

# Fat tissue after lipolysis of lipomas: A histopathological and immunohistochemical study

**Background:** Injections with Lipostabil<sup>®</sup>, a phosphatidylcholine (PDC) containing substance, have become a popular technique to treat localized fat accumulation and lipomas for aesthetic reasons. Despite its frequent use, the mechanism of action of PDC and histological changes of treated fat tissue still remain unclear. To investigate the histological changes of lipomas after treatment with PDC.

**Methods:** In all, fourteen lipomas (n = 14) in five patients presenting with multiple lipomas were treated with intralesional injections of PDC (Lipostabil<sup>®</sup>, Nettermann, Germany). Histological changes with immunohistochemical analysis of the inflammatory process were evaluated 4, 10, 24, 48 h, 10 days, 30 days and 60 days after lipolysis.

**Results:** Between 4 and 48 h after injection, histology shows a lobular neutrophilic infiltrate with partially destroyed fat cells. At day 10 the inflammatory process is accompanied by an infiltration of T-lymphocytes. After 60 days formation of macrophages with foam cells are visible, accompanied by thickened septa and capsula.

**Conclusion:** Lipolysis with PDC results in a distinct inflammatory reaction of affected fat tissue, similar to factitial panniculitis. Early destruction of fat cells may suggest the involvement of detergent or osmotic mechanisms in the process.

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Falk G. Bechara<sup>1</sup>, Michael Sand<sup>1</sup>, Klaus Hoffmann<sup>1</sup>, Daniel Sand<sup>2</sup>, Peter Altmeyer<sup>1</sup> and Markus Stücker<sup>1</sup>

<sup>1</sup>Department of Dermatology and Allergology, Ruhr-University Bochum, Bochum, Germany and

<sup>2</sup>Department of Physiological Science, University of California Los Angeles (UCLA), CA, USA

Falk G. Bechara, MD, Department of Dermatology and Allergology, Ruhr-University Bochum, Gudrunstrasse 56, 44791 Bochum, Germany  
Tel: +49-234-509-3459  
Fax: +49-234-509-3445  
e-mail: f.bechara@derma.de

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Fat dissolution with injectable phosphatidylcholine (PDC) formulations such as Lipostabil<sup>®</sup> (Nattermann GmbH, Cologne, Germany) has become a popular technique for the treatment of localized fat accumulation. Several open-label clinical trials have shown a positive effect in reduction of localized fat as well as in volume reduction of lipomas.<sup>1–5</sup>

Despite its broad usage the precise action mechanism of Lipostabil<sup>®</sup> still remains unclear. It is known that phospholipid molecules are capable of forming micelles, which may increase local fat transport and elimination of lipophilic drugs.<sup>6</sup> However, these observations are based on animal

experiments and have not been evaluated for application of PDC in human fat. Recently, Rotunda et al. described that the detergent sodium deoxycholate, a bile salt component of injectable PDC formulation, causes non-specific lysis of cell membranes and therefore may be the active ingredient for lipolysis.<sup>7</sup> However, it is unclear whether deoxycholate has a potentiating effect on PDC or acts as the only active ingredient in PDC-containing formulas. Further, histological reactions in fat tissue after injection lipolysis are poorly investigated and are only described in single case reports without evaluating chronological sequences of the changes in treated fat tissue.<sup>8</sup> These reactions are of great interest for understanding and evaluating the mechanism of action of PDC and obtaining more information on possible risks and side effects.

All authors hereby disclose any commercial associations, which might pose or create a conflict of interest with information presented in this manuscript.

The purpose of the present study was to investigate fat tissue reactions after Lipostabil<sup>®</sup> injections by histology and immunohistochemistry.

## Materials and methods

### Patients/Subjects

Five subjects (median age: 37.8 years; range: 23–60 years, three males, two females) were enrolled in the study after giving informed consent. All patients were clinically diagnosed with familial multiple lipomatosis. In all, fourteen lipomas ( $n = 14$ ) were treated. Four lipomas were located on the upper extremities, four were located on the lower extremities, four were on the back and two on the anterior abdominal wall. Exclusion criteria included single lipoma, pregnancy, lactation, history of malignancy and soybean allergy. Intramuscular lipomas and lipomas located near tendons, bones and larger nerves (e.g. ulnar nerve) were also excluded.

