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ORIGINAL ARTICLE

Layers of the abdominal wall: anatomical investigation of subcutaneous tissue and superficial fascia

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Abstract
Introduction In recent times new surgical approaches have been developed, in which subcutaneous tissue is the primary object, such as flaps and fat removal techniques, but different descriptions and abundance of terminology persist in Literature about this tissue.
Aim and methods In order to investigate the structure of abdominal subcutaneous tissue, macroscopic and microscopic analyses of its layers were performed in 10 fresh cadavers. Results were compared with in vivo CT images of the abdomen of 10 subjects.
Results The subcutaneous tissue of the abdomen comprises three layers: a superficial adipose layer (SAT), a membranous layer, and a deep adipose layer (DAT). The SAT presented fibrous septa that defined polygonal-oval lobes of fat cells with a mean circularity factor of 0.856 ± 0.113. The membranous layer is a continuous fibrous membrane rich in elastic fibers with a mean thickness of 847.4 ± 295 μm. In the DAT the fibrous septa were predominantly obliquely-horizontally oriented, defining large, flat, polygonal lobes of fat cells (circularity factor: mean 0.473 ± 0.07). The CT scans confirm these findings, showing a variation of the thickness of the SAT, DAT and membranous layer according with the subjects and with the regions.
Discussion The distinction of SAT and DAT and their anatomic differences are key elements in modern approaches to liposuction. The membranous layer appears to be also a dissection plane which merits further attention. According with the revision of Literature, the Authors propose that the term "superficial fascia" should only be used as a synonym for the membranous layer.

Keywords Superficial fascia · Hypodermis · Scarpa's fascia · Camper's fascia · Abdominoplasty · Liposuction

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Introduction

Subcutaneous tissue has recently been a subject of increasing interest. New surgical approaches have been developed in which subcutaneous tissue is the primary object, such as flaps in Plastic Reconstructive surgery, and fat removal techniques in Aesthetic surgery (abdominoplasty procedures [19] and liposuction [8, 13]), and this has led to a need for deeper knowledge of a structure which is no longer something simply to be cut through.

Subcutaneous tissue is in fact one of the organs of the human body, the structure of which has only received limited attention by anatomists in the last century, and regarding which a large gap still persists between anatomists and surgeons.

The empirical view of abdominal subcutaneous tissue, generally assumed to be well-known in surgical circles, is that of two layers of fat with differing macroscopic structure of its lobes, divided by a layer of membranous tissue (Fig. 1) [9]. Anatomical descriptions range from that of a single layer of adipose tissue, reported in most common textbooks and atlases [21, 26, 28], to more complex reports by some authors, with varying definitions of layers and terminology. This situation, however, seems to have ancient roots, as it was already lamented in the early decades of the nineteenth century [7].

The confusion surrounding subcutaneous tissues is closely connected with that on the so-called superficial fascia. As clearly described by Wendell-Smith [32], the use of the term "superficial fascia" (or fascia superficialis) is often improper and inconsistent. Because of this, the international consensus forum for agreement on official anatomical definitions abandoned this widely used and widely abused name in the latest edition of the *Termina Anatomica* [32].

Another terminological problem derives from the custom of calling some fasciae by the name of the author who first described the anatomical element in question: Antonio Scarpa described a fascial layer in the abdominal wall [29, 30], Colles described one in the perineum [5], Camper is believed to have described another [3], to quote only the best-known examples. To add to the confusion, following a single author's interpretation, the term "superficial fascia" could or could not be considered synonymous with fascia of Scarpa, Colles and Camper, etc. [17, 18, 33].

The result is that several different terms are now used to define the same structure or, vice versa, the same term is used for more than one structure by different authors [1, 32], and textbooks may even be found quoting one term with several different meanings [21].

A solution to the problem concerning both the structure and the terminology of the supra-muscular layers of the trunk wall may be beneficial in both research and clinical practice, allowing easier exchange of information between different fields and authors.

With this aim in view, we conducted a macroscopical and histological examination of the abdominal subcutaneous tissue, accompanied by in vivo studies with CT imaging techniques, to clarify its precise structure and to verify the existence in it of any fibrous layer.

