

# Clinical Efficacy of Fat Reduction on the Thigh of Korean Women through Cryolipolysis

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## Abstract

A variety of techniques such as surgical, pharmacological and electrical as well as manipulation approaches had been tried in order to remove undesirable fat on targeted area. So far, no distinguished method is considered to be most cost effective. Cryolipolysis is a new concept to reduce fat since Zeltiq machine had been introduced several years ago. Zeltiq machine, invented by Manstein Dieter, a researcher of photo-medicine group, is used to remove fat through cryolipolysis. Previous studies showed Zeltiq had a pronounced outcome to eliminate central abdominal fat in pig as well as human. Until now, no investigation was done to remove fat in human thigh lesion. Therefore, this study is conducted to investigate the clinical efficacy and safety of cryolipolysis on fat removal of thigh through cryolipolysis. Fourteen healthy premenopausal volunteered women had been conducted to assess their anthropometrical, social, and cardio-metabolic, and femoral fat amount by computer tomography, and adverse events at initial, 4 week, 12 week visit. There was no significant difference of measured fat amounts between cryolipolysis and frequency electrical treatment for 12 weeks. Minor adverse events such as pain (26.67%), bruise (20%) and numbness (20%) were noted. In conclusion, there was no difference in efficacy in removal of local fat on thigh by cryolipolysis through Zeltiq machine. Cryolipolysis was found to be safe for 12 week clinical trial.

**Keywords:** Fat; Cryolipolysis; Women; Femoral fat

## Introduction

Reduction the undesirable localized fat is becoming a major concern among many women regardless their age or race. For instance, fat treatment and removal is becoming a billion-dollar global cosmetic industry, and localized gluteal adiposity is regarded as one of the most undesirable fat in young premenopausal overweighted women. Various different therapeutic approaches were performed to treat gluteal adiposity in previous studies. While lipoplasty was purported by Gasparotti [1] to be an excellent method to improve body contouring, others [2] report increased skin dimpling after liposuction.

In addition, numerous non-invasive therapeutic approaches including endermologie, radio frequency devices and chemical lipolysis were considered as useful modalities to remove localized fat as well. Chang et al. showed promising results using endermologie for the treatment of thigh circumference reduction [3]. A decrease in mean body circumference index was not dependent on weight loss/gain of tested participants.

Sadick and Mulholland [4] evaluated 35 patients who had completed either eight or 16 treatments with radio frequency device. Blinded to the study group, a dermatologist evaluated the photographs and found 40% improvement on average. A more recent study found a statistically significant decrease in thigh circumference at 4 weeks, but no immediate change or a persistent decrease at 8 weeks post procedure [5]. Visual improvement of 50% was noted in the majority of subjects. Thirty-one percent of the subjects experienced bruising. Goldberg et al. conducted a study using a unipolar radio frequency device for cellulite treatment on subjects with higher grade cellulite on the upper thighs [6]. The subjects were treated every other week for a total of six treatments. Results obtained 6 months after the last treatment shows an average 2.45-cm reduction in thigh circumference with minimal side effects.

Park et al. reported that 12-week chemical lipolysis on the thigh had equivocal response of fat reduction in Korean women [7].

Liposuction was considered as a definitive and best outcome therapeutic option; however, it was cost-burden and invasive. Although endermologie and radio frequency method were relative less expensive,

they took more time to show the significant decrease in thigh girth. In addition, even though some evidence suggested that endermologie can reduce the thigh circumference in a dose and in a time-dependent manner, the long-term efficacy and longevity of these effects were still questionable.

A new method of non-invasive fat layer reduction called cryolipolysis [8] showed a significant reduction of a fat layer thickness in a Yucatan pig model. A cold-induced inflammatory mechanism gradually reduced fat thickness in 90 days after a 30- to 60 minute cold plate exposure to the skin surface. In vitro examination of the adipocytes' response to cold showed that the cooling of adipocytes to temperatures above freezing but below normal body temperature resulted in apoptosis - mediated cell death [9], which suggested that cryolipolysis produced an apoptotic injury in the adipose tissue. Furthermore, the subsequent inflammatory response may cause additional damage to those adipocytes not immediately affected by the cold exposure.

Inflammation of the subcutaneous tissue [10] showed different histopathology findings at different stages of development. As a result, the stage of evolution of a lesion at the time of biopsy influenced the microscopic appearance significantly.

