Aesth Plast Surg (2008) 32:111–115
DOI 10.1007/s00266-007-9031-8

ORIGINAL ARTICLE

Histologic Effects of External Ultrasound-Assisted Lipectomy on Adipose Tissue

Giuseppe A. Ferraro · Francesco De Francesco · Gianfranco Nicoletti · Ferdinando Rossano · Francesco D'Andrea

Received: 9 February 2007 / Accepted: 23 March 2007 / Published online: 20 September 2007

Abstract This study aimed to observe the effects of ultrasound waves at different frequencies on abdominal fat tissue. External ultrasound-assisted lipectomy (XUAL) via both histologic and immunohistochemic examinations was used to assess adipose tissue alterations, including cells and collagenic fibers. The results, at the immunofluorescence level, show that ultrasound used at 1 MHz with a potency of 3 W resulted in no alterations or only limited cell destruction with collagen fibers intact. In contrast, when the ultrasound was 2 and especially 3 MHz, adipocyte alterations usually were evident. Massive adipose tissue destruction, confirmed using Oil red-O staining, was observed. In addition, at the immunofluorescence level, diffuse collagen fiber retraction was detected. This was particularly evident in comparisons with biopsies of intact control samples, which showed normal adipose tissue and intact collagen fibers. The results obtained using morphologic techniques, which do not allow fixation artifacts and include collagen observations, demonstrate that with the XUAL technique, ultrasound at 1 MHz does not induce cellular alterations. In contrast, both 2- and 3-MHz frequencies are capable of causing complete fat tissue disruption, including destruction of adipose cells and collagenic fibers.

G. A. Ferraro (✉) · G. Nicoletti · F. Rossano · F. D'Andrea
Department of Orthopedic, Traumatologic, Rehabilitative, and Plastic Reconstructive Sciences, Second University of Naples, School of Medicine and Surgery, via L. De Crecchio 3, 80138 Napoli, Italy
e-mail: giuseppe.ferraro@unina2.it

F. De Francesco
Dipartimento di Medicina Sperimentale, Sezione di Istologia ed Embriologia, TESLAB, Seconda Università di Napoli, via L. Armanni 5, 80138 Napoli, Italy

Keywords Abdominal adipose tissue · External ultrasound–assisted lipectomy · Histology · Immunohistochemistry

The possibility of using ultrasound "on the surface" was hypothesized by Scuderi et al. [1] in 1987, Zocchi [2, 3] in 1992 and 1996, and Silberg [4] in 1998. Ultrasound-assisted liposuction (UAL) is a technique that can be used with suction-assisted lipectomy, the most common procedure in aesthetic plastic surgery [5–7]. The latter has been improved and modified mainly to decrease postoperative complications [8–10]. This new technique, extended to lipectomy and liposuction, is known as external ultrasound-assisted liposuction (EUA or XUAL).

The XUAL technique offers many abilities, rendering it more attractive than UAL. Actually, whereas UAL emulsifies adipose tissue due to adipocyte membrane lysis, XUAL, being less "invasive," induces a cell-to-cell contact, loosening and altering collagenic fibers. This leads to easier detachment of adipose cells, which remain unaltered, allowing them to be removed mechanically. This is possible because XUAL activity leads to fibrolysis rather than lipolysis [11–13]. Therefore, XUAL should be associated with liposuction and other techniques for body remodeling, mainly in cases of localized adiposities.

Currently, the XUAL procedure is used successfully for body and fat liposculpture. It also is used in combination with other surgical techniques for body contouring. Numerous authors have demonstrated its effectiveness in association with liposuction or a nonsurgical method, such as slimwave, for the treatment of localized adiposities.

The XUAL technique is the transcutaneous application of high-frequency ultrasound to massive infiltrated tissue, followed by traditional liposuction. Tissue irrigation is needed to make ultrasound conductibility possible and to make mechanical hydrodissection of the tissue easier. To date, a few studies have described the morphologic alterations of fat tissue as a result of UAL and XUAL.

