

A New Method to Quantify the Effect After Subcutaneous Injection of Lipolytic Substances

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Abstract

Background Increasing numbers of patients request lipolytic injection therapy for aesthetic indications. However, only the clinical results of these therapies have been published to date. In most cases, pre- and postprocedure photographs and measurements have been presented. As with every other medical procedure, it is necessary to ensure that the results of lipolytic injections are quantified on an objective and scientific basis with comparable data. **Methods** In the past, the size of fat tissue could not be measured properly with conventional ultrasound systems. High-resolution, real-time three-dimensional (RT-3D) ultrasound is a fairly new method for measuring the volume of tissue. Therefore, this study aimed to measure the interscapular fat bodies of New Zealand rabbits before and after lipolytic therapy with Lipostabil®, phosphatidylcholine and orciiprenalin (Alupent®).

Results The ultrasound-controlled injection of the lipolytic substances into the interscapular fat body ensured a precise injection. The RT-3D ultrasound data were compared with the magnetic resonance imaging (MRI) measurements performed at the same time. The greatest

decrease in volume, up to 44%, was measured with orciiprenalin (Alupent®). There was a significant correlation between the data from ultrasound imaging and MRI.

Conclusion The data suggest that RT-3D ultrasound imaging could be a simple and fast method for proving the effects on volume size after lipolytic procedures. Of the three investigated substances, orciiprenalin (Alupent®) showed the highest lipolytic effect in our animal model.

Keywords Lipolytic injection therapies · Magnetic resonance imaging · Real-time three-dimensional imaging

The subcutaneous injection of lipolytic substances such as phosphatidylcholine to reduce fat deposits has become a very popular alternative to liposuction. In fact, the Aesthetic Surgery Education and Research Foundation recently announced its approval from the U.S Food and Drug Administration (FDA) to initiate a clinical trial investigating the safety and efficacy of one type of injection lipolysis treatment. The advantages of these injection therapies are quite obvious, especially when liposuction is either not indicated or desired [1–3].

The quantity of volume reduction achieved with lipolytic drugs remains to be discussed critically [4, 5]. On the one hand, studies under in vitro conditions to investigate the mechanism of the used products, such as phosphatidylcholine solubilized in deoxycholate (Lipostabil®), still are missing. On the other hand, it is difficult to quantify the volume reduction of a local fat deposit using pre- and postprocedure photographs and simple pre- and postprocedure measurements on the surface of the body part in which lipolysis was performed [1–3]. This method is likely to be influenced by certain confounders: First the increase and decrease in volume can hardly be judged by pre- and posttherapy photographs. Differences in light

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setting, camera angle, patient body position, and patient inhalation or exhalation are just a few important factors that may falsify the results. Therefore, this type of presentation lacks a scientific basis. To ensure the quality and efficacy of these procedures on a scientific basis, the effect of these therapies must be quantified by measuring the total difference in volume before and after the procedure.

In the past, it was almost impossible to obtain reliable results using conventional ultrasound imaging to display fat tissue because the inhomogeneous echogenicity did not allow an exact differentiation of the subcutaneous structures [6]. In addition to the low definition, the volume was defined only by three parameters: length, width, and depth.

For this study, a high-resolution, real-time three-dimensional (RT-3D) ultrasound imaging system developed to guarantee optimized soft tissue visualization was used. Furthermore, it is possible with this system to produce digital slices of the tissue comparable with a magnetic resonance image (MRI) [8]. These two advantages allow a precise scan of the fat body in its volume and shape.

The current study investigated the volume measurement performed with this new ultrasound technique compared with MRI after injection of several lipolytic and nonlipolytic substances in the interscapular fat body of six rabbits. The volume of the corpus adiposum interscapulare was measured using RT-3D ultrasound imaging (Logiq 9; general electric, GE, Solingen, Germany). The MRI images were performed with the 1.5-T, Symphony, (Siemens, Erlangen, Germany) [1–4].

