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Imaging of the skin and subcutaneous tissue using classical and high-frequency ultrasonographies in anti-cellulite therapy

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Background: The development of ultrasonography allowed for skin imaging used in dermatology and esthetic medicine. By means of classic and high-frequency ultrasonographies, changes within the dermis and subcutaneous tissue can be presented.
Objective: The aim of this study was to show the possibilities of applying classic and high-frequency ultrasonographies in esthetic dermatology based on monitoring various types of anti-cellulite therapies.
Methods: Sixty-one women with cellulite were assigned to two smaller groups. One group was using anti-cellulite cream and the second group was a placebo group. The ultrasound examination was carried out before the initiation and after the completion of the treatment and evaluated epidermal echoes, the thickness of the subcutaneous tissue and the dermis, dermis echogenicity, the length and surface area of the subcutaneous tissue fascicles growing into the dermis, and the presence or absence of edemas.
Results: After the completion of the treatment, a statistically significant difference was observed. The most useful parameters were as follows: the thickness of the subcutaneous tissue, echogenicity, the surface area and length of the subcutaneous tissue, as well as the presence of edemas. The discussed changes were not observed in the placebo group.
Conclusion: Classic and high-frequency ultrasonographies are useful methods for monitoring anti-cellulite therapies.

Key words: high-frequency ultrasonography – cellulite – classic ultrasonography – ultrasonography

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FOR >20 YEARS, ultrasound sonography has been widely and successfully applied as a diagnostic procedure to depict many internal organs. Technological progress has highly contributed to the popularization of this method and allowed constructing more and more up-to-date devices, ultrasound probes and special software, resulting in a better diagnosis of a wide range of pathologies. Considering the fact that ultrasonography is inexpensive, non-invasive, repeatable and safe for a patient, we are continuously seeking new applications for this method. The creation of high-frequency ultrasound probes led to interest in imaging of the superficial tissues (1). Yanoet al. (2) were the first to write about a device for sector imaging of the skin using a 40 MHZ probe. Their studies as well as those by other authors gave rise to a new area in the application of ultrasonography in dermatology and cosmetology. Attempts were made to diagnose some benign and malignant skin lesions by means of ultrasound (3–5). Moreover, many researchers point out the usefulness of these examinations for diagnosing and monitoring the treatment of psoriasis, scleroderma and erysipelas (6–11). Ultrasonography has surpassed typical medical boundaries, playing a role in esthetic medicine, where it is used to evaluate skin lesions that have been caused by unfavorable external conditions and the process of aging (12, 13).

The subject of cellulite is of special importance in esthetic medicine. Cellulite is defined as connective tissue fibrosis and it occurs in over 85% of women (14–16). However, according to reports, the scale of this problem is increasing and cellulite is being diagnosed in men as well (17).

In women, cellulite is mainly located around the thighs and buttocks and within the abdomen, while in men, the abdomen and nape are predominant locations. Despite the fact that, to date, cellulite has not been recognized as a medical disorder, and only as an esthetic defect, the scale of this phenomenon compels to conduct studies and seek effective preventive measures and cellulite-reducing agents. In most cases, cellulite progression is evaluated by palpation and it is described using various scales, which, in the opinion of many researchers, are imprecise and unreliable (18, 19). Apart from palpation, instrumental methods are used such as termography (20) and macrophotography (21) – unfortunately, these methods have limitations and cannot be regarded as objective. The skin and subcutaneous tissue have been evaluated using magnetic resonance (MRI), computed tomography (CT) and ultrasonography (22–25). The above methods have proved to be very useful. Unfortunately, the use of CT and MRI is restricted by their high costs and limited availability. Furthermore, the exposition of a patient to ionizing radiation is an additional disadvantage of using CT. Thus, among these methods, only ultrasonography has a chance to be widely applied in cellulite evaluation.

Considering that cellulite involves subcutaneous tissue and the dermis, a cellulite examination should encompass both classic and high-frequency ultrasonographies. High-frequency ultrasonography allows for the evaluation of epidermal and dermis thickness, as well as observation of the morphology of the upper layer of the subcutaneous tissue and dermis. The classical ultrasonography enables taking precise measurements of the thickness of subcutaneous tissue.

