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

Dominic N. Reeds, MD[1], B. Selma Mohammed, MD, PhD[1], Samuel Klein, MD[1], C.B. Boswell, MD, FACS[2] and V. Leroy Young, MD, FACS[2]

[1] Center for Human Nutrition, Washington University School of Medicine, St. Louis, MO

[2] BodyAesthetic Plastic Surgery & Skincare Center, St. Louis, MO

Corresponding author: V. Leroy Young, MD
BodyAesthetic Plastic Surgery & Skincare Center
969 N. Mason Road, Suite 170
St. Louis, MO 63141
314-628-8200
leroyyoungmd@bodyaesthetic.com

ABSTRACT

Background: Phosphatidylcholine/deoxycholate (PC/DC) injections are a popular nonsurgical method to eliminate unwanted fat. The safety and efficacy of this approach is uncertain.

Objective: To evaluate the effects of PC/DC treatments on body composition, adipocyte function, and mechanisms responsible for fat loss.

Methods: This randomized, open-label study enrolled 13 women with a BMI ≤ 30 kg/m^2 and lower abdominal subcutaneous fat suitable for small-volume liposuction. Subjects were randomized by the final digit of their Social Security numbers and received between two and four PC/DC treatments, spaced 8 weeks apart. One side below the umbilicus was injected with PC/DC. The contralateral, control side received no treatment. Adipose tissue biopsies were performed on the treated side at baseline, one week after the first treatment and 8 weeks after the final treatment. The primary outcome was change in adipose tissue thickness at baseline and 8 weeks after the final treatment.

Results: Seven women completed the study. Treatment with PC/DC significantly reduced the thickness of the anterior subcutaneous abdominal fat ($p=0.004$). Adipose tissue showed rapid increases in crown-like structures, macrophage infiltration and reduced expression of leptin, hormone-sensitive lipase, adipose tissue triglyceride lipase and CD36. Plasma C-reactive protein, lipid profile, and plasma glucose concentrations were unchanged.

Conclusion: PC/DC injections can effectively reduce abdominal fat volume and thickness by inducing adipocyte necrosis. These treatments do not appear to increase circulating markers of inflammation or affect glucose and lipid metabolism.

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The use of liposuction to remove subcutaneous fat was first developed 35 years ago, and is currently the most commonly performed aesthetic surgical procedure in the United States[1]. However, liposuction is costly, often requires general anesthesia and can cause serious medical complications. Therefore, the use of less invasive, nonsurgical therapies to reduce localized fat deposits has gained interest. Subcutaneous injection of Phosphatidylcholine (PC) solubilized in deoxycholate (DC) has been purported to eliminate body fat. These injections, marketed under a variety of names such as Lipodissolve, have become increasingly popular.

The use of subcutaneous PC injection to eliminate unwanted local accumulations of fat was first proposed by Maggiori, who treated xanthelasma by using tissue injections of PC [2]. Several groups have now reported the use of this therapy to remove unwanted adipose tissue in other areas of the body, including the abdomen, thighs, buttocks, arms, and neck [3, 4, 5, 6].

Despite the widespread use of these therapies, the mechanism of action through which subcutaneous injection of PC and DC reduce fat deposits is unknown. Therefore we conducted a clinical trial to examine the effect of PC solubilized by DC on subcutaneous lower abdominal adipose tissue mass, inflammation, and the potential mechanisms responsible for fat loss in healthy volunteers.

MATERIALS AND METHODS

Study subjects

This study was conducted at the BodyAesthetic Research Center, St. Louis, MO and the Clinical Research Unit (CRU) of Washington University School of Medicine, St. Louis, MO.

Thirteen women were recruited between July and November 2008 to participate in this study. All subjects had a medical evaluation, including a history and physical examination, and blood tests including a lipid panel. Inclusion criteria included; 1) BMI <30.0 kg/m^2; and 2) presence of subcutaneous fat in the lower abdominal area suitable for small-volume liposuction. Exclusion criteria included: 1) pregnancy or breastfeeding; 2) diabetes; 3) use of agents known to affect glucose and/or lipid metabolism; 4) tobacco use; 5) known sensitivity to components of the injection formulation; 6) prior wound or infection in the treatment area. All subjects provided their written informed consent before participating in his study, which was approved by the Human Studies Committee of Washington University School of Medicine and the Western Institutional Review Board. An investigational drug exemption was obtained from the Food and Drug Administration for the use of PC/DC.

