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ORIGINAL ARTICLE BODY CONTOURING

Lower Leg Augmentation with Fat Grafting, MRI and Histological Examination

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Abstract
Introduction Nowadays, there is an increasing demand for contouring of the entire lower leg, in addition to corrections at the level of the muscle only. Accordingly, it becomes advantageous to use fat tissue for this purpose, for its availability in high quantities, potential for its easy implantation in all leg parts, biological compatibility, low antigen potential, and minimal donor morbidity.
Methods Forty-eight female patients, aged 20–54 (47.1 % younger than 35 and 52.9 % older than 35), underwent lower leg augmentation with autologous fat. We compared pre- and 1, 3, and 6 months postoperative morphological and volumetric characteristics of the recipient region by circumferential measuring of particular parts of the lower legs and evaluating the presence of live transplanted fat by MRI and histological examination.
Results The augmentation of certain circumferences of the lower leg at the 6-month follow-up examination is significantly related to preoperative circumference as well as to the quantity of infiltrated fat. Therefore, lower the baseline circumference, the greater the augmentation, and the greater the quantity of infiltrated fat, the greater the augmentation.
Conclusion Ensuring adequate technique in the transfer of fat tissue from the donor region to the recipient region of the lower leg is definitely the least invasive method of lower leg contouring. For patients with muscle dystrophies or those with injuries, this technique could become the first choice for its minimal complications. The simplicity of the procedure invites its wide application in plastic surgery.
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Keywords Lower leg augmentation · Autologous fat graft · MRI · Fat survival · Fat graft viability · Fat reabsorption · Lipo-filling · Calf augmentation

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Introduction

Harmonious legs are considered one of the most prominent beauty attributes in any woman and are defined by their length, width, and curves. So far, beautification of the lower legs was achieved by liposuction of fatty parts, whereas muscle hypotrophy was corrected with implants [1]. Today, there is an increasing demand for contouring of the entire lower leg, in addition to corrections at the level of the muscle only. Accordingly, it becomes advantageous to use fat tissue for this purpose, for its availability in high quantities, potential for easy implantation in all leg parts, biological compatibility, low antigen potential, and minimal donor morbidity [2].

In order for this technique to find its own place in filling out and contouring of legs, it is necessary to conduct an anthropological analysis of the leg's shape and adjust it to be in harmony with the rest of the body. Defining ideal proportions of the lower leg, its diameter, length, and the curve of the medial and lateral line are mathematically defined in detailed analysis by Cuenca-Guerra [3] and give a baseline for an operative plan for filling out the lower leg, in accordance with the type of hypoplasia. Von Szalay [4] mentioned an ideal circumference of the widest part of the calf to be 33–36 cm, whereas Benslimane mentioned angles [5] between the central axis of the tibia and the central axis of the femur. Defining anthropological measures is a foundation for further fine contouring, in addition to the artistic talents of the surgeon him/herself. The shape of the leg is defined by the position of the central axis of the lower leg in relation to the upper leg, the fullness created by the soleus, lateral, and medial heads of the gastrocnemius muscle, as well as the quantity and distribution of subcutaneous fat.

In our research, we compared pre- and postoperative morphological and volumetric characteristics of the recipient region using methods of circumferential measuring of particular parts of the lower legs and evaluating the presence of live transplanted fat by MRI and histological examination. In addition, we demonstrated the quantity of fat needed for contouring and filling out the lower legs in all parts.

Methods and Materials

A group of 48 patients presenting with thin or deformed lower legs were examined during the period of 2006–2015 and enrolled in the study. All patients were female, aged 20–54 (47.1 % were younger than 35 and 52.9 % were older than 35). Before the operation, the following were measured: circumference of the knee, zone below the knee, mid-calf, between calf and ankle, ankle; a body mass index and MRI of the lower extremity were obtained. Anterior, posterior, and lateral photographs were taken, and the lower extremity was marked in standing position. Filling out of the lower leg was planned in accordance to the size and shape of the upper leg and in relation to the rest of the body. Patients were followed through 1, 3 and 6 months. All pre-operative measurements were repeated during the follow-up examinations. The MRI was repeated. Tissue sampling for histological analysis was done during the 6-month follow-up. We measured the level of augmentation of target segments of the lower leg after the initial lipofilling. Many patients required additional augmentation after 6 months.

