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Gender and diet do not affect the urinary indices reflecting neuropsychiatric diseases

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ABSTRACT

We previously found that the relation between urinary contents of α1-microglobulin (α1M) and ulinastatin (UT) varies with the type of neuropsychiatric disease. In the present report, hitherto unsettled issues concerning the effects of gender and diet on the above relation were studied. In spontaneously collected urine from 22 healthy young adults (males, 11 ; females, 11) ranging from 20 to 33 years of age, the contents of α1M and UT per creatinine and the relation derived thereof were similar between males and females. In urine spot-collected after breakfast (morning urine), lunch (afternoon urine) and dinner (evening urine) for 6 consecutive days from 3 healthy middle-aged subjects, contents of α1M and UT per creatinine in the morning urine were higher, while those in the afternoon and evening urine did not differ. As for the relation between urinary contents of α1M and UT, a positive correlation and a similar regression slope were displayed in all the three time-related urine samples. Furthermore, each individual exhibited a respective constant regression slope in the correlation. These results suggest that gender and diet per se do not affect the relation between urinary contents of α1M and UT, and that any abrupt change in the correlation may reflect pathopysiological changes in the subject as observed in patients with neuropsychiatric diseases.

INTRODUCTION

In human urine, two glycoproteins, alpha-1-microglobulin (α1M ; 31 kDa) and ulinastatin (UT, also designated as bikunin ; 40 kDa) (derived from a common precursor protein in the liver)[1], are excreted. While α1M demonstrates immunosuppresive activities and UT elicits trypsin inhibitory activities[2], their mutually and physiologically relevant functions are still obscure. In the course of our studies on the relation between renal functions and urinary α1M and UT contents, we have found that the relation of urinary contents of α1M and UT varies with the type of neuropsychiatric disease[3-5] in patients without renal/hepatic dysfunctions. However, it remains unresolved whether or not gender and diet affect this relation. In addition, the respective regression slopes in the correlation between urinary contents of α1M and UT were not statistically different among healthy children (n = 19 ; age, 6-9), youths (n = 21 ; age, 20-33) and elderly subjects (n = 18 ; age, 60-87) in studies on aging[6]. These results implicate that aging per se is not a factor which affects the relation. Similar results were obtained in mice[7], a species whose urinary α1M and UT contents correlate in a fashion similar to that in humans[8].