Methods

Experimental Model

Six New Zealand white rabbits, raised for experimental purposes by Harlan Winkelmann (Borchen, Germany), were allocated randomly into three groups, with two rabbits in each group as follows: rabbits 1 and 2 (group A), rabbits 2 and 3 (group B), and rabbits 5 and 6 (group C). The rabbits received the injections in their corpus adiposum interscapulare (nuchal fat body), considered to be the best fat deposit for this experimental model [5]. All the animals were maintained with water and food ad libitum at all times. The experiment was performed in accordance with the 1975 Declaration of Helsinki. Approval was obtained from the Regulatory Board before the animal procedures were begun.

Local Injection Procedure

The rabbits were anesthetized with intramuscular injections of Alfazyne 1.5 ml (Alfasan International B.V., Woerden,

Holland), 2% xylazine HCl 20 mg/ml (CMS Chemicals Ltd., Oxford, England), Alfamine 1.5 ml (Alfasan International B.V.), and 10% ketamine 100 mg/ml (Chmeos GmbH, Regenstauf, Germany) in the animals' lateral thighs.

The procedure was performed under ultrasonic control to guarantee placement of the injection needle precisely into the fat deposit. Three different substances were used in this experiment. We disclose all relationships to the manufacturers of the applied products (Cassella med, Sanofi-Aventis Group, Paris, France).

Each of the three groups received five 1-ml injections applied with 0.30×0.12 -mm insulin needles (Braun, Melsungen, Germany). Group A received phosphatidylcholine solubilized in 5 ml of intravenous deoxycholate (Lipostabil®; Artesan Pharma, Lüchow, Germany) in a concentration of 50 mg/ml. For many years, Lipostabil® has been in off-label use for the subcutaneous lipolysis, although it is pharmacologically classified as an intravenous serum cholesterol reducer. Group B was treated with 50-mg/ml of phosphatidylcholine solubilized in ethanol (synthesized in Laboratory of Trauma and Plastic Surgery, Regensburg, Germany) using a concentration of 50 mg/ml for investigation of a possible effect from the substance in which the phosphatidylcholine is solubilized. Group C was injected with orciprenaline (Alupent®; Boehringer Ingelheim) in a concentration of 0.1 mg/ml. This substance was found to induce lipolysis in an in vitro model because of its affinity to beta-1 receptors, which induce the lipolytic pathway in fat cells. The original indication for orciprenaline is bradycardia (beta-1 receptor affinity), bronchospasm, and asthma (beta-2 receptor affinity). This drug has many systemic side effects, such as tachycardia or even cardiac dysrhythmia, especially when accidentally applied intravenously or intraarterially. Considering these dangerous side effects, there is no question that this drug is suitable only for in vitro or animal models, even if used as low-dose subcutaneous injections.

All three groups were reexamined 10 days after treatment to determine whether a change in volume had occurred. The experiment was repeated for group C because of the dramatic unexpected change in volume with orciprenaline. The repeated injection for this group was performed using the exact same technique. After 21 days, another evaluation of the change in volume was performed.

RT-3D Ultrasound Imaging

For the injection process, a high-resolution linear sound (9–14 MHz, Logic 9, GE) was used [6–11]. For volume rendering before application of the lipolytic substances, 10 digital sets of volume data were acquired for each rabbit. Data acquisition followed automatic scanning from the

lateral border of both nuchal fat body legs. Three-dimensional volume rendering was accomplished by simultaneous measurement of longitudinal and sagittal cross sections.

This technique allows scanning of the animal's fat body by creation of digital slices comparable with the slices of an MRI image. With this new ultrasound imaging, fat volumes can be measured accurately, and fat water artifacts, which we know from the MRI, are avoided. The demarcation from the other tissue (i.e., the subcutaneous fat) is more precise. The images were independently interpreted by two radiologists who had no previous knowledge of the substances injected.

MRI

Before and after every treatment, the anesthetized rabbits underwent the MRI (1.5-T, T1-weighted axial and coronal sequences). This was performed with a high-resolution surface coil (8-channel knee coil) (Fig. 1), which turned out to be the best method for avoiding artifacts. After all three orthogonal scan directions had been located, visualization of the fat body was performed using a coronal T1-weighted 3D FLASH sequence ($0.55 \times 0.55 \times 0.5\text{-mm}^3$ voxels, 140-mm field of view, 256×256 matrix, 0.5-mm thickness, 30-ms repetition time, 4.76-ms echo time, 40° flip angle).

