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Relationship between plasma uridine and urinary urea excretion

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Abstract

To investigate whether the concentration of uridine in plasma is related to the urinary excretion of urea, 45 healthy male subjects with normouricemia and normal blood pressure were studied after providing informed consent. Immediately after collection of 24-hour urine, blood samples were drawn after an overnight fast except for water. The contents of ingested foods during the 24-hour urine collection period were described by the subjects and analyzed by a dietician. Simple regression analysis showed that plasma uridine was correlated with the urinary excretions of urea ($R = 0.41$, $P < .01$), uric acid ($R = 0.36$, $P < .05$), and uridine ($R = 0.30$, $P < .05$), as well as uric acid clearance ($R = 0.35$, $P < .05$) and purine intake ($R = 0.30$, $P < .05$). In contrast, multiple regression analysis showed a positive relationship only between plasma uridine and urinary excretion of urea. These results suggest that an increase in de novo pyrimidine synthesis leads to an increased concentration of uridine in plasma via nitrogen catabolism in healthy subjects with normouricemia and normal blood pressure.

1. Introduction

Uridine is a pyrimidine nucleoside that is necessary for the synthesis of nucleic acids and plays an important role in the synthesis of glycogen. Because it is found in a considerably higher quantity than other purine and pyrimidine nucleosides in plasma, uridine may also have other physiologic activities, such as vasoconstrictive action [1,2]. We previously showed that the concentration of uridine in plasma is affected by many factors, including ethanol ingestion, fructose infusion, muscular exercise, amino acids infusion, and sucrose ingestion [3-6]. In addition to those, nitrogen catabolism must also be considered because de novo pyrimidine synthesis is related to nitrogen catabolism. In animals, nitrogen from amino acids is excreted as 1 of 3 end products (ammonia, uric acid, and urea). Of those, ammonia, which is highly toxic to all animals, is excreted directly by fish, whereas birds and land animals convert ammonia to uric acid and urea, respectively, for detoxification.

Although urea is the major end product of nitrogen catabolism in humans, the production of pyrimidine may also be related to that process, as it is synthesized using glutamine formed from ammonia and glutamate as the source of nitrogen. In the first step of urea synthesis, carbamoyl phosphate is formed from ammonia and carbon dioxide by carbamoyl phosphate synthetase I in mitochondria (Fig. 1). In contrast, in the first step of pyrimidine synthesis, carbamoyl phosphate is formed from glutamine, carbon dioxide, and adenosine triphosphate (ATP) by carbamoyl phosphate synthetase II in cytosol (Fig. 1). When carbamoyl phosphate synthesis exceeds the capacity for use in urea synthesis in mitochondria, carbamoyl phosphate escapes into the cytosol, leading to a greater increase in pyrimidine synthesis [7,8].

A previous study showed that a high-protein diet increased the urinary excretion of urea and orotic acid in rats, indicating that both urea synthesis and pyrimidine synthesis were increased after ingestion of high levels of protein [9]. Accordingly, excessive amino acid degradation may enhance the production of uridine via pyrimidine synthesis, as another study demonstrated that allopurinol decreased the concentration of uridine in plasma and increased the urinary excretion of orotic acid [10]. Allopurinol suppresses de

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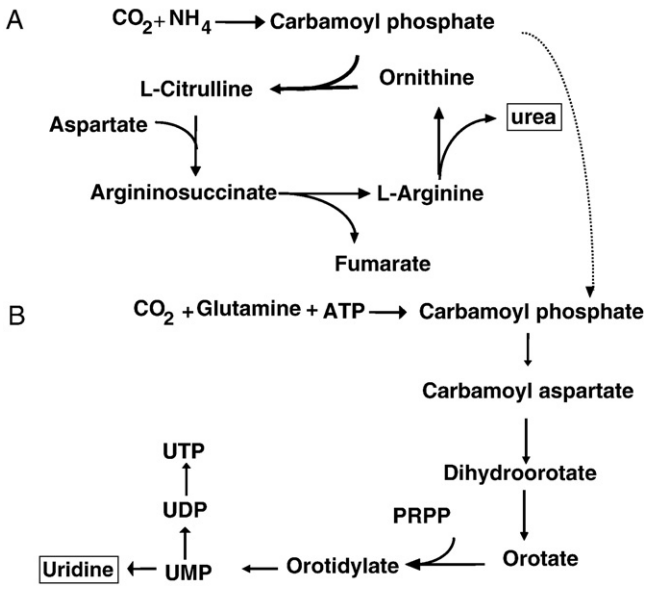

