Non-protein nitrogen substances

In addtion to proteins and peptides, serum contains other important nitrogen-containing substances. From the clinical-biochemical point of view, the most important are **urea**, **creatinine**, **uric acid**, **ammonia** and **amino acids** (*Table 1*). These components remain in solution after precipitation of serum proteins with deproteinizing reagents. The metabolism of some of them is closely related.

Table 1 . Major low molecular weight nitrogen compounds (according to Curtis et al. 1994)

Low molecular weight nitrogen substance	Source	Clinical-biochemical significance
Amino acids	proteins	▪ liver disease ▪ kidney disease ▪ hereditary disorders of amino acid metabolism
Ammonia	amino acids	▪ liver disease ▪ kidney disease ▪ hereditary disorders of ureosynthetic cycle enzymes
Urea	ammonia	▪ liver disease ▪ kidney disease
Creatinine	creatine	▪ kidney disease
Uric acid	purine nucleotides	▪ disorders of purine metabolism ▪ increased cell lysis

Examination of non-protein nitrogenous compounds in the blood and urine is important especially **for monitoring the condition of the liver**, where a substantial part of the metabolism of these substances takes place, and the **kidneys**, by which they are preferentially excreted.

Creatinine

Creatinine (cyclic amide or lactam creatine) is formed in the muscles by internal irreversible non-enzymatic dehydration and spontaneous cyclization from creatine and (after phosphate cleavage) from creatine phosphate. Creatine phosphate serves in the muscle as a source of energy for muscle contraction. Creatinine can no longer be phosphorylated and passes into the blood and is later excreted in the urine.

Creatinine is produced at a relatively constant rate in the body. Its formation is a reflection of the size of muscle mass and is stable under conditions of physical calm and meatless diet. It is excreted by the kidneys mainly by glomerular filtration, the renal tubules secrete significant amounts only at elevated blood concentrations.

Methods of determination

A simple but not entirely specific **Jaffe reaction** is used to determine creatinine. The principle is the **reaction of creatinine with picrate in an alkaline environment**. The electrophilic oxo group of creatinine allows the dissociation of the methylene group proton. The creatinine anion combines with the positively polarized carbon of the picrate ion to form a red-orange complex. In addition to creatinine, other components of biological fluids also react with picrate - *pyruvate, acetate, oxaloacetate, glucose, ascorbic acid, acetone* - so-called **Jaffé positive chromogens**. The normal values of "true" creatinine are ***9-18 µmol/l*** or lower.

Serum creatinine

Serum creatinine concentration is directly proportional to the body's muscle mass. For this reason, it is usually slightly higher in men than in women. In addition, it is affected by renal function, which is used in clinical-biochemical diagnostics.

Serum creatinine is a **good indicator of glomerular filtration** and is mainly used **to monitor the process of kidney disease (including dialysis patients)**. The relation between creatinine concentration and glomerular filtration is hyperbolic. As glomerular filtration decreases, creatinine excretion decreases. Its serum values begin to **rise** above the upper limit of normal only when the glomerular filtration rate falls below 50%. From this it is clear that the determination of serum creatinine alone is not very sensitive to the recognition of the early stage of kidney damage. For this purpose, the clearance of endogenous creatinine must be examined (see below). Conversely, with more severe glomerular damage, determination of serum creatinine concentration is a better parameter than creatinine clearance.

Other causes of increased creatininemia are rarer. These include, in particular, the release of creatinine from muscles during **acute skeletal muscle breakdown** (**rhabdomyolysis**).

Reference values of serum creatinine

- **Women: 49–90 µmol/l**
- **Men: 64-104 µmol/l**

Creatinine in urine

Creatinine production in the body is relatively constant. Its urinary excretion is also relatively constant during the day compared to other endogenous substances. In individuals with normal glomerular filtration, it is a reflection of the magnitude of muscle mass activity.