Cryolipolysis was an interesting concept that might have future applications in the reduction of cellulite and localized adiposities. There was an evidence that adipose tissue was selectively sensitive to cold injury, such as "Popsicle panniculitis [11]." The most likely mechanism hypothesized when Popsicle panniculitis was first described was that crystallization of cytoplasmic lipids in adipocytes occurred at

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temperatures well above the freezing point of tissue water. The potential for tissue-specific cold injury was first investigated and recently reported by Manstein Dieter et al. in an animal model. Black Yucatan pigs were exposed to temperatures of 20, -1, -3, -5, and -7°C for 10 minutes using the Zeltiq prototype device (Zeltiq Aesthetics, Pleasanton, CA) [12]. At 3.5 months of follow-up, some treated areas showed grossly obvious loss of several millimeters of subcutaneous fat.

The theoretical background of non-invasive cryolipolysis was to reduce fat layer through apoptotic fat cell death. Apoptosis of fat cell was triggered by maintain energy extraction rate against fat sparing dermis. A cooling intensity factor was expressed as the rate of energy extraction ( $\text{mW}/\text{cm}^2$ ) from tissue over a period of time.

Assessed by neurologic evaluation, the transient reduction in sensation occurred in six out of nine subjects. However, all sensation returned by a mean of 3.6 weeks after treatment. Biopsies showed no long-term change in nerve fiber structure. There were no lasting sensory alterations or observations of skin damage in any of the subjects evaluated. Non-invasive cryolipolysis resulted in substantial fat reduction within 2 months of treatment without damage to skin. The procedure was associated with modest reversible short-term changes [13] in the function of peripheral sensory nerves.

While most of clinical trial usage by Zeltiq machine (Pleasanton, CA, USA) in United States was to remove fat around abdomen or back fat, no clinical trial to reduce fat on thighs would be performed until now. We would like to know clinical efficacy, safety of cryolipolysis to reduce fat on the thigh by comparing to radio frequency method after just one session.

The endpoint of present study was to assess the 4-week and 12-week difference of femoral amount measured by computerizing tomography between cryolipolysis and radio frequency method on each thigh.

Therefore, we would like to compare the effect of cryolipolysis (as intervention procedure) and the radio frequency method (as sham operation) by measuring the difference of local fat cross sectional areas and circumferences of thighs of healthy Korean women, who used either one of the two products, for 12 weeks.

## Method

The protocol in the present study was reviewed and approved by the human ethnics committee of Hong Ik General Hospital. The procedure of the study was understood and the informed consents were signed by consented participants. The design of the present study was case-controlled, prospective, clinical trial. Twenty eight healthy premenopausal hospital personnel who would like to remove unwanted fat on thigh were recruited through advertisement inside local intranet. Inclusion and exclusion criteria were described as below.

### Inclusion criteria

- premenopausal healthy women
- subjects with body mass index  $\geq 18$
- subjects with visible fat on both thigh
- subjects to understand and agree to this study protocol

### Exclusion criteria

- subjects with cryoglobulinemia, cold urticaria, or paroxysmal cold hemoglobinemia
- subjects with pregnancy or lactation within 6 months or next 3 months

- subjects with any laboratory, or metabolic abnormalities
- uncooperative subjects to comply with the study protocol
- women taking any medication/ over the counter/herbal supplements to regulate weight or fat mass over 6 months
- subjects with menstrual irregularities
- subjects to change over 10% of the former weight within the past 6 months - any procedure such as liposuction, or another surgical procedure or mesotherapy to reduce fat within 6 months
- subjects with any dermatologic abnormalities on the target areas (thigh)
- subjects with any injection into the area of intended treatment (e.g. cortisone) within 6 months

Demographic and anthropometrical parameters, screening,

Anthropometrical data including weight, height, and waist, and thigh circumference were measured by a single educated nurse in order to eliminate observation bias at initial, 4<sup>th</sup> and 12<sup>th</sup> week visit. Height was measured with the stadiometer. Waist circumferences were defined as the largest perimeter at mid-level of abdomen beyond anterior superior iliac spine. Both thigh circumferences were measured by scale at mid-thigh level, the percent of body fat was gauged by body impedance analysis (In body 7.0; Biospace, Korea). Blood pressures were measured by sphygmomanometers after 5 minute of rest without caffeine intake. Socio-demographic variables (such as smoking, alcohol consumption and exercise), menstrual irregularities and medication history including birth pill and past medical history were asked on the individual questionnaire at initial visit. Besides, metabolic variables such as triglyceride, HDL cholesterol, and fasting blood glucose, and insulin were obtained. All parameters were measured at initial, 4<sup>th</sup> and 12<sup>th</sup> week visit after overnight fasting.

### Procedures and monitoring

The radio frequency method was applied on right thigh (control side) as sham procedure for 30 minutes through 3000 Hz- amplitude modulated frequency at once.

