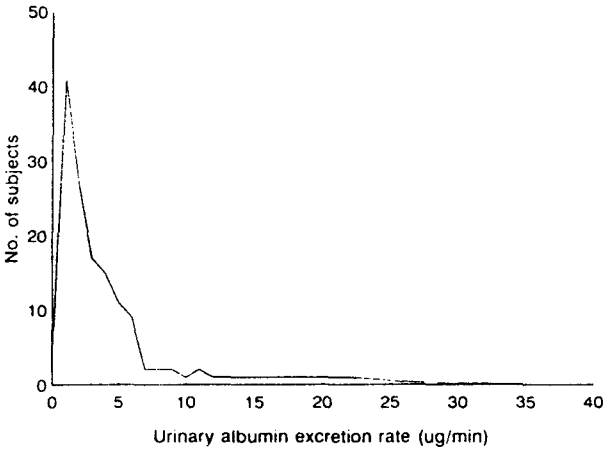

Fig. 1. Distribution of timed overnight urinary albumin excretion rate in 143 healthy children and adolescents from 10 to 18 years of age.

Results

A skewed distribution (Fig. 1) for urinary AER was found. The geometric mean was 3.2 μg/min and the 95th percentile 15.1 μg/min (Table 1).

AER was related to pubertal stage, but the pattern differed according to gender. In girls, we found an increase in AER, with a maximum at Tanner stage 4 (Fig. 2). The AER values at stage 4 were significantly higher than the values at stage 1 ($p<0.005$) and 5 ($p<0.05$). In boys, however, no significant increase in AER was found until Tanner stage 5 was reached (stage 5 versus stage 1 and stage 2, $p<0.05$) (Fig. 2).

Dividing the subjects into children < 13 years of age and adolescents (≥ 13 years) revealed a significantly increased ($p<0.01$) AER in the teenage group (Table 1). In girls, no correlation between age and AER was found (Fig. 3), in contrast to the correlation ($r=0.30$) found between these parameters in boys. Multiple linear regression analysis with AER as the dependent variable and BMI, pubertal stage and age as the independent variables was performed. In girls AER showed a weak, but significant correlation only with pubertal stage ($r=0.25$, $p<0.005$). No significant correlation with pubertal stage or age was found in boys. Furthermore, a weak negative correlation ($r=-0.13$) was found between BMI and AER.

The distribution of albumin/creatinine ratio (A/C ratio) in the material was similar to that obtained for AER (Fig. 1). When correction for urinary creatinine excretion was made, the association between AER and age and pubertal development was not seen among boys (Figs 4 and 5). In girls, however, a different pattern was observed: decreased A/C ratio in the oldest age groups (16 years versus 10, 12 and 14 years of age, $p<0.05$) and in Tanner stage 5 versus 4 ($p<0.05$).

Table 1. Albumin excretion rate (AER) and albumin/creatinine ratio (A/C ratio) in urine in healthy schoolchildren (< 13 years) and adolescents (> 13 years).

	AER (μg/min)	A/C ratio (mg/mmol)
n	143	143
Mean (SD)	4.7 (5.3)	2.1 (1.5)
Median	3.0	0.6
Range	0.2–34.6	0.2–13.5
5–95th percentile	1.1–15.1	0.3–3.0
n (< 13 years)	76	76
Mean (SD)	3.7 (3.9)	1.0 (0.9)
Median	2.4	0.7
Range	0.2–23.4	0.2–5.2
5–95th percentile	1.0–13.3	0.3–3.0
n (> 13 years)	67	67
Mean (SD)	5.8 (6.4)	1.1 (1.9)
Median	4.0	0.6
Range	1.1–34.6	0.2–13.5
5–95th percentile	1.3–20.4	0.2–3.7