Operative Procedure

Harvesting and Fat Preparation

All patients received antibiotics intravenously (cephalosporin 3rd generation) pre-operatively. All patients were operated under general anesthesia. A tumescent solution composed of 500 ml of saline solution with 1 ml (1:1000 epinephrine) was infiltrated in the abdomen and in the back. The quantity of infiltrated tumescent solution was calculated as two-thirds of planned fat tissue to be aspirated. Fat tissue was removed with 50 ml syringes and 3.5-mm-diameter cannulas. Two methods were used for fat tissue preparation: (1) rinsing with saline solution and (2) centrifugation.

Syringes with fat tissue were separated into two groups: fat tissue planned for muscle implantation was separated for rinsing, while fat tissue for subcutaneous administration was separated for centrifugation. Fat tissue rinsing was done with 0.9 % of NaCl through gauze to remove traces of blood but fibrin remains. Fat tissue for centrifugation was safely transferred to 10 ml syringes and centrifuged at 2700 rpm/3 min. Centrifugation resulted in three layers: at the bottom blood and infiltrate, fat in the middle, and oil on top. Fat was separated from liquid parts and oil, and a sample of rinsed and centrifuged fat was sent for histological analysis.

Infiltration Technique

The lower leg was positioned in a 45-degree flex position. Rinsed fat was infiltrated in small quantities through incision points while withdrawing the cannula in all muscle layers with 50 ml syringes and 3.0-mm-diameter cannulas. Below the knee, below the calf, above the ankle, and in the ankle region, fat tissue is infiltrated subcutaneously with 10 ml syringes and 1.2-mm-diameter cannulas. Filling was administered circumferentially while withdrawing the cannula, uniformly, in several subcutaneous layers and in several directions.

Massage and Bandaging

After the procedure, the lower leg was massaged thoroughly, and then an elastic adhesive bandage was placed by application of 7 × 20 cm strips in a pattern similar to roof tiles. Then 15 mmHg pressure was applied using medical grade socks.

Postoperative Procedure

During postoperative recovery, the patient was advised to elevate the legs while sitting or lying in bed. In general, usual walking activities were advised without obligatory bed rest. Elastic adhesive bandages are removed after seven days, followed by use of compression socks for 1 month. The patient took third-generation cephalosporins orally for 5 days.

MRI

An MRI of the lower legs was obtained before the operation and again 6 months after using a Simens 1.5 Tesla. Examinations were done in three layers (sagital, longitudinal, and coronar) at 3,0 to 6,0 mm intersections, within T1-weighted (typically $\leq$500 ms) relaxation times, first without, then with FS suppression. This relaxation-dependent technique is based on the different relaxation behaviors of water and fat tissue. Fat has a much shorter T1 relaxation time than other tissues. Prior to the excitation pulse of the sequence, an inversion pulse of 180° is applied which inverts the spins of all tissue and fat protons. This is followed by T1 relaxation. When choosing T1, such that the longitudinal magnetization of fat at the time when the excitation pulse is applied is zero, the fat spins will not contribute to the MR signal. STIR (Short T1 Inversion Recovery) images have an inverted T1 contrast, where the tissue with long T1 appears brighter than tissue with short T1. Volumetric analysis was done by computer software for calculation of volume and proportion of soft tissues.

Histological Analysis

Histological evaluation of fat tissue preservation, viability, and morphology of adipocytes was first done from the donor region, from a rinsed and centrifuged sample. Six months after the operation, the subcutaneous tissue was taken from the ankle or above the ankle region. Prior to the sample aspiration, a regional block anesthetic was given to avoid local infiltration, and damage of the tissue possibly caused by application of local anesthetic. Following this, the sample is taken using a 1.2 mm cannula and 10 ml Luer-Lok syringe.

