Renal Wastage of Insulin in Children with Diabetes Mellitus

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SUMMARY

In normal human beings the percentage of serum insulin excreted in the urine is constant over a wide range of values. The quantity of immunoreactive insulin found in the urine is believed to reflect the level of free insulin in the serum. Immunoreactive insulin was measured in the urine of nondiabetic children and diabetic children receiving exogenous insulin. Children with diabetes mellitus excreted greater amounts of immunoreactive insulin (18.5±8 μU./mg. creatinine) than did nondiabetic children (11.9±5 μU./mg. creatinine). This difference was statistically significant ($p<0.0005$). Children with "poor glycemic control" excreted a greater portion of their administered insulin dose than did those with "good control." The renal wastage of insulin correlated ($r=0.94$) with the duration of insulin treatment but not with the quantity administered. Antibody binding of serum insulin may explain in part these observations, but an acquired defect in the renal tubular reabsorption of insulin may also exist. Modifications in the management of diabetes that reduce the renal wastage of insulin may improve the metabolic stability of children with "poor diabetic control." DIABETES *25*:989-93, October, 1976.

In man, insulin is filtered at the glomerulus and almost completely reabsorbed by the renal tubules.[1-3] Approximately 98 per cent of the filtered insulin is reabsorbed in the kidney. Forty per cent is returned to the venous blood, while the remaining insulin is degraded in the renal parenchyma. A constant percentage of the filtered insulin is not reabsorbed and is excreted in the urine. The percentage filtered and reabsorbed remains constant over a wide range of serum insulin levels. Therefore, the quantity of insulin excreted in the urine is a useful reflection of the mean serum insulin concentration during the interval of urine collection.[2,4]

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The measurement of insulin in the urine by radioimmunoassay is a specific and reproducible technique.[5] This insulin-like immunoreactivity has been found to behave as normal insulin on gel filtration, to have an isoelectric point identical to normal insulin, and to be inactivated by treatment with insulinase.[6] Identical properties have been reported for the immunoreactive insulin (IRI) found in the urine of both treated and untreated diabetic children.[6] In contrast to the measurement of IRI in the serum of treated diabetics, quantitation of IRI in protein-free urine is not complicated by antibody interference.[5]

As a result of antibodies produced against exogenous insulin that interfere with measurement of IRI, little is known about the quantities of insulin circulating in the serum of insulin-dependent diabetic children. The dose of exogenous insulin is commonly based on the body weight of the treated individual. The resulting blood glucose concentrations and the clinical well-being of the patient are generally used to determine the appropriateness of the insulin dose. This study was undertaken to quantitate the 24-hour urinary excretion of insulin in nondiabetic and diabetic children in order to provide indirect data concerning the serum levels of unbound (i.e. filterable) insulin in diabetic children and permit comparison to the physiologic levels in nondiabetic children of similar ages.

MATERIALS AND METHODS

Fifty-four children ranging in age from seven to 18 years with insulin-dependent diabetes mellitus participated in this study. The study was performed at a camp for diabetic children.[7] Within this group, clinical manifestations of diabetes had been present from one month to 15 years (mean 4.5 years). Forty-nine of the children had clinical diabetes for more than one year. Thus, endogenous insulin production was considered to be insignificant.[8] Only one child with pro-

teinuria had evidence of renal complications. None of the children were hypertensive. A group of nondiabetic children of similar age were studied in an identical fashion.

Each individual collected all urine for 24 hours in a polyethylene bottle stored on ice. The bottles were emptied and the volumes of urine measured at 12-hour intervals. The urine was then frozen and stored at −40° C. until the time of analysis. Insulin in urine has been found to be stable for 24 hours at 4° C. and for nine months at −20° C.[5]

The completeness of the 24-hour urine collection was judged by the excretion of at least 15 mg. of creatinine per kilogram of body weight.[9] Urine creatinine was measured by the method of Hare.[10] The 24-hour excretion of protein was determined with Albumin Stix, Ames Co. Only one urine had measurable protein by this test (365 mg./24 hours). The sugar concentration in the urine up to 10 gm./dl. was determined by the Clinitest method.[11,12]

Abnormalities of renal tubular function in nondiabetic subjects have been reported to cause increased wastage of insulin in the urine. Amino acids are another substance with urinary excretion controlled by renal tubular reabsorption. An indicator of tubular function is the quantity of amino acids or alpha amino nitrogen (αAN) excreted in the urine. The amount of urinary αAN was measured spectrophotometrically by color development with ninhydrin.[13] Urinary insulin was measured by the double-antibody radioimmunoassay method of Morgan and Lazarow.[14] The apparent concentration of immunoreactive insulin in urine was not affected by the presence of glucose up to a concentration of 10 gm./dl.

