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Comparative Study of the State of Water in Various Human Tissues

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We studied the dependence of water exchange in various human tissues (skin, Achilles tendon, various types of hyaline cartilage, muscle, and muscle fascia) on the content of collagen and proteoglycans. Carbohydrates of the connective tissue do not play the major role in tissue water exchange. Collagen fibers and proteoglycan aggregates probably retain the main part of bound and free water, respectively.

Key Words: *human tissues; water; collagen; proteoglycans*

Human tissues and organs are heterogeneous systems primarily consisting of water, proteins, lipids, carbohydrates, nucleic acids, and mineral components. Water is the most mobile and variable component determining functional properties of tissues. Abnormal states of tissues (inflammation, traumas, burns, dermatosis, *etc*.) are accompanied by changes in water content and strength of its binding to structural biopolymers.

Most studies of tissue hydration were performed on animal tissues, isolated preparations (collagen, gelatin, globular proteins, and proteoglycans, PG), and biopolymers in a dissolved state [9,10, 13,14]. It remains unclear whether tissues mainly contain bound or free water. The use of various methods results in different quantitative evaluations of hydration in the same biological objects, probably because various approaches allow the study of only some of possible states of water.

Here we compared the state of water and some biochemical components (collagen and PG) in human connective and muscle tissues.

MATERIALS AND METHODS

Samples of the skin, Achilles tendon, patellar cartilage, tracheal cartilage, costal cartilage, abdominal muscle, and fascia of the transverse abdominal muscle were obtained from men (32-56 years) dying from trauma. Autopsy samples were obtained within 24 h after death.

Aquametric analysis was carried out using Fischer reagent [4]. The content of bound water was measured after 2-fold pressing of free water from the tissue (500-800 atm). Water content in the sample was measured by the Fischer method after pressing. The fraction of free water was determined as the difference between total water content and bound water content.

The mechanism of interaction between biological tissues and water was evaluated from isotherms reflecting the dependence of water vapor sorption on relative humidity of the environment. Isotherms of vapor sorption by tissue samples were constructed under static conditions. Since postmortem autolysis in native tissues leads to quantitative and qualitative changes in tissue biopolymers [5], we used tissue samples after drying with alcohol and ether.

The effective capacitance of monolayer (a_m) was calculated by the Brunauer—Emmett—Teller (BET) equation [2]. The degree of tissue swelling in water (W_S, %) was estimated from changes in the weight of samples; swelling rate constant (k) was evaluated by the method of Bolotnikov [1].

Total nitrogen concentration was measured on an element analyzer (model 1108, Carlo Erba).

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In the examined samples, the content of hydroxyproline, a marker of collagen, was measured [15]. The tissues were hydrolyzed in sealed ampoules with 6 N HCl at 120°C for 8 h.

The content of glycosaminoglycans (GAG), the carbohydrate part of PG, was evaluated by the amount of hexosamines and hexuronic acids. Hexosamine content was estimated as described elsewhere [7]. Hydrolysis was performed in 6 N HCl at 105°C for 8 h. For evaluation of hexuronic acid content, the tissues were hydrolyzed in 4 N HCl at 105°C for 4 h.

PG were removed from the Achilles tendon using amylorizin at 37°C for 24 h (enzyme/substrate ratio 1:25).

The costal cartilage was consecutively treated with 4 M guanidine hydrochloride, papain (1:20) in phosphate buffer (pH 6.2) containing 9.6 mg/mol cysteine, and hyaluronidase (1:50) in acetate-citrate buffer (pH 5.0) at 37°C for 24 h to induce disorganization of PG.

RESULTS

Muscle tissue had the highest water capacity. Total water content was maximum in this tissue, but only 26% total water was present in a bound form. The fraction of free water was 74% (Table 1).

All types of connective tissue were characterized by higher contents of bound water, especially tissues under high physical load. For example, 90% water in transverse abdominal muscle fascia was bound with tissue biopolymers. The content of free water was only 10%. The ratio of bound to free water was maximum in the fascia of the transverse abdominal muscle. The amount of bound water was high in the patellar cartilage and Achilles tendon. However, these tissues had the lowest content of free water (Table 1). It should be emphasized that these tissues are also exposed to high physical load.

