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INVESTIGATION OF VISCERAL ADIPOSE TISSUE (VAT) SURFACE USING MICROSCOPIC METHODS

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In this study, optical microscopy (OM) and scanning electron microscopy (SEM) were used to characterize visceral adipose tissue (VAT) samples collected from volunteer patients with obesity. The focus was on examining the relationship between the morphological features of VAT samples from individuals with and without metabolic diseases. The microscopic images provided insights into the key morphological characteristics of VAT layers in obese individuals. The OM images of adipose tissue from visceral layers of various obese patients with metabolic diseases revealed the formation of lipid droplets of varying sizes and oval shapes. The SEM micrographs of VAT samples from obese patients with metabolic diseases showed the presence of single circular formations, voids, and other distinct morphological features. The OM and SEM findings could potentially be used to assess metabolic symptoms and predict various diseases in patients with obesity.

Keywords: visceral adipose tissue, obesity, metabolic disorders, optical microscopy, scanning electron microscopy

1. Introduction

Adipose tissue is traditionally viewed as a storage depot for excess energy, primarily stored as triglycerides. Once considered a passive organ, adipose tissue has more recently been recognized as an active endocrine organ with significant physiological roles [1–5]. Composed mainly of adipocytes, along with pre-adipocytes, macrophages, endothelial cells, fibroblasts and leukocytes, adipose tissue is increasingly acknowledged as a key player in systemic metabolic regulation [6–10].

Adipose tissue can also be classified based on its location in the human body [11, 12]. Subcutaneous adipose tissue (SAT) lies just beneath the skin, while preperitoneal adipose tissue (PAT) is located in front of the peritoneum. Visceral adipose tissue (VAT) surrounds organs and can interfere with their function. However, there is still conflicting information regarding the relative importance of characterizing SAT, PAT, or VAT. It remains unclear which specific changes in human adipose tissue layers are most significant for predicting metabolic diseases. Nevertheless, an increasing number of studies suggest that the investigation of VAT is crucial for understanding obesity and its impact on the development of various human diseases [13–15]. For example, one study [16] highlighted gender differences in visceral obesity among patients with hepatitis C virus infection. Brown et al. [17] found that VAT is correlated with several biomarkers related to glucose homeostasis, inflammation, and lipid metabolism. Additionally, the quality and quantity of VAT are significant factors in adipocyte function and are linked to insulin resistance [18]. A relationship between VAT and the consumption of sugar-sweetened beverages has also been established [19]. The accumulation of excess VAT plays a distinct and important role in the pathophysiology of heart failure, especially in women [20], as well as in cardiovascular disease risk, type 2 diabetes [21], and even cancer risk [22]. Some findings offer new insights into VAT's role in mediating obesity-induced endothelial dysfunction [23].

Recent studies have demonstrated that the scanning electron microscopy (SEM) characterization of adipose tissue is a crucial step for potentially predicting the onset of symptoms associated with various diseases. The morphology of adipose tissue samples analyzed by SEM has been investigated in several studies [12, 24–31]. It has also been shown that a simple method of thawing cryo-stored samples effectively preserves ultrastructural features for electron microscopy analysis [27]. The microstructural characteristics observed in different layers of lyophilized adipose tissue clearly indicate that the specific area of thickening and scarring of connective tissue in obese patients with various metabolic diseases is significantly larger than in obese, healthy individuals [12, 26, 28]. Notably, the most distinct microstructural features are found in the visceral layers of adipose tissue in human body [30, 32]. Therefore, the main aim of this study was to conduct a thorough morphological investigation of visceral adipose tissue (VAT) samples taken from the obese patients with and without metabolic disease using optical microscopy (OM) and scanning electron microscopy (SEM). The comparison of OM and SEM measurements revealed unique surface morphologies in the visceral adipose tissue samples from different obese patients.

2. Experiment

Adipose tissue samples were collected from 25 volunteer patients with obesity at the Department of General Surgery, Vilnius University Hospital. Participants included men and women aged 18–65 years with a BMI greater than 30 kg/m^2. Fourteen of the participants were diagnosed with metabolic syndrome, while the remaining 11 did not have the condition. During laparoscopic gastric banding surgery, visceral samples of adipose tissue (VAT) were obtained. The research has been approved by Vilnius Regional Biomedical Research Ethics Committee, Permit No. 158200-14-722-238, and the purpose and design of the study were explained to each participant, who provided an informed consent. The adipose tissue samples were stored at −70°C prior analysis. The samples were then homogenized and lyophilized [12]. Optical imaging of the adipose tissue samples was performed using a *ZEISS* Axioscope 5 light microscope. The morphology of the adipose tissue was further analyzed using field emission scanning electron microscopy (FE-SEM, SU70, *Hitachi*). A comprehensive database of optical and SEM images from the visceral layers of adipose tissue (VAT) was compiled. Results are presented as mean ± standard deviation (SD). Statistical differences between groups were evaluated using a one-way ANOVA, with $P < 0.05$ considered statistically significant.