RESULTS

The quantities of urinary IRI excreted by diabetic and nondiabetic children are presented in table 1. The mean total 24-hour value in normal children is in close agreement with the value of 12.1 μU./mg. of creatinine reported for children of similar ages.[6]

The quantity of insulin excreted in the urine of diabetic children is significantly greater ($p<0.0005$) than that found in the urine of age-similar nondiabetic controls. The degree of glycemic control in the diabetic children was categorized by the quantity of sugar excreted in the same urine collections used for the insulin determinations. Those children who excreted more than 25 gm. of glucose in 24 hours were considered to have poor control and those excreting less than 25 gm. to be in good control.[15] The quantity of urinary insulin was significantly higher ($p<0.0005$) in the former group (table 2). Although the amount of insulin administered to both groups of children was identical (0.84 U. per kilogram), more insulin was excreted by the poor-control group. Indeed, the percentage of administered insulin excreted by the poor-control group was 52 per cent greater than the percentage excreted by the children with good glycemic control (table 2). The quantities of αAN excreted in the urine of the poor-control group did not differ significantly from that found in the urine of either the

TABLE 1

Urinary excretion of insulin
μU./mg. of creatinine ± 1 S.D.

	N	8 a.m.	to	8 p.m.	to	8 a.m.	Total	p<
Nondiabetic	33		11.6±4		12.4±5		11.9±5	
								0.0005
Diabetic	54		20.3±9.6		16.7±7.9		18.5±8	
Good control	18		14.3±7		14.1±6		14.2±6	
								0.0005
Poor control	36		23±8		17.8±7		20.5±8	

TABLE 2

Percentage of administered insulin excreted

Subjects	N	Insulin excreted mU.±1 S.D.	Administered U.	Administered U./kg.	% Excreted
Good control	18	9.24±5	29.6	0.84	0.031
Poor control	36	15.50±4	32.9	0.84	0.047

TABLE 3

Alpha amino nitrogen excretion (αAN)
mg./mg. creatinine ± 1 S.D.

	Nondiabetic	Good control	Poor control
αAN	0.142 ± 0.09 (14)	0.143 ± 0.05 (18)	0.184 ± 0.09 (32)
p value	N.S.	< 0.1 > 0.05	

good-control or the nondiabetic groups of children (table 3).

There was no correlation between the quantities of administered and excreted insulin. If, however, insulin excretion is plotted as a function of the duration of diabetes (figure 1), there is a positive correlation ($r=0.94$).

The influence of the insulin preparation administered on the pattern of insulin excretion is shown in figure 2. The excretion of insulin during the first and second 12-hour periods following a single dose demonstrates great individual variability. The data suggest that significant serum levels of insulin probably are maintained during the entire 24-hour period. The unexpected finding (nine of 22 children) of greater insulin excretion during the second 12 hours after the administration of a single dose of NPH insulin suggests that higher serum insulin levels may be present during the later time interval. Short-acting regular insulin injected with intermediate-acting NPH or lente insulins increased the apparent serum levels in that group (eight of 14) of children during the first 12 hours. However, in the other six children receiving the mixed dose of insulins, greater quantities of the hormone were excreted during the second half of the day following its administration.

DISCUSSION

The pattern of insulin excretion found in nondiabetic adolescent children is of clinical interest. In a study of normal adult subjects the daytime urinary excretion of insulin was found to be 11.9 μU./mg. creatinine and the nighttime urinary insulin excretion was 3.1±1.7 μU./mg. creatinine.[16] This observation was proposed to reflect the difference between basal insulin secretion during a nighttime period of relative fasting and the higher levels that result from dietary stimuli.[16] The rather constant amounts of insulin excreted throughout the day by the normal children described herein suggest that, in contrast to adults, certain nocturnal stimuli modulate insulin secretion in children. This is an important consideration in the management of children with diabetes mellitus, as nocturnal insulin secretion may be uniquely physiologic for children and important for the rapid growth that occurs during this stage of development. The interrelationship of nocturnal insulin production

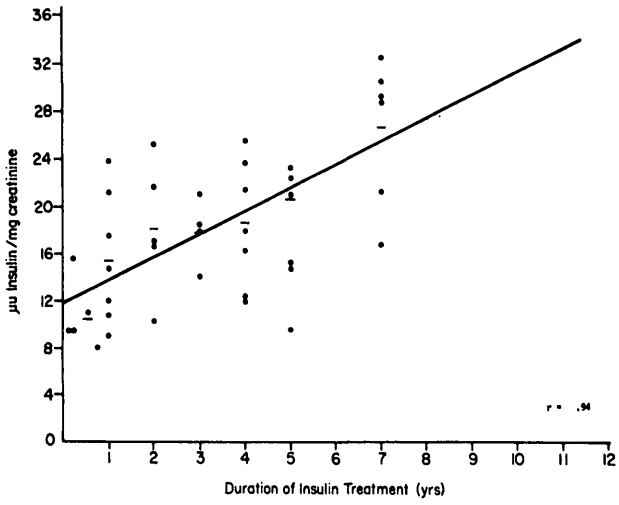

FIG. 1. Linear regression analysis of the mean 24-hour urine insulin concentration versus duration of insulin treatment. The points less than one year are considered as a group. Those years with three or fewer points were not considered.