Statistical Analysis

All data are presented as mean \pm standard error of the mean (SEM). The effect of adipose tissue volume reduction after local lipolytic therapy was statistically verified using the paired *t* test. Therefore, the sample values of all 10 nuchal fat body volume measurements for each rabbit

before and after lipolytic therapy with each substance were compared. For rabbit 4, normality testing failed, and a Wilcoxon signed rank test was applied. A *p* value less than 0.05 was considered significant, and a *p* value of 0.001 or less was considered highly significant. The results were marked in the graphs with one or two asterisks, respectively. All analyses were performed using SigmaPlot and SigmaStat (both SPSS Inc., Chicago, IL, USA).

Results

The RT-3D ultrasound-controlled injection of the lipolytic substances into the interscapular fat body ensured a precise injection into the fat tissue (Figs. 2 and 3). Therefore, intramuscular injections, which are likely to damage the muscular tissue, could be avoided. This was investigated by the pathologist who evaluated the histologic samples (Fig. 4). The pathologist was not informed about the applied substances. The decrease in the fat body's volume could be demonstrated in both the MRI images and the ultrasound scans (Fig. 5).

After Lipostabil[®] treatment (Fig. 6), rabbits 1 and 2 showed a 9% to 12% decrease in volume after 10 days. On day 0, a volume of $11.21 \pm 0.13\text{ cm}^3$ was measured for rabbit 1. On day 10, this rabbit's volume decreased to $10.54 \pm 0.09\text{ cm}^3$ ($p \leq 0.001$, paired *t* test).

Rabbit 2 showed a volume of $9.73 \pm 0.09\text{ cm}^3$ on day 0, which decreased to $8.68 \pm 0.14\text{ cm}^3$ on day 10 ($p \leq 0.001$, paired *t* test). The measurement results of the two radiologists did not differ significantly.

The treatment with phosphatidylcholine (Fig. 6) led to a volume reduction of 16% to 23%. On day 0, a volume of



Fig. 1 Anesthetized rabbit in an eight-channel knee coil during a magnetic resonance imaging (MRI) procedure

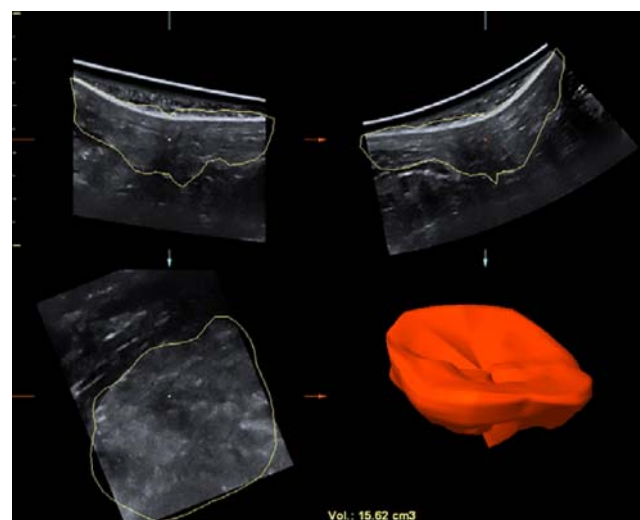


Fig. 2 Three-dimensional volume rendering of the interscapular fat body before local lipolysis and volume measurement of 15.62 cm^3 from digital source data

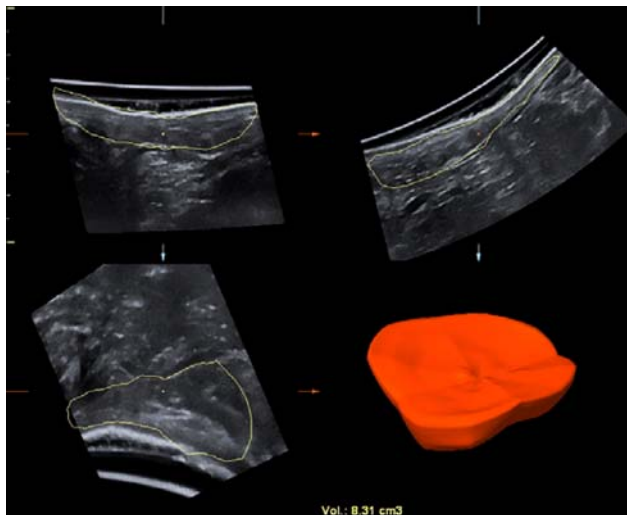


Fig. 3 Three-dimensional volume rendering of the interscapular fat body 3 weeks after local lipolysis and volume measurement of 8.31 cm^3 with significant volume reduction from 15.62 to 8.31 cm^3

