Commentary on: Metabolic and Structural Effects of Phosphatidylcholine and Deoxycholate Injections on Subcutaneous Fat: A Randomized, Controlled Trial

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In their article entitled, “Metabolic and Structural Effects of Phosphatidylcholine and Deoxycholate Injections on Subcutaneous Fat: A Randomized, Controlled Trial,” Reeds et al analyzed the effects of phosphatidylcholine and deoxycholate (PC-DC) treatments on body composition, adipocyte function, and mechanisms responsible for fat loss. Thirteen women were enrolled; only 7 completed the study. This small sample size renders statistically significant calculations virtually impossible.

The authors maintain that PC-DC injections have become increasingly popular. This may be true in Seoul, Korea, where over 6000 vials of Lipobean 1 are injected monthly into human subcutaneous fat for the purpose of fat reduction. However, in many countries around the world, the practice of injection lipolysis has been banned.2 Popularity of these injections in the United States has dwindled,3 in part due to the lack of a US Food and Drug Administration (FDA)–approved agent and also due to the lack of efficacy in treating larger body regions.4 The side effects, although not morbid, are unpleasant. Noninvasive treatments such as Liposonix (Solta Medical, Inc, Bothell, Washington) and Tite FX (Invasix, Richmond Hill, Ontario, Canada)5,6 offer much more fat reduction without the cholinergic side effects that can be seen with higher-dose PC-DC injections.

Despite the shortcomings of injection lipolysis as currently practiced, there has been a resurgence of interest in the topic,7 especially in Europe and Asia. There are many reasons for this, including the expansion of the practice of aesthetic medicine and the desire of practitioners who mainly perform injections to have an injectable solution for reduction of protrusions. If aesthetic practitioners can fill hollow regions, they may also want to reduce or take away volume. The ability to do this precisely with an injection would be an excellent addition to the cosmetic injector’s options.

The mechanism of action of PC-DC injections has been explored by several investigators, although none employed the breadth of testing presented in this study. The conjecture by Rotunda et al8 that the detergent effect of sodium deoxycholate was the mechanism of action of the drug was confirmed in a study done by Duncan et al in 2009.9 In this stem cell study, PC, normally solubilized in DC, was isolated and solubilized in inert mineral oil. Two cytotoxicity assays and 2 lipolytic assays were performed on the differentiated adipocytes. Solutions tested included PC alone, PC50-DC42, 2 concentrations of deoxycholate, the benzyl alcohol preservative, and a saline control. Phosphatidylcholine alone had no cytotoxic or lipolytic effect; the readings were equivalent to the saline control. Deoxycholate had a dose-dependent cytotoxic and lipolytic effect. Benzyl alcohol had no cytotoxic or lipolytic effect.

Interestingly, the histologic presence of “crown-like structures” is a marker for adipocyte inflammation and death in metabolic syndrome10,11; it is not a hallmark specific to injection lipolysis. Apoptosis is present in human adults as a normal occurrence. More than 50 billion cells die an apoptotic death on a daily basis in most adults.12 The caspase markers measured in this study show a decrease of caspase-3 on both the control side and the treated side at 8 weeks. Caspase-3 levels measure cells killed by this effector enzyme.13 A low level of caspase-3 would indicate that little to no actual apoptotic cell death had occurred.

Many authors note the presence of cell wall lysis14-16—an event not present with apoptosis—in their histologic evaluation of the tissue reaction to injection lipolysis. The toxic tissue injury caused by PC-DC injections causes an overwhelming oncotic effect. Commonly known as “necrosis,” tissue oncosis is caused by an inflammatory event or injury causing loss of cell respiration due to oxygen deprivation.17 The strong response of swelling, pain, and inflammation—signs of an oncotic reaction—is clear in patients who

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receive the treatment. Pyroptosis\textsuperscript{18} is a caspase-1–mediated mechanism of cell death that has features of both apoptosis and oncosis. Tissue aspirate may not provide an adequate specimen for apoptosis evaluation; cell distortion—especially of adipocytes—can mask the membrane blebs, pyknotic nuclei, cell shrinkage, and tissue collapse pattern seen with true apoptosis.\textsuperscript{19} Although both apoptosis and pyroptosis may be present—and it would have been interesting to see the caspase-1 levels in the present study—the evidence is overwhelming that toxin-induced tissue necrosis is the mechanism of the PC-DC effect.

