

Table 1. Concentration of free, peptide-bound, protein-bound and total hydroxyproline in the tissues of the Arabian sand gazelle.

Tissue	Free	Peptide-bound	Protein-bound	Total
Heart	47.51±12.25	166.3±57.07	77.47±19.17 <sup>@@</sup>	291.3±66.51 <sup>+</sup>
Skeletal muscle	32.11±11.07 <sup>*</sup>	98.12±10.32 <sup>#</sup>	118.2±23.81 <sup>@@</sup>	248.5±20.07 <sup>+</sup>
Liver	29.99±2.84 <sup>*</sup>	148.8±32.21 <sup>**</sup>	156.3±12.57 <sup>@</sup>	335.0±34.04
Kidney	22.04±6.04 <sup>**</sup>	96.77±31.66 <sup>#</sup>	188.0±11.31	282.4±47.66 <sup>+</sup>

Data expressed as means ± SD (µg/g tissue), n = 4 deers

<sup>\*</sup>Values not significant as compared to heart. ( $P > 0.05$ , Turkey's multiple comparison test).

<sup>\*\*</sup>Values significant different as compared to heart. ( $P < 0.01$ , Turkey's multiple comparison test).

<sup>#</sup>Values not significant as compared to heart, ( $P > 0.05$ , Turkey's multiple comparison test).

<sup>@</sup>Values not significant as compared to kidney, ( $P > 0.05$ , Turkey's multiple comparison test).

<sup>@@</sup>Values significant different as compared to kidney. ( $P < 0.001$ , Turkey's multiple comparison test).

<sup>+</sup>Values not significant compared to liver. ( $P > 0.05$ ), Turkey's multiple comparison test).

frozen immediately in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until processed. Tissues were homogenized in normal saline (10% g/mL) using a stainless steel Omni-Mixer homogenizer (Omni International, Inc, Gainesville, VA, USA). The homogenate was used for determination of hydroxyproline concentrations.

#### Extraction of Free, Peptide-bound and Protein-bound Hydroxyproline

Free and protein-bound Hyp was extracted by the method of Varghese, Moorhead and Wills, 1981 with slight modification. 0.5 mL of the tissue homogenate was treated with 3x5 mL portion of re-rectified absolute alcohol and centrifuged at 600xg for 10 min. The supernatants were pooled and evaporated to dryness. The residue was dissolved in 0.5 mL of distilled water and 50 µL of the extract was used for estimation of free Hyp as mentioned below. The pellets of all the samples were dissolved in 500 µL of distilled water and 50 µL of the extract was used for determination of protein-bound Hyp as described below. The peptide bound Hyp was calculated by subtracting the sum

total of free and protein bond Hyp from total Hyp content.

#### Extraction of Soluble and Insoluble Collagen Hydroxyproline

Soluble and insoluble collagen hydroxyproline was extracted by the method of Kivirikko *et al.*, 1965. Briefly, the tissue samples were homogenized (4 mL/g tissue) in a cold 0.45 M sodium chloride. The homogenate was extracted at  $4^{\circ}\text{C}$  for 24 hr with occasional stirring, followed by centrifugation at 60,000xg for 60 min. The supernatants obtained were precipitated with 4 volumes of a cold ethanol and centrifuged twice with 80% ethanol, twice with absolute alcohol, twice with ether and twice with warm ethanol-ether (1:2). The residues were gelatinized with distilled water at  $124^{\circ}\text{C}$  for three hr and after filtration a sample of gelatine solution was used for soluble collagen Hyp estimation as described below.

The precipitates obtained after centrifugation at 60,000xg were washed 3 times with 0.45 M sodium chloride and twice with distilled water, after which they were extracted absolute ethanol, ether and

Table 2. Concentration of soluble and insoluble collagen hydroxyproline in the tissues of the Arabian sand gazelle.

Tissue	Soluble collagen hydroxyproline	Insoluble collagen hydroxyproline
Heart	4.375±0.710**	47.62±11.05@@
Skeletal muscle	101.2±7.45	175.5±35.75@
Liver	44.32±7.20**	171.9±26.09@
Kidney	23.19±3.19**	303.2±59.09

Data expressed as means ± SD, (µg/g tissue) n = 4 deers

\*\*Values significant different as compared to skeletal muscle, ( $P < 0.001$ , Turkey's multiple comparison test).