Fig. 4 The histopathology shows that the needle was placed precisely in the fat tissue as seen under ultrasound control. The puncture channel and the tissue reaction are visible (arrow)

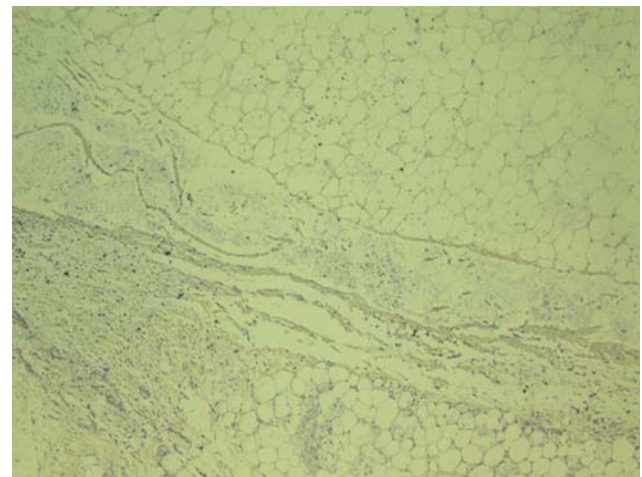
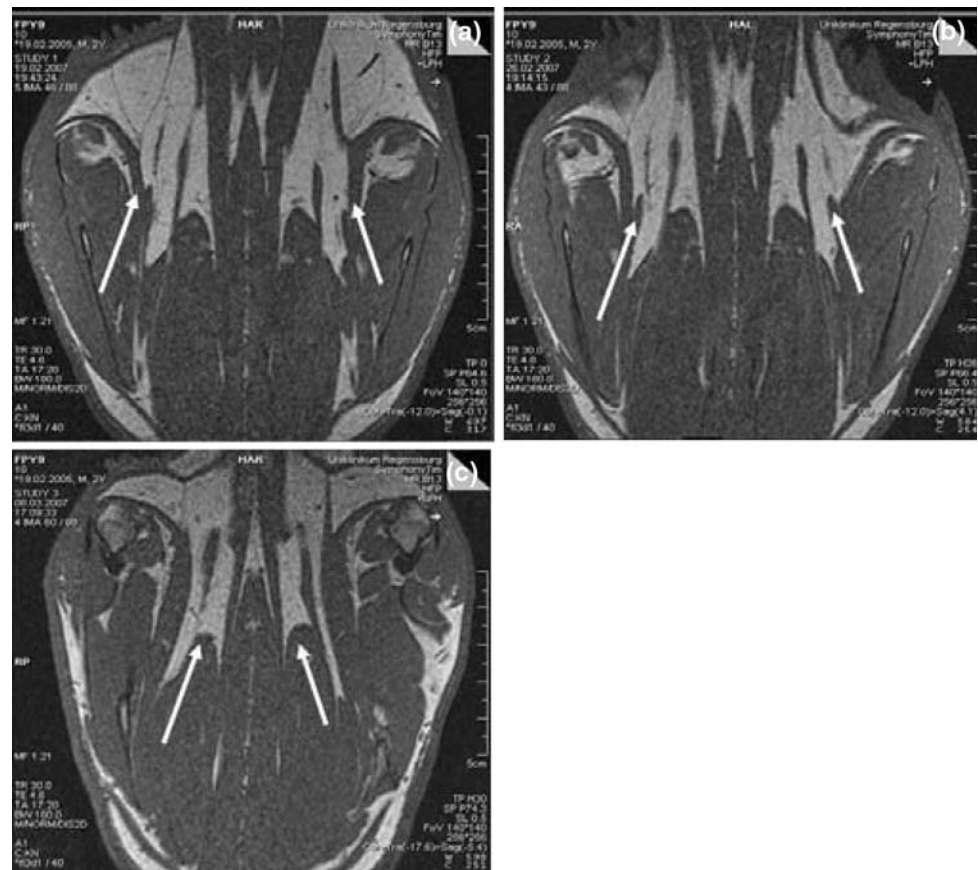