The detergent effect on cell membranes has been extensively studied by protein chemists.\textsuperscript{20} Detergents are amphipathic molecules, having a hydrophilic tail and a hydrophobic head. An ionic detergent such as deoxycholate inserts or “partitions” into the cell membrane and denatures proteins. At a lower dose of deoxycholate, the cell membrane undergoes poration and becomes “leaky.” At a higher concentration, the detergent effect causes micelle formation, with subsequent cell wall lysis.\textsuperscript{21} The addition of PC to the formula causes liposome formation rather than micelle formation.\textsuperscript{22}

Histologic effects of PC-DC injections and DC injections have appeared in the literature since 2004.\textsuperscript{7} Rotunda et al\textsuperscript{8} noted necrosis of adipocytes in porcine tissue, as well as death of skeletal muscle cells. Rose and Morgan\textsuperscript{23} noted in his human biopsies that adipocyte necrosis was present at 1 week and 2 weeks following injection. The 30-patient study by Yagima Odo et al\textsuperscript{24} showed adipocyte lysis with inflammation and phagocytosis by macrophages. Schuller-Petrovic et al\textsuperscript{25} concurred and noted dose-dependent fibroplasia in histologic specimens, as well as muscle loss and necrotic changes in the walls of small blood vessels.

The lack of tissue specificity of the PC-DC injections, or DC injections alone, is a safety concern first raised by Rotunda et al in 2004.\textsuperscript{8} Gupta et al\textsuperscript{26} performed a tetrazolium bromide assay on different tissue types to assess the cytotoxic effect of PC-DC or DC alone on preadipocytes, fibroblasts, endothelial cells, and muscle cells at 1, 2, and 3 days. The group reported similar kill rates for all cell types, showing nearly 100% cell lysis at 24 hours. Their conclusion was that these compounds act in a “nonspecific manner” and therefore may be unsafe if unintentionally injected in the wrong location.

These changes occur over time, as noted by Duncan and Golitz\textsuperscript{27} in a serial histology study performed in 2008. Biopsies were taken at 1 week, 2 weeks, and 8 weeks following patient treatment with a saline control, PC50-DC42, and DC 24 mg/mL alone. The DC solutions showed immediate cell wall lysis with ground substance formation and extensive necrosis at 1 day, with fibrosis at 2 weeks and extensive fibrosis at 8 weeks. In contrast, the onset of cell wall lysis was delayed in the PC-DC specimen and was not visible until 2 weeks postinjection. Inflammatory changes with PC-DC were noted in the fiboseptal region, but no extensive fibrosis was noted.

In that same study, Duncan and Golitz\textsuperscript{27} noted that a phenomenon called “necrobiosis” occurred at 8 weeks in the deep reticular dermis of the deoxycholate-injected specimen. The deep layer of the dermis—not initially injected during treatment—was noted to have a bluish cast to the normally pink collagen of the reticular dermal layer. This was accompanied by thickening and sclerosis of the blood vessels, with a similar pattern noted in eccrine glands and hair follicles. These findings, in addition to patient intolerance of injection-associated pain, swelling, and bruising, have led to the reduction in the concentration of PC-DC; in Europe and in Korea, the most commonly injected formula is PC25-DC12.

New uses for injection lipolysis include very small amounts of dilute PC-DC for reduction of facial features such as cheeks, nasolabial folds, marionette “poufs,” and malar bags (M. Palmer, personal communication, January 2012). Choi\textsuperscript{28} has shown excellent results in reducing supraorbital fatty bulges in Asian patients. Veterinary use includes treatment of surgically unresectable infiltrating lipomas in canines (J. Ludlow, personal communication to M. Palmer, December 2012).

The magnetic resonance imaging findings by Reeds et al are irrefutable; injections of PC and DC do indeed work for subcutaneous fat reduction. Initially quite popular, these treatments have lost favor due to the pain and downtime associated with the injections, the lack of an FDA-approved drug, and the lack of a tissue-specific response to these injections. As the popularity of aesthetic medicine grows, an increasing number of noncore practitioners will be performing cosmetic injections. Although efficacious, the lack of tissue specificity of PC-DC injections creates a concern regarding long-term safety when the drug is injected by untrained practitioners.

