Urinary insulin levels in health and disease—a concise review

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Summary

The kidneys are the most important extrahepatic site of insulin breakdown and play a significant role in regulating the systemic insulin level in normal subjects. In man, a renal arteriovenous insulin concentration difference of about 30% has been measured, and since 'insulin clearance' values are less than the glomerular filtration rates, insulin is probably removed from blood by a combination of filtration and tubular secretion. In normal subjects a constant fraction of circulating insulin is removed by the kidney. This fraction is independent of the arterial concentration but varies with creatinine clearance. Of the amount filtered, most is completely resorbed and degraded by the cells of the proximal convoluted tubules. These cells have a high insulinase content and recent evidence would point to the possible existence of a renal tubular transport mechanism for insulin. The amount of insulin excreted in the urine is small and does not exceed 2% of the filtered load. In diabetics the insulin requirements often decrease with progressive renal failure. Derangements in carbohydrate metabolism have been noted in patients with renal failure while 'insulin clearance' is elevated in uraemia, chronic and acute renal failure. In nephrotic syndrome there is no change in renal insulin excretion. In severe trauma, 'insulin clearance' is elevated in patients with normal renal function despite a relative hypoinsulinaemia for the prevailing degree of glycaemia.

Introduction

The development of accurate techniques for the measurement of insulin opened the field for the investigation of insulin levels in various body fluids.

The existence of a hypoglycaemic agent in urine which was believed to be insulin was suggested by Best and Scott in 1923. The chemical and biological properties of this agent were extensively studied by

Fisher and Noble (1923), Lawrence, Madders and Millar (1930), and Uberrack and Zell (1931). From these biological experiments it was reasonably concluded that after glucose loading insulin or an insulin derivative was excreted in the urine. Furthermore, Partos (1929) and Lawrence *et al.* (1930) did not find blood sugar lowering substance in the urine of diabetics and fasted animals, while Recordier and Andrac (1935) and Goadby and Richardson (1940) demonstrated that the exclusion of the kidneys from the circulation in rabbits led to a persistence of insulin action in blood. It was inferred that the organs were partly responsible for the elimination of insulin from the blood, thereby implying the removal of insulin via urine. In 1948, using a biological assay technique, Mirsky *et al.* (1948), quantitatively measured insulin in pooled concentrated urine of normal subjects. Yalow and Berson (1959) described an insulin specific radioimmunoassay technique for the measurement of insulin in serum which proved to be a more precise method. Using an immunoassay method, Jørgensen (1966) measured and confirmed the presence of insulin in the urine of non-diabetic and diabetic subjects with a high degree of reliability, while Rubenstein, Lowry and Fraser (1967a) used a double-antibody insulin assay to measure insulin under basal conditions and following a stimulus to endogenous insulin secretion. They found that insulin excretion per hour correlated well with the mean serum insulin level and that 'insulin clearance' appeared not to vary with changes in serum insulin concentrations but varied directly with creatinine clearance. Urinary insulin levels, as measured over an extended period of time, reflected average serum insulin levels (Jørgensen, 1966; Rubenstein *et al.*, 1967b; Jørgensen, 1969b; McArthur and Stimmler, 1966; Rubenstein and Spitz, 1968), thereby providing a useful clinical tool in the study of the factors influencing insulin regulatory mechanisms in carbohydrate metabolism.

Immunoassay procedures used for measuring

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insulin in urine are essentially the same as for serum, differing only in the preparation of the urine samples. In our laboratory, we have found the following method reliable: urine samples are preserved at -20°C before assay. The pH of the thawed urine sample is adjusted to 8.0 (± 0.1) by the addition of 0.1 N NaOH or HCl. The volume of base or acid is usually too small to require the correction of a dilution factor. Five per cent bovine serum albumin-borate buffer is added to dilute the urine sample (1 : 4) to assure negligible protein-ionic strength effect. This solution is then assayed for insulin using an immunoassay method such as the double-antibody technique previously described by Soeldner and Slone (1965).

Renal handling of insulin: *in vivo* and *in vitro* observations

The arteriovenous insulin difference varies between 30 and 55% in anaesthetized dogs following an insulin infusion (Zaharko, Beck and Blankenbaker, 1966; McCormick *et al.*, 1969). In fasting man, Chamberlain and Stimmler (1967), measuring endogenous venous insulin levels, noted a 30% arteriovenous difference. Considering the renal insulin extraction ratio ($A-V/A$) and a normal renal plasma flow of 650 ml/min, the renal 'insulin clearance'* was calculated to be 200 ml/min. In dogs, McCormick *et al.* (1969) also found the renal clearance of insulin to exceed the glomerular filtration rate.