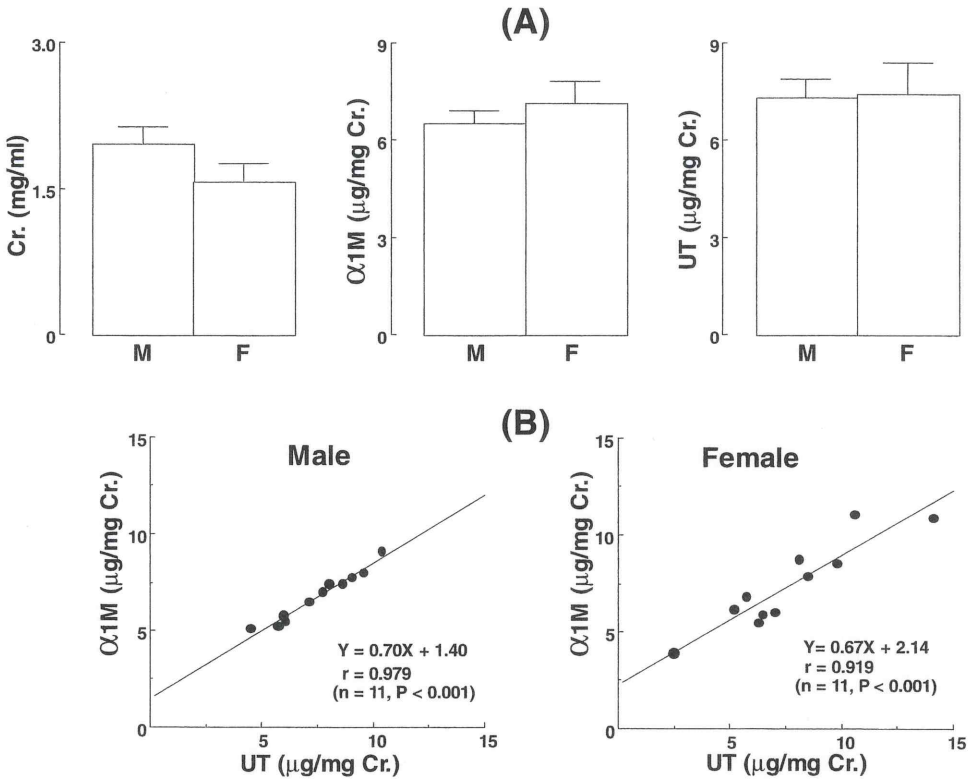

FIGURE 1. (A) Contents of creatinine (Cr.), α1-microglobulin (α1M) and ulinastatin (UT) in urine samples of males and females in youths.
(B) Relation between urinary contents of α1M and UT in males and females in youths.

In (A), each column and vertical bar represent mean ± SE of 11 subjects in males (M) and females (F). In (B), the correlation coefficient and number of subjects are represented by r and n, respectively. P value shows the significance of regression.

The present study attempted to evaluate whether or not the factors, gender and diet, affected the urinary α1M /UT relationship.

METHOD

Urine Specimens

For studies on effects of gender on the α1M /UT relation in urine, samples were collected at 1400-1600 hr from healthy youths (age range : 20-33 yr), who had undergone a routine medical check-up (respective numbers of male/female and mean±SE of their age were 11/11 and 24±1/23±1). For studies on effects of diet on the α1M /UT relation, urine specimens were collected at 1-1.5 hr after meals (breakfast, 0700-0800 hr ; lunch, 1200-1300 hr ; dinner, 1900-2000 hr) for 6 consecutive days from 3 healthy middle-aged subjects (cases 1 and 2 were males of 54 and 57 yr of age, respectively ; case 3 was a female of 49 yr of age). While all subjects followed a uniform menu for breakfast and lunch for 6 consecutive days, dinner menus were diverse and different from those for breakfast and lunch. During the experimental period, none of the subjects (cases 1, 2 and 3) encountered psychologically stressful episodes, and case 3 was in post-menopause. Informed consent was obtained from participants before the study. Urine specimens were centrifuged at 2,000*g* for 20min at 4°C to remove debris and amorphous salts prior to

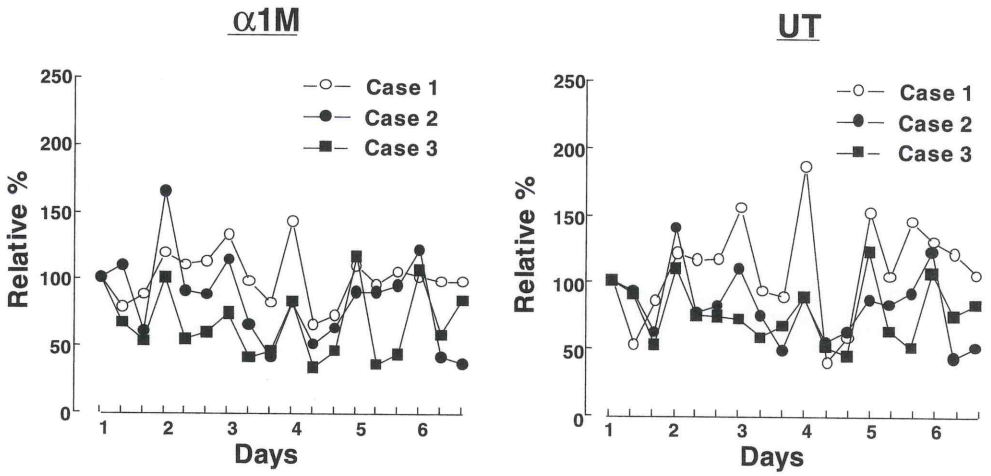

FIGURE 2. Daily changes in the contents of α1M and UT in three cases (cases 1,2 and 3 described in Method).