Tissue specimens were cut on a Leica microtome to 4 µm thicknesses and fixed in 10 % formaldehyde and embedded in paraffin wax, following routine protocols. Standard histological analysis with hematoxylin and eosin staining was done, and after that the quality, size of the fat cells and presence of vascular elements of fat tissue were determined. The assessment of the specimens was done on Nikon Eclipse microscope and the photographs were taken with a Nikon DS-FI2 camera. Morphometric measurements of the cells present on photographs were done on a control unit Nikon DS-L3.

Results

Data for the group of 48 patients were statistically analyzed (Table 1).

In all patients, fat augmentation of the lower leg was done only once; 11 patients, aged 45–54, were not completely satisfied with the level of augmentation and requested additional filling (Table 2).

If additional filling was deemed necessary, it was done 6 months after the percentage of fat survival and volume retention was assessed.

The quantity of fat necessary for augmentation of particular regions and the level of augmentation were recorded in Table 3.

In our research, it was shown that the augmentation of particular circumferences in the lower leg is, after 6 months, significantly related to the pre-operative circumference and quantity of infiltrated fat tissue in the majority of examined regions (Table 4). Accordingly, an increase in the circumference is not only related to the absolute value compared to the pre-operative circumference, but to the quantity of infiltrated fat in the respective region. On the other hand, there was no statistically significant connection between augmentation of the ankle and the area below the ankle regions and baseline circumference, in relation to either the quantity of infiltrated fat or the pre-operation circumference.

Our study shows that the augmentation of certain circumferences of the lower leg at the 6-month follow-up examination is significantly related to the pre-operative circumference as well as to the quantity of infiltrated fat. Therefore, the lower the baseline circumference, the greater the augmentation; and the more infiltrated fat, the greater the augmentation.

Table 1 Sociodemographic and clinical characteristics of the sample

	N	Min	Max	Mean	SD
Age	48	20	54	37.75	9.52
Weight (kg)	48	52	80	62.77	6.40
Weight after 6 m (kg)	48	52	79	62.03	5.99
BMI	48	19	35.60	25.45	4.07
BMI after 6 m	48	19	35.40	25.01	3.81

Discussion

Proportions of the body, as well as length and diameter of legs, are determined by etiological factors, environment, anthropological measurements in particular races, and characteristics of people living in particular meridians [6]. Restoring harmony to the lower leg as defined by numerous authors [3–5, 7] requires a new approach in contouring lower legs, fat tissue transplantation.

There are many studies mentioning fat transplantation in the face, breasts, or buttocks that include methods of implementation and tissue survival rates [2, 8–15]. Fewer authors have commented on augmentation of the lower leg with the patient's own fat tissue [16–19]. The lower leg region is well vascularized through the main arteries and veins as well as through fascial, dermal vascular plexuses, and perforators connecting them.

Table 2 Success in filling out the lower leg in one surgery

	N	%
Satisfied	37	77.08
Not satisfied	11	22.92
Total	48	100.00

This richly vascularized recipient site plays an important role in fat survival [20]. Additionally, the quality of infiltrated fat and the method of its transplantation play important roles as well [21, 22].

The fat tissue we centrifuged had preserved adipocytes, yet it had lost cell volume (Fig. 1), which is manifested in a rippled membrane, smaller diameter, as well as less, yet present vascular space.

With rinsed fat, cells have proper membranes, larger diameters, and the vascular stroma is bigger (Fig. 2).

The presence and preservation of vascular stroma in both samples show that vascularization plays a key role in tissue survival of both the rinsed and centrifuged fats and the recipient region (Fig. 3).

Follow-up MRI after 6 months shows rinsed fat that was infiltrated in the muscle region of the lower leg. Rinsed fat tissue survived and was present at MRI between the muscle fascia and muscle fibers within the muscle groups (Fig. 4).