There is increasing evidence that insulin as well as other proteins are filtered by the glomerulus and removed from the tubular fluid as it flows along the nephron (Cortney, Sawin and Weiss, 1970; Narahara *et al.*, 1958). Hardwicke and Squire (1955) noted that the filtration of proteins into the urine is inversely proportional to their molecular weight, while tracer studies using labelled insulin (mol. wt 6000) in rats (Narahara *et al.*, 1958), mice (Beck and Fedynskyj, 1967) and rabbits (Corvilain *et al.*, 1971) show a progressive and increasing concentration of the iodine isotope in the glomeruli, proximal tubular lumina and in the cells of the tubules (Narahara *et al.*, 1958; Bourdeau, Chen and Carone, 1973; Darmady, 1965). Recently, Cortney *et al.* (1970), studying the absorption mechanism of protein in renal tubules, found that the absorption of insulin along the proximal tubule was 30–50% of the injected load. Since the percentage absorbed was constant when the concentration of insulin was varied, it suggests the existence of a renal tubular transport maximum for insulin. Smaller amounts of insulin were also absorbed when injections were made in distal con-

volved tubules. The kidney thus removes a significant amount of renal artery insulin, the majority of which is freely filtered by the glomerulus while the remainder is excreted by the peritubular capillaries into the lumen of the proximal convoluted tubules from which 98% is resorbed into the tubular cells, less than 2% of the filtered load appearing in the urine. Zaharko *et al.* (1966) demonstrated that occluding the ureter, which stops glomerular filtration, decreased the renal clearance of insulin by about 50%.

The high insulinase content in the proximal tubular cells, described by Mirsky *et al.* (1949), and the diminished amounts of insulin in the renal vein, has led to the conclusion that insulin is degraded by the tubules. Thus the kidney is regarded as a major extrahepatic site for insulin catabolism (Rubenstein and Spitz, 1968; Allgood, 1961).

In animal experiments and in human subjects a constant fraction of circulating insulin is removed by the kidney which is independent of the arterial levels. The amount of insulin in the urine increases in a constant proportion to a corresponding increase in arterial insulin levels (Jørgensen, 1969a; McCormick *et al.*, 1969; Chamberlain and Stimmler, 1967; Darmady, 1965). A physiological variation in the urinary insulin output during a 24-hr period correlates with the circadian variation of insulin production (Gagliardino, 1968).

Insulin excretion is related to body size and creatinine clearance. Urinary insulin may be expressed as $\mu\text{U}/\text{mg}$ of creatinine or $\mu\text{U}/\text{kg}$ of body wt/hr. The 24-hr urinary output in adults is about 14,000 μU (Jørgensen, 1966; Jørgensen, 1969a), although mean levels ranging from 160,000 $\mu\text{U}/24$ hr to 5400 $\mu\text{U}/24$ hr have been reported in different series of normal individuals by Rubenstein *et al.* (1967b) and Najjar and Stephan (1970). Urinary excretion rises during a glucose tolerance test to levels around 800 $\mu\text{U}/\text{hr}$ (Spitz *et al.*, 1970). Some consideration should be given to the importance of measuring renal insulin clearance during steady state glucose and insulin levels. If measurements are made during the transient conditions of rising glucose and insulin levels during a GTT, falsely low clearance values are found. Rabkin and Colwell (1969) found an equilibration period of 20–30 min was required to obtain meaningful results. The insulin output in urine is low in the healthy newborn on the first day of life but increases by six-fold on the fifth day with the mean urinary levels rising to 20 $\mu\text{U}/\text{hr}$ (Lowry and Schiff, 1968). However, since body weight is not a good index of metabolic mass, because of the variable body-water content of the neonate, the correlation between urinary insulin and body weight is not simple. On the other hand, in children between the ages of 8 and 16 years, despite widely varying

* The term clearance is used in the context of:

$$\frac{U\mu\text{U}/\text{ml} \times V \text{ ml}/\text{min}}{P\mu\text{U}/\text{ml}}$$

24-hr excretions ranging between 2500 and 17,500 μU , a correlation between urinary insulin and body weight was found by Jørgensen (1969a), although some authors could only demonstrate it in children weighing 40 kg or less (Najjar and Stephan, 1970).

In the third trimester of a normal pregnancy, Trayner *et al.* (1967) were able to show that, in spite of the raised fasting serum levels which were three times higher than the normal fasting values, the renal clearance of insulin is diminished to a mean value of 0.18 ml/min during an oral glucose tolerance test. These findings were thought to be due to alterations in renal circulation or in renal tubular function occurring during pregnancy. This abnormality could also be attributed to anti-insulin effects of placental lactogen.