Urine creatinine testing can be used to check the **accuracy of a 24-hour urine collection**. Improper urine collection is one of the most common errors in the calculation of 24-hour urine losses. One of the easiest ways to verify that the collection is correct is to determine the total amount of creatinine that has been excreted in the urine in one day (creatinine waste). We compare the result with tabular values that indicate creatinine waste in the urine depending on gender, age and weight (*Table 2*). If the creatinine waste is 30 percent or more lower than the table shows, urine collection can almost certainly be described as incomplete.

Furthermore, the determination of creatinine concentration in urine is used **to standardize urinary waste** if we have only a single urine sample and collection in 24 hours is not possible or appropriate for any reason. We convert the concentration of the determined substance to 1 mmol of creatinine.

Table 2. Urine creatinine reference values depending on age and sex in µmol/kg/day.

Age	Men	Women
20–29	210±20	174±34
30–39	194±13	180±34
40–49	174±28	156±34
50–59	171±26	132±32
60–69	149±26	114±23
70–79	126±26	104±19
80–89	103±36	95±22
90–99	83±28	74±12

Reference values

- Urinary creatinine concentration (U-creatinine): **5.7-14.7 mmol/l**
- Urinary creatinine loss in 24 h (dU-creatinine): **8.8-13.3 mmo /24 h**

Clearance of endogenous creatinine

By clearance we mean a value that indicates the degree of cleansing of the internal environment by all excretory mechanisms (kidneys, liver). The following relationship applies to the excretion of low molecular weight substances that are freely filtered:

$$\mathrm{GF} \cdot \mathrm{P} = \mathrm{U} \cdot \mathrm{V},$$

where **U** is the urinary concentration of the substance, **V** is the volume of urine per time unit, **GF** is the amount of glomerular filtrate and **P** is the plasma concentration of the substance.

For substances that are excreted in the urine only by glomerular filtration, the amount of substance that passes through the glomerular membrane in a unit of time, is excreted in the urine in the same unit of time. If a quantity of **U · V** is excreted in the urine per second , then a certain (theoretical) volume of plasma must have been completely "purified" from this substance in the same time. This volume is then called clearance. (**=Clearence is a volume of plasma that has been completely purified from a certain substance per unit of time**)

By determining the clearance of different substances, we can determine different renal functions. If a substance that enters the urine only by glomerular filtration is used, the clearance value is a measure of glomerular filtration. By using substances that are excreted in the urine from both glomerular filtration and tubular secretion (e.g. para-aminohippuric acid), clearance values can be used to determine renal blood flow.

Substances excreted only by glomerular filtration can become a measure of glomerular filtration . This condition is met by **inulin**, which freely permeates the glomerular membrane and is not absorbed or secreted in the tubules. Based on **inulin clearance** measurements, the glomerular filtration rate can be accurately determined. Due to the complexity of the procedure, in which it is necessary to maintain a constant level of inulin in the plasma by continuous intravenous infusion, this method is reserved for research purposes. In routine practice, glomerular filtration is assessed based on **endogenous creatinine clearance**, which is excreted predominantly by glomerular filtration (about 90%) and its plasma concentration is normally relatively stable. Compared to inulin clearance, creatinine clearance is higher.

Examination of endogenous creatinine clearance is particularly important in patients with less severe renal impairment, in whom **glomerular filtration is reduced to 50-80%** , i.e. at a time when serum creatinine may not yet exceed the reference limits.

At higher serum creatinine levels (above 180 µmol/l), the proportion of creatinine excreted by tubular secretion increases and examination of endogenous creatinine clearance yields results that would correspond to milder renal impairment. In these cases, determination of serum creatinine is more valuable.

Determination procedure

To calculate the clearance of endogenous creatinine, it is necessary to know the concentration of creatinine in serum and urine and the volume of urine per time unit.

