Non-Invasive Cryolipolysis™ for Subcutaneous Fat Reduction Does Not Affect Serum Lipid Levels or Liver Function Tests

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Background and Objective: Cryolipolysis provides a method of non-invasive fat reduction that significantly reduces subcutaneous fat without injury to adjacent tissues. Preliminary animal and human data have suggested that cryolipolysis has no effect on serum lipid profiles or liver tests. This study was intended to more fully document any effect of this procedure on lipid and liver-related blood tests.

Study Design/Materials and Methods: Forty subjects with fat bulges on their flanks ("love handles") were treated bilaterally with a non-invasive device (Zeltiq Aesthetics, Pleasanton, CA) that precisely cools tissue to achieve a reduction in the fat layer. Serum lipid levels and liver tests were measured prior to treatment, and at 1 day and 1, 4, 8, and 12 weeks post-treatment.

Results: No meaningful changes in mean values were observed for any blood lipid level or liver test at any point over the 12-week follow-up period.

Conclusion: Cryolipolysis, when used for reduction of subcutaneous flank fat, is not associated with changes in serum lipids or liver test results. Lasers Surg. Med. 41:785–790, 2009.

Key words: cryolipolysis; body contouring; fat reduction; lipids; liver function tests

INTRODUCTION

Cryolipolysis is a new method of non-invasive fat layer reduction, which has been shown to significantly reduce fat layer thickness without damage to the skin or other surrounding tissues [1,2]. Adipocytes suffer a fatal apoptotic injury when exposed to cold, as demonstrated by studies on cultured samples [3]. Clinical studies showed that non-invasive cooling to initiate adipocyte death leads to a reduction in fat layer thickness that is evident in ultrasound measurements and visible to the eye [4–6]. The loss in volume of adipose tissue occurs gradually over time as the adipocytes are removed through an inflammatory clearing process that peaks within 2–3 months after cold exposure [1,2].

Conceivably, the process of adipocyte apoptosis and clearing of the liberated lipid could result in elevations of serum lipids. Reassuringly, however, animal studies of cryolipolysis have shown that after treatment of a large surface area, which resulted in a 30–50% reduction in fat layer thickness, serum lipid levels remained within normal limits over the subsequent 3 months [1,2]. However, there are very limited human data on the effect of cryolipolysis on serum lipids. Furthermore, apoptosis with the release of free fatty acids and adipocytokines has been postulated to play a role in the pathogenesis of non-alcoholic fatty liver disease [7]. Thus, one would like to be certain that cryolipolysis does not affect liver function tests in human subjects.

Preliminary human studies have evaluated efficacy of the cryolipolysis procedure using several measures: visible change in the surface contour, photographic assessment of baseline untreated area versus the same area post-treatment, and reduction in the fat layer thickness as measured with ultrasound [4–6]. Data for six subjects treated on a single flank or "love handle" with the Zeltiq clinical prototype device at cooling intensity factor (CIF)[1] 33 for 60 minutes showed a reduction in the size of the

[1]Cooling intensity factor: index representing the rate of heat flux into or out of tissue opposite the cooling device.

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treated love handle in comparison to an untreated contralateral control [5]. Ultrasound measurements at 2 months demonstrated a normalized[2] average fat layer reduction of 20.4% across the treated area. Blinded photographic review at 4 months demonstrated correct identification of the baseline image from a pair of baseline and post-treatment photographs 93% of the time [5]. These findings were also supported by efficacy data from a larger study of 32 subjects treated with CIF 33 for 60 minutes that resulted in an average normalized fat layer reduction of 22.4% at 4 months post-treatment [4].

A third study demonstrated similar efficacy using a range of treatment parameters (CIF 37–42, for 30 or 45 minutes) with evidence of fat layer reduction at 6 months. This study also included the collection of data on serum lipids and liver tests for 3 months following treatment. No effect on blood tests was seen over the 3-month period following treatment when a single flank (love handle) was treated with the non-invasive cooling device [6].

The current study was designed to more fully assess any effect of cryolipolysis on serum lipids and liver tests after treatment with the non-invasive cooling device in a clinically relevant manner consistent with that to be used for body contouring. Therefore, both love handles were treated and the total treatment area was approximately twice as large as that of the previous human studies.

MATERIALS AND METHODS

Bilateral treatment of the love handles was performed under a non-significant risk, IRB-approved protocol (RCRC IRB, Austin, TX).