Also, the sample we examined after 6 months from the region above the ankle (subcutaneous tissue) shows surviving centrifuged fat (Fig. 5). The cells of the recipient region are not uniform and different in size, which shows that these are young adipocytes with peripherally placed nuclei as well as small vascular spaces in the active process of proliferation.

Table 3 Descriptive analysis of clinical parameters of the sample per region

		Baseline circumference (cm)	Month 6 circumference (cm)	Fat injection (ml)	Circumference change (cm)
Under knee	R	31.47 ± 1.87	33.77 ± 1.49	34.78 ± 13.83	2.31 ± 0.90
	L	31.56 ± 2.11	33.89 ± 1.50	35.79 ± 14.31	2.33 ± 1.41
Calf	R	33.61 ± 1.73	36.27 ± 1.42	154.20 ± 45.50	2.66 ± 1.29
	L	33.60 ± 2.11	36.12 ± 1.57	157.86 ± 55.07	2.51 ± 1.26
Above the ankle	R	22.38 ± 1.17	24.17 ± 1.35	15.19 ± 4.27	1.79 ± 1.20
	L	22.51 ± 1.50	24.07 ± 1.40	15.36 ± 4.29	1.56 ± 1.29
Ankle	R	20.36 ± 1.11	21.83 ± 0.84	15.56 ± 4.50	1.47 ± 0.93
	L	20.68 ± 1.12	21.82 ± 0.98	15.79 ± 4.49	1.13 ± 0.68

Values are given as mean ± SD

L left, *R* right

Table 4 Correlation between augmentation and baseline circumference and volume of infiltrated fat in lower leg regions

	Under knee		Calf		Above the ankle		Ankle	
	L	R	L	R	L	R	L	R
IF (ml)	0.397*	0.415*	0.689*	0.542*	−0.497*	−0.275	−0.112	−0.361
BC (cm)	−0.732*	−0.664*	−0.581*	−0.545*	−0.452*	−0.505*	−0.775*	−0.728*

The values are presented as Spearman's coefficient of correlation

IF amount of infiltrated fat, *BC* baseline circumference, *R* right, *L* left

* $p < 0.05$

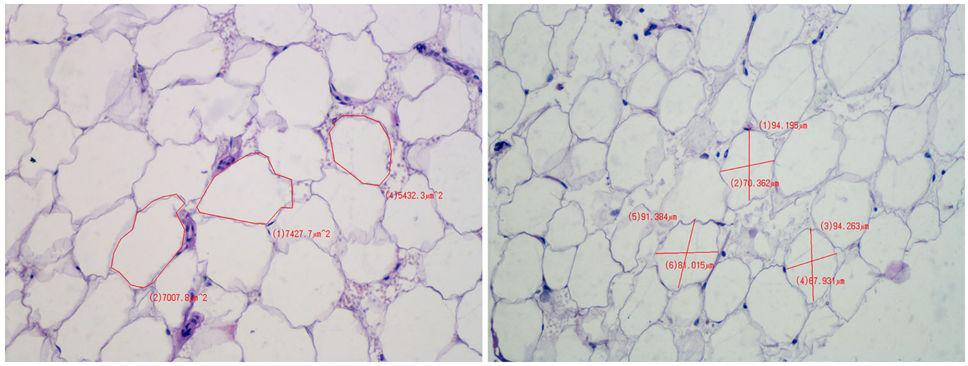

Fig. 1 Centrifuged fat. Preserved adipocytes with lost cell volume manifested in a rippled membrane, lower diameter, and present vascular space