Materials and methods

Macroscopic dissection

Macroscopic and microscopic analyses of the layers of the subcutaneous tissue of the abdominal wall were performed in 10 cadavers of differing physical constitution (4 men, 6 women; mean age 69 years; age range 48–93 years). The cadavers were neither embalmed nor frozen prior to examination. One side of the abdomen was dissected according to the following protocol. A xypho-pubic incision of the skin was performed along the para-median line on either the left or right side, randomly, exposing the lobes of subcutaneous tissue. Keeping the skin margins slightly raised and beginning at the umbilical level, the incision was deepened until the honeycomb structure of the subcutaneous tissue was found to be interrupted by a continuous whitish layer of membranous consistency. The superficial aspect of this layer was used as a dissection plane and followed laterally and medially, cranially and caudally, as far as the inguinal ligament of Poupart, the iliac crest, the inferior border of the thorax, and the linea alba, and the skin with the fat flap superficial to it was removed. A cut was then made through the membranous layer, again along the para-median line, exposing the fatty tissue beneath it. The deep aspect of the membranous layer was identified as a dissection plane, and followed caudally and laterally as far as the inguinal ligament of Poupart. Lastly, the adipose tissue thus exposed was removed,

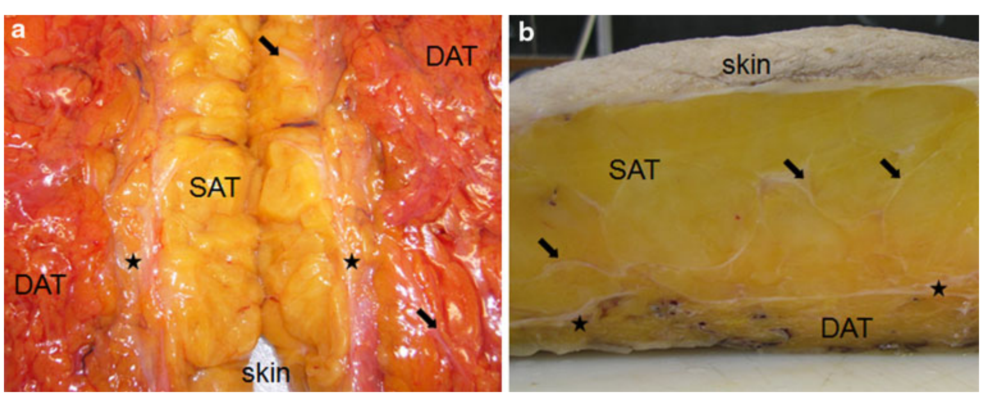

Fig. 1 "Surgical" description of abdominal subcutaneous tissue; **a** fresh full thickness specimen, reversed and cut perpendicularly to skin; **b** slice of formalin-fixed specimen. *SAT* superficial adipose tissue, *DAT* deep adipose tissue, *stars* membranous layer, *arrows* retinacula cutis

exposing the plane of the deep fascia over the muscles of the abdominal wall.

On the other side of the abdomen, full-thickness samples of subcutaneous tissue from the skin to the deep fascia were taken, as follows: (1) over the rectus sheath in the epigastrium; (2) over the rectus sheath in the hypogastrium; (3) along the mammary line in the epigastrium; and (4) along the mammary line in the hypogastrium. Samples were oriented, mounted on cardboard to avoid deformation artefacts, and fixed in a 10% formalin solution.

Histological study

Samples were embedded in paraffin and 10-μm thick sections were obtained and stained with hematoxylin-eosin, azan-Mallory and Weigert's stain for elastic fibers. Morphometric evaluation was performed with image analysis software (Qwin Leica Imaging System, Cambridge, UK). The following parameters were recorded: mean thickness of the subcutaneous tissue and membranous layer, and mean areas and circularity factors of the adipose lobules.

Further anatomical evaluations were performed on specimens from the collection of the Institute of Anatomy, to which some of the authors belong. They consist of transverse slices of three whole human bodies (2 males, 1 female, aged 3, 56 and 62 years), 5–10 cm thick, preserved in Kaiserling's solution. In these specimens, the presence of a membranous layer and its relationships with the skin and underlying tissue were analyzed.