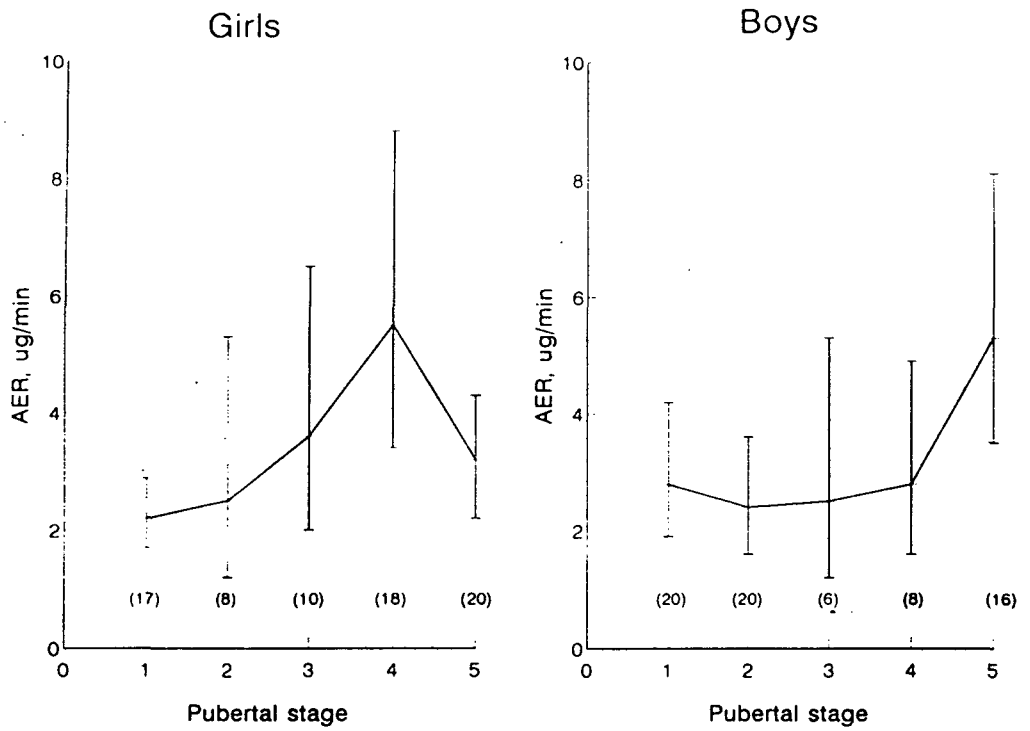

Fig. 2. Timed overnight urinary albumin excretion rate (AER) in healthy boys ($n = 70$) and girls ($n = 73$) from 10 to 18 years of age related to pubertal stage (Tanner). Geometric mean and 95% confidence intervals (number of patients) are presented.

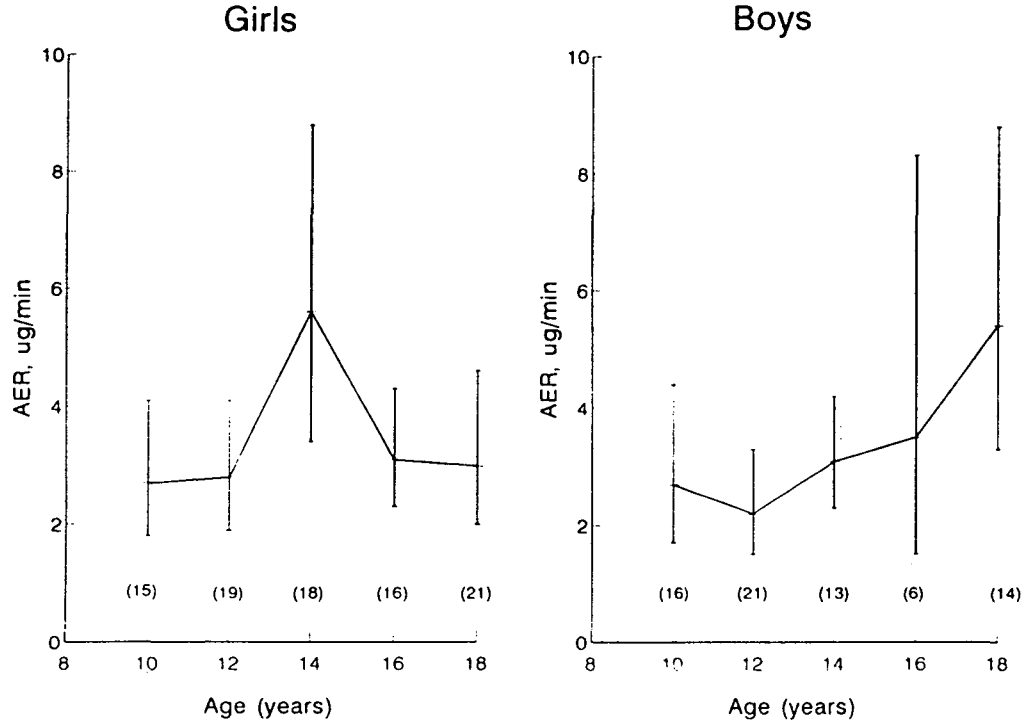

Fig. 3. Timed overnight urinary albumin excretion rate (AER) related to age in healthy boys ($n = 70$) and girls ($n = 73$) from 10 to 18 years of age. Geometric mean and 95% confidence intervals (number of patients) are presented.

