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Fascia and soft tissues innervation in the human hip and their possible role in post-surgical pain

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Abstract

Persistent symptoms, most commonly pain, may remain after otherwise successful hip replacement surgery. Innervation of fascia and soft tissues has become increasingly important in etiopathogenesis of pain, but the relative importance of the various anatomical structures in the hip region is still not known. Innervation of skin, superficial adipose tissue, superficial fascia, deep adipose tissue, deep fascia, muscles, capsule, capsule ligament, ligamentum teres, and tendon in the human hip from 11 patients and 2 cadavers were quantified by staining with anti-S100 antibody for myelin-forming Schwann cells, to obtain the percentage of antibody positivity, density and mean diameter of the nerve fibers. The skin was the most highly innervated (0.73% ± 0.37% of positive area in patients; 0.80% ± 0.28% in cadavers); the tendon was the least innervated (0.07% ± 0.01% in patients, 0.07% ± 0.007% in cadavers). The muscles (vasto-lateral and gluteus medius) were the second most innervated structure according the percentage (0.31% ± 0.13% in living humans, 0.30% ± 0.07% in cadavers), but with only a few nerves, with large diameters (mean diameter 36.4 ± 13.4 μm). Instead, the superficial fasciae showed 0.22% ± 0.06% and 0.26% ± 0.05% of positive areas in living humans and cadavers, respectively. Fasciae were invaded by networks of small nerve fibers, revealing a possible role in pain. The superficial fascia was the second most highly innervated tissue after the skin, with a density of 33.0 ± 2.5/cm^2, and a mean nerve sizes of 19.1 ± 7.2 μm. Lastly, the capsule turned out to be poorly innervated (0.09%), showing that its removal does not necessarily lead to painful consequences. Statement of clinical significance: Deeper knowledge about the innervation of the soft tissue in the human hip joint will enhance study and understanding of the best surgical procedures to follow during hip arthroplasty to reduce post-operative pain.

KEYWORDS

arthroplasty, fascia, hip joint, innervation, pain, proprioception, soft tissue

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1 | INTRODUCTION

Total hip arthroplasty (THA) is currently one of the most common and most successful orthopedic surgical procedures. However, 7% to 15% of postsurgery patients are dissatisfied,[1,2] since they continue to experience post-operative pain which prevents their return to full function and activity.[3-5] According to recent publications, persistent symptoms may remain after an otherwise successful THA. Twenty-six percent of patients were unable to return to sports for reasons related to joint replacement—most commonly pain. The main causes of post-surgical pain are well-known and include failure of fixation, instability, and damage to soft tissues associated with the trauma of the surgical procedure,[6] but the relative importance of the involvement of the various soft tissues around the hip joint is not clear, although an association between residual pain and damage to soft hip tissues after THA has been shown in magnetic resonance imaging studies. Defects of the abductor tendons and fatty atrophy of parts of the *gluteus medius* and *minimus* are seen predominantly in symptomatic patients. Even in asymptomatic patients, some soft-tissue types of damage such as tendonitis, partial tendon tears, and fatty atrophy of the anterior portion of the *gluteus minimus* have been found.[7]

Previous studies have shown that capsule preservation is a question point in arthroplastic hip surgery, involving its mechanical support to the joint,[8] its potential role in proprioceptive perception,[9] and as a pain generator.[10] Haversath et al[11] have already shown the homogenous subdivision of pain- and proprioceptive-associated markers across the joint capsule. Gáspár et al[10] suggested that pre- and post-operative pain in osteoarthritis is not correlated with the density of nerve fibers found in the capsule. Some authors have reported better post-operative satisfaction (Harris Hip Score) in cases in which the capsule was preserved and repaired, with respect to cases in which the capsule was removed, but this difference disappeared 6 months post-operatively.[12] The *ligamentum teres* also seems to play a role as a pain generator and joint lubricator.[13,14] Some studies have highlighted the presence of nociceptive receptors in the *ligamentum teres*[11] and shown evidence of pain due to damage to it, in some traumatic conditions[15] which may improve with arthroscopic debridement.[16]

However, very little is known about the innervation of the deep fascia, superficial fascia, adipose tissue, subdermal tissue, and skin round the hip joint. The presence of nociceptive fibers in fascial tissue has recently been demonstrated: Mense and Hoheisel showed evidence of nociceptors in rat thoracolumbar fascia[17] and Taguchi and coauthors demonstrated that peptidergic and non-peptidergic axons of unmyelinated C-fibers with nerve terminals are distributed in the rat crural fascia.[18] These findings help to explain the non-specific low back pain from a pathologically altered thoracolumbar fascia.[19,20] However it is still not clear whether other fasciae also play a role as pain generators and to what extent they are decisive when compared with other tissues.[21] In the present work, a first step was made using a Schwann cell marker to indirectly identify the presence of neural structures in fasciae and soft tissues. An improved knowledge of the soft tissue innervation of the hip joint may lead to better understanding of the importance of the integrity of periarticular soft tissues and the utility of surgical precautions and preservation.