Fig. 1. Urea and pyrimidine biosynthesis. A, Urea biosynthesis. B, Pyrimidine biosynthesis. UMP indicates uridine monophosphate.

novo pyrimidine synthesis by inhibiting orotidylate decarboxylase and orotate phosphoribosyl transferase, resulting in a decrease in the concentration of uridine in plasma and an increase in the urinary excretion of orotic acid. Therefore, to determine whether the plasma level of uridine is correlated with the urinary excretion of urea together with other factors that affect the plasma concentration of uridine, we conducted the present study using healthy male subjects. In addition, the relationship between plasma uridine and uric acid metabolism, and that between plasma uridine and the homeostasis model assessment of insulin resistance index (HOMA-R) were investigated, as previous studies have shown a positive relationship between plasma uridine and urinary excretion of uric acid in male patients with gout, and that of plasma uridine and insulin resistance in patients with hypertension [11,12].

2. Subjects and methods

2.1. Subjects and protocol

Forty-five men with normouricemia and normal blood pressure (systolic blood pressure [SBP] <140 mm Hg, diastolic blood pressure [DBP] <90 mm Hg) with a mean age of 48 ± 10 years and mean body weight of 70.2 ± 10.9 kg participated in the study after providing informed consent. Each subject had normal laboratory data, including serum aspartate aminotransferase, alanine aminotransferase, creatinine, urate, and plasma glucose. First, urine was collected for 24 hours from 7:00 AM; and blood was drawn the next day after an overnight fast except for water immediately at the end of the 24-hour urine collection. Each subject consumed a regular diet without ingestion of alcoholic beverages during the 24-hour urine collection period. The contents of ingested foods during that period were described by the subjects and analyzed by a dietician.

2.2. Blood and urine analyses

The plasma concentration of uridine was determined as described previously [6], whereas the urinary concentration of uridine was determined using high-performance liquid chromatography (HPLC) as follows. The HPLC system consisted of an LC-6A HPLC apparatus (Shimadzu, Kyoto, Japan), an SPD-6AV UV-VIS spectrophotometric detector (Shimadzu), a Wakosil 5C18-200 column (200 × 4.6 mm internal diameter; Wako, Osaka, Japan), and a C-R3A Chromatopac recorder (Shimadzu). Urinary uridine could not be measured in 1 step with 10 mmol/L of potassium phosphate buffer (pH 4.7) or 20 mmol/L of potassium buffer (pH 2.2) using HPLC because the retention time of uridine is the same as or close to that of many other substances in urine. However, with 20 mmol/L of potassium phosphate buffer (pH 2.2), uridine could be clearly separated from those substances in samples eluted via the column with 10 mmol/L of potassium buffer (pH 4.7).

To determine the concentration of uridine in urine, we performed 2 steps using HPLC. First, after urine without dilution was filtrated with a Chromatodisc (0.2 μm, 13A; Kurabo, Osaka, Japan), 20 μL of filtrated urine was injected into the Wakosil column in the mobile phase with 10 mmol/L of potassium phosphate buffer (pH 4.7) and a flow rate of 1 mL/min, after which samples were collected when uridine was eluted via the column. Under this condition, the elution time of uridine ranged from 10.70 to 11.30 minutes. Next, to determine the sharp peak of uridine, the collected samples were freeze-dried diluted with 40 μL of distilled water, and applied to the Wakosil column in the mobile phase with 20 mmol/L of potassium phosphate (pH 2.2); then the peak of uridine was monitored. The recovery rate of uridine was 92.9% ± 1.8% (n = 5) with this system. Other parameters, including urea, creatinine, and uric acid, were measured by our hospital laboratory. Creatinine clearance (Ccr) was calculated using the values for the 24-hour urinary excretion of creatinine and serum creatinine, whereas uric acid clearance (Cua) was calculated using the values for 24-hour urinary excretion of uric acid and serum uric acid.

2.3. Statistical analysis

Data are presented as the mean ± SD. The significance of differences between variables was analyzed using analysis of variance and a paired *t* test.