Radiological study

CT images of the abdomen of 10 subjects (4 males, 6 females; age range: 25–62 years, mean age 45 years) were analysed. Five subjects had a mean Body Mass Index (BMI) of 22.3 kg/m^2 (normal weight), and 5 a mean BMI of 34.2 kg/m^2 (obese).

The CT images were obtained from a 16-slice multi-detector CT scanner (Lightspeed16; General Electric Medical System; Milwaukee, WI, USA) with the following parameters: group 1; rotation time, 0.6 s; thickness, 5 mm; speed, 9.37 mm/rotation; rows, 16; interval, 5 mm; S-field of view, head; kV 140; mA, 310. The data were transferred to a Sun workstation and 3D reconstructions (volume rendering technique, maximum intensity projection, multiplanar reconstructions) were processed with a volume analysis program (Voxtool 3.0.54, Voxtool General Electric Medical Systems).

In each subject, we analyzed the different thickness of the two layers of the hypodermis and the membranous layer at four levels, from T10 to the femoral head. For each level, transversal sections were divided into eight sectors measured independently. For integrated anatomo-clinical evaluation, the CT images were examined jointly by two of the authors, specialists in Radiology (V.M.) and Orthopaedics (C.S.).

Statistical analysis

Results are expressed as mean values (± SD) and/or minimum and maximum values. Statistical analysis was performed with the Kruskal–Wallis test and Dunn's multiple comparison test. A $P < 0.05$ was considered to be statistically significant. Statistical calculations were carried out with Prism 3.0.3 software (GraphPad Software Inc., San Diego, CA, USA).

Results

Macroscopic dissection

Under the dermis, a first layer of adipose tissue was identified (Superficial Adipose Tissue, SAT) (Fig. 2a). It was formed of large fat lobes encased between fibrous septa in a honeycomb-like structure, and presented nearly constant characteristics throughout. These septa (retinacula cutis superficialis) appeared well-defined, mostly oriented perpendicular to the surface and mechanically strong, anchoring the dermis to the deeper planes. The fat lobes were organized in single to multiple layers, depending on fat content and thickness of the SAT in the subject. No clear border to the layer could be identified either caudally or cranially, as it appeared continuous with similarly structured layers over the inguinal ligament into the thigh and cranially into the thorax. The SAT had high structural stability and elastic properties. The fat lobes returned to their original position and shape after displacement during compression tests, performed by placing a 1-kg weight over slices of the flap and then removing it.

After removal of the SAT, a fibrous layer with a membranous appearance, apparently continuous and well organized macroscopically, was evidenced (Fig. 2). It could be followed as a dissection plane from the thorax to the inguinal ligament. It did not appear to be uniform in thickness: it was a well-defined white layer in the lower abdomen, thickening toward the inguinal ligament, where a multi-layered structure of collagen bundles following different directions was perceptible, both during dissection and in transparency after isolated raising. It lost consistency in the upper abdomen, where it was identified as a much thinner translucid collagen layer, through which adipose tissue could be seen. This membrane fused medially to the linea alba, caudally to the inguinal ligament and the osseous prominence of the iliac crest (Fig. 2b), and cranially continued into the thorax. In the lower abdomen