### Treatment/Application of Lipostabil<sup>®</sup>

Each lipoma was treated with intralesional injections of 1–4.6 ml PDC (Lipostabil<sup>®</sup>). Lipostabil<sup>®</sup> is not approved by the US Food and Drug Administration (FDA). The injection volume was calculated according to the lipoma size. The largest diameter of the lipoma was divided by two and the result was taken as injection volume in milliliters. Injections were given by the same physician under sonographic guidance. For injecting we used a 27-G needle with multiple injection points per lipoma (Fig. 1).

Extirpation and subsequent histological examination of lipomas were performed 4, 10, 24, 48 h, 10 days, 30 days, and 60 days after injection lipolysis. In all 14 lipomas ( $n = 14$ ) with two lipomas at each time of assessment were examined.

### Control group

To compare histological changes, five non-treated lipomas at comparable anatomic locations were examined.

### Histology and immunohistology

Routine histology with hemalaun–eosin staining was performed in all specimens. Additional immunohistochemical staining was performed to specify cellular inflammatory infiltrate. The following antibodies were used: CD3 (Pan T-cell-marker, NCL-CD3-PS1, Novocastra Laboratories, Newcastle, UK), CD4 (T-helper-cells, NCL-L-CD4-1F6, Novocastra Laboratories), CD8 (T-suppressor-cells, Cellule T clone C8/114B, Dako AS, Glostrup, Denmark), granzyme B

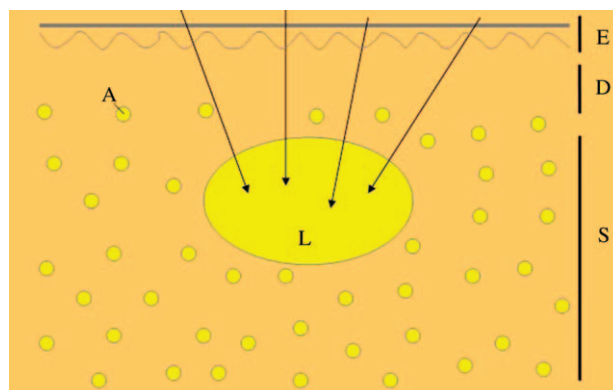


Fig. 1. Injection scheme of lipoma lipolysis. Multiple injection points are used to access a maximum of fat tissue (E, epidermis, D, dermis, S, subcutaneous fat, A, adipocytes, L, lipoma, →, direction and point of injection).

(CD 8 T-suppressor-cells, NCL-L-GRANB-B, Novocastra Laboratories), CD20 (B-lymphocytes, NCL-eD20-7D1, Novocastra Laboratories), CD68 (Macrophages, Clone PG-M1, Dako AS) as well as myeloperoxidase (neutrophilic granulocytes, A398, Dako AS). Histological slices were digitally recorded. The inflammatory reaction was determined and analyzed using an automatic software (analysis<sup>®</sup>, Soft Imaging System, Münster, Germany).

## Results

### Adipocytes and connective tissue

Initial histological changes were observed in specimens taken 4 h after injection (Fig. 2A). Adipocytes varied in form and size and appeared smaller than in the controls. The form resembled burr cells of osmotically impaired erythrocytes. This process was limited to spot-like areas within the fat tissue. The fraction of destroyed and deformed adipocytes increased with time after injection. Ten days after injection, larger areas of destroyed adipocytes became visible, with a strong variation in form and size of the fat cells.

Up to 10 days after injection no serious changes were visible within the connective tissue parts of the lipoma. Thirty and 60 days after injection we observed clearly thickened septa and a broadened capsule of the lipoma compared to the lipoma control group (Fig. 3A, B).

Although vasculitis was not observed, dilated blood vessels were present.

### Inflammatory infiltrate

The inflammatory process after injection lipolysis is presented in chronological order in Fig. 4.