Eligible subjects were men or women >18 years of age with visible fat on the flanks (love handles) and no weight change of greater than 10 lb during the preceding month. After obtaining written informed consent, subjects were screened to ensure all inclusion/exclusion criteria for study entry were met.

Potential subjects were excluded if they had recently undergone liposuction or another surgical procedure in the intended treatment area; had a history of subcutaneous injections into the area of intended treatment within the past 6 months; or had a known history of cryoglobulinemia, cold urticaria, or paroxysmal cold hemoglobinuria. Individuals unable or unwilling to comply with the study requirements; those with dermatological conditions or scars that may have interfered with the treatment or evaluation; and those taking methylxanthines or who had taken diet pills within the past 6 months were also excluded. Individuals currently enrolled in a clinical study of any other unapproved investigational drug or device and women who were pregnant or intending to become pregnant in the following 9 months were also excluded. Women who were lactating or had been lactating in the prior 9 months were excluded, as were individuals with any other condition or laboratory abnormality that could, in the opinion of the investigator, potentially affect response or participation in this clinical study, or would pose an unacceptable risk to the subject.

Prior to treatment height and weight were recorded for all subjects. Blood samples for all subjects were collected via venipuncture, processed, and analyzed by a central laboratory (Quest Diagnostics, Inc., San Jose, CA). Subjects were asked to fast for 12 hours prior to their blood draw. The following serum lipid values were obtained: cholesterol; triglycerides; and VLDL, LDL, and HDL cholesterol. The following liver-related blood tests were obtained: AST; ALT; alkaline phosphatase; total bilirubin; and albumin. Subjects with baseline laboratory values outside the reference range were excluded.

Precisely controlled cooling was applied to the treatment area. To ensure consistent thermal coupling between the skin and the applicator during treatment, a pad saturated with a coupling gel (Zeltiq Aesthetics, Pleasanton, CA) was placed on the skin surface prior to placing the applicator on the love handle tissue. The applicator of the cooling device was applied to the treatment area with a moderate vacuum pressure used to gently draw a bulge of fat into an applicator cup. Tissue drawn into the cup came into contact with two opposing cooling plates positioned on the cup's interior. The applicator was connected to a control unit that monitored the rate of heat extraction during the procedure in accordance with treatment parameters selected by the operator.

Each subject was treated on one or two sites for each love handle (depending on the size of the area to be treated) for a total of up to four treatment sites. Treatment with the Zeltiq non-invasive cooling device was applied at CIF 42 for 30 minutes. After the cryolipolysis procedure, laboratory tests for the same lipid and liver tests as taken at baseline were collected 1 day and 1, 4, 8, and 12 weeks after treatment.

The mean and standard deviation for each laboratory value were calculated for each time point. Furthermore, a repeated measures ANOVA was performed (JMP statistical software v7.0) for each analyte. Finally, mean values $\pm$ 95% confidence intervals (CIs) were graphically represented at each time point for what were considered to be the most important lipid measures (cholesterol and triglycerides) and liver tests (AST and ALT).

RESULTS

Forty subjects, 32 females and 8 males, ranging in age from 21 to 66 years were enrolled in this multi-center study. As displayed in Table 1, the mean age of the females was about 40 years and that of the males about 44 years. Mean height was about 65 in. for females and 70 in. for males, while the mean weight was about 153 lb for females and 192 lb for males. The mean BMI was 25.6 ± 3.81 and 27.7 ± 3.53 for the females and males, respectively.

Each love handle area was exposed to CIF 42 for 30 minutes. The procedure was well tolerated by all

[2]Fat layer thickness changes were normalized by subtracting the control side percent change (baseline to post-treatment) from the treated side percent change (baseline to post-treatment) to remove the influence of weight variations (i.e., gain or loss over the follow-up period).