2 | MATERIALS AND METHODS

2.1 | Sample collection

To evaluate the distribution of innervation of the hip joint, soft tissue samples (approximately 1 × 1 cm) were taken from 11 patients (three men, eight women, mean age 84.2 years, seven samples from the right limb, and four from the left), and two cadavers, both sides, right and left (Table 1), from the following layers: skin, subdermal tissue, superficial adipose tissue, superficial fascia, deep adipose tissue, deep fascia, *gluteus medius* muscle, *vastus lateralis* muscle, *vastus lateralis* tendon, iliac-femoral ligament, hip capsule and round ligament (Figure 3A).

Voluntary patients were recruited at the Orthopedic Clinic of the University of Padova. Samples were taken during surgery for partial hip replacement after traumatic femoral neck fractures, from February to April 2018. All subjects had mild osteoarthritis (grade <1) compatible with age, and were able to walk independently. They were informed about the procedure and gave their consent (Ethics Committee, Study AMOFA 3722/AO/16). Samples from two frozen cadavers were also examined within the "Body Donation Program" of the Human Anatomy Section of the Department of Neurosciences, promoted by the University of Padova. Specimens without pathological lesions (tumors, infections, history of hip surgery, rheumatological diseases) were carefully taken at the lateral and proximal regions of the thigh, following the Watson-Jones antero-lateral route.

2.2 | Immunohistochemistry by S100 antibody

All specimens from living patients and cadavers were immediately fixed in 10% buffered formaldehyde (pH 7.4) at 4°C and then embedded in paraffin. Sections of 6 µm were cut, dewaxed, and treated with blocking of endogenous peroxidases with 0.5% H_2O_2 in phosphate buffered solution (PBS; pH 7.4). After washing three times in PBS, the samples were incubated in blocking solution (PBS + 0.1% bovine serum albumin [BSA]) for 1 hour and then with primary

TABLE 1 Characteristics of the donors

	Living patients	Cadavers
Individuals (n)	11	2
Hips (n)	11	4
Side (right/left)	7/4	2/2
Age, y	84.2 [71-88]	75; 80
Sex (M/F)	3/8	1/1

antibody S100 (Polyclonal rabbit anti S100, Dako) dilution 1:4000, in BSA 0.1% at 4°C overnight. After repeated washing in PBS, incubation was performed with peroxidase-conjugated goat anti-rabbit (Jackson ImmunoResearch) secondary antibody, dilution 1:400, for 60 minutes. After washing in PBS, the reaction was finally developed with 3,3′-diaminobenzidine (Liquid DAB + substrate Chromogen System kit; Dako Corp, Carpinteria, CA), and stopped with distilled water. After nuclei counterstaining with ready-to-use haematoxylin (Dako Corp.), samples were dehydrated and mounted. To confirm the specificity of the reaction, the same protocol was performed on sections without the primary antibody (negative control). Images were acquired by using Leica DMR microscope (Leica Microsystems, Wetzlar, Germany).

2.3 | Image analysis

The Schwann cells marker S100 was used to indirectly identify the presence of neural structures in human samples. S100 positive reaction of specimens was comparatively observed in serial sections stained with immunohistochemical methods, enlargement 10×. Images were acquired through Leica DMR (Leica Microsystems) and computerized image analysis was performed with ImageJ software to quantify the percentage of antibody positivity for S100/entire enlargement of 10×. For superficial and deep adipose tissues, all the background was removed before analysis, due to the high positivity of adipocytes to the S100 antibody.

In addition, a manual count of the number of nerve fibers per area was performed, and the mean diameter of the nerves was calculated in each tissue by ImageJ software. Recognitions of axons by light microscopy can overestimate the diameter and underestimate the number of the nervous fibers, for the possible inaccurate identification of very small fibers. Our analysis permitted however the identification of axons under the 2 μm of diameter.

2.4 | Statistical analysis

Student's *t*-test was performed to verify significant differences among living patients and cadavers ($P < .05$). Tissue innervation (percentage of positivity, density, and mean diameter) were statistically analyzed by one-way analysis of variance (ANOVA) by SPSS software for all 11 layers (from skin to tendon), initially relating to 4 groups (11 living patients and 2 cadaver hips, in the 4 different combinations of the 4 hips from cadavers). Not finding significant differences the ANOVA was made for all 11 layers relating to 15 sample sizes (11 hips of living patients and 4 hips from cadavers). Multiple comparisons were performed with the LSD post-hoc test (95% confidence interval [CI]; $P < .05$).