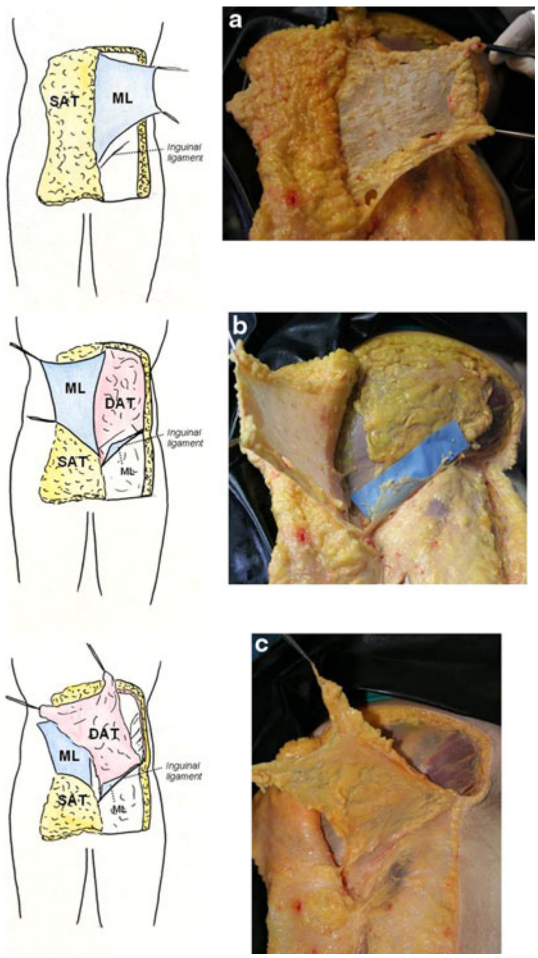

Fig. 2 Fresh cadaver dissection. Demonstration of three layers of abdominal subcutaneous tissue raised as flaps; **a** superficial adipose tissue (SAT) is overturned medially and membranous layer is isolated as a flap; **b** main part of membranous layer (ML) is overturned medially; a smaller part of it is isolated, leaving its connections to inguinal ligament (highlighted with *blue* card underneath); **c** deep adipose tissue (DAT) raised as a flap. Note different appearance of SAT, with well-defined lobes, and of DAT. Abdominal SAT is in continuity over inguinal ligament with thigh SAT, whereas DAT is interrupted by anchorage of membranous layer to inguinal ligament

on the bisiliac line, the mean resistance to traction of this fibrous layer was 2.8 kg in a transversal direction and 5.5 kg in a cranio-caudal direction.

After removal of the fibrous layer, another layer of adipose tissue was found (Deep Adipose Tissue, DAT) (Fig. 2c). It appeared very different from the SAT, particularly as its fat lobes were smaller, flatter and less well-defined, and the fibrous septa were less consistent and mostly obliquely oriented. This adipose layer showed significant variations in terms of thickness between different areas. Toward the points at which the membranous layer of the subcutaneous tissue adhered to salient structures (inguinal ligament, bony prominences, linea alba), the DAT became thinner and tended to progressively reduce its fat component, while the network of collagen fibers (retinacula cutis profunda) became stronger and more tightly packed, connecting the deep aspect of the membranous layer to the deep fascia. The DAT also showed great variations between individuals in terms of both fat content, and the thickness and strength of the retinacula cutis, which appeared partly attributable to different constitutions. Under mechanical testing, as had been done for the superficial layer of adipose tissue, the fat lobes showed a tendency toward displacement, with a limited capacity for regaining their original relationships, so that the structure more easily became disgregated. The obliquity of the septa of this layer, limited elastic properties and strength, with easy lateral displacement of lobes, explain the sliding of the subcutaneous tissue over the deep fasciae. Overall, this layer, although with greater difficulty due to the low mechanical strength of the network of membranes in which the fat lobes were immersed and to varying thicknesses, could also be raised and isolated from the underlying deep fascia which covers the muscles of the abdominal wall.

Its removal gave direct access to the plane of the superficial aspect of the deep fascia.

No further major structure could be identified in the thickness of either the DAT or SAT, apart from slightly thicker isolated oblique septa in the DAT.

As regards the lines of adherence of the membranous layer, as dissection continued in nearby regions, the superficial layer of adipose tissue was found to be in total continuity with a layer corresponding to it in terms of characteristics in the thigh and gluteus, and a similar continuity was found between the two hemi-abdomens over the linea alba. In these areas of transition, the most remarkable feature was strengthening of the retinacula cutis. Instead, the DAT compartment was clearly defined along the lines of adherence identified for the membranous layer, so that the deep adipose compartment of the abdomen appeared to be isolated from that of the thighs.

Histological study

In all the full-thickness specimens, constant morphology of the subcutaneous tissue was seen, organized in the following layers, beginning from the surface: [*skin* (*epidermis and dermis*)], superficial adipose tissue (SAT), fibrous horizontal layer of connective tissue (membranous layer), deep adipose tissue (DAT), [*deep fascia, abdominal wall muscles*].