Recently, some authors [14] have described the histologic effects of UAL on adipose tissue of the breast, stressing that UAL is a safe technique for use in breast surgery. However, their studies were limited to the histologic level, with UAL used at a frequency of 19.8 kHz and an output power of 50 to 60 W. The latter, because a limited frequency was used, does not allow for complete understanding of the morphologic effects on adipose cells. Moreover, the study was focused on the UAL, not the XUAL, technique.

Therefore, the current study aimed to observe the effects of ultrasound waves at different frequencies using the XUAL technique on abdominal fat tissue, with both histologic and immunohistochemical examinations performed to assess adipose tissue alterations, including cells and collagenic fibers.

Materials and Methods

Ultrasound comprises mechanical vibrations characterized by rarefactions and compressions of the medium in which they spread as elastic waves. The ultrasound speed of propagation is directly proportional to the density of the medium (e.g., 331 m/s in air and 1,410 m/s in water).

The structural scheme of an ultrasound device is extremely simple. It is made of a high-frequency generator, a radiofrequency transmission cable, and a probe with a piezoelectric crystal (the most modern are made of barium, or zirconium titanate). The piezoelectric crystal contained in the probe vibrates when it is stimulated by a high-frequency current, generating the ultrasonic waves (inverse piezoelectric effect). The interaction between the ultrasound and the tissues determines three main biologic effects:

1. *Mechanical effect.* This effect really is noteworthy. In fact, waves of 2.6 atmosphere are generated with a frequency of 0.8 MHz and a power of 2 watt/cm^2.
2. *Cavitation*: Bubbles are formed at high pressure in the tissue crossed by the ultrasound. As a matter of fact, in therapeutic doses and at the maximum emissions of power allowed in the European Community, cavitation should never take place.
3. *Thermal effect.* The thermal effect is reduced to a maximum of 2/3°C with 10-cm probes.

The biologic effects of ultrasound on living tissues are due to their physical characteristics, which determine a few phenomena when they come into contact with the tissue:

- *Absorption*, with attenuation of the beam of waves.
- *Penetration*, whose depth is inversely proportional to the frequency of emission. At an ultrasound frequency of 1 MHz, the depth of penetration is 7 mm into bone, 30 mm into muscular tissue, and 37 mm into skin and subcutaneous tissue.
- *Refraction and reflection*, which are well known in optical physics. These are particularly important in living tissue for the presence of the so-called "boundary layer," which is the plane of separation between two tissues of various densities on which the total biologic effects of ultrasound are amplified because of the aforementioned phenomena. The boundary layer in the tegument is evidently the fat tissue–fibrous tissue interface, so the greatest action of ultrasound takes place right on the intraadipocytary collagenic septa [10].

For this study, the new device Liposound (AMT/LUAN, Alpignano (TO), Italy) was used for XUAL. Produced for use in Europe, Liposound was chosen because it totally conforms to CE European norms approved by the Italian Ministry of Health (Law 93/42). The frequencies of emission from this equipment vary from 1 to 3 MHz. The maximum power is 3 W/cm^2 on the surface of the probe with a continuous or pulsating method emission and continuous monitoring of ultrasound emissions. The device is equipped with two power outputs that allow the simultaneous installation of two probes with different frequencies and powers of emission for the treatment of more regions at the same time. Moreover, the device is equipped with probes that have different diameters for different uses.

Eight patients (5 women and 3 men, ages 20 to 49 years) undergoing an abdominal lipectomy for diffuse abdominal lipodystrophy were treated with XUAL at frequencies of 1, 2, and 3 MHz with an ultrasound potency of 3 W/cm^2 A Klein cold physiologic solution containing 50 mg of lidocaine, 1 mg of adrenalin, and 12.5 ml of $NaHCO_3$ and left at 4°C for 30 min was used for infiltration. To improve conduction of the ultrasound through the tissue, 600 g per a 30-cm^2 area was infiltrated.