The aim of this study was to present the possibilities of imaging of subcutaneous tissue and skin using classic and high-frequency ultrasonographies in anti-cellulite therapies (our goal was not the assessment of therapy itself).

Materials and Methods

Study trial

The ultrasound study was performed from 2008 through 2010. It included 61 women aged 22–61 (mean value 43.90 years) with a cellulite diagnosis based on a palpation examination. The women participating in the study were divided into two groups. The first group ($n1 = 45$) applied an anti-cellulite cream for 30 days. The cream was rubbed into the cellulite-affected area twice a day. The second group, the control group ($n2 = 16$), took a placebo for 30 days in the form of pills.

The anti-cellulite cream used in therapy came from dermocosmetic line Pharmaceris created by the Dr Irena Eris Cosmetic Laboratory. Active ingredients in the anti-cellulite cream used were:

- proteins from *Cucurbita pepo* [INCI: hydrolyzed *Cucurbita pepo* (pumpkin) seedcake] – protect collagen fibers and counteract their degradation, allowing reinforcement of elastic skin scaffolding,
- cranberry extract [INCI: *Vaccinium macrocarpon* (cranberry) fruit extract] – rich in polyphenols – improves microcirculation, antioxidant action,
- extract from the fruit of bitter orange [INCI: Citrus aurantium dulcis (orange) extract]: provides correct desquamation of epidermis dead cells, stimulates skin microcirculation,
- unique amino acid DERMÈ™ System (INCI: L-ergothioneine) containing the functional group of L-carnitine: serves as guide for fatty acids through the mitochondrial membrane. Inside the mitochondria fatty acids are burned, thanks to which the skin retains the normal structure without accumulated, lumpy fat deposits. The placebo was also created by Dr Irena Eris laboratory.

All women participating in the research had been informed about the type of study they were taking part in, and they had given their informed consent to participate.

Devices and examination technique

The high-frequency ultrasound examinations were carried out using the US device-μScan (Institute of Fundamental Technological Research of the Polish Academy of Sciences, Warsaw, Poland) created by the team of Professor A. Nowicki from the Ultrasound Laboratory of the Institute of Fundamental Technological Research of the Polish Academy of Sciences in Warsaw. A probe with a frequency of 35 MHz with a focal length of 13 mm was applied. All device settings were constant throughout the entire study. The ultrasound images obtained were saved on a personal computer, and then analyzed using experimental software. A special software was used to measure the thickness of individual skin structures and to evaluate their echogenicity by determining the number of pixels for a given brightness range in an area selected by a researcher.

Classic ultrasound examinations were performed using a Toshiba Aplio machine (Toshiba Medical System Corporation, Otawara-shi, Japan, TMS sp.z.o.o Regional Office, Warsaw, Poland). The study used an 18 MHz linear broadband probe with the differential tissue harmonic imaging option with constant amplification values and a focus depth of 1 cm. The ultrasound pictures were saved on the device's hard drive and were used to measure the thickness of the subcutaneous tissue. The classic and high-frequency ultrasound examinations were performed twice in all women: before the initiation of anti-cellulite therapy and after its completion. The examinations were performed on the posterior part of the thigh, always at the same location.

The evaluated parameters

In the classical ultrasound examinations, the following parameters were evaluated: hypodermic thickness and the total thickness of subcutaneous tissue and the dermis.

In the high-frequency ultrasound examinations, the following evaluation parameters were evaluated: epidermal thickness, dermis thickness and echogenicity, the growth of subcutaneous tissue fascicles into the dermis, and the presence or absence of edemas within the dermis. Most of the parameters, that is, thickness of individual structures, was measured in a typical way as for ultrasound exams, by designation on the ultrasound image line between the beginning and the end of each structure. Slightly different procedures were used in the case of such parameters as echogenicity, growth of subcutaneous tissue fascicles into the dermis and also the presence or absence of edemas. Dermis echogenicity was measured using the number of pixels. On the ultrasound image, the researcher marked a selected area of the dermis region of interest (ROI) the size of which was constant in all the measurements, and then a measurement of the number of pixels in a brightness range from 10% to 100% was made. The ROI was located in the center to encompass the largest area possible of the dermis. In order to minimize errors resulting from imprecise positioning of ROI, measurements were taken on three ultrasound images of all the women, both before and after the therapy, and then the average result was used for calculations.

