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-related blood tests.

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...

1Cooling 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 contra-
lateral control [5]. Ultrasound measurements at 2 months
demonstrated a normalized 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. Indi-
viduals 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 stat-
istical software v7.0) for each analyte. Finally, mean
values ± 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

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2Fat 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)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (N)</td>
<td>40</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>40.9 ± 10.5</td>
<td>40 ± 9.1</td>
<td>44.4 ± 14.3</td>
</tr>
<tr>
<td>Height (in.), mean ± SD</td>
<td>65.7 ± 3.1</td>
<td>64.6 ± 2.4</td>
<td>69.8 ± 5.0</td>
</tr>
<tr>
<td>Weight (lb), mean ± SD</td>
<td>160.5 ± 28.8</td>
<td>152.7 ± 28.8</td>
<td>191.8 ± 27.7</td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>26.1 ± 3.90</td>
<td>25.6 ± 3.81</td>
<td>27.7 ± 3.53</td>
</tr>
</tbody>
</table>

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 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.](image)

**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

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

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

### TABLE 3. Mean Serum Liver Test Values

<table>
<thead>
<tr>
<th>Analyte (units)</th>
<th>Time</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1 day</td>
<td>1 week</td>
<td>4 weeks</td>
<td>8 weeks</td>
<td>12 weeks</td>
<td>P-value</td>
</tr>
<tr>
<td>AST-SGOT (U/L) [10–30]</td>
<td>Mean</td>
<td>19.2</td>
<td>18.1</td>
<td>20.2</td>
<td>20.1</td>
<td>19.4</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>Std dev.</td>
<td>5.5</td>
<td>5.3</td>
<td>12.3</td>
<td>8.6</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>39</td>
<td>28</td>
<td>28</td>
<td>39</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>ALT-SGPT (U/L) [6–40]</td>
<td>Mean</td>
<td>17.1</td>
<td>15.4</td>
<td>15.9</td>
<td>16.1</td>
<td>16.2</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>Std dev.</td>
<td>6.6</td>
<td>5.2</td>
<td>6.1</td>
<td>5.9</td>
<td>6.9</td>
<td>6.4</td>
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<td>39</td>
<td>28</td>
<td>28</td>
<td>39</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L) [33–115]</td>
<td>Mean</td>
<td>56.0</td>
<td>55.3</td>
<td>57.5</td>
<td>56.6</td>
<td>55.3</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>Std dev.</td>
<td>15.3</td>
<td>18.7</td>
<td>15.1</td>
<td>17.0</td>
<td>14.9</td>
<td>17.0</td>
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<td>28</td>
<td>28</td>
<td>39</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl) [0.2–1.2]</td>
<td>Mean</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Std dev.</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<td>0.3</td>
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<td>28</td>
<td>28</td>
<td>39</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>Albumin (g/dl) [3.6–5.1]</td>
<td>Mean</td>
<td>4.5</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
<td>4.5</td>
<td>4.4</td>
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<tr>
<td></td>
<td>Std dev.</td>
<td>0.3</td>
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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.
REFERENCES


