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 hypoglycaemia 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 these 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 radioimmunooassay 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 Stimpler, 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...
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 Stimmmer (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 (Corvillain 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 convoluted 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 (Jorgensen, 1969a; McCormick et al., 1969; Chamberlain and Stimmmer, 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 μU/mg of creatinine or μU/kg of body wt/hr. The 24-hr urinary output in adults is about 14,000 μU (Jorgensen, 1966; Jorgensen, 1969a), although mean levels ranging from 160,000 μU/24 hr to 5400 μU/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 μU/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 20μU/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...
24-hr excretions ranging between 2500 and 17,500 \( \mu U \),
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 altera-
tions 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 improve-
ment 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 \( \mu U \) (Jørgensen, 1966; Jørgensen, 1969). In
obese diabetics urinary insulin is somewhat lower
than in normal subjects, although this is not statisti-
cally significant. This is not the case in siblings
of childhood-onset diabetics, which tend to excrete
higher amounts of insulin in urine (McArthur and Stimmer, 1966). Insulin \( \alpha \) 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 carbo-
hydrate 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 dis-
appearance 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 de-
gradation of insulin is mainly dependent on function-
ing renal tissue (Rubenstein and Spitz, 1968). In
patients with chronic renal failure and uremia 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 Stimmer, 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 pre-
dominantly '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 sub-
jects. In the trauma patients, it rose throughout the
baseline 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'
hypoinsulinemia was found, the injured subjects
being 'pseudodiabetic' (Meguid et al., 1974). The
total urine insulin and the urine insulin concentra-
tions 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
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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.

References


BECK, L.V. & FEDYNIEWSKI, N. (1967) Evidence from combined immunoassay and radioautography procedures that intact insulin 125I molecules are concentrated by mouse kidney proximal tubule cells. Endocrinology, 81, 475.