3. Results

3.1. Laboratory data, diet content, and urinary excretion of urea, uric acid, and creatinine

The plasma concentrations of creatinine, uric acid, urea, and fasting blood glucose were within their reference ranges

Table 1
Subjects characteristics (N = 45)

Age (y)	48 ± 10
Body weight (kg)	70.2 ± 10.9
Height (cm)	169 ± 6.6
BMI (kg/m^2)	24.5 ± 3.2
SBP (mm Hg)	122 ± 20
DBP (mm Hg)	75 ± 11
Serum creatinine (μmol/L)	63 ± 8
Serum uric acid (μmol/L)	345 ± 51
Serum urea (μmol/L)	5.0 ± 1.1
Plasma uridine (μmol/L)	3.88 ± 0.70
Serum total cholesterol (mmol/L)	5.32 ± 0.74
Serum HDL cholesterol (mmol/L)	1.26 ± 0.28
Serum triglyceride (mmol/L)	1.66 ± 0.77
Fasting blood glucose (mmol/L)	5.25 ± 0.45
IRI (μU/mL)	8.2 ± 4.5
HOMA-R (mg/dL × mg/dL)	1.86 ± 0.92

BMI indicates body mass index; HDL, high-density lipoprotein; IRI, immunoreactive insulin.

in all subjects (Table 1). The urinary excretion of urea, uric acid, and creatinine, as well as dietary contents during the 24-hour urine collection period, is shown in Table 2.

3.2. Relationship between plasma uridine and other parameters

In the present healthy subjects, the plasma concentration of uridine was correlated with the 24-hour urinary excretion of urea ($R = 0.41$, $P < .01$, Fig. 2), as well as with the urinary excretion of uric acid ($R = 0.36$, $P < .05$) and uridine ($R = 0.30$, $P < .05$), Cua ($R = 0.35$, $P < .05$), and ingested purine ($R = 0.30$, $P < .05$). In contrast, plasma uridine was not correlated with age; body weight; height; body mass index; SBP; DBP; serum creatinine; serum uric acid; serum urea; plasma uridine; serum total cholesterol; serum high-density lipoprotein cholesterol; serum triglyceride; fasting blood glucose; immunoreactive insulin; HOMA-R; Ccr; or intake of protein, fat, and carbohydrates.

Using plasma uridine as a dependent variable and the values for 24-hour urinary excretion of urea, uric acid, Cua, and ingested purine as independent variables, multiple regression analysis was performed. As a result, plasma uridine was shown to be correlated with the 24-hour urinary excretion of urea ($P < .05$), but not with the other parameters (Table 3).

Table 2
Urinary excretion of uric acid, urea, and uridine; clearance of uric acid and creatinine; and intake of protein, fat, and carbohydrates by subjects over 24 hours

Uric acid/body surface (mmol/m^2)	2.58 ± 0.71
Urea/body surface (mmol/m^2)	213 ± 60
Uridine/body surface (μmol/m^2)	1.32 ± 0.74
Cua (mL/min)	7.8 ± 2.5
Ccr (mL/min)	112 ± 20
Protein (g)	85.5 ± 16.8
Fat (g)	63.4 ± 18,1
Carbohydrates (g)	267.1 ± 48.0
Purine nitrogen (mg)	145.1 ± 44.1

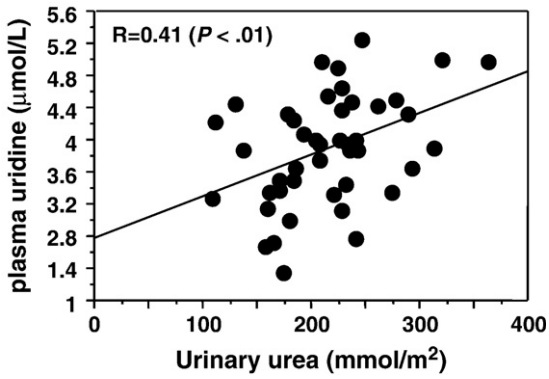

Fig. 2. Relationship between urinary urea and plasma uridine.