3. Results and discussion

Surprisingly, optical microscopy studies have already revealed differences in adipose tissue between patients with and without metabolic syndrome. Samples were collected from several sites of the visceral adipose tissue, typically from three distinct locations. Figure 1 shows the optical microscopy images of adipose tissue taken from different sites in the patients without clearly identified metabolic diseases. Single lipid droplets were observed only in a few specimens of adipose tissue taken from the visceral layers of obese patients without metabolic diseases. No other specific or distinctive features were noted in the optical microscopy images of those samples.

In contrast, a completely different situation is observed in the patients with metabolic syndrome. The amount of lipid droplets varies across different patient samples. Some of the oval-shaped lipid droplets range in size from 30 to 90 μm, while others are smaller than 30 μm. A few specimens contained larger droplets, ranging from 140 to 200 μm in size. Figure 2 shows the optical microscopy images of adipose tissue taken from different sites in the patients with diagnosed metabolic diseases.

Figure 3 presents the overall distribution of lipid droplets observed in the optical microscopy images from all patients with metabolic syndrome. The images reveal a generally semi-regular distribution of lipid droplets. Stephens et al. [33] suggested an association between increasing weight loss and the number/size of lipid droplets in the adipose tissue of cancer patients. The authors concluded that both the number and size of lipid droplets increase in the presence of cancer, and this increase is further amplified with weight loss and the reduction of adipose mass in other body compartments.