Fig. 5 Magnetic resonance imaging (MRI) of the corpus adiposum interscapulare (a) before, (b) 10 days after, and (c) 3 weeks after local lipolysis. Sagittal imaging shows the volume reduction effects after lipolysis (arrows)



$13.09 \pm 0.22 \text{ cm}^3$ was measured for rabbit 3. The volume was $10.97 \pm 0.15 \text{ cm}^3$ on day 10 ($p < 0.05$, Wilcoxon signed rank test). The volume for rabbit 4 decreased from $14.18 \pm 0.12 \text{ cm}^3$ on day 0 to $10.83 \pm 0.16 \text{ cm}^3$ on day 10 ($p \leq 0.001$, paired t test). The measurements of the two radiologists did not differ significantly.

Group C, treated with orciprenalin (Alupent®) (Fig. 6), showed a decrease in volume of 15 to 22% after 10 days. The volume lowered in rabbit 5 from $12.90 \pm 0.13 \text{ cm}^3$ on day 0 to $10.32 \pm 0.13 \text{ cm}^3$ on day 10 ($p \leq 0.001$, paired t test). Rabbit 6 showed a decrease from $15.58 \pm 0.16 \text{ cm}^3$ on day 0 to $12.95 \pm 0.15 \text{ cm}^3$ on day 10 ($p \leq 0.001$,

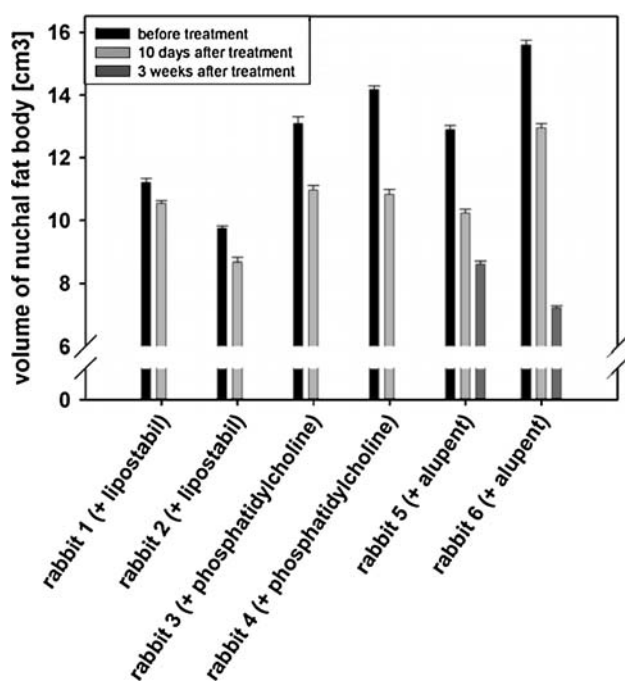


Fig. 6 Graphic results from ultrasound volume measurements of the interscapular fat body (nuchal fatbody) of six rabbits after percutaneous injections of three various lipolytic substances (■) before injection, (□) after 10 days, and (▨) after 3 weeks in two cases after a second injection

paired *t* test). The measurement results of the two radiologists did not differ significantly.

In Group C the treatment with orciiprenlin (Alupent®) (Fig. 6) was repeated. Rabbits 5 and 6 therefore underwent the injection procedure again on day 10. This led to a decrease in volume of 19 to 44% until day 21 compared with the measurements on day 0. The volume of the interscapular fat body measured on day 21 was $8.62 \pm 0.10 \text{ cm}^3$ in rabbit 5 and $7.21 \pm 0.07 \text{ cm}^3$ in rabbit 6 ($p \leq 0.001$, paired *t* test). The measurement results of the two radiologists did not differ significantly [2, 3].