Measurement of the growth of subcutaneous tissue fascicles into the dermis was made by measuring the length and calculating the surface that was growing into the dermis subcutaneous tissue fascicles cover. The measurement of the length of the subcutaneous tissue fascicles growing into the dermis was taken by marking the base of a fascicle on the ultrasound image. Next, its length was measured and the center was determined. Then, the length was measured starting from the center of the base and ending at the tip of the subcutaneous tissue fascicle growing into the dermis. In order to determine the ROI, the selected area was outlined by hand using experimental software tools and the surface area of the subcutaneous tissue fascicle growing into the dermis was calculated. The presence or absence of edemas was assessed by two independent researchers by analyzing the collected material. In addition both before the initiation of the anti-cellulite therapy and after its completion, assessment of the stage of cellulite according to Nürnberger–Müller scale and measurement of thigh circumference in the place of ultrasound exam was made.

Statistical Analysis

The *t*-Student test and the non-parametric Wilcoxon Matched Pairs Signed-Ranks were used for verification of the results. The assumed statistical significance level was $\alpha = 0.05$. All calculations were performed using the STATISTICA 8 program. The results obtained are summarized in Table 1.

Results

Classic ultrasound examinations revealed significant changes in the parameters studied. The thickness of the subcutaneous tissue reducing, which was confirmed by statistically significant results on the *t*-Student test. The average thickness of the subcutaneous tissue before the therapy in the group that was using anti-cellulite cream was 13.84 mm and after the therapy only 11.32 mm, which is synonymous with reducing its thickness by 18.20%. No statistically significant changes were noticed in the placebo group (Fig. 1).

The analysis of the total thickness of subcutaneous tissue and the dermis showed that as a result of the anti-cellulite therapy used, the thickness of these two layers decreased. The observed differences were statistically significant. Similar to the case of the thickness of subcutaneous tissue, no significant differences were noted in the women taking the placebo. (Table 1, Fig. 1).

TABLE 1 The results in two groups before and after anti-cellulite therapy

Parametres	Cream ($n = 45$)				Placebo ($n = 16$)			
	$\bar{x}$ before therapy (mm)	$\bar{x}$ after therapy (mm)	*t*-Student' (*t*)/Wilcoxons' test (*Z*)	*P*	$\bar{x}$ before therapy (mm)	$\bar{x}$ after therapy (mm)	*t*-Student' (*t*)/Wilcoxons' test (*T*)	*P*
Classic USG								
Thickness of subcutaneous tissue	13.85	11.32	$t = 7.29$	0.0000	17.14	16.88	$t = 1.08$	0.296
Thickness of subcutaneous tissue and dermis	15.41	13.01	$t = 6.56$	0.0000	18.81	18.56	$t = 1.42$	0.174
High-frequency USG								
Thickness of epidermis	0.16	0.14	$Z = 3.37$	0.0007	0.34	0.34	$T = 9$	0.398
Thickness of dermis	1.66	1.36	$t = 9.41$	0.0000	1.72	1.74	$t = 2.11$	0.522
Length of a subcutaneous tissue fascicle	0.82	0.47	$Z = 5.37$	0.0000	0.82	0.79	$t = 2.07$	0.565
Surface area	0.80	0.40	$Z = 5.67$	0.0000	0.72	0.69	$t = 1.49$	0.157
Echogenicity	17486.71	18343.24	$Z = 5.63$	0.0000	16902	16898.20	$T = 50$	0.570
Presence/absence of edemas	0.76	0.24	$Z = 3.87$	0.0001	0.69	0.69	$T = 28$	—
Palpation								
Thigh circumference at the place of US examination	56.74	54.58	$Z = 5.65$	0.0000	58.09	57.88	$t = 2{,}087$	0.054
Stage of cellulite according to Nürnberger–Müller scale	2.33	1.40	$Z = 5.37$	0.0000	2.69	2.50	$T = 0$	0.108

n, number of women in test; $\bar{x}$, arithmetic mean; *t*, value of the *t*-Student test; *T*/*Z*, value of the Wilcoxon test (*T* – in trial <25, *Z* – in trial >25) *P*, statistically significantly level for the Wilcoxon test or *t*-Student test.