After anamnesis, objective examination, photography, design, planning of surgical participation and recordings, general anesthesia, and placement of cannulas, each patient underwent a "superwet" infiltration uniformly by means of needles from 0.70 mm × 30 mm (22 G – 1¼ ml) and then the XUAL application. Two 10-cm probes with frequencies ranging from 1 to 3 MHz and a power of 3 W/cm^2 were applied to the cutaneous surface through an ecographic gel used to assist in the treatment and make good contact with the skin to favor the conduction of the ultrasound. Ultrasound in continuous emission to 100% of the power of escape was administered for 20 min by means of a slow but

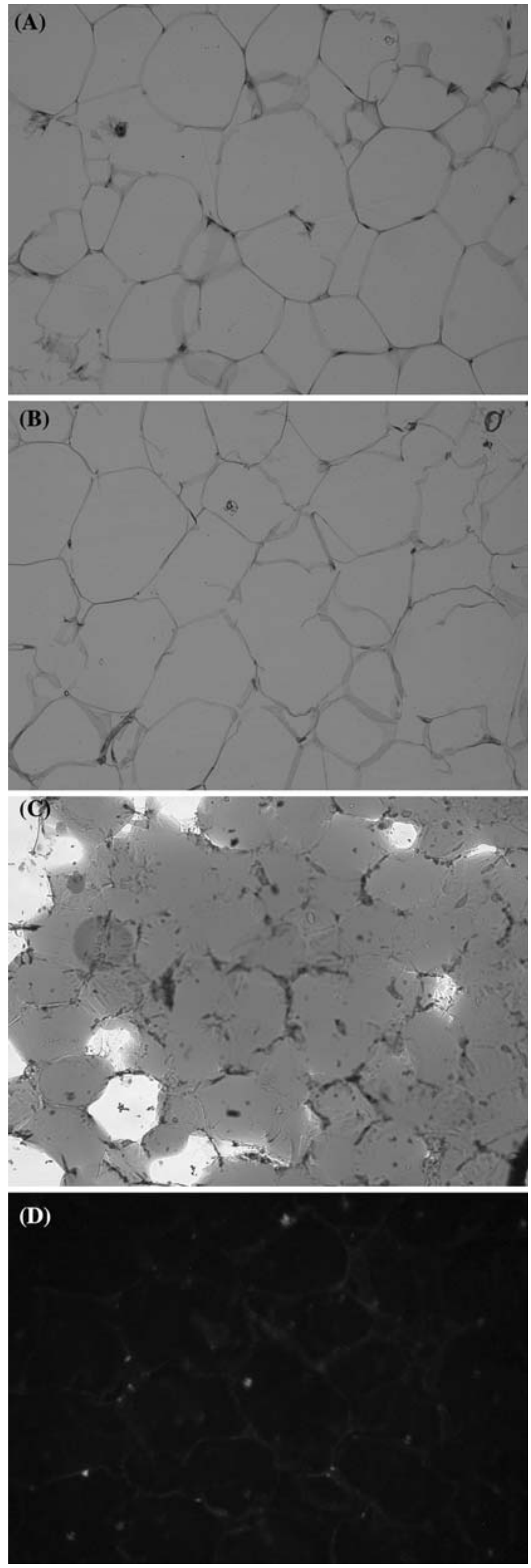

Fig. 1 (**A**) Abdominal adipose biopsy of a patient treated with ultrasound at a frequency of 1 MHz. Adipocytes are intact at this level (hematoxylin–eosin staining; original magnification, ×100). (**B**) Same sample with Mallory's trichromic stain (original magnification, ×200). (**C**) Abdominal adipose biopsy of a patient treated with ultrasound at a frequency of 1 MHz. Oil red-O staining shows that tissue is almost intact (original magnification, ×200). (**D**) Abdominal adipose biopsy of a patient treated with ultrasound at a frequency of 1 MHz. Immunofluorescence PE (Phycoerythrin) staining for collagen type 1 fibers shows that they are of normal appearance (original magnification, ×200)

continuous rotation. Skin temperature was measured by means of a thermical probe linked to a digital analyzer (FLUKE 17; Fluke Corporation, Everett, WA, USA).