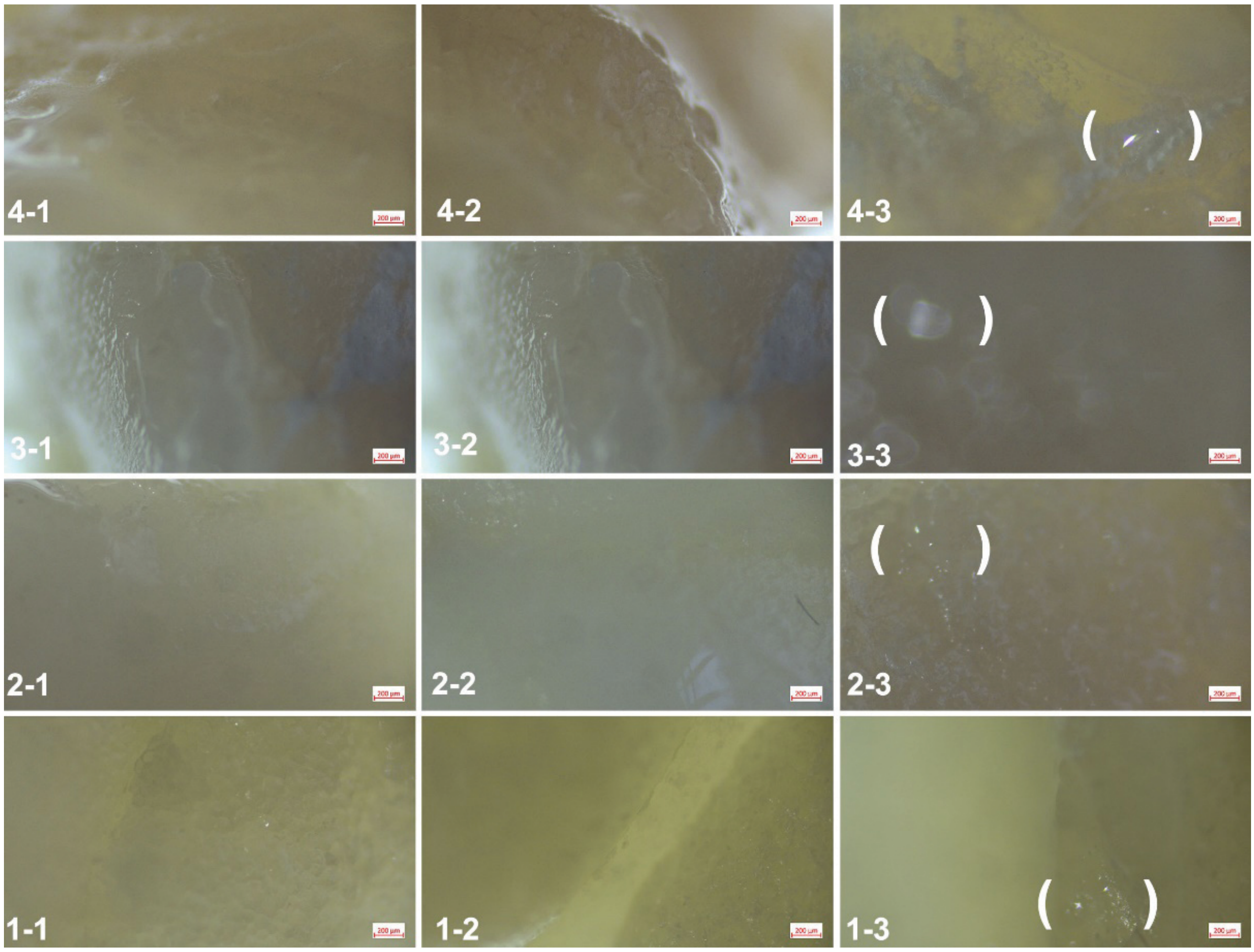

Fig. 1. The OM images of adipose tissue obtained from the visceral layers of obese patients (1, 2, 3 and 4) without metabolic diseases. Lipid droplets can be seen within parentheses of 1–3, 2–3, 3–3 and 4–3.

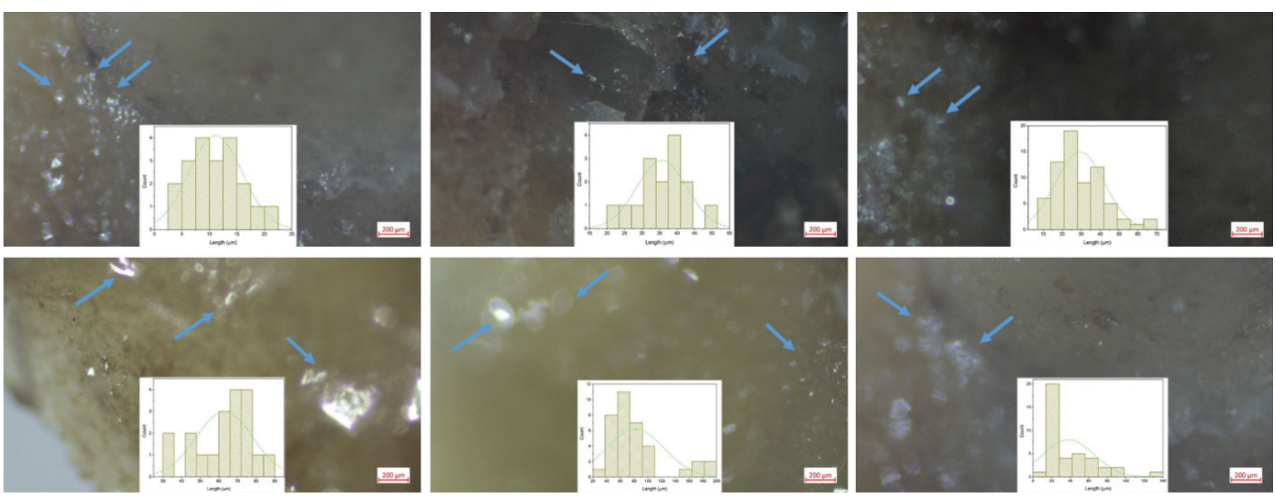

Fig. 2. The OM images of adipose tissue obtained from the visceral layers of various obese patients with metabolic diseases. The insets show the diagrams of the lipid droplet size distribution.

Characterizing visceral adipose tissue layers using optical microscopy provides valuable insights, not only into the condition of oncological patients but also for the early diagnosis of diseases such as incipient alcoholic cirrhosis of the liver [34]. Moreover, optical microscopy can serve as an effective tool for identifying whether a patient with obesity has metabolic disorders.