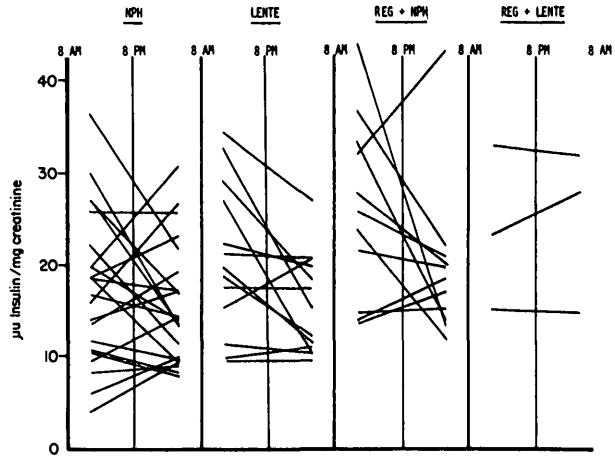

FIG. 2. The concentration of insulin in urine collected during the first and second 12 hours after insulin administration. The insulin preparation administered is indicated at the top of each column.

to the secretion of growth hormone, prolactin, and luteinizing hormone, which also appear to be sleep-related, remains to be determined. Children may require higher nighttime serum insulin levels than adults in order to achieve their growth potential.

Greater urinary insulin excretion was found in diabetic rather than in nondiabetic children. This could result from (1) the administration of supraphysiologic doses of insulin, (2) altered insulin utilization, or (3) an abnormality in the renal disposal of this hormone.

How closely the administered insulin dose of 0.84 U./kg./day approaches the physiologic quantity of insulin secreted during a 24-hour period is unknown at this time. However, in this study those children with good glycemic control as a group received the same dose of insulin (U./kg.) as did those with poor control. This observation plus the poor correlation between the quantities of insulin administered and excreted indicate that the size of the administered dose is not a major factor accounting for the differences noted in urinary insulin excretion.

Serum binding of insulin has been reported as a mechanism for improving the metabolic control of children with diabetes.[17] The children with good control were found to have greater concentrations of total serum insulin than did those with poor control. Both patient groups had similar levels of free (unbound) insulin, with the difference being a greater portion of antibody-bound insulin in those children with good control.[17] Intravenously administered insulin was observed to be cleared more rapidly by children with poor control than by those in good control.[17] These observations can explain the differences in urine insulin excretion observed between the good- and poor-control groups. Protein-bound insulin is not filtered and, therefore, is not excreted by the kidney.[5] Although the free serum insulin levels are similar in both well- and poorly controlled diabetics, the higher concentrations of bound insulin in those with good control as well as the greater quantities of urinary insulin in those with poor control suggest a greater turnover and loss of insulin in children with poor glycemic control.

Serum binding of insulin may also alter the expected activity of the various insulin preparations. Measurement of serum IRI in newly treated diabetic individuals reveals that peak levels of insulin following the administration of NPH insulin occur within five to nine hours, with return to pretreatment levels by 15 hours.[18] The pattern of insulin excretion in the chronically treated children in this study, however, suggests that serum levels of free insulin are constant throughout the 24-hour period and that in some instances peak serum concentrations may occur later than previously reported.[18] Approximately 40 per cent of the children treated with a single dose of NPH insulin excreted more insulin in the second 12 hours than during the first 12 hours following insulin administration. This observation may reflect an effect of the acquired insulin antibodies.

If the lack of serum insulin binding explains the greater urinary insulin loss in the children with poor glycemic control, it becomes difficult to explain the tendency toward increased renal wastage of insulin with increasing duration of the disease. This observation implies an acquired defect. Renal tubular reabsorption of insulin is a critical factor in controlling the quantity of urinary insulin.[3] Newly diagnosed diabetic subjects and nondiabetic controls have similar rates of renal insulin clearance,[19] but, as previously noted, insulin has been observed to be cleared more rapidly by subjects with poorly controlled diabetes.[17] The αAN excretion in the urine of the two groups was only suggestive of tubular dysfunction. However, the recent observation of a unique structural change in the renal tubules of individuals with long-standing diabetes suggests that a functional defect may also exist. This may be a defect that affects the reabsorption of insulin to a greater degree than it does amino acids. The finding of the same accumulation of IgG and albumin in the tubular basement membranes in nondiabetic donor kidneys following transplant into a diabetic host[21] supports the acquired nature of that tubular defect.

The renal wastage of insulin in children with diabetes is a heretofore unrecognized management problem. This acquired defect may explain some of the management problems that are so often attributed to the uncooperative child with diabetes. Whether this is a manifestation of differences in insulin binding, differences in the renal tubular reabsorption of insulin, or a combination of both is uncertain. These observations suggest that modifications in management that reduce renal insulin loss may improve metabolic management in those children who now have poorly controlled diabetes.

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