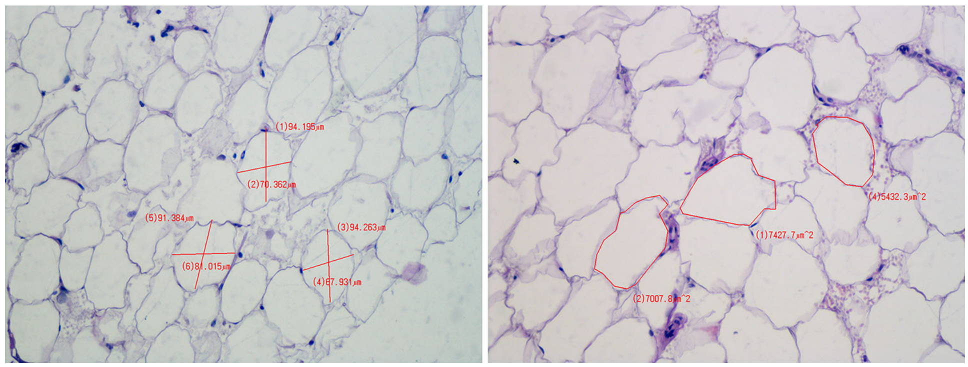

Fig. 2 Rinsed fat. Cells with proper membranes, larger diameter and bigger vascular stroma compared to centrifuged fat

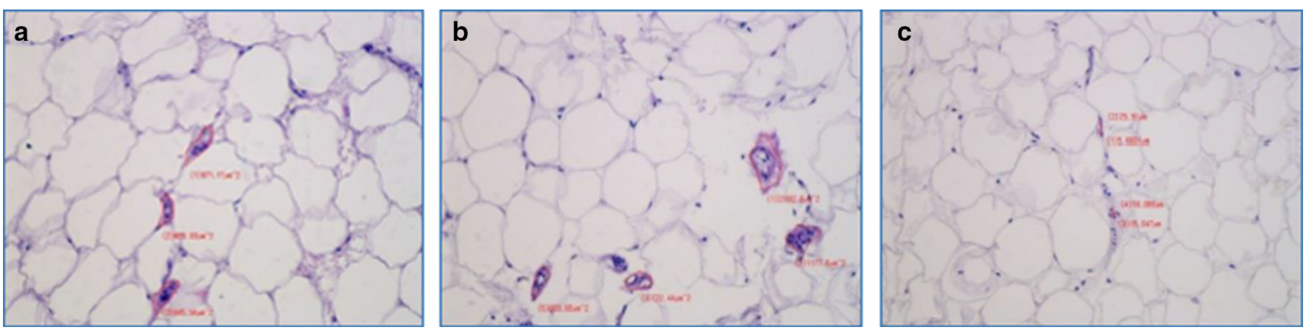

Fig. 3 Vascular stroma. **a** Centrifuged fat. **b** Rinsed fat. **c** Sample of subcutaneous fat surviving after 6 months

The particular significance of the technique of filling out the lower leg with fat tissue lays in the fact that it gives an ideal contour to the entire leg as well as the muscle, which agrees with the contour of the entire leg. The results achieved with one filling coincide with the standards of ideal leg proportions [4, 7]. There are no complications such as those in placing sub-fascial or sub-muscular implants, like postoperative hematomas, displacing or misplacing implants, capsular contracture, seromas, double contours, or compartment syndromes [3, 16], all leading to patient dissatisfaction and additional surgeries.

We measured patients' legs 1, 3, and 6 months after surgery. After 3 and 6 months, there was no change in circumferences in all regions of the lower leg, meaning that in the period between 3 and 6 months after surgery there was no resorption of the fat. Infiltrated fat showed a high level of stability 3–6 months after surgery; therefore, it can be concluded that the process of fat resorption ended at 3 months. The fat resorption rate has been calculated in regard to circumference change from month 1 to 6. The fat resorption rate was variable among the regions and in regard to the side of the body. It ranged from under the knee (left: 22.53 %, right: 24.11 %), in the calf (left: 26.47 %, right 26.75 %) to the lowest in the ankle, and above the ankle region. The highest variability was also noted in the ankle (left: 20.58 %; right: 15.63 %) and above the ankle area (left: 22.43 %; right: 14.18 %), in regard to the side of the body.