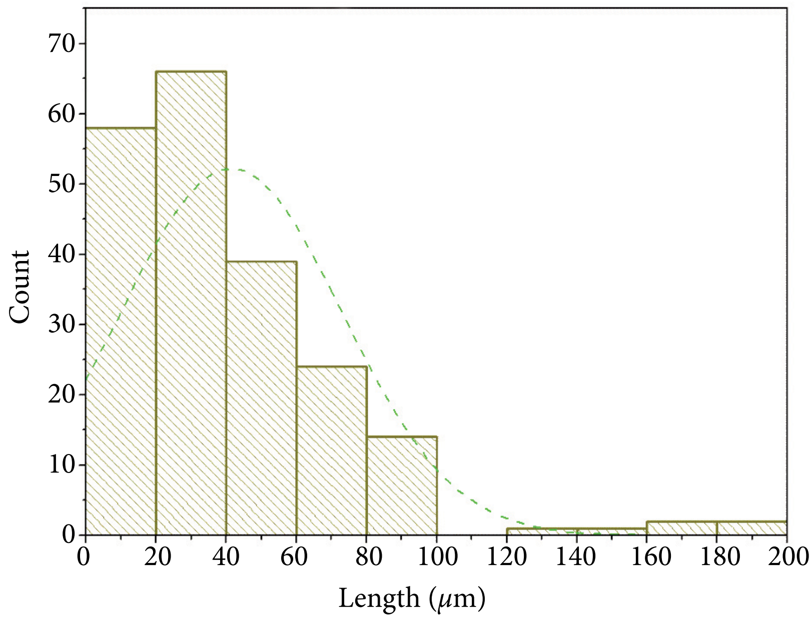

Fig. 3. The size distribution diagram of lipid droplets observed in the OM images of adipose tissue obtained from visceral layers of various obese patients with metabolic diseases.

The morphological features of the same VAT samples from the human body, both with and without metabolic disease, were analyzed using SEM. However, the SEM examination did not reveal any distinct morphological features in the VAT samples from patients without metabolic syndrome. Only single circular formations were occasionally observed in certain areas of the visceral adipose tissue. Representative SEM micrographs are shown in Fig. 4, which displays adipose tissue from the visceral layers of obese patients (1, 3, 4, 5, 6 and 7) without metabolic disease, showing detectable single circular formations. The distribution of these circular formations across all 21 samples from 7 patients without metabolic syndrome is presented in Fig. 5. As seen, the size of circular formations ranges from 7 to 22 μm, with the majority of particles measuring approximately 10–12 μm.

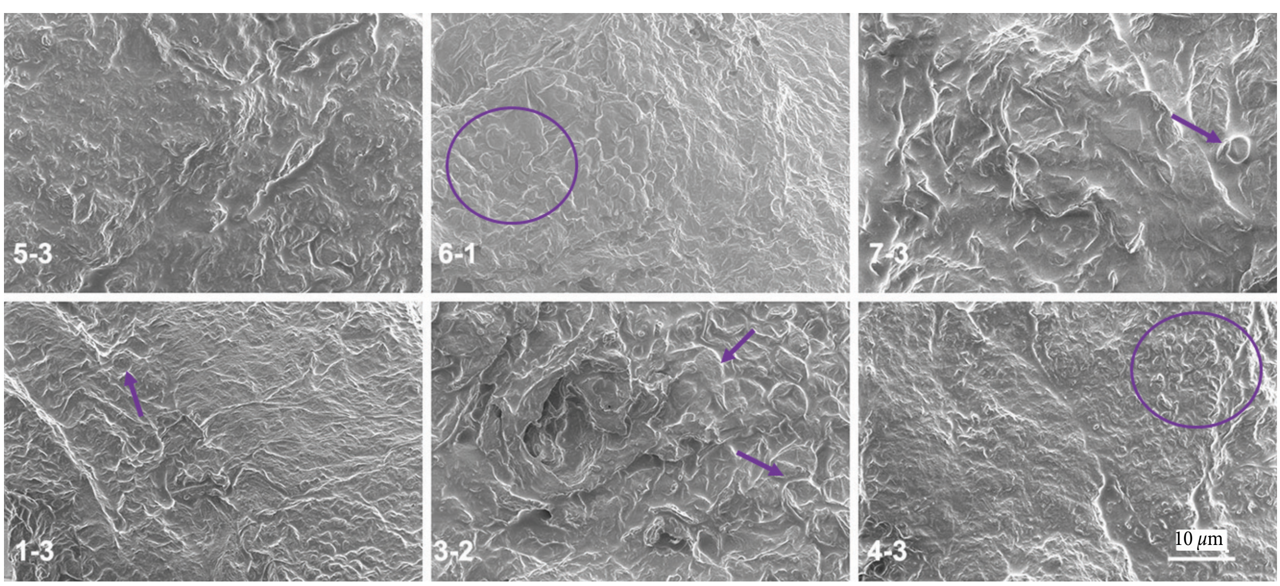

Fig. 4. The SEM micrographs of adipose tissue obtained from the visceral layers of obese patients (1, 3, 4, 5, 6 and 7) without metabolic diseases with detectable single circular formations.