The patient usually collects urine for 24 hours. Urine collection error can be reduced by shortening the collection period to 6 or 12 hours. The patient urinates just before collection (this urine is not yet collected). Fluid intake is not limited during the collection period. Exactly at the time when the collection ends, the examinee urinates into the collection container for the last time. To complete collection, the patient should be instructed to urinate into the collection container before each stool. At the end of the collection, the volume is measured to the nearest 10 ml, the urine is mixed well and a sample is taken in which the creatinine concentration is determined. At the end of the collection period, we also take blood for serum creatinine analysis. At the request for endogenous creatinine clearance, the patient's height and body weight and the exact volume of urine with the length of the collection period should be provided.

Clearance calculation

Endogenous creatinine clearance is calculated according to the formula:

$$\mathrm{Cl_{kr}}\ (\mathrm{ml/s}) = \frac{\mathrm{U} \cdot \mathrm{V}}{\mathrm{P}},$$

where **U** is the urinary creatinine concentration in mmol/l, **V** is the urine volume over time (diuresis) in ml/s, **P** is the plasma (serum) creatinine concentration in mmol/l.

The clearance values obtained in this way are difficult to compare between different patients and with reference ranges - they depend on the total area of the glomerular membrane, which is different for each pacient. However, the filter surface is assumed to be proportional to the body surface area. Therefore, the clearance value is corrected to the so-called **ideal body surface**, i.e. 1.73 m^2. The value of the examined person's body surface area is found in the tables on the basis of the patient's body weight and height data or can be calculated according to the formula:

$$A = 0,167 \cdot \sqrt{m \cdot l},$$

where **0.167** is the empirical factor (dimension $\mathrm{kg}^{-\frac{1}{2}} \cdot \mathrm{m}^{\frac{3}{3}}$), m patient weight in kilograms and **l** height in meters.

The calculation of the **corrected creatinine clearance** is as follows:

$$\mathrm{Cl_{cr.correc.}}\ (\mathrm{ml/s}) = \mathrm{Cl_{cr}} \cdot \frac{1,73}{\mathrm{pacient'ssurfaceinm^2}}$$

1,73 m^2 is the standard body surface.

Clearance estimation

Creatinine clearance estimation according to Cockroft and Gault

Endogenous creatinine clearance can be estimated from serum creatinine concentration without the need to collect urine by calculation using a formula (Cockcroft and Gault), which includes some factors affecting glomerular filtration - age, sex and body weight of the patient as an indirect indicator of muscle mass.

Calculation for men:

$$\mathrm{Cl_{cr}}\ (\mathrm{ml/s}) = \frac{(140 - \mathrm{age\ [years]}) \cdot \mathrm{weight\ [kg]}}{44,5 \cdot \mathrm{serum\ creatinine\ [\mu mol/l]}}.$$

Calculation for women:

$$\mathrm{Cl_{cr}}\ (\mathrm{ml/s}) = 0,85 \cdot \frac{(140 - \mathrm{age\ [years]}) \cdot \mathrm{weight\ [kg]}}{44,5 \cdot \mathrm{serum\ creatinine\ [\mu mol/l]}}.$$

Estimation of creatinine clearance using the MDRD equation

Recently, the estimation of creatinine clearance according to Cockcroft and Gault has begun to be replaced by a more reliable calculation using the so-called MDRD equation, which was proposed in 1999 by Levey and colleagues. It is an empirical equation based on data large multicenter study investigating the influence of diet on renal disease (*Modification of Diet in Renal Disease* - MDRD). The basic equation has the form:

$$\mathrm{Cl_{cr}} = 2,83 \cdot (0,0113 \cdot \mathrm{serum\ creatinine})^{-0,999} \cdot \mathrm{age}^{-0,176} \cdot (2,8 \cdot \mathrm{serum\ urea})^{-0,17} \cdot (0,1 \cdot \mathrm{serum\ albumin})^{0,318}$$

For women, the value calculated in this way must be multiplied by a factor of 0.762.

The results of this estimation correspond very well to the measured values, especially in patients with reduced glomerular filtration. None of the estimations is appropriate for patients with normal or only slightly reduced renal function.