It was postulated that acid PG of the connective tissue matrix play the major role in water binding and retention. Published data show that isola-

TABLE 1. Water Content in Human Tissues ($M\pm m$)

Sample	Water content, %			Bound H_2O/free H_2O
	total	bound	free	
Skin	64.6±1.7	35.7±0.9	28.9±2.4	1.24
Achilles tendon	68.1±2.1	46.5±1.4	21.6±2.1	2.15
Achilles tendon after GAG removal	71.3±1.9	43.4±0.4	27.9±2.5	1.56
Costal cartilage	57.4±0.6	34.5±0.7	22.9±1.4	1.51
Costal cartilage after GAG removal	61.0±1.1	29.2±1.2	31.8±1.7	0.92
Tracheal cartilage	65.8±0.6	34.8±1.5	31.0±2.0	1.12
Patellar cartilage	71.1±0.7	51.8±1.2	19.3±1.4	2.68
Abdominal muscle fascia	55.3±1.2	49.8±2.1	5.5±1.4	9.05
Abdominal muscle	76.8±1.5	20.1±1.3	56.7±2.1	0.35

Note. Here and in Tables 2 and 3: mean values of 10-25 tissue samples are presented.

TABLE 2. Composition of Human Tissues (g/100 g dry tissue, $M\pm m$)

Sample	Hydroxyproline	Hexosamines	Hexuronic acids	Total nitrogen
Skin	8.12±0.68	0.41±0.07	0.46±0.05	15.37±0.66
Achilles tendon	9.80±0.53	0.55±0.05	—	17.45±0.21
Achilles tendon after GAG removal	—	0.10±0.10	—	—
Costal cartilage	4.56±0.36	5.18±0.19	4.30±0.10	13.28±0.22
Costal cartilage after GAG removal	4.10±0.49	3.90±0.19	3.21±0.27	—
Tracheal cartilage	2.41±0.23	5.06±0.49	4.96±0.31	12.40±0.06
Patellar cartilage	6.79±0.38	4.78±0.20	4.83±0.20	13.71±0.17
Abdominal muscle fascia	9.31±0.69	1.09±0.26	1.97±0.12	17.11±0.42
Abdominal muscle	1.00±0.11	0.25±0.01	0.53±0.02	16.32±0.38

Note. Here and in Table 3: dash, not measured.

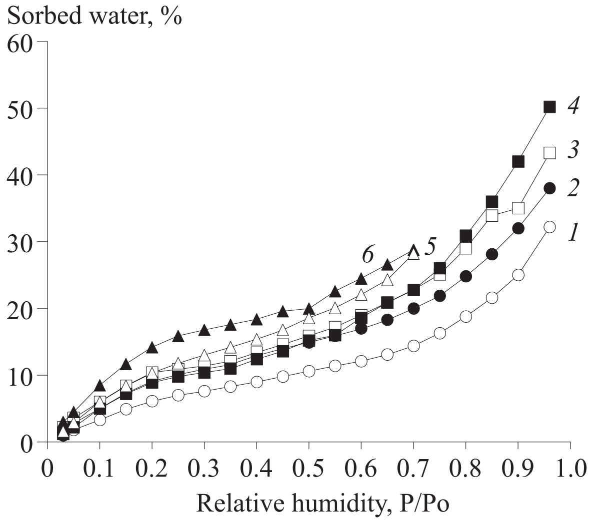

Fig. 1. Isotherms of water vapor sorption by human tissues: muscle (*1*); costal cartilage (*2*); Achilles tendon (*3*); skin (*4*); costal cartilage after GAG removal (*5*); and Achilles tendon after GAG removal (*6*).

ted PG infinitely swell in water [14]. However, no correlation was found between water content in various types of the connective tissue and GAG level (Tables 1 and 2). Despite relative close values of total and bound water content, various types of connective tissue differed by the content of GAG and hydroxyproline. The skin, tendon, and fascia were characterized by higher content of hexosamines and hexuronic acids (by 9-10 times) and lower amount of collagen (by 2-3 times) compared to cartilage tissue.

Three types of cartilage tissue (patellar cartilage, tracheal cartilage, and costal cartilage) differed by water content, but had the same amount of hexosamines and hexuronic acids. However, the samples of cartilage tissue differed by collagen content. The greater was collagen content in the cartilage, the higher was the amount of bound water. It can be hypothesized that collagen fibers are responsible for retention of bound water, while PG aggregates retain free water.