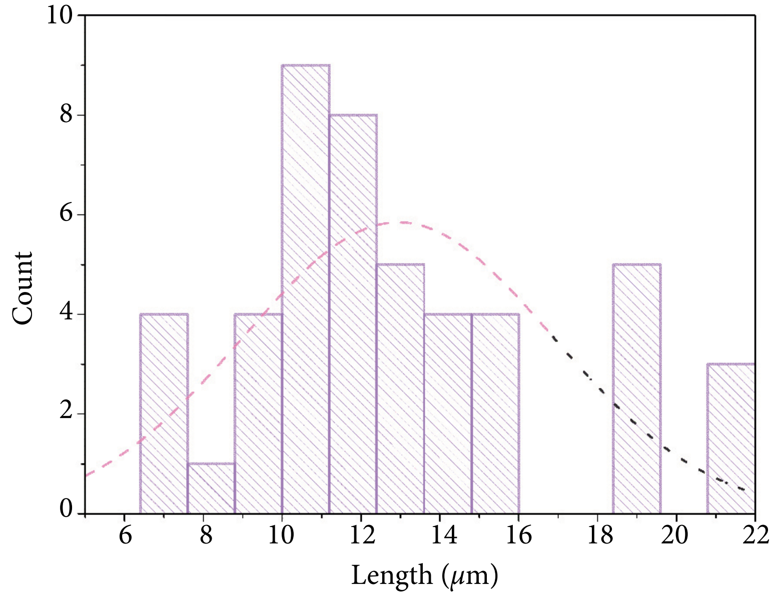

Fig. 5. The size distribution diagram of the circular formations observed in the SEM micrographs of visceral adipose tissue from various obese patients without metabolic diseases.

Interestingly, circular formations are also present in the SEM images of obese patients with metabolic diseases (Fig. 6). The size of most particles is less than 10 μm, and it does not differ significantly from that observed in the VAT samples without metabolic syndrome. However, the quantity of these particles is significantly higher in the adipose samples of obese patients with metabolic diseases, particularly in patients 5 and 6. Recent SEM studies have shown similar dynamic morphological changes in adipose tissue, revealing that the number and size of lipid circular formations are closely related to the abundance of mitochondria [35]. It is known that mitochondrial diseases are clinically classified based on the affected organ systems and symptoms. Therefore, these specific morphological features of VAT in obese patients could be useful for identifying mitochondrial diseases and