The exact proportions of the lower leg should be in agreement with the upper leg and the entire body. Defining

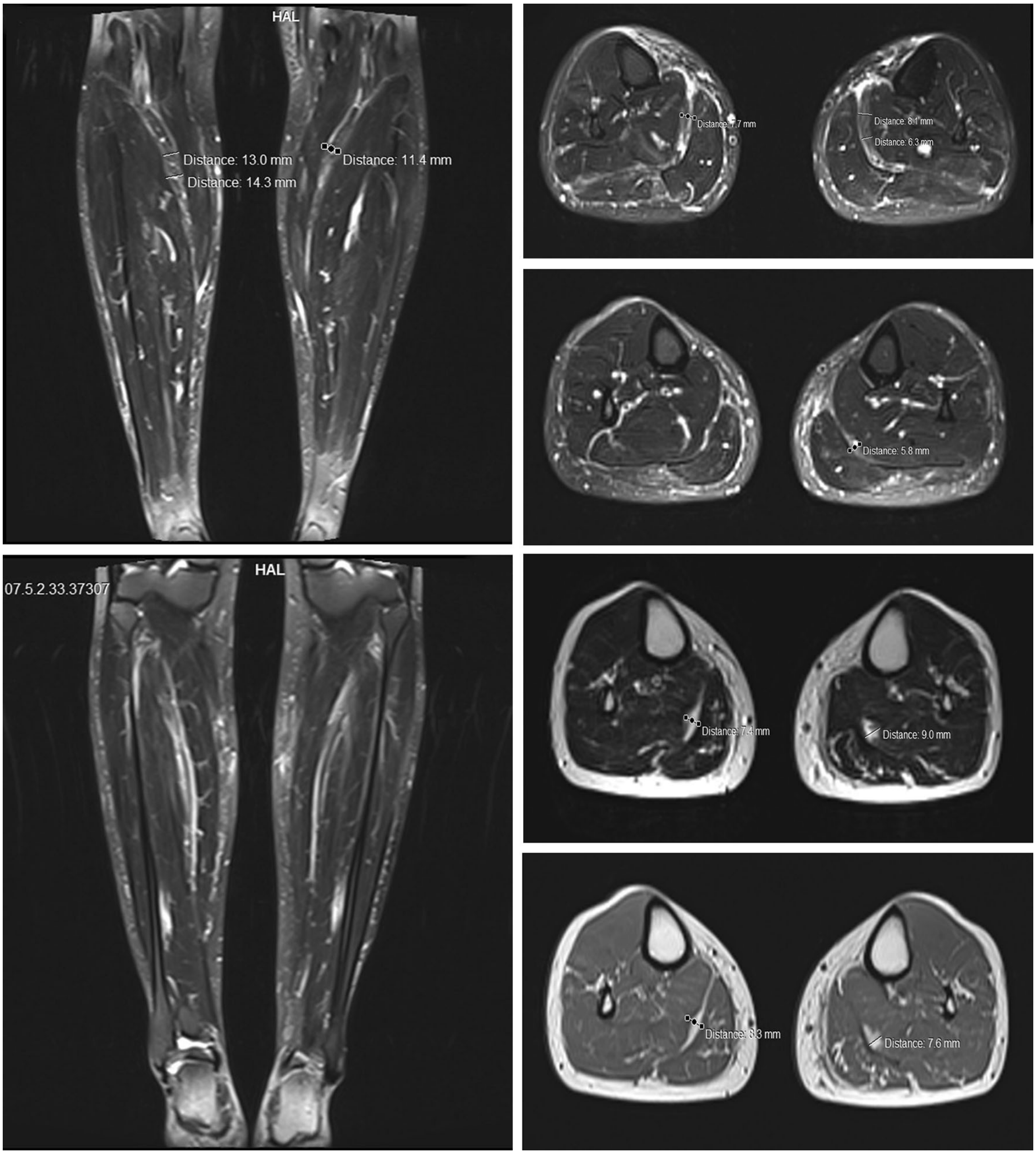

Fig. 4 MRI 6 months after. Survived rinsed fat infiltrated in muscle region of lower leg present between muscle fascia and muscle fibers within muscle groups