Physiological values of creatinine clearance

Glomerular filtration decreases with age:

Physiological values of Cl_{cr} [ml/s]

Age	13–49	50–59	60–69	70 and more
Women	1,58–2,67	1,0–2,1	0,9–1,8	0,8–1,3
Men	1,63–2,6	1,2–2,4	1,05–1,95	0,7–1,0

The ideal age-related creatinine clearance can be found according to the equation:

$$\mathrm{Cl_{cr}} = -0,00946 \cdot \mathrm{age\ [years]} + 2,118$$

The patient's clearance should not differ by ± 30%.

Glomerular filtration based on serum level of cystatin C

Cystatin C is a 120 amino acid protein produced by a variety of tissues in different amounts. It serves as one of the most important inhibitors of extracellular cysteine proteases. The rate of synthesis of this protein is practically constant, it is not affected by inflammation, catabolism or diet. Due to its low molecular weight (about 13,000), it is freely filtered through the glomerular membrane. It is then completely resorbed and degraded in the proximal tubules. Thus, **plasma cystatin C concentration is a measure of glomerular filtration and urinary concentration is a measure of proximal tubule failure**. Cystatin C concentrations can be determined by immunochemical methods. The reference range so far varies according to the specific analytical technique used, but a uniform calibration methodology is expected. The cystatin C assay has some advantages: it detects early stages of glomerular damage, 24-hour urine collection, which is a common source of error, is not required, and non-specific reactions do not distort the analysis (creatinine does). Although this test is relatively expensive and is still reserved for research purposes, it is expected that it will expand the repertoire of commonly used renal function tests in the future.

Fractional excretion

The amount of a substance excreted in the final urine depends on glomerular filtration (i.e. the amount of the substance that enters the primitive urine), tubular secretion and resorption. For simplicity, we limit further interpretation to substances that are not excreted by tubular secretion at all or whose tubular secretion is insignificant.

The proportion of the substance filtered into primitive urine that is eventually excreted in the final urine is referred to as fractional excretion (FE) . The FE value of a substance is between 0 and 1 (or we can express it as 0 to 100%); if zero, this means that the substance is completely resorbed in the tubules, if FE is 100%, all filtered substance is excreted in the final urine. The "mirror" quantity to FE is tubular resorption (TR), i.e. the proportion of a substance resorbed from primitive urine by tubular cells. Assuming that tubular secretion is insignificant, the following applies:

$$\mathrm{FE} + \mathrm{TR} = 100\%$$

The general formula for calculating the fractional excretion is given by the ratio of the clearance of the test substance and the glomerular filtration rate:

$$\mathrm{FE}_x = \frac{\mathrm{U}_x \cdot \mathrm{V}}{\mathrm{P}_x \cdot \mathrm{GF}}$$

Glomerular filtration can be estimated as the clearance of endogenous creatinine. In a fraction, the urine volume per time unit is truncated, so to calculate the fractional excretions, we only need to know the concentration of the substance in the urine and plasma and the concentration of creatinine in the urine and plasma. There is no need to collect urine, which is often burdened with error.

$$\mathrm{FE}_x = \frac{\mathrm{U}_x \cdot \mathrm{P_{cr}}}{\mathrm{U_{cr}} \cdot \mathrm{P}_x}$$

(x is a substance of concern, U is a urinary concentration of the test substance, P is a plasma (serum) concentration of the test substance. Serum and urine concentrations of the test substance as well as creatinine must be in the same units.)

To assess renal function, it is useful to determine the fractional excretions of Na^+, K^+, Cl^-, phosphates and water.

The fractional excretion of water is calculated according to the formula:

$$\mathrm{FE_{H_2O}} = \frac{\mathrm{V}}{\mathrm{GF}}$$

After establishing the creatinine clearance for glomerular filtration and canceling out, we get a simple formula:

$$\mathrm{FE_{H_2O}} = \frac{\mathrm{P_{cr}}}{\mathrm{U_{cr}}}$$

Normal value $\mathrm{FE_{H_2O}}$: 0.01–0.02, i.e. **1–2 %**. We encounter increased values in:

- diabetes insipidus
- excessive fluid intake
- kidney tubular cell damage

Tubular water resorption

From the values of endogenous creatinine clearance and the amount of urine excreted in 1 second, we can calculate the value of tubular reverse water resorption (TR). The difference between glomerular filtration and the volume of definitive urine per time unit (s) is equal to the volume of water that is resorbed per second in the renal tubules.