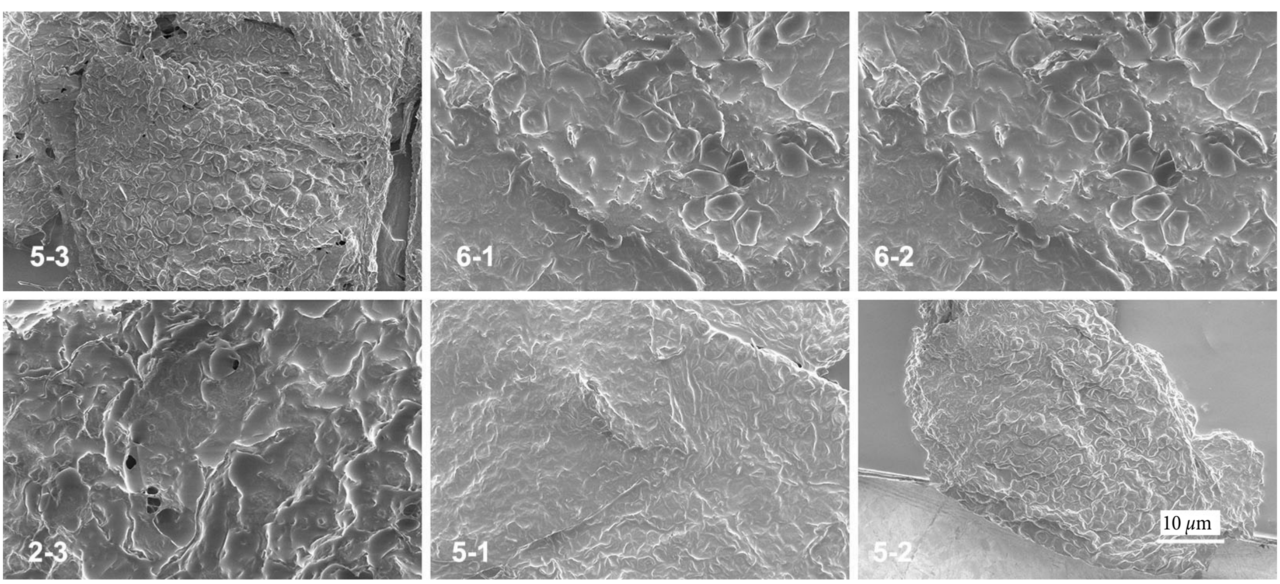

Fig. 6. The SEM micrographs of adipose tissue obtained from the visceral layers of obese patients (2, 5 and 6) with metabolic diseases with detectable single circular formations.

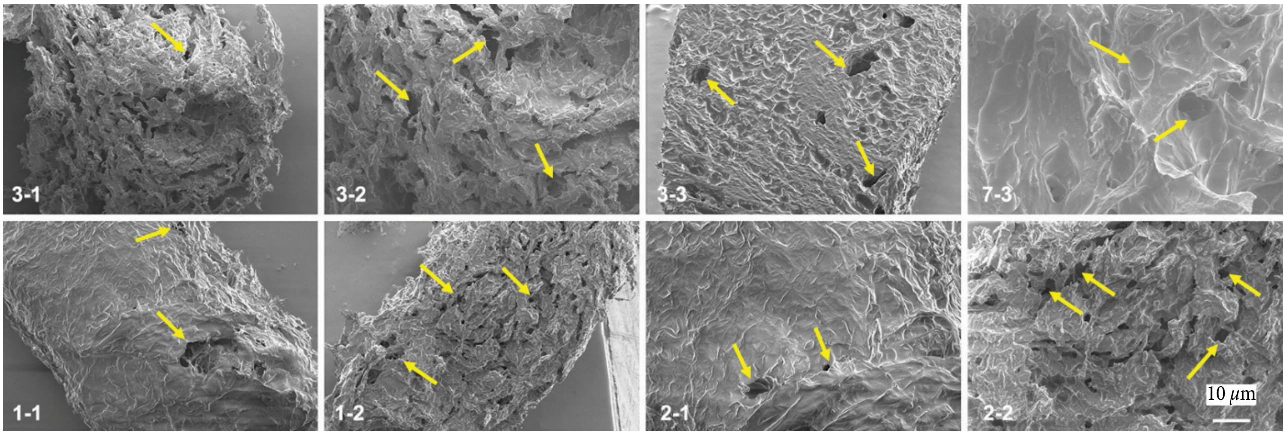

Fig. 7. The SEM micrographs of adipose tissue obtained from the visceral layers of obese patients (1, 2, 3 and 7) with metabolic diseases, showing detectable voids.

exploring potential connections between metabolic syndrome and symptoms of neuromuscular disorders. SEM studies have also demonstrated that adipose-derived stem cell sheets hold potential for use in various therapies [36]. Thus, when selecting adipose tissue for this purpose, a careful consideration is necessary.

Additionally, voids of various sizes were observed on the surface of some VAT samples from the patients with metabolic syndrome. The representative SEM images illustrating the formation of voids are shown in Fig. 7. In some VAT samples (1–1, 2–1, 3–3 and 7–3), isolated foci of voids or holes are observed.