Urine samples were respectively collected at 1-1.5h after breakfast, lunch and dinner for 6 consecutive days. Each specimen was divided by the respective contents of α1M and UT after breakfast on day 1 collected urine, and data are represented in the ordinate. Three points per day respectively show the relative urinary contents of α1M and UT after breakfast, lunch and dinner.

storage at −50°C.

Measurements of Urine Indices

Creatinine contents in the urine were measured with a photometric assay based on Jaffé reaction (Wako creatinine kit, Wako Chemicals, Osaka). Urinary contents of α1M and UT were measured with an ELISA method, whereby galactosidase-labelled goat anti-rabbit IgG (Biotrin International, Dublin) of the former and latter was interacted with rabbit anti- α1M IgG (Dako Co., Copenhagen) and rabbit anti-UT IgG[9], respectively. Standard α1M (Dako Co., Copenhagen) and UT (a gift from Mochida Pharmaceutical Co., Tokyo) were used as references. Urinary contents of α1M and UT were expressed as μg/mg creatinine to correct for the dilution rate of urine samples.

Statistical Analyses

Statistical significance was verified by the Student's t-test for the urine indices (creatinine, α1M and UT) in youths and by the factorial analysis of varience (ANOVA) test followed by Fisher's Protected Least Significant Difference (PLSD) for the urine indices of cases 1, 2 and 3 for the 6-day period. Values where $p<0.05$ were considered statistically significant.

RESULTS

The contents of creatinine, α1M and UT did not differ significantly between males and females in youths (Figure 1, A). In the urinary α1M /UT relation, a positve correlation was observed in both males and females, and the regression slope in males was not significantly different from that of females (Figure 1, B).

During the 6-day period, urinary contents of α1M and UT per se varied with day and time of urine collection (morning, afternoon or evening) (Figure 2). When the urinary contents of α1M and UT were classified into three groups (morning, afternoon and