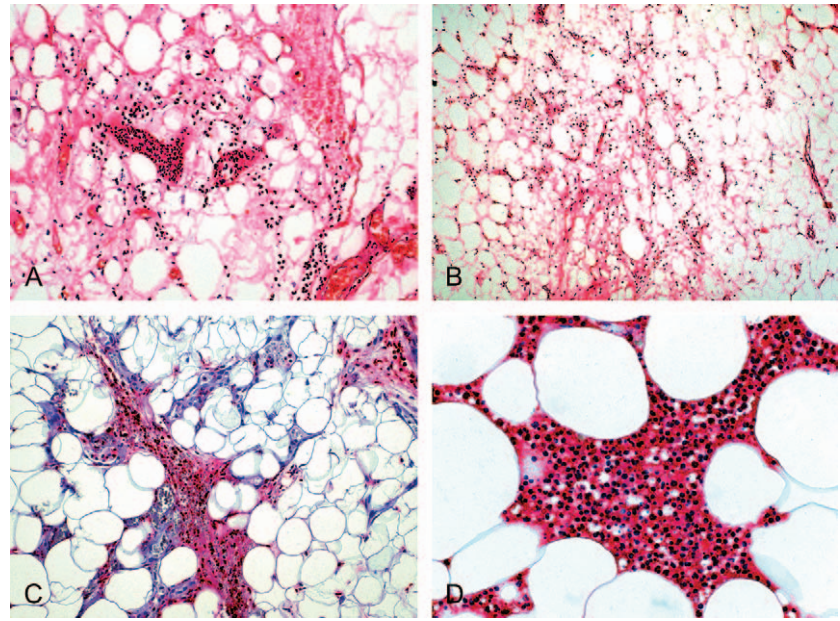


Fig. 2. (A) 4 h after injection of Lipostabil®. Visible lobular neutrophilic panniculitis accompanied by dilated and congestive capillaries within the fat lobule (hemalaun–eosin  $\times 20$ ). (B) 10 h after injection. Lobular neutrophilic panniculitis accompanied by adipocytes varying in form and size (HE  $\times 10$ ). (C) 24 h after injection. Immunohistology (myeloperoxidase) revealing the dense infiltrate of neutrophils ( $\times 20$ ). (D) Higher magnification ( $\times 40$ ) 24 h after injection. Adipocytes differ in size and reveal an atypical form.

Four hours after injection a lobular suppurative panniculitis was observed. Within the first 48 h of injection an increase of inflammatory infiltrate was noticeable, almost completely consisting of neutrophilic granulocytes (Figs 2A–D, 3C). Additionally, extravasal erythrocytes were partially present. With time following injection, the composition of the inflammatory components changed toward a lymphocyte and macrophage infiltration (Fig. 5A). Besides neutrophilic granulocytes,  $CD3^+$  lymphocytic cells (Pan-T-cell-marker) were observed at day 10, with a  $CD4^+$  T-helper-cell/ $CD8^+$  T-suppressor-cell ratio of 1.7( $CD4$ ):1( $CD8$ ). At this stage, an increase of destroyed adipocytes and the develop-

ment of lipophages with the presence of foam cells can be observed. At 30 and 60 days, respectively, after injection of PBC the previously described neutrophilic granulocytes are missing. They are replaced by a macrophage infiltrate with massive lipophage-granuloma and foam cells arranged in a lobular fashion (Fig. 5B, C). In late-stage findings, 30 and 60 days, after injection we observed about 5–10% of  $CD8^+$  T-suppressor lymphocytes, confirmed by positive staining with granzyme B (Fig. 5D).

In all specimens the inflammatory process and destruction of adipocytes was limited to focal parts of the fat tissue with some areas of the lipoma being completely unaffected.