TABLE 1. Subject Age, Height, Weight, and Body Mass Index (BMI)

Characteristic	Overall	Female	Male
Subjects (*N*)	40	32	8
Age (years), mean ± SD	40.9 ± 10.5	40 ± 9.1	44.4 ± 14.3
Height (in.), mean ± SD	65.7 ± 3.1	64.6 ± 2.4	69.8 ± 2.0
Weight (lb), mean ± SD	160.5 ± 28.8	152.7 ± 28.8	191.8 ± 27.7
BMI, mean ± SD	26.1 + 3.90	25.6 ± 3.81	27.7 ± 3.53

subjects. Consistent with previous human studies, bruising, erythema, and numbness at the treatment sites were common immediately after cold application; in most cases they were rated as "minor." By 1 week symptoms and signs had largely resolved. A total of three adverse events were reported in two subjects, none of which were rated as serious: (1) a 48-year-old woman experienced transient anxiety, nausea, and abdominal bloating shortly after the procedure; (2) a week later she complained of pruritis and generalized numbness which the investigator felt was not related to the procedure; and (3) a 38-year-old man reported pain at the treatment site 1 week following the procedure, which was successfully treated with ibuprofen. All three adverse events resolved without sequellae.

An example of baseline and post-treatment images from a subject in this study is presented in Figure 1A,B. A circle marker in each image indicates the common feature used to measure the relative fat layer thickness change in this pair of images. Note that a clinically apparent reduction in fat layer was seen despite the fact that the subject gained 5.4 lb from pre-treatment to the 6-month follow-up.

Laboratory results are shown in Tables 2 and 3 and Figures 2–5. Note that because of poor compliance at one study site some subjects did not have blood drawn on Day 1 and Week 1; once this problem was identified subsequent blood draws included virtually all subjects.

Serum Lipids

Table 2 displays the mean values for the serum lipid analytes at each time point. Repeated measures ANOVA showed no statistically significant changes for any lipid other than HDL cholesterol. Figures 2 and 3 graphically represent mean values ± 95% CIs for what are considered the two most important serum lipids: cholesterol and triglycerides.

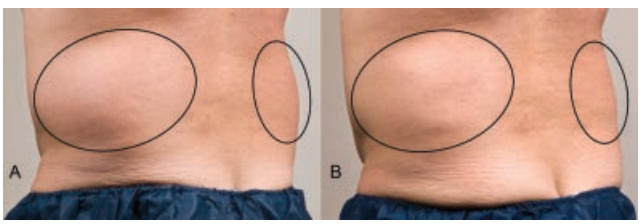

Fig. 1. **A**: Rotated view of a baseline photograph of a subject treated on both flanks. The circles indicate the regions to be treated. **B**: Rotated view of a 6-month follow-up of the subject in (A). The circles indicate the areas that were treated.

To be sure that the laboratory results were not influenced by the missing subject data at Day 1 and Week 1, further analyses of serum lipids were done for those subjects with values for every time point. In this subgroup analysis ($n = 18$, data not shown), the pattern was entirely consistent with those for the whole group.

Serum Liver Tests

Table 3 displays mean values for the liver-related tests at each time point. Repeated measures ANOVA showed no statistically significant changes from baseline for any test. Figures 4 and 5 graphically represent mean values ± 95% CIs for AST and ALT over time, the two liver tests most sensitive to hepatocellular damage. Analysis of the subset of 18 subjects with data at every time point similarly showed no significant changes from baseline (data not shown).

DISCUSSION

Cryolipolysis causes fat layer reduction as a result of apoptotic injury of adipocytes. The amount of injury is sufficient to cause a diminution of the fat layer that is visible and measurable, as has been demonstrated in clinical studies with a non-invasive cooling device [4–6]. This study using similar treatment conditions applied to a larger area representative of clinical practice for body contouring (i.e., treatment of both love handles) demonstrates that the procedure does not affect important blood chemistry values: serum lipid levels and liver tests remained virtually unchanged from baseline to all subsequent time points. The 12 weeks over which blood was drawn encompassed the peak time of the inflammatory process within the fat layer, so that blood test changes subsequent to 12 weeks as a result of the procedure would be extremely unlikely.

Of all the repeated measures ANOVA performed, the only "significant" *P*-value was for HDL. This is explained by the tendency for HDL values in this data set to decrease very slightly between baseline and the first few subsequent time points (maximum mean decrease of <4 mg/dl). This is most likely a chance occurrence, and in any case is of no clinical significance, particularly since mean HDL values remained well above the lower limit of the reference range (46 mg/dl) at all time points. Furthermore, the final several mean values were virtually identical to that of baseline.