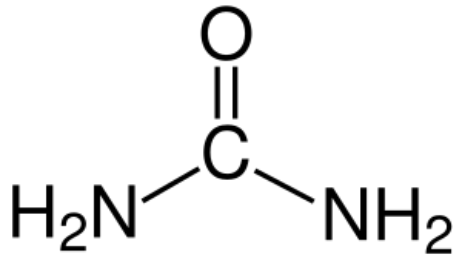
$$\mathrm{TR_{H_2O}} = \frac{\mathrm{Cl_{cr}} - \mathrm{V}}{\mathrm{Cl_{cr}}}$$

V is the volume of definitive urine in ml excreted in 1 s.

Normal value $\mathrm{TR_{H_2O}}$: 0.988–0.998, i.e. **98.8-99.8%**. Decreased values indicate a disorder of water reabsorption, e.g. in diabetes insipidus.

Urea

Urea is the most quantitatively important degradation product of amino acids and proteins. It is formed in the liver from ammonia released by deamination reactions in amino acid metabolism. It **diffuses well through cell membranes**, so its concentration is the same in both plasma and intracellular fluid.



Urea structure

It is excreted from the body mainly by the kidneys, namely by glomerular filtration and tubular resorption, which is variable (=It is lower with increased diuresis and increases with reduced diuresis).

Blood urea concentration depends on **dietary protein content, renal excretion and hepatic metabolic function** (Tab.).

Some causes of changes in serum and urine urea levels

Elevated serum urea	Decreased serum urea
Renal inpairment	Low protein diet
High protein diet	Liver impairment
Increased protein catabolism	Late pregnancy (increased need for protein during fetal growth)
Dehydration	

Serum urea levels may increase with increased protein intake. 5.74 mmol (0.34 g) of urea are formed from 1 g of protein. Increased urea concentration without changing other low molecular weight nitrogenous substances (especially creatinine) is a sign of intense protein catabolism, which increases during starvation, febrile conditions, malignancy. Protein catabolism is reduced in children, so urea levels are significantly lower. Serum urea concentration increases during kidney disease, which is accompanied by a significant reduction in glomerular filtration (below 30%), while in such cases the creatinine concentration is also increased. The determination of urea is not suitable for detecting incipient glomerular filtration disorders. However, it is important in patients on regular dialysis treatment.

When liver function fails, urea synthesis decreases and thus its plasma concentration decreases.

Based on the urea concentration in serum and urine, a nitrogen balance can be calculated.

Reference values

- **Serum concentration (S-urea): 1,7–8,3 mmol/l**
- **Urine losses (dU-urea):**

  330-600 mmol urea (20-35 g) is excreted in the urine in 24 hours in adults, depending on dietary protein intake and protein catabolism.

  $$\mathrm{dU_{urea}\ [mmol/24\ hours]} = \mathrm{U_{urea}\ [mmol/l]} \cdot \mathrm{diuresis\ [l/24\ hours]}$$

Methods of determination

Urea is determined in biological fluids either directly or indirectly as ammonia. In an indirect determination, urea is first catalytically cleaved by enzyme **urease** to form carbon dioxide and ammonia, which is converted to ammonium ion in an aqueous medium. The amount of ammonia formed is then determined by the **Berthelot reaction**. Ammonium ion with sodium hypochlorite and phenol or salicylate catalyzed by sodium nitroprusside forms a colored product.

The recommended routine method uses the conversion of α-ketoglutarate to glutamate to determine the ammonium ions formed in the urease reaction. The reaction is catalyzed by **glutamate dehydrogenase**, which is coupled to the oxidation of $NADH^+ + H$ to NAD^+ (Warburg optical test).