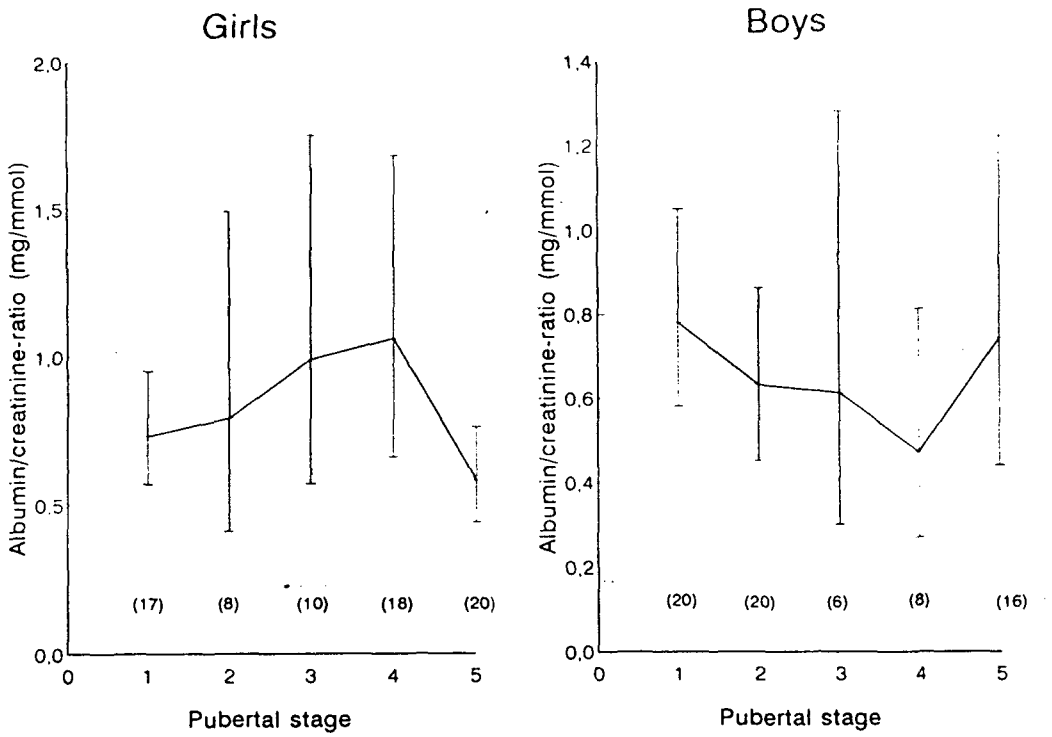

Fig. 4. Urinary albumin creatinine ratio in healthy boys ($n = 70$) and girls ($n = 73$) from 10 to 18 years of age related to pubertal stage. Geometric mean and 95% confidence intervals (number of patients) are presented.