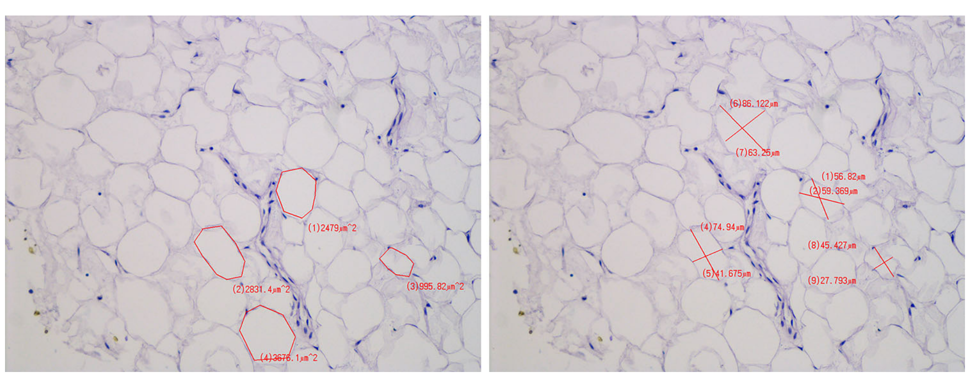

Fig. 5 Survived centrifuged fat in the sample from recipient region 6 months after