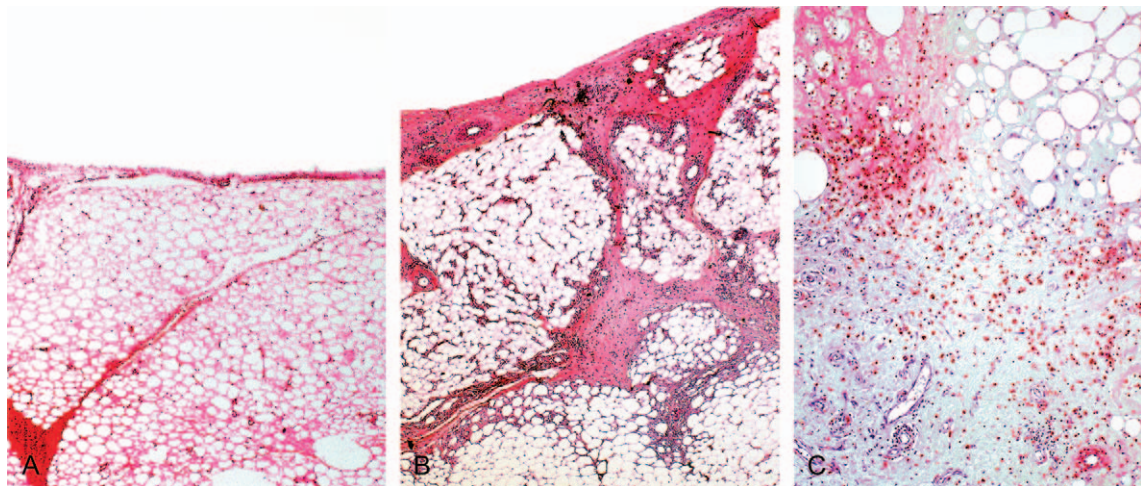


Fig. 3. A) Non-treated lipoma from controls. Note the thinner capsule and septa compared to Fig. 2B ( $\times 10$ ). B) 60 days after injection. Note the thickened capsule and septa (hemalaun–eosin  $\times 10$ ). C) Intense neutrophilic infiltrate 48 h after injection (immunohistology, myeloperoxidase staining) with dilated blood vessels ( $\times 20$ ).



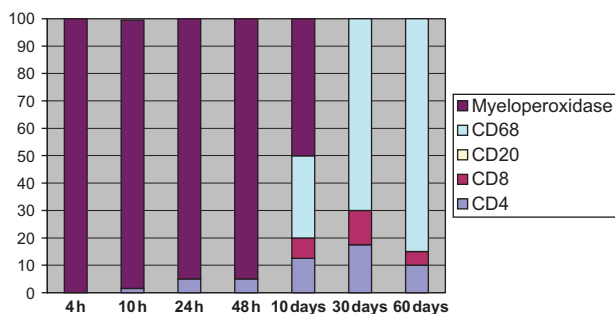


Fig. 4. Composition of inflammatory process in fat tissue after injection lipolysis. Shifting from neutrophilic to lymphocytic and macrophage infiltrate over time after injection (CD3, Pan T-cell-marker, CD 4, T-helper-cells, CD8, T-suppressor-cells, CD20, B-lymphocytes, CD68, macrophages, myeloperoxidase, neutrophilic granulocytes; y-axis: percent, x-axis: time after injection, h, hours, d, days).

## Discussion

The off-label use of PDC containing substances (e.g. Lipostabil<sup>®</sup>) for intralesional application in subcutaneous fat is a popular and widely used technique to dissolve localized fat accumulations.<sup>1,2,3</sup> In 2001, Rittes reported its indication for aesthetic purposes using PDC for correction of lower lid bulging due to prominent fat pads.<sup>2</sup> 'Buffalo hump' lipodystrophy, fat accumulations on the waist and the hip as well as lipolysis of lipoma have also been reported as possible indications for dissolution with PDC.<sup>3-5,9</sup> Despite its broad use, the mechanism of action of PDC and the histological changes induced by its injection in vivo have not been investigated in detail. To date Lipostabil<sup>®</sup> is not approved by the US FDA and its usage has to be critically evaluated by physicians. Theoretically, phospholipid molecules could form

micelles leading to enhanced local fat transport within the treated fat tissue.<sup>10</sup> Suzuki et al. reported a decrease of non-esterified fatty acid after intravenous administration of PDC.<sup>6</sup> Another theory, recently postulated by Rotunda et al., described that the detergent sodium deoxycholate, a component of injectable PDC formulation, causes non-specific lysis of cell membranes and therefore may be the active ingredient for lipolysis.<sup>7</sup> However, this theory has not been analyzed conclusively. Possible synergetic effects of PDC and detergents cannot be evaluated now and have to be further investigated.