Superficially, it could appear that there was a slight tendency for triglyceride values to increase from a baseline mean of 82 mg/dl to between 91 and 93 mg/dl at subsequent

TABLE 2. Mean Serum Lipid Values

Analyte (units) [reference range]	Time						
	Baseline	1 day	1 week	4 weeks	8 weeks	12 weeks	*P*-value
Cholesterol (mg/dl) [125–200]							
Mean	173.3	171.2	174.4	172.1	175.2	177.1	0.6286
Std dev.	23.1	27.3	23.8	25.7	25.9	26.5	
N	39	30	28	39	38	38	
Triglycerides (mg/dl) [<150]							
Mean	82.1	84.7	93.4	90.8	92.6	93.2	0.2218
Std dev.	30.3	45.9	37.2	44.8	47.5	40.0	
N	39	30	28	39	38	38	
HDL cholesterol (mg/dl) [≥46]							
Mean	67.0	64.4	63.3	64.0	66.3	66.7	0.0296*
Std dev.	11.4	10.6	12.0	11.9	12.4	11.6	
N	39	30	28	39	38	38	
LDL cholesterol (calc) (mg/dl) [<130]							
Mean	89.8	89.8	92.4	89.9	90.4	91.8	0.9903
Std dev.	18.9	21.6	20.8	20.4	21.6	23.7	
N	39	30	28	39	38	38	
VLDL cholesterol (mg/dl) [5–35]							
Mean	16.5	17.1	18.6	18.2	18.5	18.6	0.2987
Std dev.	6.0	9.2	7.6	9.0	9.5	8.1	
N	39	30	27	39	38	37	

* A *P*-value < 0.05 is considered statistically significant.

TABLE 3. Mean Serum Liver Test Values

Analyte (units) [reference range]	Time						
	Baseline	1 day	1 week	4 weeks	8 weeks	12 weeks	*P*-value
AST-SGOT (U/L) [10–30]							
Mean	19.2	18.1	20.2	20.1	19.4	19.6	0.8101
Std dev.	5.5	5.3	12.3	8.6	6.6	6.6	
N	39	28	28	39	39	38	
ALT-SGPT (U/L) [6–40]							
Mean	17.1	15.4	15.9	16.1	16.2	15.9	0.9368
Std dev.	6.6	5.2	6.1	5.9	6.9	6.4	
N	39	28	28	39	39	38	
Alkaline phosphatase (U/L) [33–115]							
Mean	56.0	55.3	57.5	55.6	55.3	57.1	0.5264
Std dev.	15.3	18.7	15.1	17.0	14.9	17.0	
N	39	28	28	39	39	38	
Total bilirubin (mg/dl) [0.2–1.2]							
Mean	0.7	0.6	0.6	0.6	0.7	0.7	0.4119
Std dev.	0.2	0.2	0.2	0.2	0.3	0.3	
N	39	28	28	39	39	38	
Albumin (g/dl) [3.6–5.1]							
Mean	4.5	4.4	4.4	4.4	4.5	4.4	0.4792
Std dev.	0.3	0.2	0.2	0.3	0.2	0.2	
N	39	28	28	39	39	38	

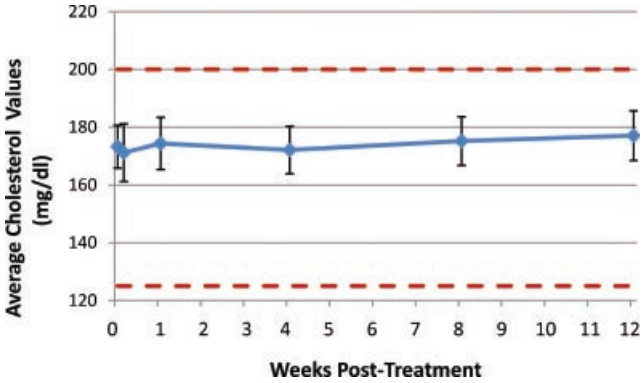

Fig. 2. Plot of mean cholesterol values reveals no significant change over time. Further, mean values ± their 95% confidence interval (denoted by the error bars) remain within the reference range (shown as dashed lines).