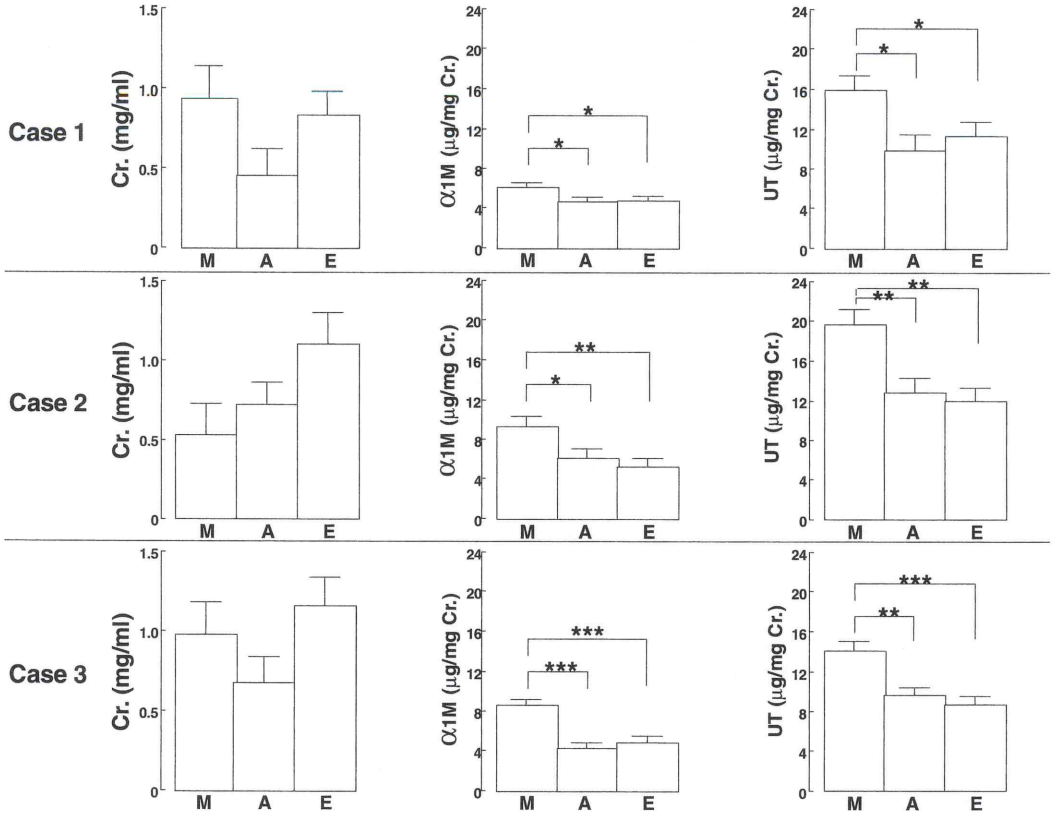

FIGURE 3. Urinary contents of creatinine (Cr.), α1M and UT in the morning (M), afternoon (A) and evening (E).

Urine specimens were respectively collected at 1-1.5 h after breakfast (morning urine ; M), lunch (afternoon urine ; A) and dinner (evening urine ; E) for 6 consecutive days. Each column and vertical bar represent mean ± SE of 6 urine specimens in all cases. Statistical significance (* : $p<0.05$, ** : $p<0.01$, *** : $p<0.001$) was verified by the factorial analysis of varience (ANOVA) test followed by Fisher's PLSD.

evening), contents in the morning were significantly ($p<0.001 \sim p<0.05$) higher than those in the other two groups. Urinary contents of α1M and UT in the afternoon were not significantly different from those in the evening, and creatinine contents per se did not statistically differ among these three groups (Figure 3). Regarding the relation between urinary contents of α1M and UT, a positive correlation was displayed in all cases tested on three group series. In addition, the respective regression slopes in urine of three groups (morning, afternoon and evening) were almost constant when reviewed on an individual basis (respective regression slopes in the morning, afternoon and evening urine were 0.21, 0.21, 0.21 in case 1 ; 0.63, 0.61 and 0.62 in case 2 ; 0.55, 0.57 and 0.54 in case 3) (Figure 4).

DISCUSSION

Regarding gender-related differences in the urinary contents of α1M and UT in humans, the contents of α1M in urine collected over a 24-hr period (urine-24hr) are reportedly higher in males than females ranging from 20 to 30 yr of age[10], whereas the contents of α1M per creatinine do not differ