Other adipose tissue samples (1–2, 2–2, 3–1 and 3–2) exhibit deep cavities carved into the surface, displaying a characteristic dendritic morphology. The overall distribution of voids observed in the SEM images of all 21 samples from 7 patients with metabolic syndrome is depicted in Fig. 8. The length of the voids ranges approximately from 5 to 50 μm, with the largest voids (10–20 μm)

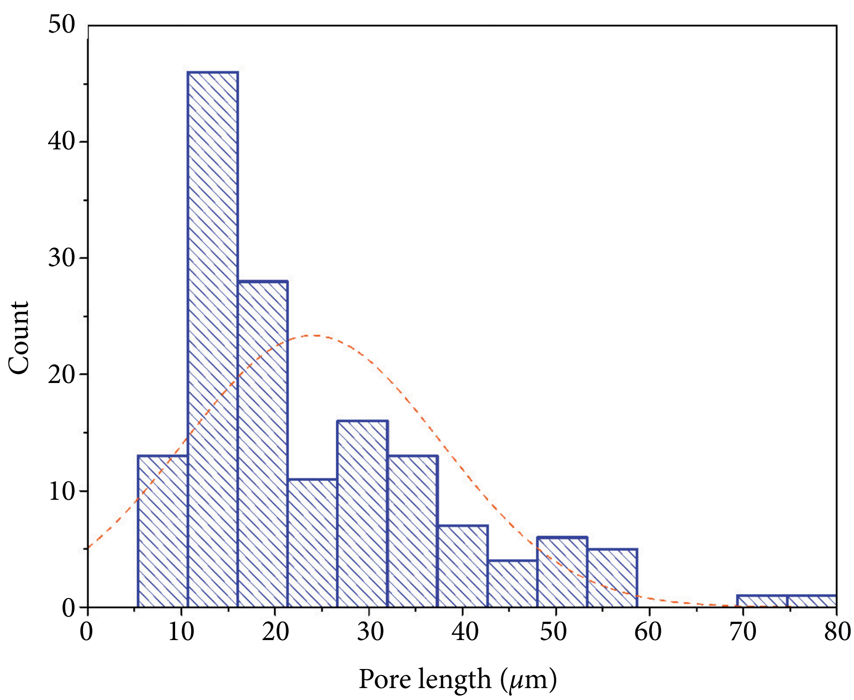

Fig. 8. The size distribution diagram of voids observed in the SEM micrographs of visceral adipose tissue from various obese patients with metabolic diseases.

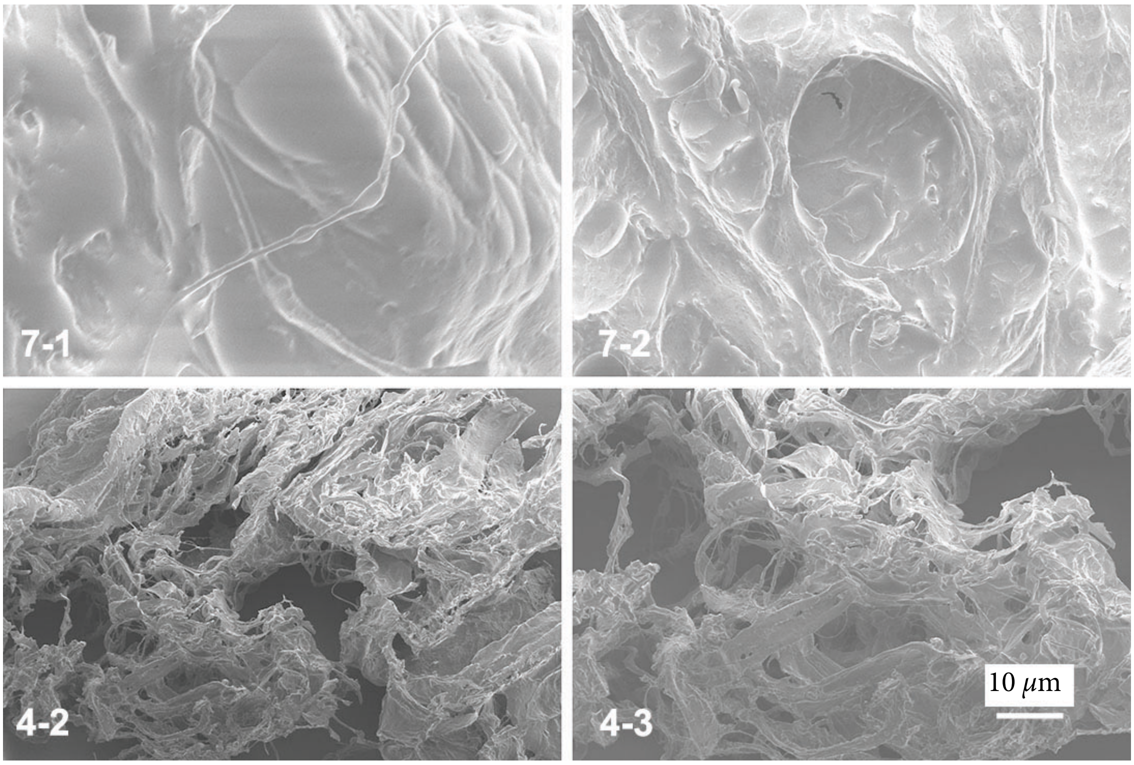

Fig. 9. The SEM micrographs of adipose tissue obtained from the visceral layers of obese patients (4 and 7) with metabolic diseases, showing individual morphological features.

constituting the majority of these distinct morphological features. The SEM analysis thus revealed significant differences in the morphological characteristics of VAT samples from individuals with and without metabolic disease [37].