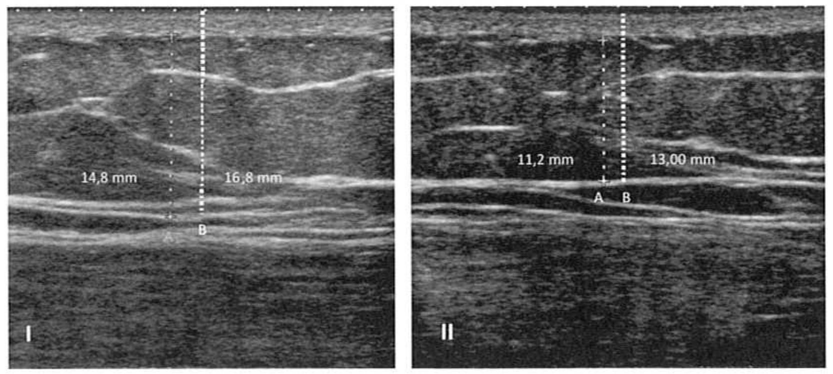

Fig. 1. Thickness of the subcutaneous tissue A and the total thickness of the subcutaneous tissue and dermis B. I, before therapy; II, after therapy.

The analysis of the high-frequency ultrasound examination results displayed changes in most parameters in the group of patients using the anti-cellulite therapy. The measurements of epidermal echo thickness showed that epidermal thickness decreased after therapy. The observed differences were statistically significant (Table 1). The reduction of epidermal thickness after completion of the therapy was about 17%, which probably results from the fact that the cream was rubbed into the thighs twice a day, leading to the mechanical shedding of the epidermis and also one of its ingredients induced shedding of the corneous layer. No differences in epidermal thickness were observed in the placebo group before and after completion of the therapy (Table 1, Fig. 2). Reduction of the thickness of the dermis in the group of women using the cream was also observed by the ultrasound effect of the therapy (Fig. 2). After the completion of the therapy, reduction of the thickness of the dermis by about 18% was observed. These changes were not seen in the placebo group (Table 1). The measurement of the length of subcutaneous tissue fascicles

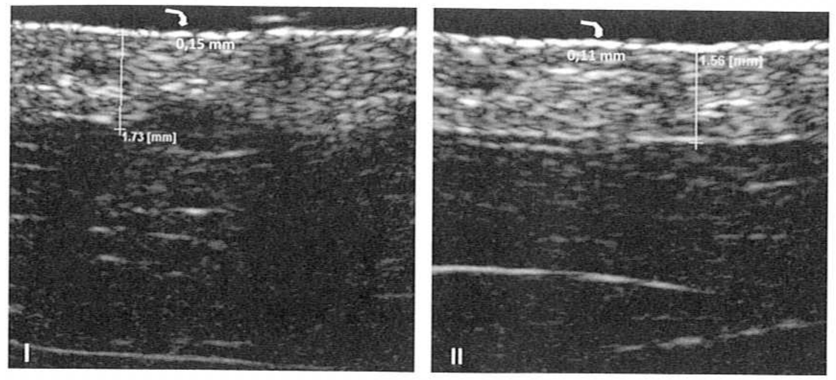

Fig. 2. Dermis and epidermis thickness. I, before therapy; II, after therapy.

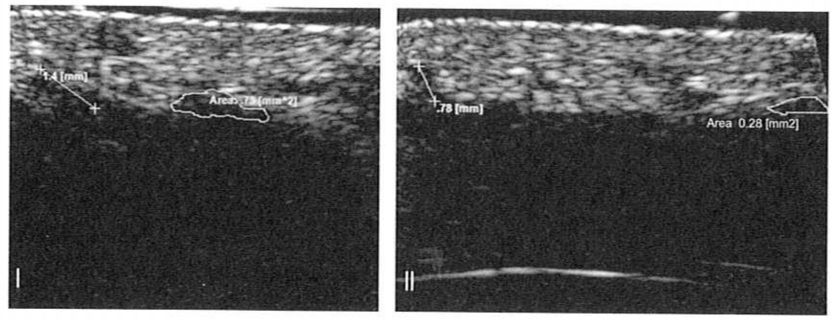

Fig. 3. The length and surface area of subcutaneous tissue fascicles into the dermis. I, before therapy; II, after therapy.