The superficial adipose tissue presented fibrous septa (retinacula cutis superficialis) connecting the dermis with the membranous layer. These septa defined polygonal-oval lobes of fat cells with a mean circularity factor of 0.856

(±0.113) (Fig. 3), made up of collagen fibers and abundant elastic fibers.

The membranous layer was a fibro-elastic tissue in which elastic fibers were abundant and well organized, and showed an undulating course (Fig. 4). Overall, it had a mean thickness of 847.4 ± 295 µm, but this increased in a proximo-caudal direction, with a mean value of 551 µm in the epigastrium, and 1,045 µm in the hypogastrium. Histologically, the membranous layer is formed of various sublayers of fibro-elastic tissue with a mean thickness of 66.6 ± 18.6 µm, with points of interconnection between the layers. Irregular islands of thin sublayers of fat cells (mean thickness 83.87 ± 72.3 µm) may be deposited between layers of collagen fibers. Therefore, while macroscopically the membranous layer appears and can be isolated as a well-defined membrane, microscopically its structure is better described as lamellar, or like a tightly packed honeycomb.

In the deep adipose layer, the fibrous septa were predominantly obliquely-horizontally oriented (retinacula cutis profonda), connecting the membranous layer to the deep fascia of the rectus abdominis or external oblique muscle, defining large, flat, polygonal lobes of fat cells (circularity factor: mean 0.473, SD: 0.07) (Fig. 3).

Vascular and nervous structures were observed in the deep adipose layer and crossing the membranous layer.

Radiological study

In axial CT scans, a relatively hyperdense tortuous line (the membranous layer) was identified within the context of hypodense fibro-adipose tissue, and appeared as a continuous line from the spine to the linea alba (Fig. 5), dividing the subcutaneous tissue into two layers and confirming macroscopic observations: superficial and deep. At CT, the membranous layer had a mean thickness of 2.31 (±0.6) mm, but varied according to subject: in particular, in obese subjects, it measured 3.19 (±0.8) mm and in normal-weight ones 1.43 (±0.8) mm. The thickness of the membranous layer also changed in the various sectors of the transversal sections, being thicker in the dorsal region (peak values of 4.41 mm in obese subjects and 2.1 mm in normal-weight ones) rather than in the anterior region (peak values 1.52 and 0.95 mm, respectively). No significant differences among the examined levels in the cranio-caudal direction were identified.

SAT and DAT showed different behavior in terms of local accumulation. In the SAT, thickness was quite uniform all round the trunk. The DAT tended to be thin anteriorly, and especially antero-laterally over the obliquus externus muscle, and presented maximum thickness postero-laterally at the level of the flanks, where a kind of “fat accumulation pouch” was found. In addition, both SAT and DAT thickness varied according to subject: in obese subjects, the SAT had a mean thickness of 17.18 mm (range 6–35 mm) and the DAT 18.5 mm (range 10–35 mm); in normal-weight subjects, the values were 3.66 mm (range 1–10 mm) and 3.14 mm (range 0.5–8 mm), respectively. In both the slim and obese, the DAT thickness significantly increased progressively, from T10 to the femoral head, whereas SAT thickness increased in the same direction only in obese subjects, and not in slim ones.

Discussion

According to our observations, three layers can be identified under the dermis in the subcutaneous tissue of the abdominal region: a superficial adipose layer (SAT), a membranous layer (ML), and a deep adipose layer (DAT). Beneath these layers lies the deep fascia enveloping the abdominal wall muscles. This simple description of the subcutaneous tissue of the abdomen finds good correspondence with that reported by several authors [1, 10–12, 18, 21–25, 27], but is apparently in strong contrast with that of others [2, 6, 14, 15, 16, 31]. This may most probably be attributed to variations in the consistency of the deep compartment more than to the membranous layer, which we never observed, although, microscopically, we did