**Disclosures**

The author declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

**REFERENCES**

1. Jeong ES. Clinical use of phosphatidylcholine/deoxycholate in a Seoul, Korea obesity clinic. Presented at: PC/DC Workshop, MIPS; September 1, 2012; Seoul, Korea.
2. Non-surgical lipolysis banned in France. http://www .consultingroom.com/blog/184/non-surgical-lipolysis-banned-in-france. Accessed January 2, 2013.
3. Laddy S. Masterpharm Bulletin. http://www.masterpharm .com. April 12, 2012.
4. Palmer M, Curran J, Bowler P. Clinical experience and safety using phosphatidylcholine injections for the localized reduction of subcutaneous fat: a multicenter, retrospective UK study. J Cosmet Dermatol. 2006;5:218-226.
5. Gadsden E, Aguilar MT, Smoller BR, Jewell MT. Evaluation of a novel high-intensity focused ultrasound device for ablating subcutaneous adipose tissue for noninvasive body contouring; safety studies in human volunteers. Aesthetic Surg J. 2011;31(4):401-410.
6. Mulholland S. Electroporation effect of radiofrequency based energy assisted noninvasive fat reduction using the
Tite FX device. Presented at: IMCAS Asia; October 4-6, 2012; Hong Kong.
7. Duncan D. Current status of injection lipolysis. Presented at: IMCAS Asia; October 4-6, 2012; Hong Kong.
8. Rotunda A, Suzuki H, Moy RL, Kolodney MS. Detergent effects of sodium deoxycholate are a major feature of an injectable phosphatidylcholine formula used for localized fat dissolution. *Dermatol Surg.* 2004;30(7):1001-1008.
9. Duncan D, Rubin JP, Golitz L, Badylak S, Kesel L, Duncan D. Refinement of technique in injection lipolysis based on scientific studies and clinical evaluation. *Clin Plast Surg.* 2009;36(2):195-209, v-vi; discussion 211-213.
10. Altintas M, Azad A, Nayer B, et al. Mast cells, macrophages, and crown-like structures distinguish subcutaneous from visceral fat in mice. *J Lipid Res.* 2011;52(3):480-488.
11. Aporian C, Bigiorna S, Mott M, et al. Adipose macrophage infiltration is associated with insulin resistance and vascular endothelial dysfunction in obese patients. *Arterioscler Thromb Vasc Biol.* 2008;28:1654-1659.
12. Apoptosis. www.wikipedia.org/wiki/apoptosis. Accessed January 2, 2013.
13. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ.* 1999;6(2):99-104. http://www.nature.com/cdd/journal/v6/n2/abs/4400476a.html. Accessed January 2, 2013.
14. Bechara FG, Sand M, Sand D, et al. Lipolysis of lipomas in patients with familial multiple lipomatosis: an ultrasonography-controlled clinical trial. *J Cutan Med Surg.* 2006;10(4):155-159.
15. Duncan D, Rotunda A. Injectable therapies for localized fat loss: state of the art. *Clin Plast Surg.* 2011;38(3):489-501.
16. Koper D, Horejsi R, Werner S, Moeller R. Injection lipolysis for reduction of saddlebag trochanteric bulges—half side controlled pilot study. *J Dtsch Dermatol Ges.* 2008;6(4):287-290.
17. Majno G, Joris I. Apoptosis, oncrosis, and necrosis: an overview of cell death. *Am J Pathol.* 1995;146(1):3-15.
18. Bergsbaker T, Fink S, and Cookson B. Pyroptosis: host cell death and inflammation. http://www.nature.com/nrmicro/journal/v7/n2/abs/nrmicro207. Accessed January 2, 2013.
19. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol.* 2007;35(4):495-516.
20. Thermo Scientific. www.piercenet.com. Accessed January 2, 2013.
21. Micelles. www.elmhurst.edu/~chm/vchembook/558michelle.html. Accessed January 2, 2013.
22. Wowra B, Cremer K, Stricker H, Zeller WJ. Intraneoplastic application of metrizamide-containing liposomes: kinetic studies with computed tomography. *J Neurooncol.* 1992;14(1):9-18.
23. Rose PT, Morgan M. Histological changes associated with mesotherapy for fat dissolution. *J Cosmet Laser Ther.* 2005;7(1):17-19.
24. Yagima Odo ME, Cece LC, Odo LM, Natrielli A. Action of sodium deoxycholate on subcutaneous human tissue: local and systemic effects. *Dermatol Surg.* 2007;33(2):178-188.
25. Schuller-Petrovic S, Wolkart G, Hofler G, et al. Tissue-toxic effects of phosphatidylcholine/deoxycholate after subcutaneous injection for fat dissolution in rats and a human volunteer. *Dermatol Surg.* 2008;34(4):529-542.
26. Gupta A, Lobocki C, Singh S, et al. Actions and comparative efficacy of phosphatidylcholine formulation and isolated sodium deoxycholate for different cell types. *Aesthetic Plast Surg.* 2009;33(3):346-352.
27. Duncan D, Golitz L. Tissue toxic effects of sodium deoxycholate dominant formulas for injection lipolysis. Presented at: FSPS Annual Meeting; December 5, 2008; Palm Beach, FL.
28. Choi H. Use of Lipobean formula for reduction of excessive subcutaneous fat in the Asian face. Presented at: MIPS Conference; September 1, 2012; Seoul, Korea.