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Glycosaminoglycans of normal and scarred fascia

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Fascia is a connective tissue structure which surrounding muscles forms a soft framework of the body and, at the same time, plays an important role in the locomotive system. The main structural elements of fascia are collagen fibres which ensure considerable mechanical strength, while proteoglycans, stabilize collagen fibres, being also responsible for hydration of fascia.

Proteoglycans are macromolecules composed of glycosaminoglycan (GAG) chains covalently bound to a protein core. The individual glycosaminoglycans consist of repeating disaccharide units of *N*-acetylated hexosamine (glucosamine or galactosamine) and either hexuronic acid (glucuronic acid or iduronic acid) or galactose [1, 2].

The composition and content of the fascia glycosaminoglycans is still not fully known. Reconstruction of damaged fascia leads to the formation of a scar which differs in composition and properties from the normal tissue. Scarred fascia disturb the locomotive system. The clinically observed impairment of elasticity of the altered tissue could be caused by changes in the content and properties of glycosaminoglycans. These alterations could lead to accretions between the muscle and fascia [3, 4]. So far, biochemical properties of the scarred fascia glycosaminoglycans have not been evaluated. Therefore, the aim of the present study was to investigate, both qualitatively and quantitatively, glycosaminoglycans in normal and scarred fascia.

Samples of normal fascia were taken at autopsy from 10 individuals of either sex ranging in age from 35 to 65 years. Samples of scarred fascia were removed from 18 patients aged from 29 to 62 years at reoperation on femoral fracture. Reoperations were performed 1.5 to 2.5 years after the first operation. Glycosaminoglycans were isolated from the tissue according to Suszka & Chmiel [5] and separated by precipitation with cetylpyridinium chloride at increasing Na_2SO_4 and $MgCl_2$ concentration as described by van Amerongen *et al.* [6]. The obtained fractions (IA, IB, II and III) were assayed for total hexosamine, glucosamine, galactosamine [7] and hexuronic acid contents [8]. The electrophoretic pattern of glycosaminoglycans of each fraction was analyzed. The samples containing the same amount of hexuronic acids, were placed on cellulose acetate strip using 0.044 M aluminium sulphate solution. Electrophoregrams were stained with Alcian Blue 8GS. Glycosaminoglycan content was evaluated densitometrically. Statistical significance (at $P < 0.05$) was estimated by the Student's *t*-test.

It was found that the first two fractions, IA and IB, isolated from the normal and scarred fascia did not contain any glycosaminoglycans. Fraction II contained a large amount of dermatan sulphate (DS) and smaller quantities of heparan sulphate (HS) and chondroitin-4--sulphate (Ch-4-S). Dermatan sulphate of the normal tissue differed from that of altered fascia in electrophoretic mobility (Fig. 1). Furthermore, small amounts of hyaluronic acid (HA)