Zeltiq System (Zeltiq Aesthetics Inc, Pleasanton, CA) was developed. This device consists of a control console, with a treatment applicator attached by a cable. A thermal coupling gel is placed on the area to be treated, and the applicator is then applied. Tissue is drawn into the cup-shaped applicator with a moderate vacuum to optimally positioning the tissue between 2 cooling panels; this allows for more efficient cooling of the tissue. A cooling intensity factor (CIF) is then selected by the treating clinician. The CIF is an index value representing the rate of heat flux into or out of tissue opposite the cooling device.

Treatment with the cold exposure for up to 60 minutes then begins. The energy extraction rate, or cooling, is controlled by sensors that monitor the heat flux out of the treated areas and is modulated by thermoelectric cooling cells. Following completion of the treatment, the system automatically stops the cold exposure and the clinician releases the vacuum. Depending on the surface area to be treated, multiple applications may be necessary to effectively expose the entire area to cryolipolysis. Zeltiq machine as intervention procedure was carried out on left inner thigh (treated side) for 1 hour through cool sculpting procedure with CIF (Cooling Intensity Factor: -73  $\text{mW}/\text{cm}^2$ ).

Diet and energy expenditure were monitored by food diary though 24 hour recall method. The photographs were taken at initial, 4<sup>th</sup> week

and 12<sup>th</sup> week visit. Histological examination for skin was carried out at initial and 12<sup>th</sup> week; questionnaires [14] for satisfaction were obtained at 12<sup>th</sup> week, and adverse events were monitored at each visit.

### Statistical analysis

The Wilcoxon signed-rank test was applied to evaluate the difference of anthropometrical, cardio-metabolic variables, and circumferences, and cross-sectional areas of both thighs at initial, 4th week, and 12th week visit. Pearson correlation analysis was done to assess the socio-demographical, anthropometrical, metabolic variables with difference of fat amount in thigh. SPSS packages for window version 15 (Chicago, Illinois, USA) was performed, and a probability value of less than 0.05 was considered as statistically significant at both sides.

Variable	Mean ± Standard deviation
Age (Years)	28.57 ± 7.81
Body Mass Index (Kg/m <sup>2</sup> )	23.12 ± 3.17
Percent of body fat (%)	31.93 ± 4.80
Smoking (%)	0/14 (0)
Alcohol consumption (%)	8/14 (64.3)
Exercise (%)	7/14 (50)
Weight (kg)	60.58 ± 7.71
Waist circumference (cm)	28.06 ± 2.71
Hip circumference (cm)	37.41 ± 2.18
Systolic pressures (mmHg)	116.43 ± 12.16
Diastolic pressure (mmHg)	74.29 ± 8.52
Fasting blood glucose (mg/dl)	88.93 ± 5.18
Total cholesterol (mg/dl)	174.50 ± 24.59
Triglyceride (mg/dl)	71.93 ± 24.60
HDL Cholesterol (mg/dl)	64.64 ± 11.85

Table 1: Initial baseline socio-demographic variables.

Variable	Initial	12 <sup>th</sup> week	P-values
Weight(Kg)	60.58 ± 7.71	60.96 ± 8.52	0.039
Percent of body fat (%)	31.93 ± 4.80	32.93 ± 6.26	0.510
BMI(Kg/m <sup>2</sup> )	23.12 ± 3.17	23.26 ± 3.63	0.184
Circumference of Left thigh(inches)	22.26 ± 1.82	22.30 ± 1.99	0.529
Fat cross-sectional areas of Left Thigh(mm <sup>2</sup> )	13548.36 ± 3099.55	13282.81 ± 3864.89	0.433
Circumference of Right Thigh(inches)	22.56 ± 1.69	22.71 ± 1.98	0.915
Fat cross-sectional areas of Right Thigh(mm <sup>2</sup> )	14426.26 ± 3526.21	14019.12 ± 6840.86	0.470

Data were expressed as Mean ± Standard Deviation

Table 2: Comparison of circumferences and Fat cross-sectional areas of both thighs between initial and 12<sup>th</sup> week.

Variable	Initial	12 <sup>th</sup> week	P-values
Weight(Kg)	60.58 ± 7.71	60.96 ± 8.52	0.039
Percent of body fat (%)	31.93 ± 4.80	32.93 ± 6.26	0.510
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Data were expressed as Mean ± Standard Deviation

Table 3: Comparison of circumferences and perimeters of both thighs between initial and 12<sup>th</sup> week.