The presence of bias for axons diameter quantification is replicated in all our analyses, and it does not influence the results from a statistically point of view.

3 | RESULTS

The choice of anti-S100 as neuronal marker permitted to obtain good reactions even in the cadaver samples, being fully comparable with those from living patients.[22-24] In fact, nerve elements were observed in all samples analyzed, and the anti-S100 reaction for myelin-forming Schwann cells showed the same innervation trend in both living patients and cadavers (Figure 1): the *t*-test between them did not produce any significant differences for any tissue (data not shown).

Then the statistical analysis by one-way ANOVA, in all 15 analyzed hip samples, was performed to analyze statistically significant differences in percentage of innervation, density or mean diameter of nerve fibers (Table 2). As expected, the skin was the most highly innervated tissue (0.73% ± 0.37% in patients; 0.80% ± 0.28% in cadavers), and was significantly different from all other tissues examined (Table 2). Instead, tendons were the least innervated (0.07% ± 0.01% in patients, ±0.007%

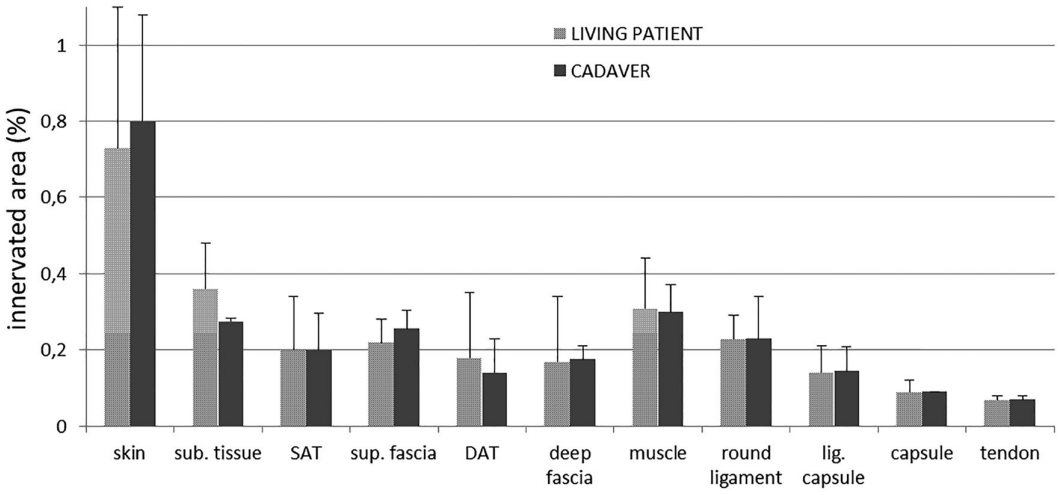

FIGURE 1 Mean percentages of innervated areas of various tissues (skin, subdermal tissue, superficial adipose tissue, superficial fascia, deep adipose tissue, deep fascia, muscle, round ligament, ligament of capsule, capsule, tendon) from living patients and human cadavers

TABLE 2 One-way ANOVA: multiple comparisons for the three parameters (percentage of innervated area, mean diameter of nerves and density) for all 11 layers (from skin to tendon), relating to 15 sample sizes (11 hips of living patients, 4 hips of cadavers)