Urease catalyzed reaction:
$Urea + H_2O + 2\ H^+ \longrightarrow 2\ NH_4^+ + CO_2$

Glutamate dehydrogenase catalyzed reaction:
$2\ NH_4^+ + \alpha\text{-ketoglutarate} + NADH + H^+ \longrightarrow L\text{-glutamate} + NAD^+ + H_2O$

Determination in kidney disease

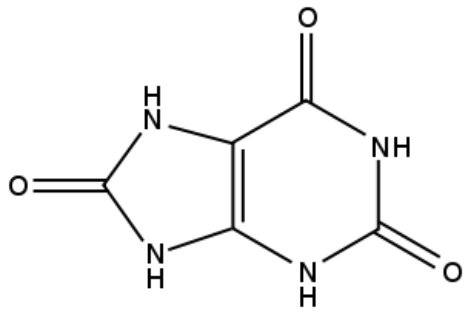
Urea concentration depends on its production (i.e. dietary protein intake, tissue catabolism and liver function). Urea is excreted by glomerular filtration and its serum concentration will therefore increase even in renal failure. However, this is a relatively insensitive parameter, the rise above the upper limit of the reference range usually occurs only when the glomerular filtration decreases by more than 75%.

On the other hand, serum urea is a good indicator of renal hypoperfusion - in addition to a decrease in glomerular filtration, urea reabsorption in the tubules increases and its serum level increases much faster than, for example, creatinine concentration. It is stated that in the case of prerenal type of renal failure (e.g. renal hypoperfusion, very often due to dehydration), the ratio of serum urea to creatinine in µmol/l is higher than 160.

Uric acid

Uric acid is the end product of purine metabolism in humans.

During catabolic processes, nucleic acids derived from the cell nuclei of the body and food are broken down into nucleotides, nucleosides and bases, which are in the final phase partially converted by the enzyme **xanthine oxidase** to uric acid. At this level, the degradation of purine bases in humans and primates is complete. In other mammals, uric acid is further converted by uricase to allantoin, which is more soluble in water than uric acid. Some of the purine bases are used for **nucleotide resynthesis** (salvage pathway reactions) using the enzymes **hypoxanthine-guanine phosphoribosyltransferase** (HPRT) and **adenine-phosphoribosyltransferase** (APRT).



Uric acid structure

The total uric acid content in the body is **approximately 1 g**. Uric acid comes from three sources:

- from food nucleotides
- from the breakdown of tissue nucleoproteins
- from biosynthesis

However, uric acid is not only a waste metabolite of purines, but its **antioxidant effects** protect cells from the action of oxygen radicals. The kidneys are responsible for 75-80% of uric acid excretion (see below). The remaining part of uric acid (20–25%) is eliminated by the gastrointestinal tract, where it can be further degraded by bacteria to NH_3 and CO_2.

Properties

Uric acid is a **poorly water-soluble** compound . At pH below 5.5, which is found in urine, most uric acid molecules are in **undissociated** and therefore less soluble form. Uric acid can then form crystals or stones. Cold helps to reduce the solubility of uric acid. As the pH increases, its solubility increases.

At a **physiological pH of 7.4**, it is present mainly in ionized form and forms Na^+ and K^+ (sodium or potassium) urate, which are more soluble in aqueous solution. Oxidative cleavage with concentrated **nitric acid** can be used to detect uric acid. The reaction opens the imidazole ring of purine and the two product molecules condense to purple acid, whose salts are colored. Addition of ammonia to the purple acid produces *murexide* (ammonium salt of the purple acid). **Murexide reactions** are used to detect uric acid in the analysis of urinary stones.

Serum uric acid

The concentration of uric acid in plasma depends on the intake of purines by food, the intensity of self-production and its excretion. Elevated plasma uric acid concentrations - i.e. hyperuricemia - are of particular clinical importance. This occurs during overproduction or reduced uric acid excretion. In hyperuricaemia, urate concentrations may exceed their solubility.