Besides the lack of evidence-based scientific data concerning the mechanism of action, studies investigating histological changes of fat tissue after injection with PDC containing formulas are also missing. A review of the English literature resulted in only two reports about the reactions after injection lipolysis with PDC and one report about histological changes after a lipolysis attempt with dextrose.<sup>8,11</sup>

These reports cited only give a histological view on fat tissue several weeks after injection showing a lymphocytic panniculitis with destroyed adipocytes, focal fibrosis and an infiltrate consisting of macrophages. The early phase of histological reactions and the chronological sequences of the inflammatory process have not been described so far.

In our lipoma specimens we observed a lobular neutrophilic panniculitis within the first 4 h after injection with PDC. Comparing these histological changes with other inflammatory disorders of subcutaneous fat, we noted a remarkable similarity to factitial panniculitis, which is induced by injection or implantation of foreign substances. A variety of drugs have been described to cause factitial

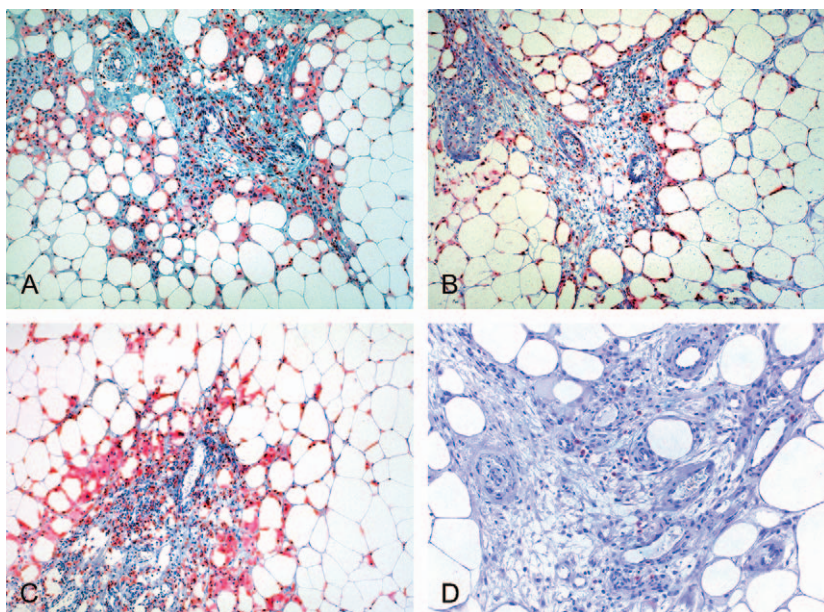


Fig 5. (A) 10 days after injection. Change from solely neutrophilic inflammation to infiltration of CD4<sup>+</sup> T-helper-cells. Immunohistology. (×20). (B) 30 days after injection. Beginning formation of CD68<sup>+</sup> macrophages. Immunohistology (×20). (C) 60 days after injection. Dense macrophages (CD68<sup>+</sup>) infiltrate. Immunohistology (×20). (D) 60 days after injection. Visible infiltrate of about 5% T-suppressor-cells. Immunohistology with granzyme B (×20).

panniculitis such as povidone, meperidine, pentazocine and vitamin K<sub>1</sub>.<sup>12–16</sup> Moreover, substances for cosmetic use like silicone or Polymethylmethacrylate-microspheres<sup>17–21</sup> or even self-injection of unsuspected substances such as acids, alkalis or lard by psychiatric patients with personality aberrations are capable of causing factitial panniculitis.<sup>22</sup> Histological findings of factitial panniculitis are remarkably similar to our observations, typically presenting an inflammatory infiltrate composed predominantly of neutrophils in early lesions. During the course of inflammation, the infiltrate changes toward a granulomatous inflammation in late-stage lesions.<sup>23</sup> Like in our patients, late-stage lesions of factitial panniculitis are also characterized by foamy histiocytes and surrounding fibrosis.