Study protocol

Subjects were randomized to receive PC-DC subcutaneous injection therapy (Medisca, Inc., Plattsburgh, NY and formulated by MasterPharm, Richmond Hill, NY) to either the right or left abdomen, below the umbilicus. The phosphatidylcholine was derived from soybean lecithins, half of which was composed of phospholipids. The sodium deoxycholate is a bile salt used to keep the PC soluble as it passes through the manufacturer's sterile filtration system and ensure that the PC remained in an injectable form without precipitating out of solution. This formulation was specifically chosen because it is the most commonly used commercially available formula in the United States. Each ml of the treatment formula contained 50 mg PC, 42 mg DC, and 8 mg benzyl alcohol, which was added as a preservative. Subjects were randomized according to the final digit of their Social Security numbers; those with an even last digit received treatment on

the right side of the lower abdomen, and those with an odd last digit were treated on the left side. The contralateral side of the abdomen received no treatment and served as a control.

Before each treatment session, standardized photos of the abdomen were obtained as patients stood in the right lateral, left lateral and anterior-posterior positions using a high-resolution digital camera with a 50 mm lens. Each subject had a minimum of two and a maximum of four PC-DC treatment sessions, spaced 8 weeks apart.

The maximum dose of PC given at any treatment session was limited to 2500 mg, which is the highest dosage recommended for minimizing potential side effects, such as nausea or diarrhea while maximizing the therapeutic response[7]. Prior to injection, a grid was drawn on the abdominal treatment area and marked into 1.5-cm squares as symmetrically as possible. Each 1.5 cm square site received 0.5 ml of the PC-DC solution injected into the center of each grid square. To increase the reproducibility of grid placement at future treatment sessions, the distance from the inferior border of the grid to the floor was measured and recorded so it could be duplicated later. All injections were delivered by using a standard 27 gauge, 13 mm needle attached to a 10 cc syringe. Subjects were counselled to maintain their normal lifestyle and body weight for the duration of the study.

Body composition was assessed at baseline and 8 weeks after the final treatment session. Total body fat and fat-free mass were determined by using dual energy x-ray absorptiometry (DXA) (Hologic QDR 4500, Waltham, MA). Abdominal subcutaneous and intraabdominal adipose tissue masses were evaluated by using magnetic resonance imaging (3-T magnet, Siemens, Erlangen, Germany).Three cross-sectional images were obtained: at the L3-L4 inter-vertebral space, above L3-L4 inter-vertebral space, and below the L3-L4 inter-vertebral space. Consistent slice localization was accomplished by using a rigid landmark (i.e., the iliac crest) to

position the subject in the machine and by using coronal scouting images to identify the site for image acquisition. Intra-abdominal and abdominal subcutaneous adipose tissue volume (cm^3) was determined by using the Analyze® software (Mayo Clinic, Rochester, MN).

Abdominal subcutaneous adipose tissue biopsies were obtained three times during the study: 1) approximately 1 week before the first PC-DC injection from the control side of the abdomen ; 2) 1 week after the first treatment session from the treatment area; and 3) 8 weeks after the final treatment session from both the treated and control sides. Adipose tissue was obtained by needle aspiration. The biopsy site was anesthetized with 1% lidocaine and adipose tissue was aspirated by using a 10 ml syringe with a 14-gauge needle. Tissue samples were vigorously irrigated with iced saline and one sample was flash frozen in liquid nitrogen, and a second sample was placed in formalin for subsequent histological analysis. Quantification of the number of crown-like structures and macrophage dispersal were performed as previously reported [8].

Blood samples were obtained during fasting conditions at baseline, 1 week after the first PC-DC treatment, and 8 weeks after the final treatment session to measure plasma lipids, and the plasma concentrations of insulin, glucose, leptin, adiponectin, TNFα and IL-6.

Safety assessments

Subjects received a diary that listed common side effects and were asked to record which (if any) of these side effects they experienced during the first week after treatment. Subjects were seen 1 week after each treatment session for follow-up. Blood samples were obtained at baseline, 1 week after the first PC-DC treatment, and 8 weeks after the final treatment session to

determine plasma lipids, and the plasma concentrations of glucose, hormone and inflammatory markers.

Study subjects had a final visit at 24 weeks after the last study treatment session. At the final follow-up visit body weight, abdominal circumference and skin fold thickness measurements were obtained. Both the subject and surgeon completed questionnaires to assess body contour and degree of improvement in localized fat deposits, compare baseline and final photographs, and patient satisfaction.

Analyses of samples

Plasma lipids were measured by using commercially available kits. Plasma insulin was measured by ELISA (Immulite®, Diagnostic Products Corp., Los Angeles, CA). Plasma interleukin-6 (IL-6), C-reactive protein (CRP), and leptin was measured using ELISA (Quantikine Immunoassay kits, R&D Systems Inc., Minneapolis, MN).