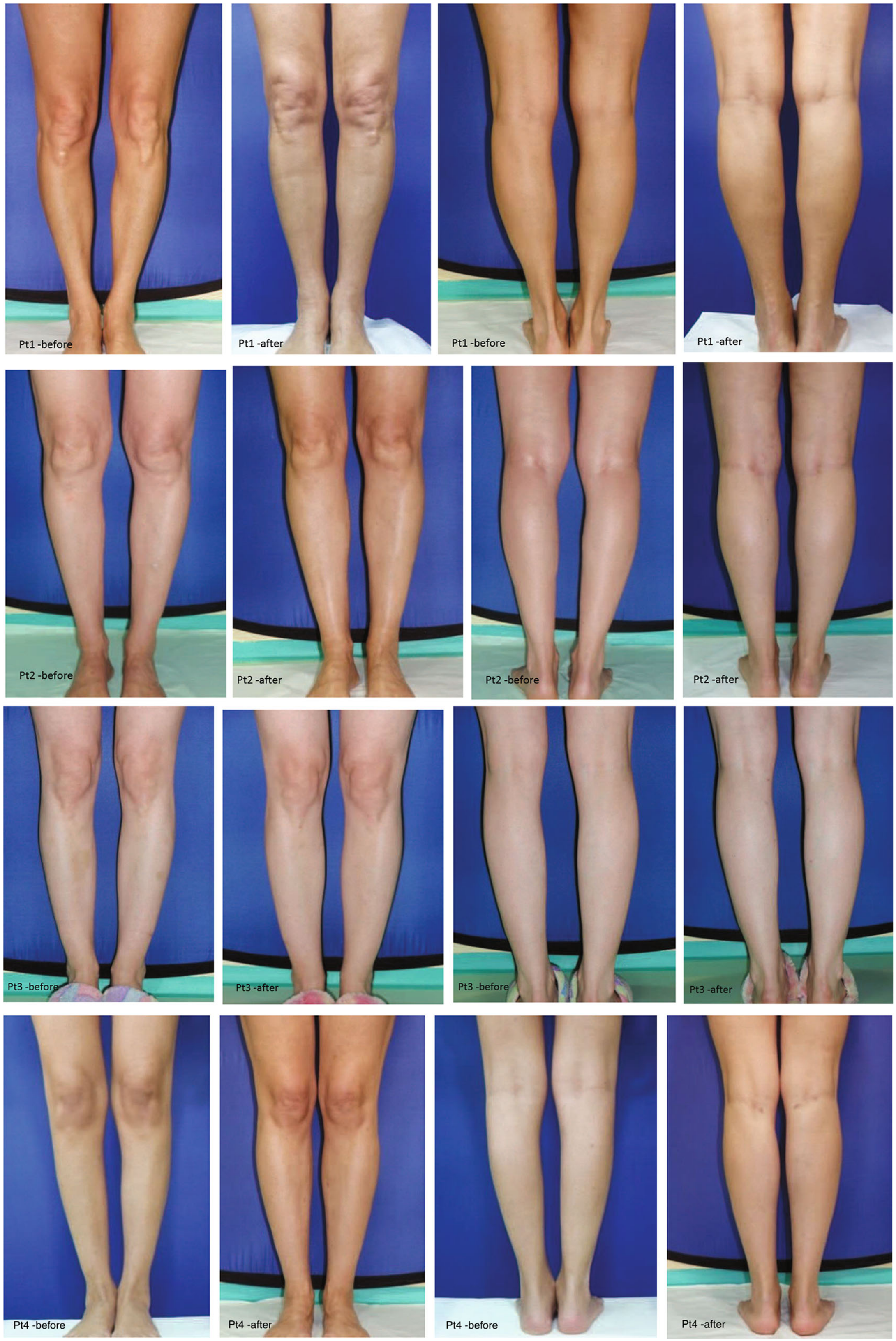

Fig. 6 Anterior and posterior appearance of the lower leg before the operation and 6 months after

ideal proportions [3, 6, 7, 16] is a prerequisite for election of the appropriate technique of placing implants, filling out the muscle, or combining these two procedures.

The patient photos (Fig. 6) show significant improvement in the way the lower leg looks as well as balancing the harmony with the upper leg.

The establishment of the technique of fat transplantation as well as its standardization brings numerous possibilities in body part contouring, while restoring its congruity. This technique allows patients to be fully mobile in the post-operative period, without any need for adaptation and getting accustomed to something new as with implants. We selected a uniform sample for the results to be as precise as possible—all women, similar age, weight, height, and socio-economic status. The research, in particular, focuses on women only, with augmentation of both lower legs, in a large sample and through parallel histological and MRI analyses. MRI volumetric studies and biopsies proved helpful in delineating survival of freely transplanted fatty tissue [22].

In this research, there was significant correlation between the quantity of infiltrated fat and the increase in circumference; however, it is essential to assure that the quantity of infiltrated fat in some areas of the lower leg does not compromise blood flow in any way. Fat injection in small amounts and in multiple layers and from different directions provides better fat survival and better volume to the recipient region [21].

One of the potential risks of both lipofilling and liposuction procedures is developing FES (fat embolism syndrome) caused by damage to large blood vessels [23, 24]. In our research, there were no complications such as embolism or hematoma. To minimize potential risks of fat embolism, liposuction was performed carefully and patiently, while lipofilling was performed with cannulas with small holes, and fat was injected while withdrawing the cannula. Also, during the surgical procedure and 24 h following the procedure, all patients were maintained on lower extremity compression systems to reduce the incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE). Post-operatively, 15 mmHg compression socks were used by all patients for 1 month, and all patients were on early mobilization.

In cases of trauma, multiple operations of the lower leg and various neuromuscular diseases, this technique will definitely ensure better survival of fat by infiltration of smaller quantities after 3 months, when the process of resorption is finished [15, 16]. In this way, the appearance of both legs can be completely uniform. We also noticed that adequate compression and dynamic postoperative care decrease leg swelling, thus producing better results in fat tissue survival and preserving the desired circumference. Securing safe fat transfer and avoiding contamination play important roles in safety of the operation as well. Some older patients required additional augmentation because aging causes a change in metabolic cell processes that reduces the ability of positive cell adaptation.

Conclusion

Transfer of fat tissue from the donor region to the recipient region of the lower leg is definitely the least invasive method of lower leg contouring. For patients with muscle dystrophies or those with trauma injuries, this technique could become the first choice for its minimal complications. The simplicity of the procedure invites its wide application in plastic surgery.

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