@Values significant different as compared to kidney. ( $P < 0.01$ , Turkey's multiple comparison test).

@@Values significant different as compared to kidney. ( $P < 0.001$ , Turkey's multiple comparison test)

ethanol-ether and gelatinized as above. A sample of gelatine solution was used for insoluble collagen Hyp estimation as described below.

#### Determination of Hydroxyproline Concentration

Hydroxyproline was measured by the modified alkaline hydrolysis method of Reddy and Enwemeka, 1996. Briefly 50 µL of homogenate sample was added into sodium hydroxide (2 N final concentration) the mixture solution was then hydrolyzed by heating in boiling water bath for about 3-4 hr. 900 µL of 56 mM chloramine T reagent was added to the hydrolyzed sample and oxidation was allowed to proceed at room temperature for 25 min. Then 1000 µL of 1 M Ehrlich's reagent (p-dimethylaminobenzaldehyde) was added to the oxidized sample and the chromophore was developed by incubating the samples at 65 °C for 20 min. The absorbance was read at 550 nm using Ultrospec 2000 UV/visible Spectrophotometer (Pharmacia Biotech Ltd, Science Park, Cambridge, England). The Hyp concentration in the samples was calculated from the standard curve of Hyp. Total collagen content was calculated from

Hyp concentration assuming that Hyp constitute 12.5% collagen (Edwards and O'Brien, 1980).

#### Statistically Analysis

Data from each sample were run in duplicate. The Hyp concentration and collagen content were expressed as means ± SD µg/g wet weight tissue, for n = 4 animals. The Hyp level and collagen content in various tissues were compared using one-way ANOVA analysis followed by Turkey's test for multiple comparison test. Bartlett's test was used for homogeneity of variances. Spearman correlation analysis was used to examine the association between variables. Values were considered significant if  $P < 0.05$ . Statistical analysis was performed by means of GraphPad Prims® package for personal computers (GraphPad™ Software, San Diego, USA).

## Results

Table 1 shows means ± SD of free, peptide bound, protein bound and total Hyp concentration in various tissues of Arabian sand gazelle. Heart had the highest

collagen in calf skeletal muscle was reported by McClain, 1973. Kidney had significantly the highest concentration of insoluble collagen Hyp and heart the lowest concentration of insoluble collagen Hyp. This variation in the soluble and insoluble collagen Hyp may reflect the differences in the collagen structure and composition.

In conclusion, significant differences were observed in Hyp concentration and the soluble and insoluble collagen levels in the tissues of this species. We speculate that these differences could be due to the variation in the turn over rate of collagen metabolism in this species.

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## تقدير تركيز هيروكسي برولين الكولاجيني الكلي، الحر، المرتبط بالببتيد المرتبط بالبروتين ، الذائب وغير الذائب في أنسجة الريم العربي

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الهدف من هذه الدراسة هو تقدير تركيز هيروكسي برولين الكولاجيني الكلي، الحر، المرتبط بالببتيد، المرتبط بالببتيد، المرتبط بالبروتين، الذائب وغير الذائب في أنسجة الريم العربي. النتائج أوضحت أن هناك تباين ملحوظ في التركيز بين الأنسجة المختلفة. يحتوي القلب على أعلى تركيز في الحر، ثم يليه العضلات المخططة ومن ثم الكبد والكلية. بينما أعلى تركيز للمرتبط بالببتيد وجد في القلب أيضا ولكن يليه الكبد ومن ثم العضلات المخططة. أيضا بينت الدراسة أن أعلى تركيز للمرتبط بالبروتين وجد في الكلية يليها الكبد ومن ثم العضلات المخططة والقلب. أن العضلات المخططة تحتوي على أعلى تركيز في الذائب، بينما الكلية تحتوي أعلى تركيز في غير الذائب لهيروكسي برولين الكولاجيني. إن هذا التباين في تركيز لهيروكسي برولين الكولاجيني في جميع أشكاله بين الأنسجة المختلفة ربما ناتج من الاختلاف في معدل تكوين وأيض الكولاجين في هذه الأنسجة.