Some histological features seen in our collective also resemble to the so-called encapsulated fat necrosis (*syn.* mobile encapsulated lipoma). This term is used for a well-circumscribed fat necrosis sometimes accompanied by calcification, surrounded by thin to thick fibrous tissue.<sup>24</sup> Trauma and subsequent reduced blood supply are thought to be the histogenic factor; however, they are only found in approximately one-third of described cases in the literature. The typically degenerated or necrotic tissue is occasionally accompanied by inflammatory cells. Whether in early stages of encapsulated fat necrosis inflammation is a typical feature remains unclear. Therefore, only late stage histopathological findings of lipolyzed lipomas are comparable to this rare form of fat necrosis.

Interestingly, destruction and deformation of adipocytes also are present in the early phase after injection in our collective. From the authors' point of view this could be explained by immediate reactions of adipose tissue to the detergent contained in PDC formulas or by osmotic processes, as recently described by Rotunda et al.<sup>7</sup> This damage of adipocytes could lead to the consecutive lobular suppurative panniculitis. We were able to show that the inflammatory process is limited to focal areas within the lipomas during all chronological stages. Some parts of the lipoma are not affected by the inflammatory process and the destruction of adipocytes. In contrast to a panniculitis caused by internal disease, it appears that the PDC-induced panniculitis involves circumscribed areas with a subsequent lysis of adipocytes. The spot-like areas are most likely caused by the multiple injection scheme. To obtain a consistent distribution of PDC this technique was chosen. However, it seems that only parts of the lipoma were lipolyzed. Precise data about the diffusion capacity of PDC are missing, but due to our results we assume that the effect is strictly limited to the area of injection. The extravasated erythrocytes, which we observed, can be explained by the

trauma of the injection needle as in the control group no pronounced extravasated erythrocytes were found. Signs of vasculitis as another possible cause for extravasated erythrocytes could not be observed.

Our results are limited due to the fact that lipomas differ biochemically from normal mature fat with increased levels of lipoprotein lipase in lipomas.<sup>25</sup> Therefore, our results may not be completely transferable to injection lipolysis of fat tissue for aesthetic body contouring and further studies are necessary.

### Relevance to clinical findings

The histological findings occurring a few hours after injection correspond to our clinical observations. In our study all patients reported a mild burning accompanied by sensation of heat and erythema at the treated body sites in the early phase after injection. The burning sensations started approximately 1 h after injection and were most intense 4 to 12 h after intralesional application of Lipostabil®. These clinical findings seem to reflect the beginning of the neutrophilic panniculitis.

Histology revealed that only focal, spot-like areas of fat tissue were affected by the inflammatory process. We speculate that this may be one reason why despite the frequent and widespread use of PDC no corresponding observations of scarring are described in the literature. The limited inflammatory reaction might not be extensive enough to result in macroscopically visible complications. Recently Kopera et al. reported on the lipolysis of lipomas. However, they did not investigate side effects or complications of this method.<sup>5</sup> Our study group also evaluated the clinical outcome of lipoma lipolysis by means of ultrasound control. We could show minor complications such as hematoma in 27%, and pain limited to pressure on the treated sites in 40%. No severe complications occurred.<sup>26</sup> However, in the authors' opinion it should be postulated that side effects like scarring and post-inflammatory hyperpigmentation of the treated regions have to be considered as possible side effects of injection lipolysis. The limited focal panniculitis is possibly also responsible for clinical observations that a successful lipolysis needs multiple treatment cycles. The affected fat tissue after one injection cycle may be too small to get a macroscopically evident volume reduction.

Focusing on changes within the connective tissue we found an increase of septal and capsula thickness, corresponding to previous reports.<sup>8</sup> The panniculitis appears to induce a fibrotic process in subcutaneous connective tissue supporting the clinical observation of consolidated lipomas after injection lipolysis. A positive aspect of the broadened capsula was reported by the board-certified surgeons who extirpated the lipomas. They reported an easier

preparation and extirpation due to the clear demarcation of lipoma and surrounding subcutaneous tissue compared to the control group. Due to the reduced size a shorter incision with a subsequent smaller scar were reported by the surgeons.

In summary, we found that injections with PDC-containing formulas induce a factitial panniculitis. Although no major complications were found in the cases we studied, we assume that standard complications of panniculitis (e.g. postinflammatory hyperpigmentation and scarring) should be discussed with the patient before therapy and careful consideration of PDC use for fat dissolving is strongly advised.

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