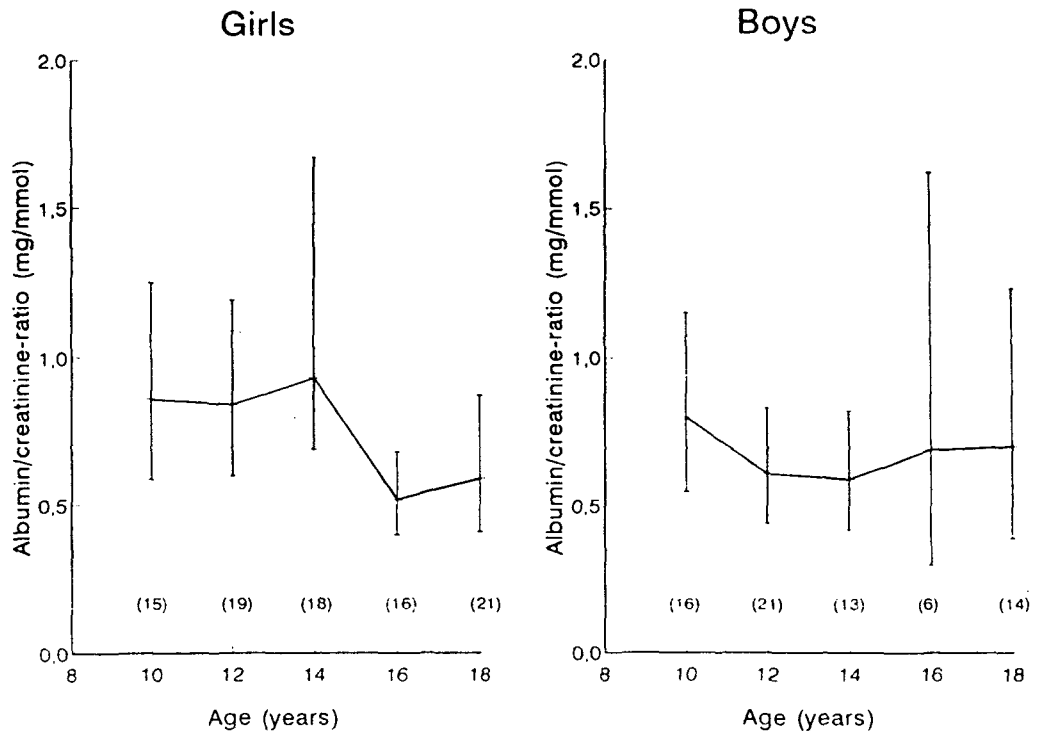

Fig. 5. Urinary albumin creatinine ratio related to age in healthy boys ($n = 70$) and girls ($n = 73$) from 10 to 18 years of age. Geometric mean and 95% confidence intervals (number of patients) are presented.

Table 2. Subjects grouped according to urinary albumin excretion rate (AER) and albumin/creatinine ratio.

	Albumin/creatinine ratio		
	≤2.5 mg/mmol	>2.5 mg/mmol	Total
AER ≤15 μg/min	134	1	135
AER >15 μg/min	1	7	8
Total	135	8	143

Sensitivity: (7/1+7)=0.88; specificity: (134/134+1)=0.99; positive prediction value: (7/1+7): 0.88; negative prediction value: (134/134+1)=0.99.

Table 3. Subjects grouped according to urinary albumin excretion rate (AER) and morning urinary albumin concentration.

	Albumin concentration		
	≤20 mg/l	>20 mg/l	Total
AER ≤15 μg/min	122	13	135
AER >15 μg/min	1	7	8
Total	123	20	143

Sensitivity: (7/1+7)=0.88; specificity: (122/122+13)=0.90; positive prediction value: (7/7+13)=0.35; negative prediction value: (122/122+1)=0.99.

Two screening methods for elevated AER (>15 μg/min = the 95th percentile) were evaluated. First, there was a strong correlation between A/C ratio and AER ($r=0.90$). The 95th percentile for A/C ratio was 3.0 mg/mmol. We used, according to the guidelines for nephropathy in the declaration of St Vincent (22), 2.5 mg/mmol as the screening level and found a sensitivity of 0.88, specificity of 0.99, a positive prediction value of 0.88 and a negative prediction value of 0.99 (Table 2). Second, a correlation ($r=0.88$) was found between albumin concentration and AER, but a large number of false positive values resulted in a low positive prediction value (0.35) (Table 3).

Discussion

In the present study, we found a significant association between AER and pubertal development stage in non-diabetic children and adolescents. Previous studies have not addressed this question directly. However, a Danish study relating AER to body surface and Tanner's stages, found that in Tanner stages 2–4, the ratio between AER and body surface gradually approached the level found in stage 5 (12). Another study found a relationship between AER and stimulated serum growth hormone concentration (9). In the present study, girls appeared to have a peak AER at Tanner stage 4, whereas in boys, we saw no increase in AER until they reached full maturity at Tanner stage 5.