	Skin	Sub tissue	Sup fascia	SAT	Deep fascia	DAT	Muscle	Round ligament	Ligament of capsule	Capsule	Tendon
Innervated area (%)											
Skin	/	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Sub tissue	**0.0**	/	0.11	0.06	**0.02**	**0.01**	0.61	**0.03**	**0.0**	**0.0**	**0.0**
Sup fascia	**0.0**	0.11	/	0.72	0.49	0.44	0.39	0.80	0.24	0.07	0.11
SAT	**0.0**	0.06	0.72	/	0.77	0.70	0.25	0.86	0.45	0.17	0.20
Deep fascia	**0.0**	**0.02**	0.49	0.77	/	0.93	0.14	0.58	0.63	0.25	0.28
DAT	**0.0**	**0.02**	0.44	0.70	0.93	/	0.12	0.51	0.70	0.29	0.31
Muscle	**0.0**	0.61	0.39	0.25	0.14	0.12	/	0.24	0.06	**0.02**	**0.03**
Round ligament	**0.0**	**0.03**	0.80	0.86	0.58	0.51	0.24	/	0.25	0.06	0.12
Ligament capsule	**0.0**	**0.03**	0.23	0.45	0.63	0.70	0.06	0.25	/	0.45	0.45
Capsule	**0.0**	**0.0**	0.07	0.17	0.25	0.29	**0.02**	**0.06**	0.45	/	0.86
Tendon	**0.0**	**0.0**	0.11	0.20	0.28	0.31	**0.03**	**0.12**	0.45	0.86	/
Mean diameter											
Skin	/	0.35	0.10	0.63	0.09	0.07	0.43	0.07	**0.01**	0.08	0.62
Sub tissue	0.35	/	**0.02**	0.59	**0.02**	0.49	0.79	**0.01**	**0.0**	**0.02**	0.29
Sup fascia	0.10	**0.02**	/	**0.03**	0.62	**0.0**	**0.01**	0.89	0.22	0.69	0.75
SAT	0.63	0.59	**0.03**	/	**0.04**	0.16	0.75	**0.02**	**0.0**	**0.03**	0.44
Deep fascia	0.09	**0.02**	0.62	**0.04**	/	**0.0**	**0.02**	0.68	0.68	0.90	0.57
DAT	0.07	0.49	**0.0**	0.16	**0.0**	/	0.28	**0.0**	**0.0**	**0.0**	0.12
Muscle	0.43	0.79	**0.01**	0.75	**0.02**	0.28	/	**0.01**	**0.0**	**0.01**	0.35
Round ligament	0.07	**0.01**	0.89	**0.02**	0.68	**0.0**	**0.01**	/	0.25	0.76	0.70
Ligament capsule	**0.01**	**0.0**	0.22	**0.0**	0.68	**0.0**	**0.0**	0.25	/	0.52	0.37
Capsule	0.08	**0.02**	0.69	**0.03**	0.90	**0.0**	**0.0**	0.76	0.52	/	0.61
Tendon	0.62	0.29	0.75	0.44	0.57	0.12	0.35	0.70	0.37	0.61	/
Density, n/cm^2											
Skin	/	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Sub tissue	**0.0**	/	0.38	0.18	0.55	0.29	0.12	0.39	0.79	**0.04**	0.10
Sup fascia	**0.0**	0.38	/	0.64	0.77	0.86	0.53	0.86	0.48	0.25	0.47
SAT	**0.0**	0.18	0.64	/	0.44	0.77	0.91	0.47	0.22	0.52	0.84
Deep fascia	**0.0**	0.55	0.77	0.44	/	0.64	0.34	0.86	0.70	0.15	0.30
DAT	**0.0**	0.29	0.86	0.77	0.64	/	0.66	0.71	0.37	0.34	0.60
Muscle	**0.0**	0.12	0.53	0.91	0.34	0.66	/	0.35	0.14	0.55	0.92
Round ligament	**0.0**	0.39	0.86	0.47	0.86	0.71	0.35	/	0.51	0.12	0.30
Ligament capsule	**0.0**	0.79	0.48	0.22	0.70	0.37	0.14	0.51	/	**0.04**	0.12
Capsule	**0.0**	**0.04**	0.25	0.52	0.15	0.34	0.55	0.12	**0.04**	/	0.62
Tendon	**0.0**	0.10	0.47	0.84	0.30	0.60	0.92	0.30	0.12	0.62	/

Note: In the table are reported the *P*-values (CI 95%, significant values $P < .05$ in bold).
Abbreviations: ANOVA, analysis of variance; DAT, deep adipose tissue; SAT, superficial adipose tissue.

in cadavers). The capsule was also very poorly innervated (0.09%, ±0.03 in living patients, ±0.0 in cadavers). The superficial fascia showed more (although not statistically significant) innervation, with respect to the deep fascia (0.22% ± 0.06%, in living patients; 0.26% ± 0.05%, in cadavers, for the superficial fascia; 0.17% ± 0.17% and 0.18% ± 0.04, for the deep fascia). Superficial adipose tissue (SAT) also showed a descriptively higher percentage than deep adipose tissue (DAT), although it was not statistically significant: 0.2% ± 0.14% and 0.2% ± 0.1% of positivity in SAT, 0.18% ± 0.17% and 0.14% ± 0.09%, in living patients and cadavers for DAT. Muscles showed high percentages of positivity to S100 (0.31% ± 0.13% and 0.30% ± 0.07%), whereas the ligament of the capsule was poorly innervated (0.14% ± 0.07% in patients and 0.15% ± 0.06% in cadavers).