Fat tissue RNA was extracted from frozen adipose tissue using the RNeasy total RNA kit (Qiagen Inc., Valencia, CA). The first-strand cDNA was generated by reverse transcription using total RNA. Real-time RT-PCR was performed using the ABI PRISM 7700 Sequence Detection System and the TaqMan kit (Applied Biosystems, Foster City, CA). Gene expression of multiple target genes for adipocyte inflammation (IL-6, TNF-α, MCP-1) [9, 10]; adipocyte production (leptin, adiponectin) [11]; adipocyte function (ATGL, CD36 , FAS, and HSL) [12, 13]; macrophage infiltration and vascularization (EMR1, ITGAM, and VEGF) [14] and fibrosis (COL4A1, COL6A1, COL6A3) [15, 16] were measured by using quantitative real time RT-PCR, using gene-specific primers. Gene expression of several different apoptosis markers (an initiator caspase (Casp8); an effector caspase (Casp3); the Fas receptor-ligand complex (CD95/Fas)); and GRP78, a marker of

endoplasmic reticulum (ER) stress [17, 18], were also analyzed by RT-PCR using gene-specific primers.

Statistical analyses

The primary outcome was change in adipose tissue thickness between the treated and untreated side. Secondary outcomes were changes in plasma lipids, markers of inflammation (IL-6, TNFα) and changes in adipose tissue mRNA expression. Primary and secondary outcomes were tested by using repeated measurements of analysis of variance (ANOVA) with post-hoc testing, when appropriate, using a Bonferroni correction for multiple comparisons. A paired t-test was performed to compare the differences in measures between the untreated and treated side at the 8 week post-treatment time point. A related-samples Wilcoxon Signed Ranks test was conducted to determine differences in the count of dispersed macrophages and crown-like structures. We also examined changes in body weight, abdominal circumference, skin fold measurements, patient diaries, aesthetic evaluations, and patient satisfaction. P values of ≤ 0.05 were considered statistically significant.

RESULTS

Participant flow

Study participant flow is shown in Figure 1. Subjects were recruited starting in July 2008 and the study was completed in December 2009. After obtaining informed consent, thirteen female subjects received a physical examination, medical interview, routine blood tests and a lipid panel at the IRU of Washington University School of Medicine. Two subjects withdrew from the trial after the evaluation at the IRU-- one because her husband did not want her to participate and the other subject simply decided she did not want to participate--and did not progress to treatment.

The 11 remaining subjects had an average age of 43.6 years at the time of enrollment. After the first treatment, three subjects withdrew from the study, one because of the treatment's disruption to daily activities, one because of a job change, and one because of family issues. Another subject withdrew after Treatment 3 following a family health emergency. Thus, seven women completed all study visits in the experimental portion of the study and the final evaluation at the IRU. Six of the seven chose to have the same treatments on the control side because of abdominal asymmetry. The seventh subject decided that liposuction would be quicker and better for her. The average number of injections on one side of the abdomen per treatment was 71 (range, 27-124 injections), for an average PC/DC dose of 888 mg (range, 337.5-1550 mg).

Subject characteristics

BMI (26.5 ± 1.2 vs 26.2 ± 1.0 kg/m^2) and age (44 ± 2 vs 44 ± 2 yrs) were not different between the 11 randomized subjects and 7 completed subjects, respectively. In this paper we report on the data for the 7 subjects who completed the study. There were no changes in body weight, plasma lipid profile, glucose and insulin concentrations, leptin, liver enzymes, circulating markers of inflammation (IL-6, CRP), or white blood cell count during the study (Table 1). Hematocrit significantly decreased ($p< 0.021$) between baseline and Visit 1, possibly due to repeated blood sampling. This difference was not observed at the final study visit.

Body composition and fat distribution

As expected, there were no changes in whole body adiposity (Table 2). The thickness of the anterior (32.8 ± 4.0 vs 28.7 ± 3.4 cm, $p=0.004$) and lateral (24.0 ± 4.1 vs 21.7 ± 4.0 cm, $p<0.001$)

abdominal subcutaneous fat was greater before than after treatment, respectively. Although there was no change in subjects' measured abdominal circumference over time, there was a significant difference of 9.1 mm in subcutaneous fat measured by skinfold thickness between treated and control sides at the end of the experimental study (p=0.032). Representative photographs and MRI images are shown in Figures 2 and 3, respectively.