The control tissue was not subjected to XUAL treatment. It underwent removal of fat tissue via lipectomy rather than lipoaspiration to avoid any mechanical damage. Biopsies were subdivided into two parts and fixed in formalin or stored at –80°C. Tissues fixed in formalin then were dehydrated in alcohol, clarified in xylene, and embedded in paraffin. Sections (5 micron thick) were stained with hematoxylin–eosin and Mallory's trichromic stain and observed using light microscopy. Some sections were washed in 0.1 M of phosphate-buffered saline (PBS) with 3% bovine serum albumin (BSA), then challenged with collagen type 1 mouse antihuman antibodies (Santa Cruz, CA, USA). The secondary antibody was a fluorescein isothiocyanate (FITC)-conjugated or Phycoerythrin-conjugated goat antimouse (Santa Cruz, CA, USA).

As a control condition, the same sections were treated with the secondary antibody. Stained sections were analyzed under a fluorescence microscope (Olympus BX41; Tokyo, Japan) to verify the effect of the ultrasound on adipocytes and collagen fibers. The remaining biopsies, kept at –80°C, were sectioned using a cryostat and stained with Oil red-O, which can show neutral fats, avoiding histologic artifacts.

Results

The results show that when the XUAL-treated tissue is examined in comparison with a control untreated tissue, several different effects on both adipose cells and collagen fibers are detected. In fact, sections from patients treated with an ultrasound frequency of 1 MHz and a potency of 3 W showed no cell destruction at the light microscope level and partial conglomeration of collagenic fibers (Fig. 1A and B).. In particular, it was difficult to distinguish artifacts detected in both the treated and control samples from putative ultrasound destruction. Oil red-O staining, performed on cryosections, confirmed that in only a few cases, adipocytes presented with rounded membranes, and that in limited cases, cells were detached from each other (Fig. 1C). In fact, immunofluorescence showed that these fibers were either intact or conglomerated when the ultrasound was applied at 1 MHz (Fig. 1D).