In a later analysis, the mean diameter of the nervous structure was studied layer by layer. Table 2 lists the statistical analysis and Table 3 mean values with standard deviations. The highest size values were found for muscle (36.4 ± 13.4 μm), DAT (35.2 ± 7.5 μm), SAT (30.6 ± 6.7 μm) and subdermal tissue (30.1 ± 9.5 μm). The smallest nerves were found in the capsule ligament (15.3 ± 4.0 μm) and deep fascia (15.5 ± 4.7 μm).

Table 4 shows density analysis (mean number of nervous structure per cm^2). The skin had the highest density (64.0 ± 5.2/cm^2), with statistically significant differences ($P < .05$) with respect to all

TABLE 3 Mean diameter (μm) and standard deviations of nervous structure layer by layer (ImageJ software)

	Diameter, μm
Skin	26.9 ± 10.2
Subdermal tissue	30.1 ± 9.5
SAT	30.6 ± 6.7
Superficial fascia	19.1 ± 7.2
DAT	35.2 ± 7.5
Deep fascia	15.5 ± 4.7
Muscle	36.4 ± 13.4
Tendon	19.7 ± 2.6
Ligament of capsule	15.3 ± 4.0
Capsule	17.2 ± 4.9
Round ligament	18.7 ± 5.5

other soft tissues (see Table 2). The superficial fascia was the second innervated tissue (33.0 ± 2.5/cm^2). The capsule and tendon had the lowest density, with mean numbers per cm^2 of 7.5 ± 4.2 and 11.0 ± 0.8, respectively. Generally, subcutaneous tissues (SAT, superficial fascia, DAT, according to the classification of Lancerotto and coauthors[25]) showed a descriptively higher innervation density than deep soft tissues: among the latter, the capsule ligament was the first innervated tissue and the deep fascia the second, with densities of 22.0 ± 5.1 and 19.0 ± 5.0/cm^2, respectively.

Figure 2 shows the overall analysis of size of the nerve bundles and density of innervation layer by layer: SAT, DAT, and muscle had few nerves (14.5 ± 1.6, 15.0 ± 6.3, and 12.0 ± 6.1/cm^2, respectively) but were larger (30.6 ± 6.7, 35.2 ± 7.5, and 36.4 ± 13.4 μm). Instead, the superficial fascia showed the opposite: higher density (33.0 ± 2.5 nerves/cm^2) with smaller size (19.1 ± 7.2 μm). The deep fascia also showed small nerves, with mean diameters of 15.5 ± 4.7 μm. Statistical analysis showed that the difference in the innervation percentages was not significant between muscle and either superficial fascia or deep fascia: however, when the size of the nervous structures was analyzed, the *P*-values were .01 and .02, respectively, showing a significant difference in the diameter of the nerves crossing these tissues (Table 2).

TABLE 4 Mean density (number/cm^2) and standard deviations of nervous structures in each soft tissue of hip joint region

	Number/cm^2
Skin	64.0 ± 5.2
Subdermal tissue	24.0 ± 1.4
SAT	14.5 ± 1.6
Superficial fascia	33.0 ± 2.5
DAT	15.0 ± 6.3
Deep fascia	19.0 ± 5.0
Muscle	12.0 ± 6.1
Tendon	11.0 ± 0.8
Ligament of capsule	22.0 ± 5.1
Capsule	7.5 ± 4.2
Round ligament	17.8 ± 4.2

The subdermal tissue and the skin revealed homogenous mean nerve sizes (30.1 ± 9.5 and 26.9 ± 10.2 μm, respectively), but with very high density in the skin (64.0 ± 5.2 nerves/cm^2). The largest positive structures were Pacini's and Ruffini's corpuscles (data not shown). The round ligament, ligament of the capsule, hip capsule, and tendon showed the same mean nerve diameters:18.7 ± 5.5, 15.3 ± 4.0, 17.2 ± 4.9, and 19.7 ± 2.6 μm, respectively.

The qualitative images (Figure 3) show the innervation distribution and reflect the results described above. For example, the muscles contained very large nerves (Figure 3H), whereas in the fasciae, in both superficial (Figure 3E) and especially deep fascia (Figure 3G), innervation was distributed as a dense network with thin nerve endings. The skin (Figure 3B) and subdermal tissues (Figure 3C) revealed large corpuscles, especially close to the subcutaneous glands and blood vessels. In adipose tissues (SAT and DAT; Figures 3D and 3F, respectively) some large nerves crossed various adipocyte areas. Lastly, the *vastus lateralis* tendon, iliac-femoral ligament, hip capsule and round ligament (Figure 3I-L, respectively) showed totally negative areas, and more innervated areas with small and medium-sized nerves.