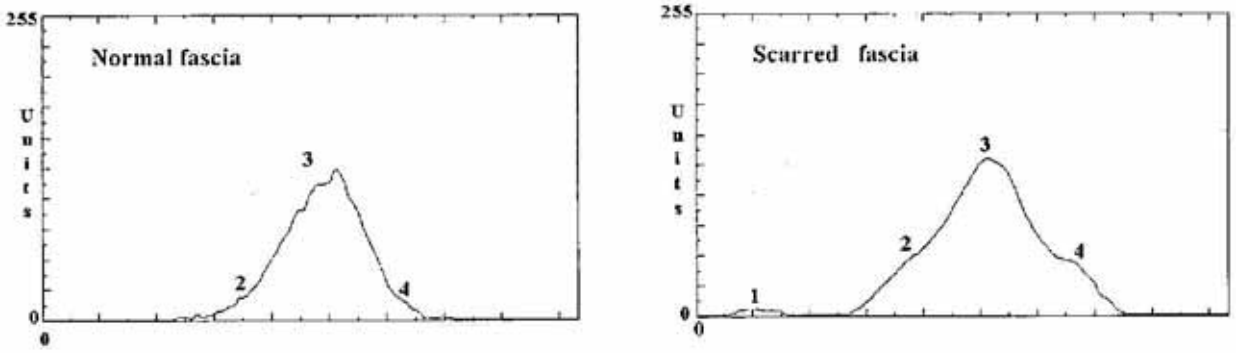

Fig. 1. Densitograms of fraction II glycosaminoglycans isolated from normal and scarred fascia.
1, Hyaluronic acid; 2, heparan sulphate; 3, dermatan sulphate; 4, chondroitin-4-sulphate.

were found in scarred fascia. Figure 2 shows the proportions of individual glycosaminoglycans in fraction II isolated from normal and scarred fascia. Fraction III contained exclusively heparan sulphate. The amount of this GAG in scarred fascia was three times higher than in control tissue. The increased electrophoretic mobility and enhanced binding of the scarred fascia heparan sulphate with the dye used for staining the electrophoregrams may suggest a rise in sulphation of this GAG.

The analysis of GAG composition demonstrated large differences in the amount of hexosamines and hexuronic acids in particular fractions (Fig. 3). Fraction IA isolated from either normal or scarred fascia contained large amounts of hexosamines and hexuronic acids. Fraction IB was composed almost exclusively of glucosamine and hexuronic acids although their quantities were negligible compared to fraction IA. Galactosamine was not found in fraction IB. Fraction II contained both hexosamines and uronic acids, their quantities in the scar tissue being three times higher than in control fascia. Fraction III of both tissues contained similar amounts of hexosamines and hexuronic acids. Hexosamines were represented exclusively by glucosamine in this fraction.

As can be seen from the results obtained glucosamine is a main fascia hexosamine. Significant increase in hexosamine content but mainly of galactosamine in the scarred fascia is accompanied by an enhanced level of hexuronic acids and elevated water content, as compared to the normal tissue (Fig. 4). Thus it may be concluded that the scarred fascia contained much more of glycosaminoglycans than nor-

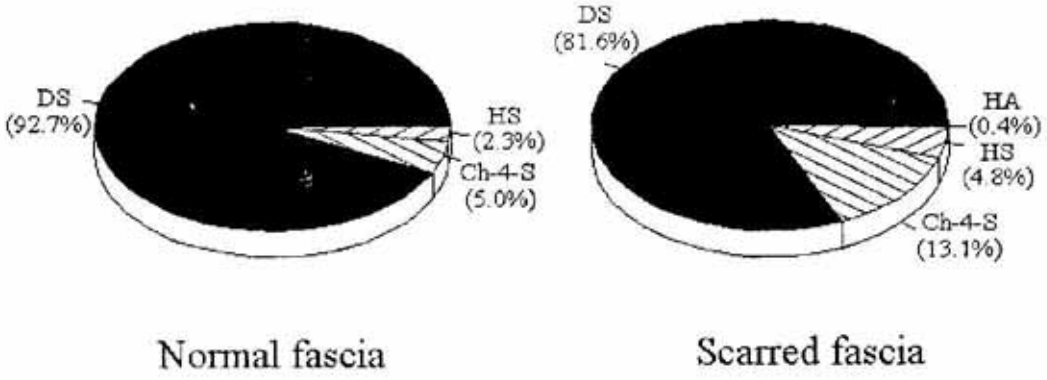

Fig. 2. Proportional contents of fraction II glycosaminoglycans isolated from normal and scarred fascia.
DS, dermatan sulphate; Ch-4-S, chondroitin-4-sulphate; HS, heparan sulphate; HA, hyaluronic acid.