Adipose tissue histology: There were no changes in adipocyte diameter, volume, or lipid content after PC/DC injections (Table 3). At 1 week after the first treatment, there were significantly more dispersed macrophages (p=0.015) (Table 4), and a trend towards increased crown-like structures compared to baseline (p = 0.083) (data not shown). However, by 8 weeks after the final treatment there was no difference in these measures between the treated and untreated sides.

Adiopocyte gene expression: Monocyte chemotactic protein-1 (MCP-1) expression was greater at 1 week after the first treatment (p=0.04) and 8 weeks after the final treatment (p=0.049) than baseline (Table 5). Adipocyte vascular endothelial growth factor (VEGF) was lower at 1 week (p= 0.004) after treatment, but was no longer different 8 weeks after the final treatment from baseline or from the untreated side. The expression of macrophage marker integrin alpha M (ITGAM) did not change during the study.

Adipocyte metabolic and hormonal gene expression (Table 5): Leptin expression was almost 80% lower at 1 week after the first treatment than baseline (p=0.02) and tended to remain lower at 8 weeks after the final treatment than baseline (p=0.1). At 8 weeks after the final treatment, leptin expression was lower in the treated than the untreated side (p=0.05). Changes in expression of several genes related to lipid uptake and metabolism by adipocytes were noted.

Expression of hormone-sensitive lipase (HSL), decreased by ~75% from baseline (p =0.004) 1 week after the first treatment and was lower 8 weeks after the final treatment than at baseline (p= 0.03) and the untreated side (p=0.05). Adipose triglyceride lipase (ATGL) was lower at 1 week after the first treatment (p= 0.03) and 8 weeks (p = 0.02) after the final treatment and was markedly lower than the untreated side (p=0.03). CD36 expression was not significantly different 1 week after the first treatment (p=0.16), but was significantly lower at week 1 as compared to 8 weeks after the final treatment (p=0.05).

Fibrosis

No changes in expression of collagen type IV, alpha 1 (COL4A1) and collagen type VI, alpha 1 (COL6A1) genes (Table 6) were found. Expression of the collagen type VI, alpha3 (COL6A3) gene tended to be greater at 1 week after the first treatment than baseline (p=0.08) but was not different from baseline values 8 weeks after the final treatment.

Apoptosis

Caspase 8 expression was greater at 1 week after the first treatment than baseline (p= 0.02) and was slightly greater 8 weeks after the final treatment than the untreated side (p=0.05). Caspase 3, CD95/FAS and GPR78 expression were unchanged during the study (Table 7).

Patient satisfaction

Patient satisfaction with the treatment protocol was high. All participants reported that they were glad that they had the treatments. Six out of 7 participants: reported seeing a visible difference in the treated side; preferred the treatment side and thought the amount of fat seemed less; elected

to receive a similar treatment on the control side; would recommend the treatment to others. Two out of seven participants said that they wished they could have had suction-assisted lipectomy instead of the injection protocol.

No serious adverse events (SAE) were reported during the trial. Typical side effects reported in the treatment area were those expected based on reports in the literature: edema, erythema, pain, stinging or burning sensation, tenderness to touch, bruising, and temporary nodules or lumps. Less frequent were itching and brief episodes of facial flushing, nausea, diarrhea, hyperpigmentation and contour irregularity. Most of these tended to resolve within 1 week, with swelling and tenderness sometimes lasting into the second week following treatment. Pain following treatment was generally limited to a few days and was usually treated by over-the-counter medications, but narcotics were also made available and sometimes used.

DISCUSSION

The purpose of the present study was to carefully evaluate the effect of PC/DC treatment on glucose and lipid metabolism and plasma markers of inflammation. Further, we wished to examine the effect of treatment on adipose tissue histology and gene expression in addition to aesthetic measures of efficacy. Treatment with PC/DC subjectively and objectively reduced abdominal adipose tissue, and was well tolerated. Seven days after treatment with PC/DC there was increased local gene expression of the macrophage chemotactic factor (MCP-1), and increased macrophage dispersal and crown-like structures. In addition to causing a transient local inflammatory infiltrate, PC/DC treatment reduced markers of lipid uptake (CD36), triglyceride metabolism (HSL, ATGL), and adipose-tissue associated hormones (leptin). These data suggest

that PC/DC treatment effectively reduced local adipose tissue depots, increased tissue inflammation and reduced fat mass by adipocyte necrosis. The treatment had no effects on glucose and lipid metabolism or circulating inflammatory markers.

To our knowledge, this is the first study to sequentially evaluate the effects of PC/DC injection on adipose tissue histology and gene expression in a cohort of human subjects.