4 | DISCUSSION

This work, for the first time, analyses all the soft tissues, from the skin to the capsule, of the hip joint, aiming at better understanding of the amount and distribution of its innervation and its potential role in nociception and proprioception. We believe that, in general, not enough attention is paid to the breaking and removal of certain structures in hip replacement surgery and also in post-operative rehabilitation. For example, the decision to perform a capsulectomy may be based on the surgeon's personal choice according to experience, on the surgical path (which is essential in the postero-lateral pathway) and the need for wider exposure.[26-29]

The first important deduction that we can make starting from our results is that the analysis, by calculating the means of the percentage of the innervated area, is misleading during interpretation of the results. Analysis of the number and size of the nervous structures turned out to be necessary for a clearer interpretation, because each tissue has different functional characteristics and therefore shows differences in innervation.

Surely there are several important limitations of our study. First, the S100 stain is a nonselective neuromarker: it is preferentially distributed in myelin-forming Schwann cells, so it does not differentiate among axon diameter and types of nerves. However, it has been demonstrated a precise and predictable proportionality between the diameter of an axon and the thickness of the myelin that surrounds it,

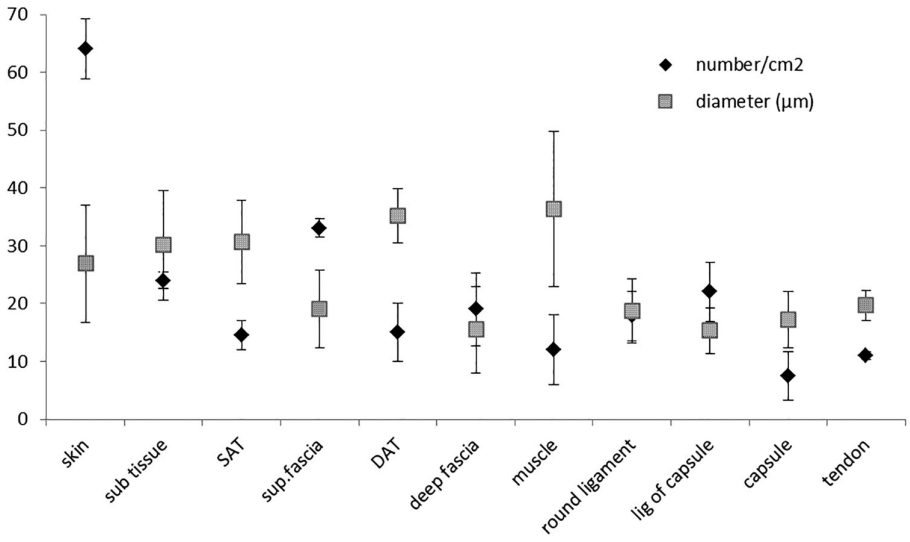

FIGURE 2 Mean number of nerves for each tissue (number/cm^2), and mean size (µm)

by the axonal Neuregulin-1 signals.[30] Second, the use of a marker for the Schwann cells can enable accurate quantification of the number of nerve fibers or the diameter of individual nerve axons. On the other hand, regarding the function of the nerves, no conclusions can be drawn via the S100 marker, if not topographically, based on the diameter and localization of the nerves. Further, this is an exploratory study with a small sample size, specially of cadavers. In order to carry out the *t*-test between living and cadavers we analyzed both sides, right and left, of the two cadavers, to reach the minimum number of four samples to be used for the *t*-test. The anti-S100 reaction showed the same innervation trend in all the hips of the donors without any significant difference in any tissue. These results permitted us to analyze all together the 15 samples by one-way ANOVA.

Despite the study needs to be deepened, our results show that, as expected, the skin is the most highly innervated structure, with a density of 64.0 ± 5.2 nervous structures per cm^2 of tissue: this confirms how important the skin is in its tactile, nociceptive and thermoregulatory roles.[31] The muscles (*vasto-lateral* and *gluteus medius*) were the second most highly innervated tissues, according to the percentages of positivity (0.31% ± 0.13% and 0.30% ± 0.07% in living humans and cadavers, respectively) and the innervation was composed of large nerves bundles (mean number 12.0 ± 6.1/cm^2, mean diameter 36.4 ± 13.4 µm), presumably motor nerves.[32]

Good maintenance of muscular structures during Watson-Jones surgery and along minimally invasive pathways allows better functional recovery and good post-operative proprioception.[33,34]