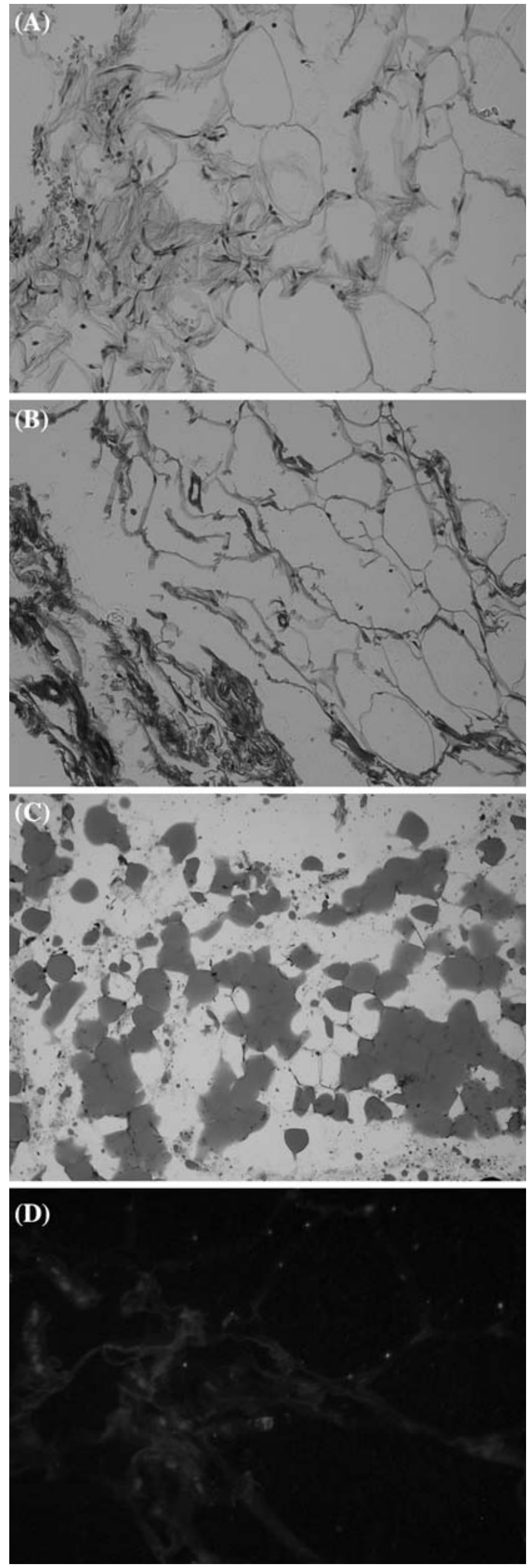

Fig. 2 (**A**) Abdominal adipose biopsy of a patient treated with ultrasound at a frequency of 3 MHz. Adipocytes are disrupted at this level (hematoxylin–eosin staining; original magnification, ×200). (**B**) Same sample with Mallory's trichromic staining (original magnification, ×100). (**C**) Abdominal adipose biopsy of a patient treated with ultrasound at a frequency of 3 MHz. Oil red-O staining shows that adipose tissue is widely deranged (original magnification, ×200). (**D**) Abdominal adipose biopsy of a patient treated with ultrasound at a frequency of 3 MHz. Immunofluorescence PE staining for collagen type 1 fibers shows that they are completely retracted (original magnification, ×200)

In contrast, ultrasound at 2 and 3 MHz with a potency of 3 W resulted in adipocyte alterations, but mainly in the biopsies of patients who had ultrasound at 3 MHz. In fact, these latter patients experienced large adipose tissue destruction (Fig. 2A and B), confirmed with the Oil red-O staining. Complete derangement of fat tissue was observed in the biopsies of these patients (Fig. 2C). In addition, diffuse collagen fiber retraction at the immunofluorescence level was observed (Fig. 2D). This was particularly evident in comparison with the biopsies of intact controls, which showed normal adipose tissue and intact collagen fibers (Fig. 3).

Discussion

For patients with moderate obesity, XUAL is a good method for localized fat removal [15]. This technique can be used for fat tissue removal while avoiding effects on other vital structures. To date, few studies have examined the effects of XUAL on adipose cells. Only one report has described the histologic effect of the UAL technique on breast fat tissue [13]. The reported study focused on adipose and breast tissue, but did not take into consideration that adipose cells are subjected to many histologic artifacts. The only way to assess their structural derangement is to cryopreserve fresh tissue at –80°C, cryosection the fragments, and stain them using Oil red-O. In addition, no observations were performed for collagen fibers. This is important when XUAL [16] is used because although fat tissue derangement may involve the whole architecture, it affects collagen primarily.

The simple observation of cell-to-cell detachment, which has been reported as cell contact loosening, does not establish that an effective disruption has occurred. In addition, no studies have been performed to date with different XUAL frequencies, which can help in understanding the appropriate frequency for fat disruption.

We therefore performed this study to assess the level of fat disruption and the involvement of collagenic fibers in relationship to XUAL MHz frequencies that lead to a complete adipose tissue derangement. Using hematoxylin-eosin and Mallory staining, we found that biopsies of patients treated with 1 MHz do not show clear cell destruction at the light microscopic level. Actually, within sections, it was difficult to distinguish between artifacts of

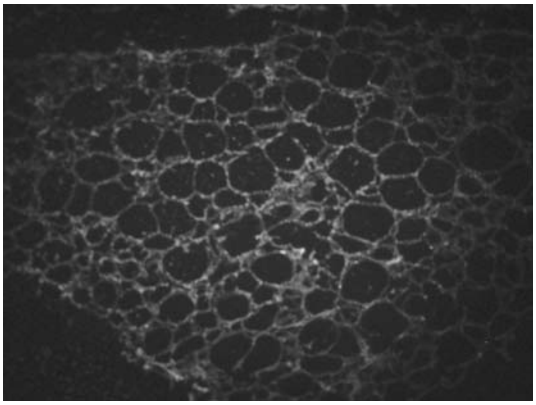

Fig. 3 Abdominal adipose biopsy of a patient treated without ultrasound treatment. Immunofluorescence FITC (fluorescein isothiocyanate) staining for collagen type 1 fibers shows that they are of normal appearance (original magnification, ×100)