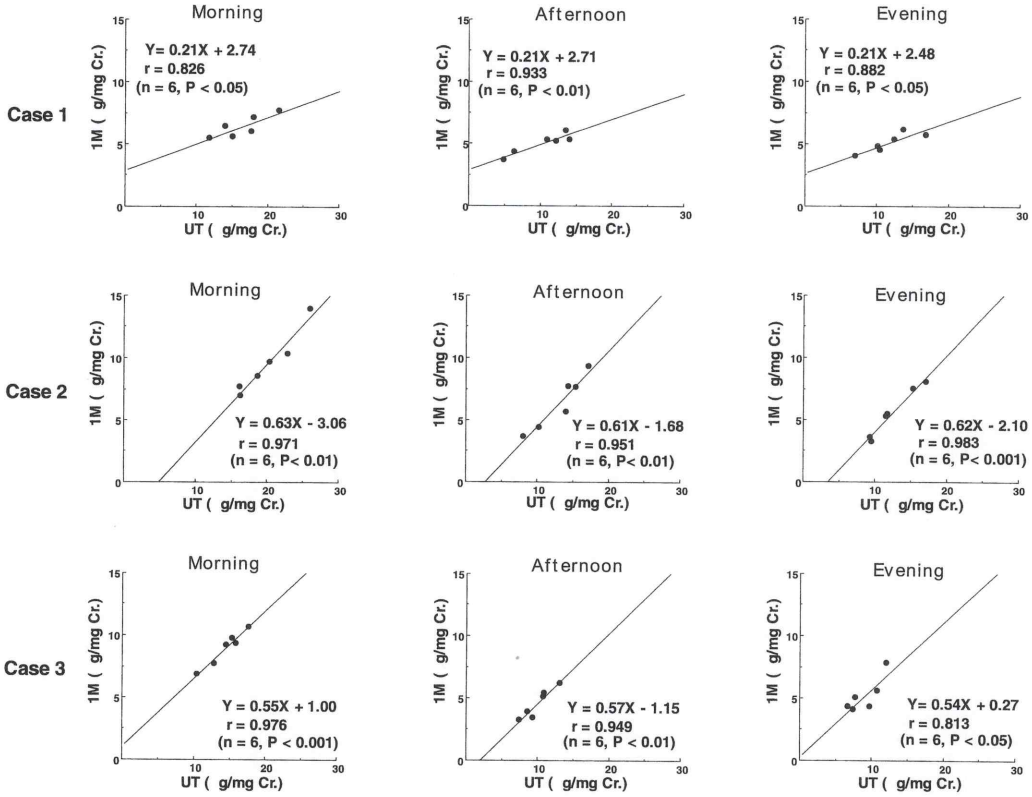

FIGURE 4. Relation between the contents of α1M and UT in urine samples collected in the morning, afternoon, and evening.

The correlation coefficient and number of urine specimens are represented by r and n, respectively. Cr represents creatinine in the urine. P value shows the significance of regression.

between genders in subjects with age ranges of 18-20, 21-30, 31-40, 41-50 and 51-77 yr[11]. As for UT, a slightly higher (statistically insignificant) content of UT/urine-24hr in males than females from 28 to 38 yr of age has been documented[12]. In the present study of α1M and UT contents per creatinine and the relation thereof in spontaneously collected urine from youths ranging from 20 to 33 yr of age, gender-related differences were not observed. Similar results were obtained in our previous studies in mice[7]. These results suggest that gender per se is not a factor that affects the urinary α1M /UT relation.

As for diet, contents of α1M and UT in the afternoon urine collected after lunch were similar to those in the evening urine collected after dinner in spite of difference in the menu between lunch and dinner. Meanwhile, contents of α1M and UT in the morning urine collected after breakfast were higher than those after lunch or dinner. From these findings, factors other than diet were probably responsible for the change in urinary contents of α1M and UT. In this regard, glucocorticoids may serve as one of the factors affecting the urinary contents of α1M and UT based on the following findings. In plasma, α1M exists in both free (α1M) and IgA- α1M complexed form[13], while UT exists not only in free (UT) and IgG-UT complexed forms[14], it also serves as a component for matured forms of inter-α-inhibitor, pre-α-inhibitor and inter-α-like inhibitor[15]. Among the varied forms in plasma,

only free forms of α1M and UT are found in urine[13,16]. In a gene responsible for synthesis of the precursor protein of α1M and UT, glucocorticoid-responsive elements[17] relating to glucocorticoid-associated fluctuations of UT in urine[9,18] have been noted. Although the effects of glucocorticoids on individual contents of the different forms of α1M and UT in plasma have not yet been established, glucocorticoids influence the contents of immunoglobulins in plasma[19]. The contents of glucocorticoids in plasma are reportedly higher in the morning compared with those in the afternoon or evening[20]. Taken together, high contents of α1M and UT in the morning urine were probably associated with the high content of plasma glucocorticoids in the morning.

As for the urinary α1M /UT relation, a positive correlation and an almost constant regression slope were displayed in all the three time-related urine groups, notwithstanding the difference in contents of α1M and UT between those in the morning and afternoon or evening. Furthermore, each subject displayed an individually characteristic regression slope in the correlation. These results advocate that any abrupt change in the slope may provide useful information on pathophysiological changes in the subject, an observation evidenced in patients with neuropsychiatric diseases[3-5]. As our results demonstrate that aging[6,7], gender and diet do not affect the relation between urinary contents of α1M and UT, variations in the relation (depending on the type of neuropsychiatric diseases) may reflect neuropsychological differences underlying the various psychiatric diseases in humans.

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