

SHORT REPORTS

Urinary Proline to Hydroxyproline Ratio Varies with Age

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Received February 5, 1979

Abstract. Urinary excretion of proline and hydroxyproline was studied in five groups of subjects, viz. controls, pituitary dwarfs, familial dwarfs, patients with fibrodysplasia ossificans progressive and patients with generalized scleroderma. The ratio Pro/Hyp in the urinary fraction precipitated with 5 parts of acetone (1+5 fraction) was close to one in children and youngsters in all the groups, while in adults it was higher than two. Total Pro/Hyp does not give an accurate index of age.

Key words: Proline/Hydroxyproline ratio; Age variation

It is well known that an increased biosynthesis and catabolism of collagen occurs in adolescence (2, 5). Due to the biosynthetic interrelationship between proline (Pro) and hydroxyproline (Hyp) in collagen, insofar as Pro is the imino acid precursor of all Hyp, the possibility of a parallel increase in the urinary excretion of both imino acids was considered. In previous work at this laboratory (2, 4) it was shown that the Hyp content in a urinary polypeptide precipitable with acetone in the proportion of 1 ml urine to 5 ml acetone ("1+5 fraction"), was a better marker of growth than was total Hyp. We have now performed studies on the urinary excretion of total Pro and Hyp as well as their presence in the 1+5 fraction. In children and adolescents the molar ratio Pro/Hyp in the 1+5 fraction is very close to 1, increasing from the third decade of life onward. When the molar ratio of total Pro/total Hyp was used, the results were less constant. As in

Table I. Urinary excretion of proline and hydroxyproline as a function of age

Values represent mM imino acid excreted per 24 hours

Subjects	Decade	No. of subjects	No. of samples	Proline		Hydroxyproline		Molar ratio	
				Total	1+5 fraction	Total	1+5 fraction	Total Pro/Hyp	1+5 Pro/Hyp
Normal controls	1.	1	1	0.33	0.13	0.34	0.11	0.97	1.18
	2.	9	9	1.27±0.06	0.42±0.90	0.88±0.02	0.41±0.01	1.49±0.49	1.03±0.12
	3.	3	3	0.66±0.02	0.25±0.09	0.37±0.10	0.11±0.03	1.74±0.20	2.18±0.34
	4.	2	2	0.59±0.16	0.22±0.07	0.35±0.07	0.08±0.03	1.63±0.15	2.86±0.14
	5.	1	1	0.75	0.26	0.41	0.06	1.83	4.33
	6.	3	3	0.59±0.02	0.25±0.02	0.33±0.03	0.05±0.01	1.79±0.13	4.94±1.01
Pituitary dwarfs	1.	1	1	0.35	0.14	0.26	0.12	1.35	1.16
	2.	7	7	0.76±0.34	0.31±0.10	0.54±0.24	0.29±0.15	1.38±0.06	1.11±0.22
Familial dwarfs	1.	1	1	0.40	0.11	0.31	0.11	1.29	1.00
Fibrodysplasia ossif. progr.	2.	1	4	0.82±0.31	0.37±0.13	0.51±0.26	0.28±0.10	1.58±0.28	1.19±0.05
	3.	1	5	0.39±0.08	0.29±0.08	0.14±0.04	0.072±0.01	1.42±0.01	2.00±0.04
Generalized scleroderma	2.	3	8	0.64±0.27	0.23±0.13	0.50±0.19	0.18±0.12	1.26±0.13	1.45±0.35
	3.	4	11	0.58±0.15	0.20±0.02	0.38±0.11	0.08±0.02	1.59±0.36	3.01±1.01
	4.	4	9	0.54±0.14	0.27±0.04	0.28±0.10	0.09±0.02	1.66±0.03	2.31±0.04
	5.	7	12	0.62±0.16	0.32±0.12	0.32±0.13	0.09±0.03	1.53±0.27	3.32±1.07
	6.	6	16	0.48±0.10	0.24±0.03	0.25±0.10	0.06±0.02	1.55±0.02	3.19±0.93

scleroderma patients we had found an increased urinary excretion of Hyp in the 1+5 fraction (2), they were included in the present study.

MATERIAL AND METHODS

Patients

Five groups of subjects were studied.

Group I. Normal controls. The group included one child, 9 youngsters and 9 adults.

Group II. Pituitary dwarfs. The group consisted of one child and 7 youngsters of abnormally short stature in whom a growth hormone analysis (insulin-arginine stimulation) had shown deficiency.

Group III. Familial dwarf. One child of abnormally short stature, in whom a growth hormone analysis showed normal growth hormone response.

Group IV. 24 scleroderma patients grouped according to their age.

Group V. Two patients with fibrodysplasia ossificans progressive, one in the second decade and the other in the third decade of life.

Urine samples

Twenty-four-hour urine samples were collected. The subjects had an unrestricted food intake.

Analyses

Analyses of Hyp and Pro were performed *ad modum* Blumenkrantz & Asboe-Hansen (1, 3).

Total Hyp and total Pro. One ml of filtered urine was hydrolysed in 6 N HCl at 110°C for 16 hours. After hydrolysis, samples were evaporated under vacuum at 65°C. The evaporated samples were dissolved in 2 ml phosphate-citrate buffer (1) and 0.5 ml was diluted with 9.5 ml of the same buffer. The samples were centrifuged and the supernatant used for the assays of total Hyp and Pro.

Hydroxyproline-containing peptides. One ml urine was added to 5 ml acetone. The samples were left at 4°C for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The supernatant was aspirated by vacuum and the pellet hydrolysed with 6 N HCl at 110°C for 16 hours, after the small amount of acetone remaining in the pellet had been allowed to evaporate. Samples were evaporated, dissolved and analyzed as indicated above.

RESULTS

1+5 fraction: Pro/Hyp molar ratio

Normal children and teenagers had a urinary excretion ratio for Pro/Hyp close to one. From the third decade on, there was an increase in the urinary ratio Pro/Hyp due to the more marked decrease of the latter. This was valid for the five groups of subjects studied (Table I).

Total Pro/total Hyp molar ratio

Although the urinary excretion of total Pro and Hyp followed approximately the same pattern as that of the same imino acids in the 1+5 fraction, the latter were the more constant. No significant differences in this ratio were noted between children, youngsters and adults belonging to group I.

DISCUSSION

In a previous study we found an increased excretion of Hyp and hydroxylysine (Hyl) in the 1+5 fraction in adolescents and in patients with active generalized scleroderma (2, 4). Considering that the high molecular weight peptides precipitable in the 1+5 fraction derive from collagen, as shown by the content of Hyp and Hyl, it is worth noting that, in the first and second decade of life, Hyp and its precursor Pro maintain a constant relationship in the aforementioned fraction. This is true of the five groups of subjects studied and is not affected by the conditions mentioned.

The molar ratio Pro/Hyp in the 1+5 fraction is quite an accurate index of age, which is not true of the molar ratio total Pro/total Hyp. Total Pro or Hyp represent quite a heterogeneous population of peptides and free imino acids, while in the 1+5 fraction there are only polypeptides of high molecular weight (2).

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