One of our new results is that the superficial and deep fascia are penetrated throughout by a network of small nerve fibers (33.0 ± 2.5/cm^2, mean size 19.1 ± 7.2 µm in the superficial fascia, and 19.0 ± 5.0/cm^2, mean diameter 15.5 ± 9.4 µm, in the deep fascia). The superficial fascia is presumably rich in autonomic fibers, a point that will have to be explored in the future. The thin but huge network of nerve fibers in all directions confirms its probable role in proprioception, due to the capacity to perceive variations of tension in a complex tridimensionality, integrating signals from longitudinal, transversal and vertical axes.[23] Mense and coauthors[35,36] have repeatedly stated that the deep fascia has a dense sensory nervous network, including nociceptive fibers, and also affirmed that the lack of proprioceptive corpuscular receptors in the thoracolumbar fascia does not preclude its role as a proprioceptive structure, because some free nerve endings may function as proprioceptors. Hoheisel et al[35] also showed that chronic inflammation of the fascia can increase the density of substance P-positive fibers, presumably nociceptive, supporting the role of the deep fascia in peripheral sensitization. These authors have also shown that the thoracolumbar fascia is innervated inhomogeneously, according to layer (inner layer adjacent to the multifidus muscle; middle layer; outer layer). However this is the first work which compares layer by layer, by an absolute value of density of innervation, all the structures of the hip joint region, highlighting its possible role in pain.

Another work analyzed the mean number of free nerve endings per area (1 × 1.5 cm) with anti-S100 antibody, in the human upper limb[24]: the flexor retinaculum turned out to be the most innervated structure (53.55 free nerve endings per area) confirming its perceptive function, whereas the lacertus fibrosus, as a tendinous expansion, was less innervated (27.36 free nerve endings per area), playing a mechanical role in transmitting tension. The bicipital fascia and the antebrachial fascia (48.57 and 44.37 free nerve endings per area, respectively) confirmed their important role in peripheral motor coordination and proprioception. This study was in line with our results that show more innervation in fasciae with respect to tendon structure, according the differing roles of the various structures.

Our results show that it is important to preserve fascial structures carefully during hip surgery. It has been clearly demonstrated that damage to fasciae always causes inflammatory reactions which promote the healing process of collagen fibers, that may be