The main hormonal changes during puberty of interest in this context are the increased levels of growth hormone, insulin and sex steroids. The increased growth hormone secretion is partly responsible for decreased insulin sensitivity observed in the same period (23). Neither growth hormone nor insulin have been shown to affect AER (24). In rats (female BB with spontaneous insulin-dependent diabetes and male Sprague Dawley with streptozocin-induced diabetes), however, castration prevented increased vascular permeability of ^{125}I-labelled albumin in different organs (25). This indicates that sex steroids do modulate albumin permeation in general and may partly explain the results of the present study. Obviously one has to be cautious in interpreting the data. The increased albumin permeation was in diabetic rats (25) and not in healthy non-diabetic adolescents.

Studies in non-diabetic children and adolescents have shown a relationship between age and AER (12, 14). In our study there was no clearcut relationship between age and AER. In girls, the eldest subgroup had AER levels in the same range as the youngest. An inexplicable peak in AER for girls at 14 years of age underlines the lack of AER–age association. In boys, a tendency towards a correlation between age and AER was found, but when multiple linear regression analysis with BMI, age and pubertal stage as independent variables was applied, the correlation did not reach statistical significance. Studies in young insulin-dependent patients have shown an association between AER and age, puberty or both (9, 10, 17, 26). Our results question if the findings in diabetic patients are related to normal physiological changes or express aspects of diabetic metabolic or hormonal dysfunction.

Screening for AER has so far been of interest only in patients with diabetes mellitus. However, in non-diabetic adults, AER is associated with hypertension and increased mortality risk (27, 28), but this aspect has not been illuminated in children and adolescents.

It is commonly accepted that the amount of urinary creatinine excretion is a measure of total muscle mass (29) and thus related to the stage of pubertal development. In the individual patient, the urinary creatinine excretion is rather constant. When we related albumin to creatinine excretion, we found that the urinary A/C ratio in boys was not associated with either age or pubertal stage, whereas in girls a trend towards decreased values was observed for A/C ratio in the older age groups and for subjects who had reached Tanner pubertal stage 5.

Since AER is time consuming and cumbersome to perform, we wanted to evaluate morning albumin concentration (MAC) and A/C ratio as screening methods. According to our results, >15 μg/min was taken as an appropriate level for elevated urinary AER. Both A/C ratio and MAC correlated well with AER ($r=0.90$ and $r=0.88$, respectively). MAC yielded a

considerable percentage of false positive results and therefore either repeated tests or unnecessary high numbers of timed measurements are needed. However, by using one of the new semiquantitative methods (30, 31), which give a quick "bedside" result, this disadvantage is partly reduced as several tests may be performed easily. The fact that A/C ratio varies only slightly with age or puberty in non-diabetics, makes it a practical screening method for AER in children and adolescents and probably even in patients with diabetes mellitus.

We have found a significant relationship between puberty and elevated urinary AER in non-diabetic healthy children and adolescents. The pattern observed seems to be different in girls and boys. The A/C ratio appears to be the best *screening* method for elevated urinary AER in children and adolescents.