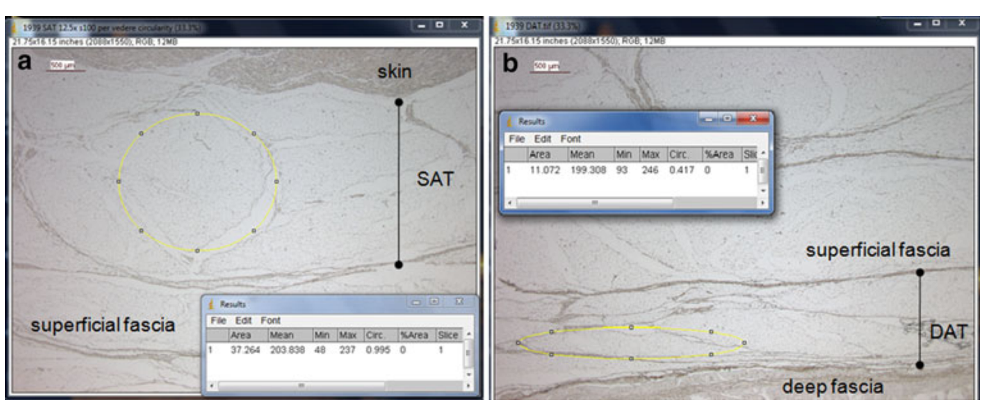

Fig. 3 Calculations of circularity factor for fat lobes of SAT (**a**) and DAT (**b**)

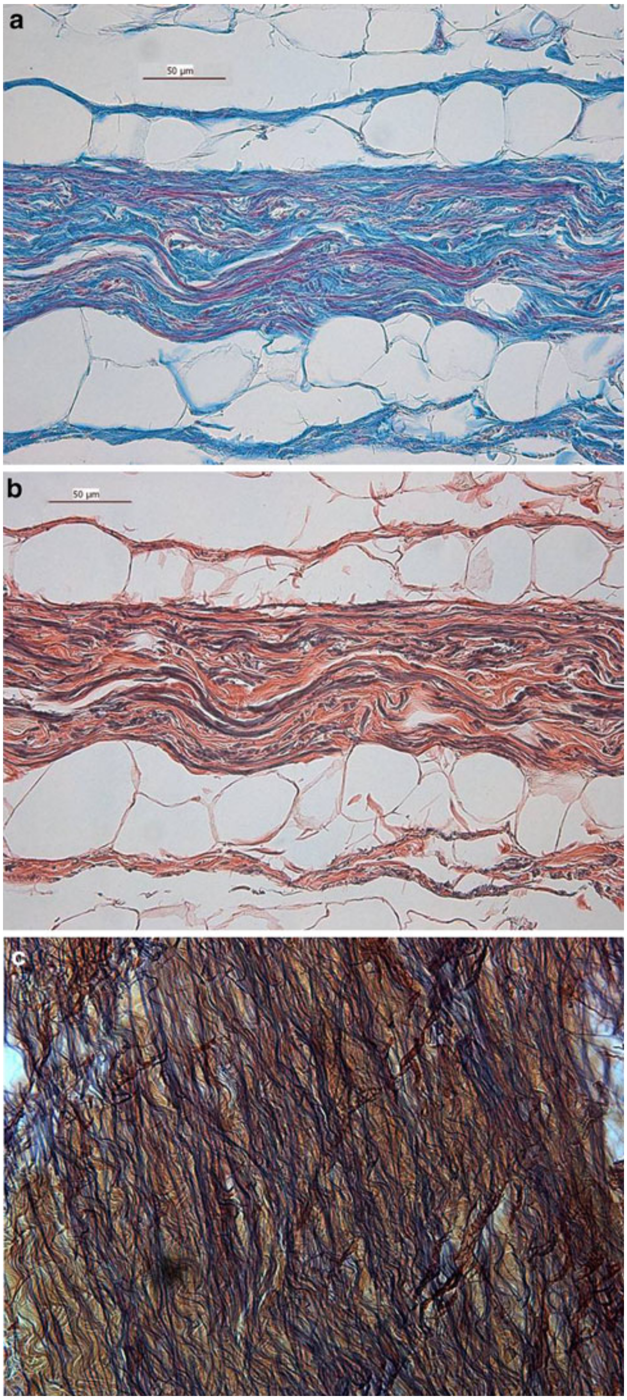

Fig. 4 Histology of membranous layer; **a** (azan-Mallory) and **b** (van Gieson): perpendicular sections of membranous layer. Note richness in wavy elastic fibers (stained *red* in **a**, *black* in **b**) and presence of a few adipose cells in thickness of membranous layer. **c** transversal section of membranous layer, van Gieson stain: extreme richness in wavy elastic fibers (stained *black*) which all appear to be oriented in a preferential direction