We found that PC/DC reduced abdominal adipose tissue volume in the treated areas. These findings are in agreement with most, but not all, prior studies using PC/DC injections [19, 20 6]. The majority of the patients in these studies saw improvements with treatment, but in all cases there were some patients who were nonresponders to the treatment. In contrast, Tawfik et al [21] failed to see any improvement in lower eye lid appearance after multiple injections of PC/DC in a randomized, double-blind, placebo-controlled study in 45 healthy adults.

It is likely that DC is predominantly responsible for the reduction in adipose tissue mass in subjects treated with PC/DC. Salti et al [22] compared the effects of injections of PC/DC or DC into outer thigh subcutaneous fat in 40 female subjects. Injections of PC/DC were administered on 4 occasions over an 8 week period to one outer thigh and a comparable dose of DC was placed in the opposite thigh. After 8 weeks of treatment, an overall reduction in fat was seen in 91.9% of subjects. There was no difference in fat loss between the sides, suggesting that DC was the active component. Similarly, others have reported no difference in efficacy in submental fat between subjects treated with PC/DC or DC alone after a 4-week intervention [23].

The mechanism(s) through which PC/DC reduces adipose tissue mass are unclear; both adipocyte apoptosis and increased lipolysis have been proposed. We found that injection of

PC/DC rapidly caused an increase in crown-like structures, expression of caspase-8 and macrophage chemotactic factors. These changes were accompanied by a reduction in genes associated with the metabolic and hormonal activity of adipocytes (i.e. leptin, ATGL, HSL). These results suggest, in agreement with the literature, that PC/DC induces adipocyte dysfunction, necrosis and macrophage infiltration causing fat loss. These findings are in agreement with several prior studies that have examined the cellular effects of local treatment with PC/DC [24]. Klein et al examined the in-vitro effects of PC/DC on lipolysis and cell viability in 3T3-L1 adipocytes [25]. DC alone and PC/DC both produced dose-dependent cell death in 3T3-L1 adipocytes while PC alone had no effect. Neither PC alone nor the PC/DC combination induced lipolysis. Gupta et al [26] found similar effects in treated 3T3-L1 adipocytes, fibroblasts, neonatal human dermal microvascular endothelial cells, and fetal human skeletal muscle cells.

Inflammation has been closely associated with impaired insulin sensitivity, and adverse effects on plasma lipids. We systematically examined the effects of treatment on glucose and lipid metabolism, and on markers of inflammation. Fortunately, despite robust increases in inflammation in the treated adipose tissue, there were no changes in plasma glucose or cholesterol. Further, we saw no change in the plasma concentration of C-reactive protein, suggesting that whole-body inflammation was not dramatically affected by the intervention.

Overall, the PC/DC treatments were well-tolerated and produced highly significant improvements in adipose tissue mass in the treated areas. As expected, some discomfort and bruising occurred following treatment but there were no major adverse events. Several authors have reported case series for PC/DC treatment to multiple anatomical areas. In 2006 Hasengschwandtner [5]reported the results of the Network Lipolysis group which included 400

physicians from 29 countries in 2004. At that time, the Network Lipolysis database had data on 5000 patients and side effects experienced from a total of 753 treated patients which included pain at the injection site, bruising, itching, burning, redness, swelling, sensitivity to touch, dents, nodules, and cysts. Results of over 10,000 PC treatments administered during a 13-month period from a network of 39 UK doctors specially trained to administer the injections revealed that 73.8% of patients reported either being very satisfied or satisfied with the treatments [27]. Local side effects in these patients including swelling, erythema, burning/stinging, pain, tenderness and bruising, which were described as very mild or mild by most patients. Systemic side effects were reported in 3% of cases and included diarrhea, nausea, dizziness/light headedness and intermenstrual bleeding. These data suggest that PC/DC injections have minimal risk when administered by trained physicians.

In summary, we have shown in this small study that injections of PC/DC can effectively reduce abdominal fat volume and thickness, with no serious adverse effects in healthy adult females. We believe that the ideal candidate for injection lipolysis will be an individual desiring treatment of small areas of excess fat or localized deposits, such as the correction of post-lipoplasty contour irregularities or asymmetry. Injection lipolysis is a tool for those patients who wish to have less invasive procedures and/or are afraid of anesthesia. However, patients need to be aware that achieving desired results may take several months.

SUGGESTED READING

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DISCLOSURES

Doctors Klein, Mohammed, and Reeds have no disclosures.

Dr. Boswell is a consultant and investigator for Allergan, Inc. (Irvine, CA) and an investigator for Kythera Biopharmaceuticals (Calabasas, CA) and RXi Pharmaceuticals (Worcester, MA).

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Figure 1- Participant flow