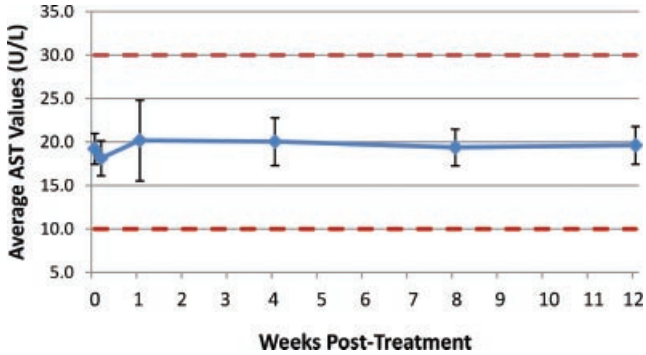

Fig. 4. Plot of mean AST values reveals no significant change over time. Further, mean values ± their 95% confidence interval (denoted by the error bars) remain well within the reference range (shown as dashed lines).

time points. However, this small difference is neither clinically nor statistically significant, and is well below 150 mg/dl, the upper limit of the reference range. Furthermore, it is widely appreciated that triglyceride levels vary markedly from day to day in normal subjects [8].

The lack of effect of the cryolipolysis procedure on lipids and liver tests was confirmed by analyzing the subset of subjects that had data available for every time point. This analysis confirmed that sporadic missing data for some patients was quite unlikely to have obscured any changes from baseline values for any blood test.

It is not surprising that cryolipolysis has no effect on lipid levels; the resorption of fat after cryolipolysis occurs at a very slow rate, as had been demonstrated with histologic evaluation of treated tissue [1,2] and ultrasound assessment of the fat layer reduction [4–6] over time. Even with suction lipectomy, which causes much more rapid destruction and liberation of lipid than does cryolipolysis (much of it remaining inside the subcutaneous cavity after the suction procedure is performed) the effect on serum lipids is minor and very transient. For example, in a study involving the removal of a mean of 1,470 cm^3 of fat—far more than is destroyed with the cryolipolysis procedure—serum cholesterol and triglycerides were mildly increased from baseline at 20 minutes and 1 hour, and had returned nearly to baseline values by 4 hours after the procedure [9]. Furthermore, humans are able to clear very substantial lipid loads without discernable changes in serum values [10,11]. Finally, it is well documented that over the first few months after major liporeduction procedures serum lipid levels are actually reduced [12,13].

In conclusion, cryolipolysis with the Zeltiq device was performed bilaterally on the flanks to reduce the prominence of love handles in 40 subjects. Serial measurement of serum lipids and liver tests for 12 weeks following the procedure showed no meaningful changes from baseline for any analyte. Cryolipolysis appears to be quite safe and well tolerated.

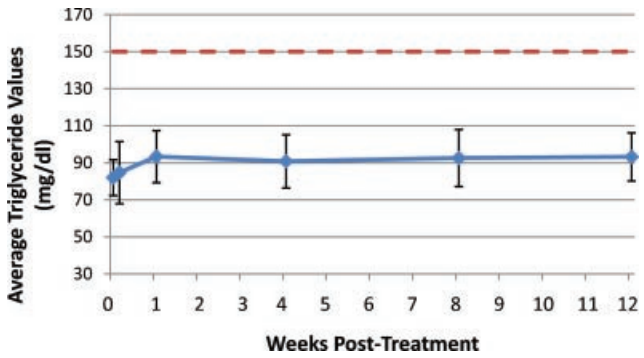

Fig. 3. Plot of mean triglyceride values reveals no significant change over time. Further, mean values ± their 95% confidence interval (denoted by the error bars) remain well below the upper limit of the reference range (shown as a dashed line).

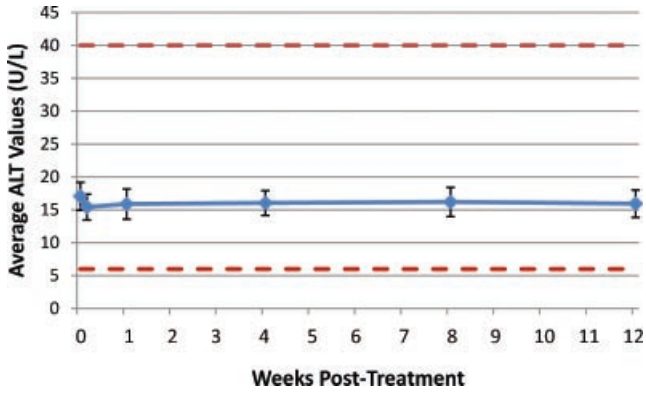

Fig. 5. Plot of mean ALT values reveals no significant change over time. Further, mean values ± their 95% confidence interval (denoted by the error bars) remain well within the reference range (shown as dashed lines).

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