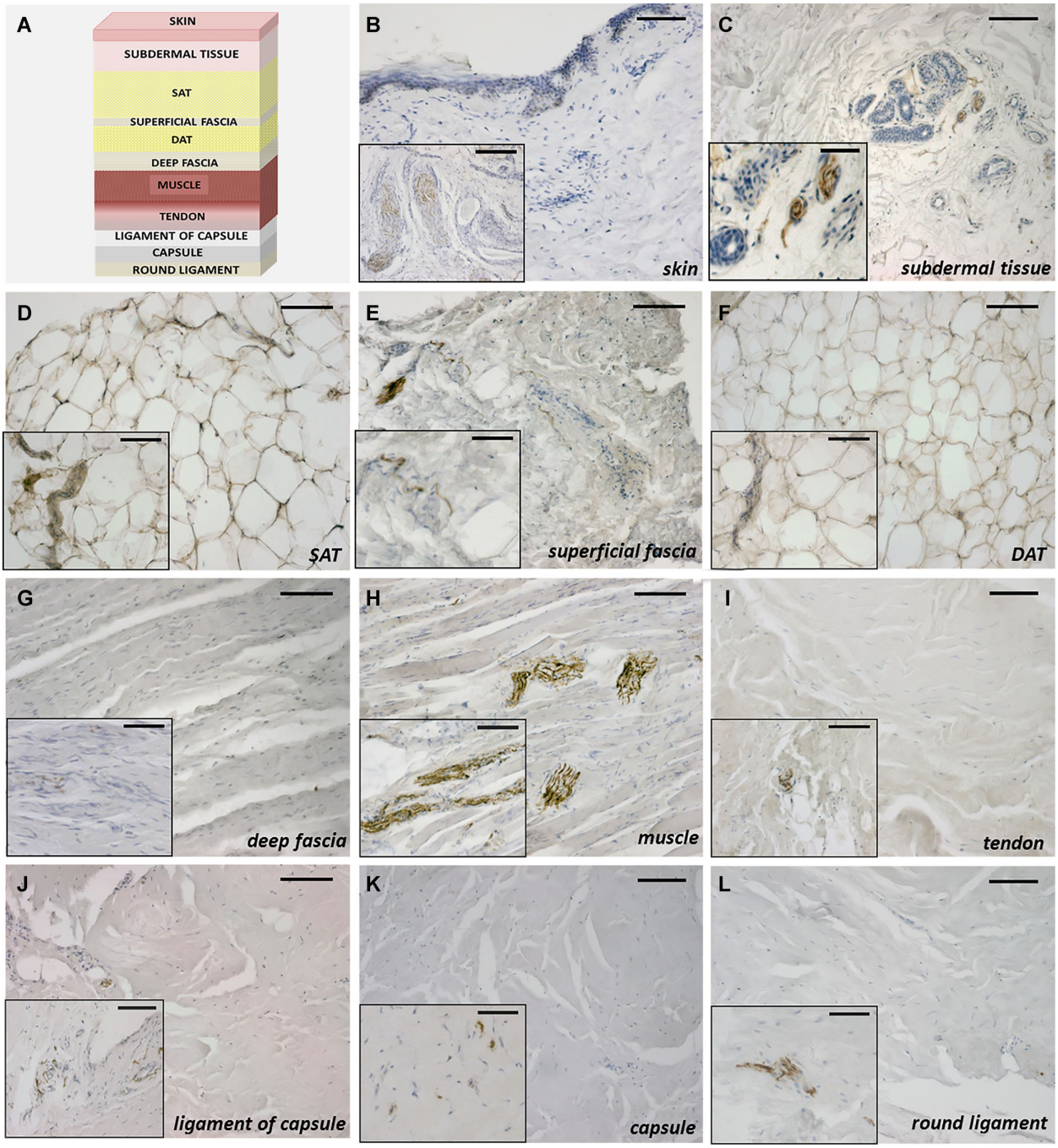

FIGURE 3 Lay-out of soft tissues analyzed in human hip region (A); immunohistochemistry with S100 antibody layer by layer: skin (B), subdermal tissue (C), superficial adipose tissue (SAT, D), superficial fascia (E), deep adipose tissue (DAT, F), deep fascia (G), muscle (*gluteus medius* muscle and *vastus lateralis* muscle, H), *vastus lateralis* tendon (I), iliac-femoral ligament (J), hip capsule (K), and round ligament (L). Scale bars: 50 μm. Insets: more detailed pictures of tissues, scale bars: 25 μm [Color figure can be viewed at wileyonlinelibrary.com]

remodeled and realigned: the fascia is affected by muscle traction because, during contraction, part of the force generated is transmitted to the surrounding fascia along clearcut lines of force.[37] If the fascia is damaged, the collagen fibers may not be remodeled along the correct lines, leading to fibrosis.[38]

As regards other investigated tissue, we can state that the round ligament shows a descriptively high positivity (0.23% ± 0.06% in living patients and 0.23% ± 0.11% in cadavers), due to the greater diameter of the nerves (18.7 ± 5.5 μm) and not to a higher number of positive structures (17.8 ± 4.2/cm^2). Our findings match with those of many

other authors who continue to recommend ligament sutures, especially in pediatric pathologies such as congenital hip dysplasia, rather than their removal, to preserve the proprioceptive component, and also in view of its importance in preventing postsurgery dislocations.[39] The capsular ligament also has a higher percentage of innervation with respect to the capsule itself (with a statistically different density of innervation), confirming that the ligaments can play a role in proprioception.

Instead, the capsule and the tendon, show the smallest ranges of innervation (less than 0.1%), with low densities per area: $7.5 \pm 4.2/cm^2$ and $11.0 \pm 0.8/cm^2$. The tendon must be preserved without any damage, due to its essential mechanical functions,[40] but low innervation may demonstrate the lesser role of this structure as pain generator. As regards the capsule, Hughes et al[29] suggest maintaining the capsular structure as well, improving proprioception and relieving pain, especially during the first postsurgery weeks. However, our results confirm that capsulectomy in hip replacement surgery does not particularly influence patients' pain, matching the results of Gáspár et al[10] who evaluated 22 joint capsules excised after arthroplasty surgery. They identified nerve fibers in only 16 capsules, without correlation of postsurgery pain, and suggested that pain due to osteoarthritis of the hip was not related to the density of nerve fibers in the joint capsule: the density of innervation did not show any significant correlation with scores of pre- and post-operative pain, disability, or changes in scores. Instead, we emphasized the fact that, until now, little importance has been given to the subcutaneous fascial tissues: they are crossed by a dense nervous network and therefore deserve attention and further in-depth studies to understand their role in the autonomic system, in proprioception and in pain.

AUTHOR CONTRIBUTIONS

All the authors have made their contribution to this work and agree with the contents of the manuscript, approving the final version. CF wrote the work and is responsible about immunostaining technique and data analysis; AP contributed to data analysis and editing of the manuscript; LP contributed to all staining techniques and images acquisition; CF and CP contributed to sample processing and images acquisition; CB was responsible for sample collection and contributed to editing the manuscript; RDC worked on data interpretation and editing the manuscript; CS was responsible for research design and work coordination, and revised the whole manuscript.

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