observe moderate infiltration of the membranous layer by small fat lobes. Besides, the various descriptions of the subcutaneous tissue deriving by radiologic studies (ecography in the first, CT and MR in the second) could be related to artifacts when interpreting the images (e.g., the multi-layering of the membranous layer may be due to the appearance and disposition of the fibrous septa, which may appear thicker than they really are in a single slice). A similar bias may affect cross-sectional studies, which do not take into account the 3D deformation to which the membranous layer is subject in some regions, due to either gravity and strength of retinacula cutis, or to the adiposity of the lobes, besides the fact that, in some regions of the body, the superficial and/or deep compartments may indeed lose their adipose component.

Our results report a great discrepancy between the thickness of the membranous layer measured on histological sections and on CT images. This could be probably due to the fact that the treatments done to obtain the histological sections implies a dehydration of the tissue, and so the obtained measures could underestimate the real thickness of the membranous layer. At the contrary, the low resolution of the method of measurement of the CT images and the strong hyperintensity of the membranous layer respect to the adipose tissue could give overestimate values.

The clear differentiation of the subcutaneous tissue into two layers, with a membranous layer interposed, is an important feature in the surgical approach to the subcutaneous tissue. The membranous layer, the DAT and SAT create a sliding system that absorbs the mechanical stimulations applied to the skin or generated by the muscular contractions. In this way the subcutaneous tissue guarantees an autonomy between the skin and the muscles, and so between the exteroception (depending to the skin receptors) and the proprioception (depending to the receptors inside the muscles and deep fascia). It is probable that a scar could create an adhesion between skin, membranous layer and deep fascia, and so every muscular contraction could propagate also to the skin, activating the cutaneous receptors. Vice versa, every stimulation of the skin could be transmitted to the underlying structures. This may explain the importance which correct layer reconstruction of the subcutaneous tissue has in affording only slight complications after closure of abdominal surgery wounds.

Further studies are necessary to evaluate the relationships of the nerves and vessels with these planes, i.e., to understand if the perforating vessels pierce the superficial fascia or if they branch in this layer before spreading their distal terminals in the subdermal plexus, and if the nerves run axially in the plane of the superficial fascia or not.

The membranous layer appears to be a dissection plane which merits further attention. It is indeed an important plane, according to some authors specializing in abdominoplasty techniques [20]. But, as it separates two different layers, and is a constrained passage for vessels and nerves