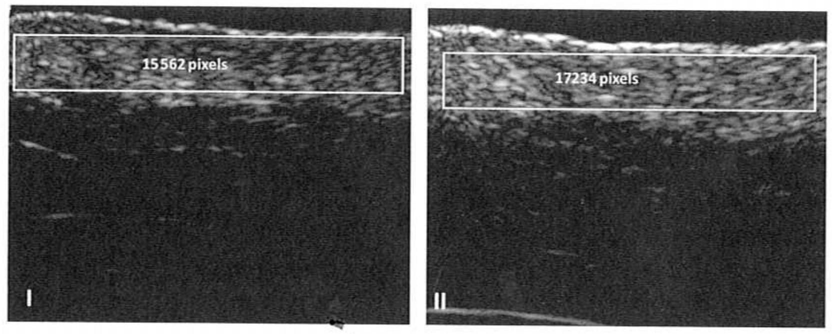

Fig. 4. Dermis echogenicity. I, before therapy; II, after therapy.

growing into the dermis showed that the fascicles shortened in all patients subjected to therapy (Fig. 3). The differences observed are statistically significant. The difference in the length of the fascicles before and after the therapy was 41%. In the group taking the placebo, a small shortening of subcutaneous tissue fascicles growing into the dermis was also observed, but the reported difference was not statistically significant (Table 1). The analysis of the results of the surface area of subcutaneous tissue growing into the dermis showed that the application of anti-cellulite therapies resulted in a reduction of this area. The difference obtained in the group that was using the anti-cellulite cream was statistically significant (Table 1, Fig. 3). No changes were observed in the placebo group, which was confirmed by a statistically insignificant result on the *t*-Student test (Table 1).

The comparison of echogenicity before and after the anti-cellulite therapy displayed an increase of dermis echogenicity. All differences were statistically significant (Table 1, Fig. 4). In the placebo group, echogenicity did not change. Moreover, the analysis of the results showed that the number of women with dermis edemas decreased after the application of anti-cellulite therapies (Fig. 5). In the group using the anti-cellulite cream, 75.55% of the women demonstrated swellings before therapy and only 24.4%

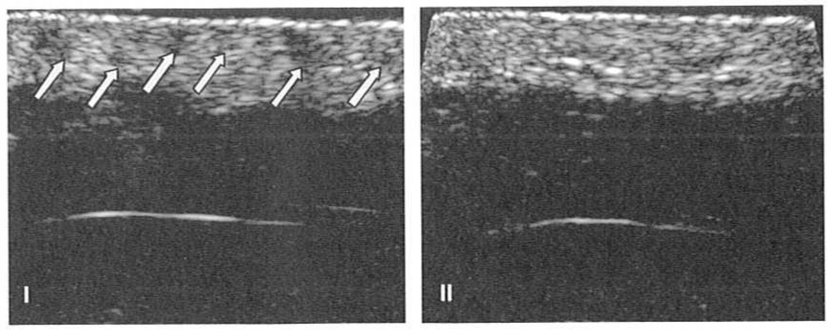

Fig. 5. The presence of edemas before therapy (I), absence of edemas after therapy (II).

of the women after completion. In the placebo group, the number of women who developed swellings before and after placebo administration was the same.

The analysis of the data obtained showed a statistically significant variability in the case of such parameters as thigh circumference in the place where an ultrasound exam was conducted. The average circumference before starting the anti-cellulite therapy was about 56.74 cm in the group that was using anti-cellulite cream and 58.09 cm in the placebo group. After finishing the therapy in the group that was using cream, the average circumference was 54.58 cm and in the placebo group, it was 57.72 cm. The observed difference in thigh circumference is statistically significant in comparison with the group that was using the anti-cellulite cream (Table 1).