Reference values

Serum uric acid concentration (fS-uric acid):

- women **120–340 µmol/l**
- men **120–420 µmol/l**

Overproduction

- Overproduction of de novo purine synthesis associated with elevated uric acid levels is found in some genetic defects in purine metabolism, such as partial or complete hypoxanthine-guanine phosphoribosyltransferase deficiency (Lesch-Nyhan syndrome). It reduces the reuse of purine bases, which are therefore increasingly degraded to uric acid. Another genetic defect leading to increased uric acid production is increased phosphoribosyl diphosphate synthetase activity.
- Increased uric acid production accompanies anticancer treatment (chemotherapy with cytostatics, radiation), during which there is a more intense breakdown of cells. Purine bases released during nucleic acid degradation are metabolized to uric acid. Similarly, some hematological diseases associated with excessive neoplasia (polycythemia vera) or increased cell lysis (leukemia, hemolytic anemia) are accompanied by hyperuricemia.
- Increased intake of a diet rich in purines (e.g. offal, meat, legumes, to a lesser extent also chocolate, cocoa, coffee) leads to overproduction of uric acid. Healthy kidneys may not be able to compensate for uric acid overload by more intense excretion, and uricemia then rises.
- Alcohol consumption increases uricemia by inhibiting uric acid secretion by the kidneys. Decreased uric acid excretion is later replaced by increased uricosuria.

Reduced excretion

Decreased uric acid excretion is one of the most common causes of hyperuricaemia.

- In patients with hyperuricemia, tubular uric acid secretion is often reduced; the cause is unknown.
- A decrease in renal excretion of uric acid accompanies conditions associated with decreased glomerular filtration and tubular dysfunction (e.g. uric acid competition for tubular excretory mechanisms with lactate or keto acids - see below).

Uric acid in urine

Most of the uric acid is excreted by the kidneys (75-80%), where it is freely filtered by the glomerulus (it is minimally protein bound) and then most is reabsorbed in the proximal tubule. It is then secreted into the distal part of the proximal tubule and resorbed again by post-secretory reabsorption. About **0.6 g of uric acid per day** (3.6 mmol/day) is normally excreted in the urine on a low-purine diet, and values are higher on a normal diet - around **0.8 g/day** (5.0 mmol/day). Tubular uric acid secretion may be inhibited by concomitant increased excretion of other organic acids such as acetoacetic acid, β-hydroxybutyric acid, lactic acid and some drugs.

Uric acid is a significant risk factor for both the excretory urinary tract and the renal parenchyma.

Due to the poor solubility of uric acid, there is a risk of **urate urolithiasis** due to its increased urinary excretion. Individuals with permanently more acidic and concentrated urine pose a special risk. Urate stones are most often made up of pure uric acid, sometimes sodium urate. Ammonium urate stones may form in slightly alkaline urine, which is usually in the presence of a urinary tract infection.

Sodium urate crystals can also **precipitate in the renal interstitium** and cause an inflammatory reaction (chronic interstitial nephritis).

Acute renal failure is relatively rare, which can occur with a sudden rise in uric acid in the blood (e.g. cytostatic treatment in patients with leukemia) when urine is concentrated (dehydrated) at an acidic pH. These circumstances create conditions for the formation of **uric acid crystals in the distal renal tubules and kidney collecting ducts**, which can block urine outflow (acute uric acid nephropathy).

Urine uric acid testing is important especially in patients with elevated serum uric acid levels and in patients with urolithiasis.

The amount of uric acid in urine can be expressed in several ways:

1. By measuring the concentration in the morning urine sample. According to the results of uric acid concentration in the morning sample, the drinking regime in patients with urolithiasis is adjusted in order to reduce its urinary concentration during this period.
2. As the amount of uric acid excreted in 24 hours. It is analyzed in a urine sample taken from a mixed all-day collection. Examination of uric acid waste in 24 hours is useful for distinguishing hyperuricaemia from uric acid overproduction and decreased excretion.
3. As the uric acid/creatinine ratio in a random urine sample that does not require all-day urine collection (so-

called Kaufman Index - IK).