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References

1. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria is a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. Lancet 1982;1:1430–2
2. Parving HH, Oxenbøll B, Svendsen PAA, Sandahl Christiansen J, Andersen AR. Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. Acta Endocrinol 1982;100:500–5
3. Mogensen CE. Microalbuminuria as a predictor of clinical diabetic nephropathy. Kidney Int 1987;31:673–89
4. Feldt-Rasmussen B, Mathiesen ER, Jensen T, Lauritzen, Deckert T. Effect of improved metabolic control on loss of kidney function in type I (insulin-dependent) diabetic patients: an update of the Steno studies. Diabetologia 1992;34:164–70
5. Mogensen CE. Prediction of clinical nephropathy in IDDM patients. Alternatives to microalbuminuria? Diabetes 1990; 39: 761–7
6. Østerby R. Glomerular structural changes in type I (insulin-dependent) diabetes. Consequences, causes and prevention. Diabetologia 1992;35:803–12
7. Walker JD, Close CF, Jones SL, et al. Glomerular structure in type I (insulin-dependent) diabetic patients with normo- and microalbuminuria. Kidney Int 1992;41:741–8
8. Bangstad H-J, Østerby R, Dahl-Jørgensen K, et al. Early glomerulopathy is present in young, Type I (insulin-dependent) diabetic patients with microalbuminuria. Diabetologia 1993; in press
9. Salardi S, Cacciari E, Pascucci MG, et al. Microalbuminuria in diabetic children and adolescents. Relationship with puberty and growth hormone. Acta Paediatr Scand 1990;79:437–43
10. Gibb DM, Dunger D, Levin M, Shah V, Smith C, Barratt TM. Early markers of the renal complications of insulin dependent diabetes mellitus. Arch Dis Child 1989;64:984–91
11. Martin P, Walton C, Bodansky HJ, Stickland MH. Increased urinary excretion of transferrin in children with type I diabetes mellitus. Diabetic Med 1990;7:35–40
12. Mortensen HB, Martinelli K, Norgaard K, et al. A nationwide cross-sectional study of urinary albumin excretion rate, arterial blood pressure and blood glucose control in Danish children with type I diabetes mellitus. Diabetic Med 1990;7:887–97
13. Rowe DJF, Bagga H, Betts PR. Normal variations in rate of albumin excretion and albumin to creatinine ratios in overnight and daytime urine collections in non-diabetic children. BMJ 1985;291:693–4
14. Davies AG, Postlethwaite RJ, Price DA, Burn JL, Houlton CA, Fielding BA. Urinary albumin excretion in school children. Arch Dis Child 1984;59:625–30
15. Hutchison AS, Paterson KR. Collecting urine for microalbumin assay. Diabetic Med 1988;5:527–32
16. Nathan DM, Rosenbaum C, Protasowicki VD. Single void urine samples can be used to evaluate quantitative microalbuminuria. Diabetes Care 1987;10:414–18
17. Cowell CT, Rogers S, Silink M. First morning urinary albumin concentration is a good predictor of 24-hour urinary albumin excretion in children with type I (insulin-dependent) diabetes. Diabetologia 1986;29:97–9
18. Osberg I, Chase P, Garg SK, et al. Effects of storage time and temperature on measurement of small concentrations of albumin in urine. Clin Chem 1990;36:1428–30
19. Smith ST. Nonprotein nitrogen. In: Bishop, Duben-Von Lamfen, Fody eds Clinical Chemistry, Principles, Procedures, Correlations, 1st Edn. Philadelphia: Lippincott, 1985:414–16
20. Sanford B. Pharmaceutical Statistics. Practical and Clinical Applications. New York: Marcel Dekker Inc, 1984:109–10
21. Elston RC, Johnson WD. Essentials of Biostatistics. Philadelphia: FA Navis Company, 1987:249–50
22. Krans HMJ, Porta M, Keen H. Diabetes care and research in Europe: the St Vincent Declaration Action Programme. WHO, Regional office of Europe, 1992: 29–32
23. Bloch CA, Clemons P, Sperling MA. Puberty decreases insulin sensitivity. J Pediatr 1987;110:481–7
24. Viberti GC, Wiseman MJ. The kidney in diabetes: significance of the early abnormalities. Clin Endocrinol Metab 1986;15:753–82
25. Williamson JR, Chang K, Tilton RG, et al. Increased vascular permeability in spontaneous diabetic BB/W rats and in rats with mild versus severe streptozocin-induced diabetes. Diabetes 1987;36:813–21
26. Dahlquist G, Rudberg S. The prevalence of microalbuminuria in diabetic children and adolescents and its relation to puberty. Acta Paediatr Scand 1987;76:795–800
27. Yudkin JS, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Lancet 1988;II:530–3
28. Damsgaard EM, Froland A, Jorgensen OD, Mogensen CE. Microalbuminuria as a predictor of increased mortality in elderly people. BMJ 1990;300:297–300
29. Holliday MA. Body composition, metabolism, and growth. In: Holliday MA, Barratt TM, Vernier RL eds Pediatric Nephrology 2nd Edn. London: Williams and Wilkins, 1987
30. Bangstad H-J, Try K, Dahl-Jørgensen K, Hanssen KF. New semiquantitative dipstick test for microalbuminuria. Diabetes Care 1991;14:1094–7
31. Bangstad H-J, Helgerud R, Prestegård E, Dworsky E, Cornelissen L, Smol AM. Evaluation of a new rapid immunometric test for detection and monitoring of microalbuminuria: comparison to a turbidimetric method. 13th WONCA World Conference 1992: PO237

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