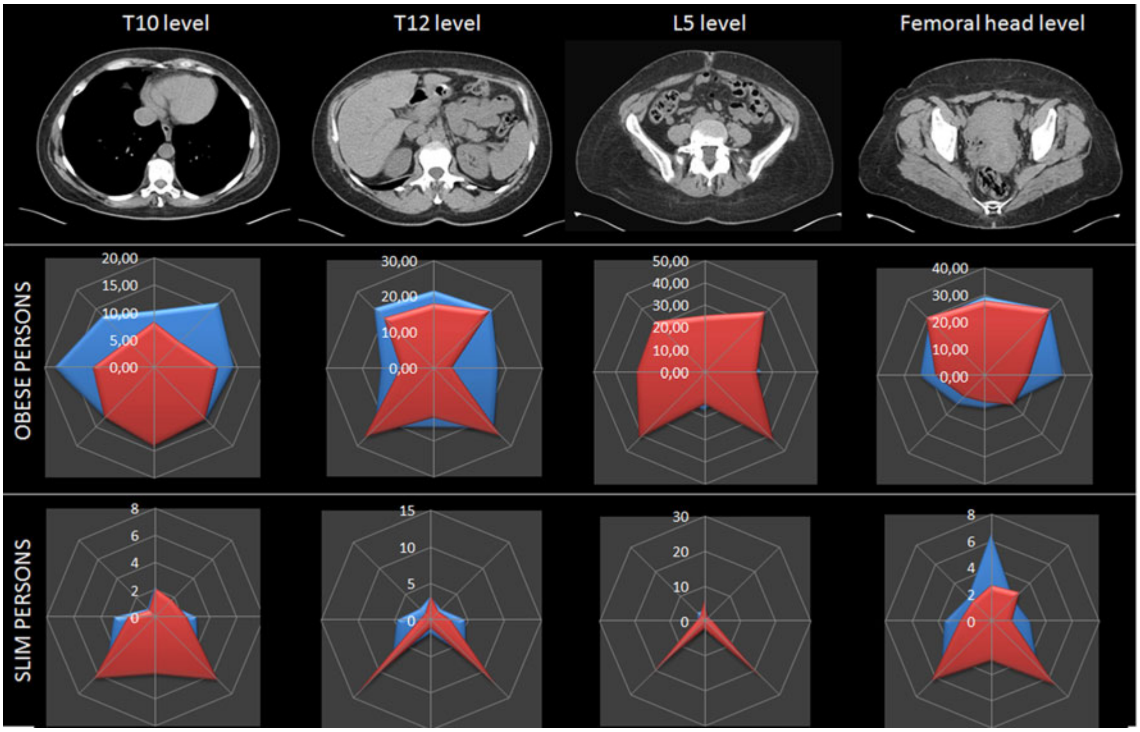

Fig. 5 CT scans of abdominal subcutaneous tissue. Graphs show mean thickness of two layers of subcutaneous tissue and of membranous layer at four levels, from T10 to femoral head. *Blue* area: thickness of DAT; *red* area: that of SAT. In obese subjects, DAT at T10 level is thicker in anterior region and in slim subjects SAT is thicker in posterior region. At T12 and L5 levels in obese subjects, DAT is homogeneously thick at all points, whereas in slim subjects SAT is usually little represented, except at points 4 and 6, where it noticeably thickens. At level of femoral head in obese subjects, both SAT and DAT are thicker in anterior region than in posterior one, whereas in slim subjects SAT is thicker in posterior region

going from one layer to the other, it may also offer new solutions in flaps surgery, i.e., with adipocutaneous propeller flaps superficial to the DAT, or isolated ml-DAT flaps. In addition, the distinction of SAT and DAT and their anatomic differences are key elements in modern approaches to liposuction as "liposculpture". It is well-known that, in order to reduce irregularities, the two should be approached differently, with large cannulas for aspiration of DAT and thin ones for SAT. However, the histological characteristics of the septa and of the membranous layer, with their richness in elastic fibers, may also be important in the "contraction effect" of the skin resulting from inflammatory phenomena involving collagenous structures caused by liposuction, particularly with the Ultrasound Assisted technique.

The richness and organization of the elastic component of the membranous layer suggests that it plays a major role in the mechanical homeostasis of the subcutaneous tissue, and perhaps also participates in the balancing of outward directed abdominal pressure.

An interesting point which should be further investigated is the variation in the membranous layer in slim and obese people. Although the membranous layer appears to undergo a duplication in thickness with obesity, the fat layers both quadruplicate, and the thickness and density of the retinacula is reduced as the fat lobes increase in size, leading to altered anatomical and mechanical relations. This may have major implications according to modern theories on the "adipose organ", which stress the importance of relations with the septal structures and nearby tissues in determining the functions and metabolism of adipocytes [4].

In conclusion, according to our observations, three layers should be identified in the subcutaneous tissue of the abdomen: a superficial adipose layer (SAT), a membranous layer, and a deep adipose layer (DAT). The term "superficial fascia" should only be used as a synonym for the membranous layer, and all other eponyms for it should be abandoned. Indeed, comparison with the works of other authors covering other regions of the body suggests that, besides regional physiological variations, this scheme may be considered as generally describing the structure of the subcutaneous tissue throughout the body.

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Conflict of interest The authors declare that they have no conflict of interest.

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