4. Uric acid clearance. Examination of uric acid clearance helps to distinguish whether the cause of hyperuricaemia is a metabolic disorder or a change in its renal excretion. We use the formula for the calculation:

$$\mathrm{Cl_{UA}\ [ml/s]} = \frac{\mathrm{U_{UA} \cdot V}}{\mathrm{P_{UA}}},$$

where Cl_{UA} is the uric acid clearance, U_{UA} is the uric acid concentration in the urine (mmol/l), P_{UA} is the plasma uric acid concentration (mmol/l), V is diuresis (ml/s).

5. By determining the fractional excretion of uric acid. There is general information about transport processes in the renal tubules. The role of renal tubular cell dysfunction in hyperuricaemia can be inferred by fractional uric acid excretion. It can be tested in a randomly taken urine and blood sample, in which we examine the concentration of uric acid and creatinine. We calculate it according to the formula:

$$\mathrm{FE_{UA}} = \frac{\mathrm{U_{UA} \cdot P_{creat}}}{\mathrm{U_{creat} \cdot P_{UA}}},$$

where FE_{UA} is the fractional excretion of uric acid, U_{UA} is a concentration of uric acid in the urine in mmol/l, P_{UA} is serum (plasma) uric acid concentration in mmol/l, P_{creat} is serum (plasma) creatinine concentration in mmol/l, U_{creat} is a concentration of creatinine in the urine in mmol/l.

Reference values

- Uric acid losses by urine in 24 hours (dU-uric acid)

  3,6 mmol/24 h (low-purine diet)
  5,0 mmol/24 h (normal diet)

- Uric acid clearance:

  0,07–0,22 ml/s

Methods of determination

Most modern methods for determining the concentration of uric acid use the enzyme uricase, which converts uric acid to allantoin, hydrogen peroxide and carbon dioxide. The decrease in uric acid concentration in the reaction mixture can be determined directly by measuring the loss of absorbance at 290-293 nm. This method is based on different absorption spectra of allantoin and uric acid. Unlike uric acid, allantoin formed in the uricase reaction does not show an absorption peak at 290–293 nm. Another possibility is an indirect determination using the product - hydrogen peroxide for another peroxidase-catalyzed coupled reaction. By **oxidative coupling** (which is used as an indicator reaction, is a special type of coupling between aromatic amines and phenols. The hydrogen peroxide formed in the previous reaction is used for the peroxidase catalyzed oxidation. Oxidative coupling is the essence of the determination of other important analytes in biological material such as glucose, cholesterol or triacylglycerols) usually 4-aminoantipyrine with a suitable phenol derivative (in our case N-ethyl-N-(2-hydroxy-2-sulfopropyl)-m-toluidine) produces a quinoneimine dye whose color intensity is proportional to the concentration of uric acid in the sample. Ascorbic acid interferes with the determination. Its effect is suppressed by the presence of ascorbate oxidase in the reaction mixture.

Gout (arthritis urica)

Gout is a serious manifestation of a disorder of uric acid metabolism. It is characterized by an increased concentration of uric acid in extracellular fluids and in various tissues. When the solubility of urates is exceeded, their crystals fall out of solution and settle mainly in low-blooded tissues - e.g. in the soft tissues of the joints. There they cause an inflammatory reaction and condition degenerative changes in the joint. In chronic gouty arthritis, urate causes the formation of so-called *gouty tophi* - nodular formations containing centrally deposited urate crystals, which are surrounded by inflammatory cells and fibrous tissue. Manifestations of gout are repeated attacks of acute arthritis, in which sodium urate crystals are found in the leukocytes of the synovial fluid.

Source

Links

With the consent of the authors taken from https://el.lf1.cuni.cz/p45355481/

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