4. Discussion

Several previous studies have demonstrated that the liver of various mammals can respond to increasing levels of ammonia, which can be caused by high protein intake, by increasing de novo pyrimidine synthesis [7-9]. Urinary urea excretion reflects ammonia production in humans because most is converted to urea and excreted in urine. Accordingly, an increase in urinary urea excretion may suggest an increase in de novo pyrimidine synthesis. Other studies have shown that the concentration of 5-phosphoribosyl-1-diphosphate (PRPP) in mouse livers is elevated by protein intake, leading to increased pyrimidine nucleotide synthesis [13,14]. Those

Table 3
Multiple regression analysis

Analysis of variance					
Source	*df*	Sum of square	Mean square	F value	*P* value
Regression	5	5.805	1.161	3.110	.0186
Residual	39	14.561	0.372		
Total	44	20.366			
Predictor	Coefficient	SE	Standard coefficient (β)	T value	*P* value
Constant	2.148	0.471	2.148	4.565	.0001
UUN	0.004	0.002	0.333	2.181	.0357
UUA	−0.098	0.236	−0.103	−0.417	.6789
UUR	0.107	0.138	0.116	0.774	.4434
Cua	0.082	0.062	0.299	1.332	.1907
Purine	0.002	0.002	0.143	0.943	.3513

Plasma uridine was a dependent variable, whereas UUN, UUA, UUR, purine, and Cua were independent variables.
UUN indicates 24-hour urinary excretion of urea nitrogen; UUA, 24-hour urinary excretion of uric acid; UUR, 24-hour urinary excretion of uridine.

results strongly suggest that uridine monophosphate is produced in great amounts from increased carbamoyl phosphate via orotic acid and orotate monophosphate, then degraded to uridine. However, there is no known study of the relationship between uridine and urea.

In the present study, we measured the concentrations of uridine in plasma and urine and investigated the relationship between plasma uridine and urinary urea excretion in healthy subjects. Our multiple regression analysis results showed a positive relationship between them when plasma uridine was used as a dependent variable. Because the urinary excretion of uridine was correlated with the urinary excretions of urea and uric acid (data not shown), multiple regression analysis was performed using the urinary excretion of uridine as a dependent variable, with the urinary excretions of urea and uric acid and level of uridine in plasma as independent variables. Our results showed no significant relationship between the urinary excretion of uridine and those independent variables (data not shown), suggesting that the urinary excretion of uridine does not reflect the production of uridine because nearly all uridine is reabsorbed via nucleoside transporters in the kidneys. In addition, they suggest that an increase in de novo pyrimidine synthesis leads to an increase in pyrimidine degradation, resulting in an increase in the concentration of uridine in plasma.

It was previously reported that plasma uridine levels are elevated in gouty patients with increased excretion of uric acid (overexcretion hyperuricemia) [11], suggesting that de novo pyrimidine synthesis and/or pyrimidine degradation is enhanced in relation to increased production of uric acid in gouty patients with overexcretion hyperuricemia, which is mainly caused by enhanced de novo purine synthesis and excessive ATP consumption, such as ethanol ingestion and rigorous muscular exercise. In enhanced de novo purine synthesis, PRPP synthesis is increased, leading to an increase in de novo pyrimidine synthesis because PRPP is used as a substrate in that process. The resulting enhancement of de novo pyrimidine synthesis may accelerate pyrimidine degradation, leading to increased production of uridine. On the other hand, after excessive ATP consumption, the phosphorylation of uridine diphosphate (UDP) to uridine triphosphate (UTP) decreases because UDP is converted to UTP, using ATP as a phosphate donor. Thus, abrupt consumption of ATP leads to an increase in UDP, resulting in increased pyrimidine degradation, which causes an overproduction of uridine [3,4]. However, in the present subjects, multiple regression analysis found no relationship between plasma uridine and 24-hour urinary excretion of uric acid. This finding suggests that there is no significant amount of loss of ATP and/or enhanced PRPP synthesis in healthy subjects with normouricemia. In a recent study, plasma uridine levels were high in hypertensive patients and correlated with HOMA-R [12], suggesting that plasma uridine may be a marker of insulin resistance in hypertensive patients because extracellular uridine enhances both UTP-glucose accumulation and glycogen synthesis to increase glucose utilization in peripheral insulin resistance. In contrast, in the present study, we did not find a relationship between plasma uridine and HOMA-R; thus, HOMA-R may not be related to plasma uridine in healthy male subjects with normal blood pressure.

In conclusion, we found a positive relationship between the concentration of uridine in plasma and urinary excretion of urea, which strongly suggests that increased de novo pyrimidine synthesis via nitrogen catabolism leads to an increased concentration of uridine in plasma in healthy subjects with normouricemia and normal blood pressure. Although the concentration of uridine in plasma is affected by a number of factors, its physiologic functions outside of vasoconstriction activities remain undetermined, except for vasoconstriction activities; and additional examinations are needed.

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