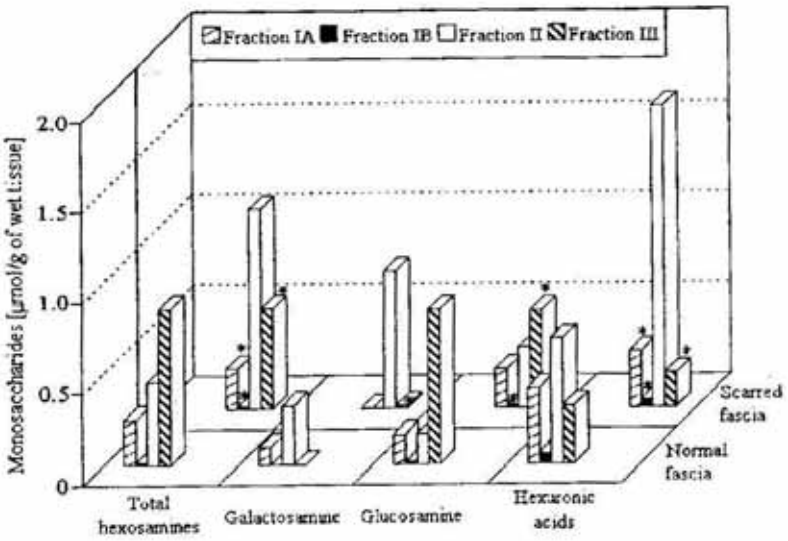

Fig. 3. Glycan monosaccharide content in fraction IA and IB, and glycosaminoglycan monosaccharide content in fraction II and III.
All fractions were isolated from the normal and scarred fascia. * Statistically insignificant.

mal fascia and this lead to an increase in tissue hydration. It should be emphasized that the changes described are not influenced by the post mortem processes. Firstly, acid hydrolases degrading the extracellular glycosaminoglycans are enclosed within the lysosomes, so both the lysosomal and cell membranes separate the enzymes and their substrates [9]. Secondly, lysosomal disruption and cell lysis are the long lasting processes therefore during 24 h after individual death there is a small possibility of the significant GAG desintegration.

Both normal and scarred fascia contain mainly dermatan sulphate and heparan sulphate. In the latter tissue a significant relative increase in the Ch-4-S content was accompanied by a slight decrease in the DS content. A similar shift in glycosaminoglycan pattern in the skin scars was previously described [10–13]. Our results suggest also that the scarred fascia heparan sulphate is characterized by a higher degree of sulphation. It cannot be excluded that this phenomenon concerns another form of sulphated GAG (probably of DS) of the scarred fascia. An increase in GAG sulphation in the skin scars was also observed [14, 15].

The enhancement in proteoglycan biosynthesis in scarred fascia accompanied by alter-

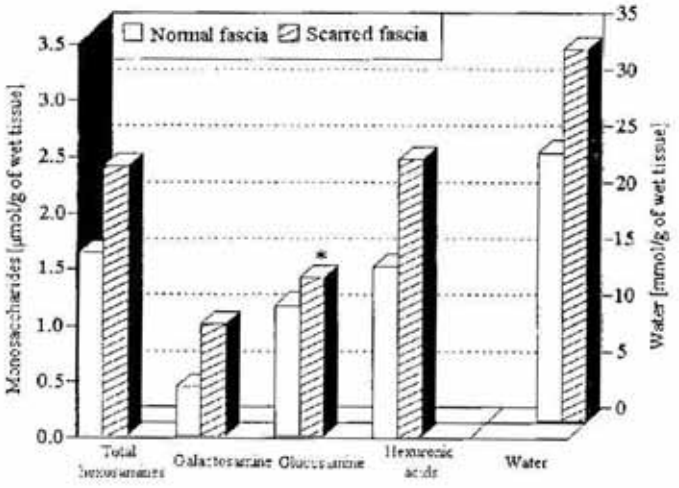

Fig. 4. Total monosaccharide content in four fraction and water content from normal and scarred fascia.
* Statistically insignificant.

ations in the glycan component structure may lead to disturbed interaction between connective tissue fibrillar proteins and proteoglycans, followed by impairment of the physiological functions of fascia.

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