Distinctive morphological features were observed in the SEM images of VAT samples from patients 4 and 7 (Fig. 9). The spherical, cloud-like surface is characteristic of the adipose tissue from patient 4. This spider-web-like morphology has not been previously observed in VAT samples. In contrast, the adipose tissue from patient 7 is very dense, with long, bead-like structures on its surface. This study focused solely on the visceral adipose tissue of obese patients. The fact that only a single subject was investigated limits the ability to generalize the findings. Furthermore, this study should be regarded as an exploratory methodological work that could be expanded for future investigations on a larger scale.

4. Conclusions

In this study, optical microscopy (OM) and scanning electron microscopy (SEM) were employed to further characterize the lyophilized visceral adipose tissue (VAT) from obese volunteers with and without metabolic diseases. The results revealed morphological differences in VAT between the specimens from individuals with and without metabolic syndrome. Single lipid droplets were detected in only a few specimens of adipose tissue obtained from the visceral layers of obese patients without metabolic diseases. No other distinct or specific features were observed in the OM images of those samples. In contrast, the lipid droplet content increased in the VAT samples from patients with metabolic diseases, with variation between individual patient samples. The size of some oval-shaped lipid droplets ranged from 30 to 90 μm, while others were smaller than 30 μm. Only a few specimens contained larger droplets, measuring between 140 and 200 μm. The SEM images revealed circular formations in both groups of obese patients, with most particles measuring less than 10 μm. However, the number of these particles was significantly higher in the adipose samples from the obese patients with metabolic diseases. Additionally, voids of varying sizes were observed on the surface of VAT samples from the patients with metabolic syndrome, with lengths ranging from approximately 5 to 50 μm. These observations indicate that the surface of VAT tissue contains notable morphological features linked to significant biomedical information. The OM and SEM analyses led to the conclusion that the microstructure of VAT is associated with metabolic changes in the human body and VAT could be regarded as an active organ. This study serves as an exploratory methodological approach, which could be expanded in future investigations on a larger scale.

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VISCERALINIO RIEBALINIO AUDINIO (VRA) PAVIRŠIAUS TYRIMAS MIKROSKOPINIAIS METODAIS

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Santrauka

Šiame tyrime optinė mikroskopija (OM) ir skenuojanti elektroninė mikroskopija (SEM) buvo naudojamos visceralinio riebalinio audinio (VRA) mėginiams, paimtiems iš nutukusių savanorių, sergančių ir nesergančių metabolinėmis ligomis. Pavienių lipidų lašelių OM vaizduose aptikta tik keliuose riebalinio audinio mėginiuose, gautuose iš nutukusių pacientų, nesergančių metabolinėmis ligomis, visceralinių sluoksnių. Šių mėginių OM vaizduose nepastebėta jokių kitų aiškių ar specifinių požymių; priešingai, medžiagų apykaitos ligomis sergančių pacientų VRA mėginiuose nustatytas padidėjęs lipidų lašelių kiekis, o atskirų pacientų mėginiuose jis buvo nevienodas. SEM vaizdai atskleidė, kad abiejose nutukusių pacientų grupėse aptikta apvalių darinių, kurių dauguma dalelių buvo mažesnės nei 10 μm. Tačiau šių dalelių skaičius buvo gerokai didesnis nutukusių, medžiagų apykaitos ligomis sergančių pacientų riebalinio audinio mėginiuose. Be to, metaboliniu sindromu sergančių pacientų VRA mėginių paviršiuje pastebėta įvairaus dydžio tuštumų, kurių ilgis svyravo nuo maždaug 5 iki 50 μm. OM ir SEM tyrimų rezultatai potencialiai galėtų būti naudojami vertinant medžiagų apykaitos simptomus ir prognozuojant įvairias nutukimu sergančių pacientų ligas.