The evaluation of the MRI images as single slices of the interscapular fat bodies showed an average decrease in volume of 9.7% in group A, 17.5% in group B, and 20.6% in group C after 10 days [4].

Discussion

Although this study was performed with a rather small group of six animals, the changes in volume of the corpus adiposum interscapulare are significant (Fig. 6). Based on the results of this study, RT-3D ultrasound imaging is an appropriate method for providing evidence of a decrease in volume after the injection of lipolytic substances.

The efficacy of this new method is quite obvious. It is a simple noninvasive procedure without any side effects, and

it is reproducible, allowing repeated applications. Furthermore, the expenses for ultrasound scans are rather reasonable compared with the costs of an MRI, although the accuracy and scientific evidence for this specific query are equal.

On the other hand, this method also has some failure risks. Two investigators may have different results depending on their level of training as well as the angle or pressure of the ultrasound head positioning on the tissue. These confounders were eliminated by having two independent physicians perform the ultrasound procedure, both of whom had practiced proper handling of the RT-3D system.

It can be concluded that although the RT-3D ultrasound method is not entirely independent of external influences, it has many advantages over pre- and posttreatment photographs and surface measurements.

The experiment demonstrated that a reduction in the volume of fat tissue after injection of our samples with lipolytic drugs can be observed after a period of 10 days. However, the mechanism of this decrease in fat tissue still remains unknown and requires further investigation. This is especially important because an increasing number of patients have been treated with Lipostabil.

Considering the increasing demand for lipolytic injection therapies in the field of aesthetic medicine, the RT-3D ultrasound could be a new method for comparing the clinical potential of various lipolytic drugs on a scientific basis. Furthermore, the ultrasound-controlled precise injection into the fat tissue prevents damage caused by false intramuscular injections.

References

1. Ablon G, Rotunda AM (2004) Treatment of lower eyelid fat pads using phosphatidylcholine: clinical trial and review. *Dermatol Surg* 30:422–427
2. Rittes PG (2003) The use of phosphatidylcholine for correction of localized fat deposits. *Aesth Plast Surg* 27:315–318
3. Hasenschwandtner F (2005) Phosphatidylcholine treatment to induce lipolysis. *J Cosm Dermatol* 4:308–313
4. Rittes PG (2006) Injection of phosphatidylcholine in fat tissue: experimental study of local action in rabbits. *Aesth Plast Surg* 30:474–478
5. Salles AG (2006) Histologic response to injected phosphatidylcholine in fat tissue. Experimental study in a new rabbit model. *Aesth Plast Surg* 30:479–484
6. Lell M, Wenkel E, Aichinger U, Schulz-Wendtland R, Bautz W (2003) Einsatz des 3D Ultraschalls bei der Stanzbiopsie unklarer Mammaläsionen. *Ultraschall Med* 24:126–130
7. Rose Sc, Nelson TR, Deutsch R (2004) Display of 3-dimensional ultrasonographic images for interventional procedure: volume-rendered versus multiplanar display. *J Ultrasound Med* 23:1465–1473
8. Merz E, Welter C (2005) 2D and 3D Ultrasound in the evaluation of normal and abnormal fetal anatomy in the second and third trimester in a level III center. *Ultraschall Med* 26:9–16

9. Prantl L, Pfister K, Kubale R, Schmitt S, Stockhammer V, Jung W et al (2007) Value of high-resolution ultrasound and contrast enhanced US pulse inversion imaging for the evaluation of the vascular integrity of free-flap grafts. *Clin Hemorheol Microcirc* 36:203–216
10. Jung EM, Kubale R, Jungius K-P, Jung W, Lenhart M, Clevert D-A (2006) Vascularization of liver tumors: preliminary results with coded harmonic angio (CHA) phase-inversion imaging, 3D power Doppler and contrast medium-enhanced B-flow with second-generation contrast agent (Optison). *Clin Hemorheol Microcirculation* 34:483–497
11. Jung EM, Jungius K-J, Rupp N, Gallegos M, Ritter G, Lenhart M et al (2005) Contrast enhanced harmonic ultrasound for differentiating breast tumors: first results. *Clin Hemorheol Microcirculation* 33:109–120