The same conclusion was made in the study of human tissue hydration. Sorption capacity of the tissue depends on its chemical composition and structure.

Figure 1 shows isotherms of water vapor sorption in the skin, muscle, tendon, and costal cartilage. Monomolecular sorption was revealed at low relative humidity (up to 0.25-0.30). In this state of hydration, each hydrophilic group of tissue polymers is bound to 1 molecule of water. The value of a_m, which serves as an important characteristic for the structure of biopolymer or tissue, is calculated from this region of isotherm by the BET equation. The region of isotherm at high relative humidity is characterized by the formation of multilayers and capillary sorption on each active site. Ttissues with considerable number of pores and cavities were capable of binding greater amount of water.

Study of binding and retention of vaporized and liquid water showed that the monolayer of muscle tissue bound the lowest amount of water. Muscle tissue mainly contains globular proteins, whose hydration degree is much lower compared to that of fibrillar proteins. The value of a_m was maximum in the Achilles tendon. This tissue consists of 90% parallel collagen fibers (Table 3).

The degree of water consumption by polymers from the liquid phase is higher compared to the vapor phase. These differences are associated with various mechanisms for the pore filling. Fluid fills all pores, including large cavities. Sorption primarily occurs in thin pores. All types of examined tissues bound and retained a greater amount of water during swelling in the liquid phase (compared to sorption of vaporized water).

For evaluation of the role of PG in the interaction of connective tissue with water in the tendon and costal cartilage some PG complexes were destructed with mucolytic enzymes. Treatment of the Achilles tendon with amylorizin exhibiting no collagenase activity, but possessing amylolytic and proteolytic properties led to destruction of 82% he-

TABLE 3. Swelling and Sorption of Water Vapors by Human Tissues (*M*±*m*)

Sample	Water vapor sorption, %		Swelling	
	a_m	a_{max}	W_S, %	$K \times 10^{-4}$
Skin	11.49	38.0±3.6	66.4±2.9	150±6
Achilles tendon	20.48	—	66.7±0.5	12.0±2.0
Achilles tendon after GAG removal	14.04	—	69.1±2.8	31.8±0.7
Costal cartilage	14.74	43.4±2.8	62.9±1.2	2.3±0.6
Costal cartilage after GAG removal	12.65	51.0±2.1	67.3±1.2	1.3±0.1
Muscle	8.40	32.8±2.9	—	—

xosamines, which disturbed integration and buffering of the matrix and disintegration of collagen fibers into individual fibrils.

As differentiated from the tendon, it was difficult to remove GAG from the costal cartilage. First, molecular bonds in the carbohydrate-protein complex of PG were modified by consecutive treatment with guanidine hydrochloride and papain. And second, a part of PG was destructed with hyaluronidase. This procedure removed 25% of hexosamines and hexuronic acids from cartilaginous tissue.

Disorganization of the main substance in cartilaginous tissue and tendon was accompanied by changes in tissue hydration (Table 1). We revealed a decrease in the amount of water, which was in contact with active groups of tissue biopolymers. Hence, the number of vaporized water-binding functional groups decreased after GAG destruction. This conclusion was derived from the decrease in a_m. However, structural changes in collagen fibers provided tissue swelling in liquid and vaporized water.

Coefficient k reflects the packing density of structural components in the examined tissues. The rate of water permeability was highest in the skin. The swelling rate of the Achilles tendon and tendon was lower by 12.5 and 65.2 times, respectively.

Coefficient k increased by 3 times after PG destruction in the main substance of the tendon and formation of empty cavities in the tissue. It was surprising that the coefficient k decreases after GAG removal from the costal cartilage. We hypothesized that partial removal of PG, which serves as a screen between structural elements of the connective tissue skeleton, is probably followed by adhesion of collagen fibers and compaction of the cartilaginous tissue. This decreases the rate of water diffusion into tissues at the early stages of swelling.

We conclude that despite destruction of carbohydrate-protein complexes in the main substance of connective tissue and decrease in the number of hydration sites, the tissue is capable of binding and retaining a considerable amount of water in the interstructural spaces.

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