fixation and effective adipocyte destruction, which usually is detectable in control samples. Therefore, we cryofixed some biopsies and after cryosectioning used Oil red-O for staining. This technique avoided fixation artifacts, giving us more reliable results. When frequencies of 2 and especially 3 MHz were used, a diffuse adipocytic derangement was observed using both traditional and Oil red-O stainings with light microscopy. In addition, immunofluorescence staining for collagen types 1 and 2 showed that these fibers were intact with an ultrasound frequency of 1 MHz, whereas when the ultrasound frequency was either 2 or 3 MHz, a diffuse collagen fiber retraction was detected, in addition to a diffuse adipocyte alteration.

In conclusion, when an ultrasound frequency of 1 MHz was used, no alterations or only limited adipocytic alterations were observed, which did not include collagen fibers. Therefore, ultrasound can be used in aesthetic medicine at this frequency. In fact, tissue can be removed easily with this technique, then reused for reconstruction. In addition it can be used in the nonbloody treatment of localized lipodystrophies (1° Pannicolopatia Edemato, Fibro Sclerotica (PEFS) or Cellulitis). Ultrasound frequencies of 2 and especially 3 MHz can lead to complete fat tissue disruption, including adipose cells and collagenic fibers. Their use can be helpful in common techniques used to perform plastic surgery for body contouring. These ultrasound frequencies are particularly indicated for the treatment of fibrous and inveterate cellulitis (2° and 3° PEFS).

References

1. Scuderi N, De Vita R, D'Andrea F, et al. (1987) Nuove prospettive nella liposuzione: La lipoemulsificazione. Giorn It Chir Plast Ric Est 2:1
2. Zocchi M (1992) Ultrasonic liposculpturing. Aesth Plast Surg 16:287–298
3. Zocchi M (1996) Ultrasonic-assisted lipoplasty. Clin Plast Surg 23:575
4. Silberg BN (1998) The use of external ultrasound assist with liposuction: Aesth Surg J 16:284
5. Illouz YG (1996) History and current concepts of lipoplasty. Clin Plast Surg 23:721
6. Kesselring UK (1997) Notes on the history of the adoption of liposuction (letter). Plast Reconstr Surg 99:595
7. Springer RC (1996) Liposuction: An overview. Plast Surg Nurs 16:215
8. Bolivar de-Souza-Pinto E, Erazo IPJ, Prado-Filho FSA, et al. (1996) Superficial liposuction. Aesth Plast Surg 20:111
9. Pinto EB, Indaburo PE, Muniz AC, et al. (1996) Superficial liposuction: Body contouring. Clin Plast Surg 23:529
10. Toledo LS (1996) Syringe liposculpture. Clin Plast Surg 23:683
11. Wilkinson TS (1999) External ultrasound-assisted lipoplasty. Aesth Surg J 19:124
12. Gasperoni C, Salgarello M, Gasperoni P (2000) External ultrasound used in conjunction with superficial subdermal liposuction: A safe and effective technique. Aesth Plast Surg 24:253–258
13. Gasperoni C, Salgarello M (2000) The use of external ultrasound combined with superficial subdermal liposuction. Ann Plast Surg 45:369
14. Walgenbach K-J, Riabikhin AW, Galla TJ, et al. (2001) Effect of ultrasonic-assisted lipectomy (UAL) on breast tissue: Histological findings. Aesth Plast Surg 25:85
15. Rosenberg GJ, Cabrera RC (2000) External ultrasonic lipoplasty: An effective method of fat removal and skin shrinkage. Plast Reconstr Surg 105:785
16. Mendes FH (2000) External ultrasound-assisted lipoplasty from our own experience. Aesth Plast Surg 24:270