Palpation exam and assessment of stage of cellulite according to Nürnberger–Müller scale showed that in the trial using the anti-cellulite cream, there was a reduction of the stage of cellulite. Reduction of this stage after finishing the therapy was found in 84.44% of those tested using the cream. This difference was statistically significant. In the placebo group, there were no significant differences. (Table 1)

Discussion

Technological progress has been achieved in inexpensive, non-invasive and safe ultrasonography, an important element of medical diagnostic imaging (1). In recent years, more and more attempts have been made to apply it in dermatology and esthetic medicine, which is a rapidly developing field (19, 21, 24).

The results of the research presented above explicitly indicate that both classic and high-frequency ultrasonographies can be applied in the examination of the skin. Among the parameters determined by means of classic ultrasonography, the most relevant was subcutaneous tissue thickness, which decreased after the anti-cellulite treatment. Microcirculation improvement was probably responsible for reducing subcutaneous tissue thickness.

All parameters evaluated using high-frequency ultrasonography, except for the epidermal thickness, may be regarded as useful in anti-cellulite therapy monitoring. Measuring epidermal thickness creates a possibility of miscalculation because the layer is very thin. Additionally, the ultrasound image of the epidermis consists of the echo created between the ultrasound gel and the skin surface as well as the epidermal echo (1). A hyperechoic fascicle visible on the ultrasound image is not the epidermis 'sensu stricto'; therefore, an objective measurement in this case is impossible.

We know from our experience that the most useful parameters of cellulite evaluation are the surface area and length of the subcutaneous tissue fascicles growing into the dermis, echogenicity and the thickness of the dermis. The growth of the subcutaneous tissue fascicles into the dermis is typical of cellulite and this fact is emphasized by many authors (26, 27). Measuring fascicle length before and after the therapy explicitly indicates whether the applied therapy is effective. However, our experience shows that in many examined women, the subcutaneous tissue does not grow into the dermis in a typical way – in the form of the so-called 'teeth.' We often observed the growth of subcutaneous tissue fascicles extensively at the base, but not reaching the deep layers of the dermis. In these cases, the measurement of the fascicle length was not objective. This resulted in the introduction of a second parameter: the measurement of the surface area of the subcutaneous tissue growing into the dermis. The analysis of fascicle length and the surface area of the subcutaneous tissue fascicles growing into the dermis revealed a decrease in the length and surface area after therapy completion, leading to an improvement of the skin condition and a decrease in cellulite progression. This change can be observed on the ultrasound image as the smoothing of the level between the dermis and the subcutaneous tissue, just as an image of healthy skin.

The level of dermis echogenicity also turned out to be a useful parameter to monitor anti-cellulite therapy. The therapy led to a noticeable increase in the echogenicity level, probably resulting from the reconstruction of collagen, which is a solid structure and has a tendency to increase the echogenicity of an image. In the scientific literature, we can find studies dedicated to dermis echogenicity (12, 13, 19, 28, 29). However, the studies covered the issues of changes in dermis echogenicity connected with age and photo-aging. Researchers took separate echogenicity measurements of the upper and the lower layer of the dermis (12, 13, 19). Gniadecka's examinations (12, 13) showed that in patients subjected to excessive exposure to the sun, changes tend to locate predominantly in the upper part of the dermis and can be observed as increased echogenicity. As far as photo-aging is concerned, a relationship of echogenicity of the upper dermis layer with the lower layer increases proportionally to the degree of skin destruction. In our study, the echogenicity measurement of both dermis layers was taken together.

The measurement of the dermis thickness is also an important cellulite evaluation parameter. A decrease in dermis thickness after the application of anti-cellulite therapy is evidence of successful therapy (24, 26).

The presence or the lack of swellings was also a useful parameter in monitoring therapy. The results obtained explicitly indicate that after the therapy, the number of women with dermis swellings diminished significantly. The reduction of swellings after anti-cellulite therapy has been confirmed in other studies (24, 26). However, in the case of this parameter, there are difficulties in the appropriate interpretation of the ultrasound images, because swelling can be easily confused with the dermis blood vessels. It seems essential that this parameter is evaluated by several independent researchers or the assessment is based on a dynamic image (movie file).

On the basis of our studies and literature reports, we can conclude that classical and high-frequency ultrasonographies are useful methods for the evaluation and monitoring of anti-cellulite therapies. Considering the advantages of ultrasound examinations, this method can be widely used in esthetic medicine. However, further studies are necessary to create examination standards.

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