Renal handling of insulin: pathological conditions

As early as 1886, Stokvis noted that following the development of nephropathy there was an improvement in the clinical condition of diabetic patients (Gagliardino, 1968). In 1951, Zubrod, Eversole and Dana (1951), in a review of 190 cases from Johns Hopkins Hospital, found that the onset of the Kimmelstiel-Wilson syndrome was accompanied by a decrease in insulin requirements. The insulin excretion in urine is found to be lowered in non-obese adult diabetics without impairment of the renal function and the mean daily output is in the range of 8800 μU (Jørgensen, 1966; Jørgensen, 1969). In obese diabetics urinary insulin is somewhat lower than in normal subjects, although this is not statistically significant. This is not the case in siblings of childhood-onset diabetics, who tend to excrete high amounts of insulin in urine (McArthur and Stimmler, 1966). Insulin α chain, which is produced by the action of glutathione-insulin transhydrogenase on insulin, is normally eliminated in urine in quantities five times greater than insulin. The 24-hr urinary elimination of this protein is also higher in diabetics (Varandani, 1970).

In obesity the urinary insulin levels are greatly increased (30,300 $\mu U/24$ hr), but levels fall steadily during starvation and rise again upon eating (Jørgensen, 1969a; Hellier, 1970; Lowy *et al.*, 1966).

Several authors have reported abnormal carbohydrate metabolism in patients with renal failure (Briggs *et al.*, 1967; Cerletty and Engbring, 1967; Friis and Hindberg, 1969; Horton, Johnson, and Lebowitz, 1968; Lowrie *et al.*, 1969; Lowrie *et al.*, 1970), suggesting that insulin antagonism, relatively decreased insulin secretion and impaired insulin degradation are contributory factors to abnormal carbohydrate metabolism in the uraemic state (Spitz *et al.*, 1970). Rabkin *et al.* (1970) provided direct evidence that renal insulin uptake virtually ceases in diabetic subjects with a glomerular filtra-

tion rate under 20 ml/min. Serum has little if any insulin deactivation properties. The rate of disappearance of labelled insulin from plasma is delayed in uraemic patients (O'Brien and Sharp, 1967; Silvers *et al.*, 1969) and does not significantly differ from the disappearance rates seen in anephric patients (Corvilain *et al.*, 1971). This suggests that the degradation of insulin is mainly dependent on functioning renal tissue (Rubenstein and Spitz, 1968). In patients with chronic renal failure and uraemia the urinary loss of insulin is markedly increased, reaching mean values above 1000 $\mu U/hr$ (Spitz *et al.*, 1970; Rubenstein *et al.*, 1967a; Lowy *et al.*, 1971). In acute renal failure (Chamberlain and Stimmler, 1967), or during its recovery, the urinary insulin clearance is elevated. The same occurs in patients with Fanconi syndrome or cadmium nephropathy in which the disease affects almost exclusively the renal tubules. On the other hand, nephrotic syndrome, a predominantly 'glomerular' disease, causes little or no disturbance in the renal excretion of insulin (Lowy *et al.*, 1971).

In a case of islet cell adenoma of the pancreas, the urinary insulin loss was found to be increased, but after the surgical removal of the tumour, the 24 hr output returned to normal levels (Willner and Weinstein, 1950).

The excretion of insulin by the kidneys 48 hr following trauma was examined by Meguid, Aun and Soeldner (1975). Six severe trauma patients and five matched control subjects were studied. Blood and urine samples for insulin and glucose were obtained at 2 hr intervals during a control period and during the infusion of 5% glucose in water at 100 ml/hr for 6 hr. The mean baseline serum insulin levels in trauma patients were significantly higher than the mean serum insulin value in control subjects. In the trauma patients, it rose throughout the glucose infusion reaching a value which differed significantly from the mean serum insulin in the normal subjects. However, when the serum insulin-plasma glucose ratio was calculated, a 'relative' hypoinsulinaemia was found, the injured subjects being 'pseudodiabetic' (Meguid *et al.*, 1974). The total urine insulin and the urine insulin concentrations were significantly higher in the injured patients before and during the glucose infusion. The insulin clearance in the trauma patients was significantly greater than in control subjects. No significant difference was found in the urea nitrogen and creatinine clearance between the two groups, their renal function being the same.

Wide and Thoren (1972) investigating renal excretion of insulin and other polypeptides for 4-5 days following surgery found these to be increased when compared to preoperative measurements. These observations were attributed to a decrease in

tubular resorption, although arteriovenous differences were not measured.

On the basis of the reviewed data, it would appear that renal tubules exhibit the physiological property of a transport maximum for insulin. An increase in insulin levels in urine is found when circulating insulin concentrations exceed this threshold or when tubular function, and hence insulin degradation, is impaired.

The knowledge gained from the determination of insulin in urine has contributed to our understanding of carbohydrate metabolism. The non-invasive sampling procedures together with the direct relationship between mean urinary and serum levels of insulin are some of the advantages of its use in routine studies, especially in infants and small children.

However, the major contributions derived from urinary insulin studies include: (1) a more precise understanding of the physiology of insulin handling by the kidney; (2) a suggestion that insulin has an effect on renal metabolism; (3) an explanation for the sudden decrease in insulin requirements in diabetics who develop renal failure; (4) its potential use in the estimation of free insulin levels in patients with insulin antibodies in plasma; (5) its use as a sensitive marker for proximal tubular disorders.

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