## Results

Four subjects were eliminated for disagreeing with the protocol and menstrual irregularities at the initial interview. Nine subjects were further excluded as those with abnormal laboratory finding; fifteen final subjects were selected as healthy normal subjects. A total of fourteen subjects were analyzed because one subject violated the protocol at 4th week. Data was expressed as mean ± standard deviation.

### Initial baseline demographic characteristics

Mean age was 28.57 ± 7.81 year; mean body mass index was 23.12 ± 3.17 Kg/m<sup>2</sup>. The average percentage of body fat was 31.93 ± 4.80 %. All 14 subjects were non-smokers, 8 subjects (64.3%) regularly consume regular alcoholic beverage, and 7 subjects (50%) did not exercise regularly (Table 1).

### Comparison of the cross sectional fat areas, girth of both thighs between initial and 4<sup>th</sup> week

Observed were increases of cross sectional fat areas of thigh by either radio frequency method (0.2%) or Zeltiq machine (3.1%) respectively. The measured cross sectional fat areas in the treated (left) thigh increased from 13548.36 mm<sup>2</sup> to 13968.61 mm<sup>2</sup>, whereas the thickness decreased from 22.26 to 22.21 inches. The measured cross sectional fat areas in the control (right) thigh increased from 14426.26 mm<sup>2</sup> to 14455.19 mm<sup>2</sup>, while the thickness decreased from 22.56 to 22.46 inches.

### Comparison of the cross sectional fat areas, girth of both thighs between initial and 12<sup>th</sup> week

The mean of thickness of control (right) thigh increased from 22.56 to 22.71 inches, whereas that of measured cross-sectional fat area decreased from 14426.26 mm<sup>2</sup> to 14019.12 mm<sup>2</sup>. The average of thickness of treated (left) thigh increased from 22.26 to 22.30 inches, while that of measured cross sectional fat areas decreased from 13548.36 mm<sup>2</sup> to 13282.81 mm<sup>2</sup>. The fat reducing efficacy of cryolipolysis (-19.55%) seemed to be lower than that of radio frequency method (-28.2%). The observation suggested no significant statistical differences between the two methods (Tables 2 and 3).

### Satisfaction

Several different questions were asked to assess the satisfactory degree related with Zeltiq procedure through questionnaire survey (Table 4). No one showed this procedure to have effect on their popularity, romantic relationship nor making friendship. 35.7% experienced better in mood. 28.6% felt more self-confidence, and 57% were satisfied with the procedure, but 78.6% found no change in their appearance. 64.3% received generally positive comments from others and their future plan were affected by the procedure. 78.6% suggested this aesthetic procedure to anyone. Half of them answered to apply the cosmetic procedure due to same problem. 71.4% of them did not want to apply procedure. In general, half of them satisfied.

### Adverse events

No major adverse events were noted for past 12 weeks. Numbness, pain, and swelling were some examples of minor adverse events. Four out of 14 patients (28.6%) experienced mild (7.1%), moderate (14.3%) and severe (7.1%) pain at time of procedure. Three among them reported somewhat moderate bruit (21.4%). Blood tinged stool was notified in one subjects (7.1%).

### Change of metabolic variables

No significant statistical difference of metabolic variables such as

S.No	Question items
1.	Was there any change in your popularity? Decreased Not changed Increased
2.	Was there any change in your mood? Worse No change Better
3	Was there any change in your ability to make friends? Decreased Not changed Increased
4	Was there any change in your ease at having romantic relationships? Decreased Not changed Increased
5	Was there any change in your self-confidence? Decreased Not changed Increased
6	How did you find your appearance? Worse No change Better
7	Were you satisfied with the procedure? No Yes
8	Which kind of comments did you receive? Generally negative Generally positive
9	How did the procedure affect your future plan? Negatively Not affected Positively
10	Did you suggest aesthetic procedure to anyone? No, I did not No occasion Yes, I did
11	Did you have procedure or want to have it because same problem? No, I did not I want I had
12	did you have procedure or want to have it because of any other problem? No, I did not I want I had

Table 4: Questionnaire to assess satisfaction.

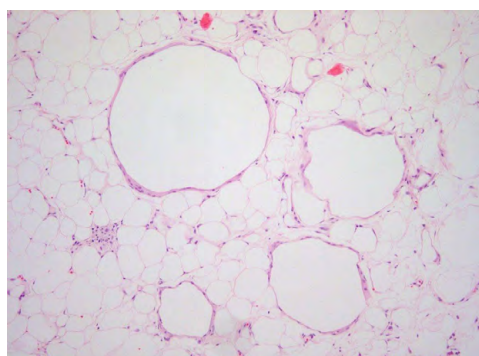


Figure 1: Histological findings in eleven subjects.

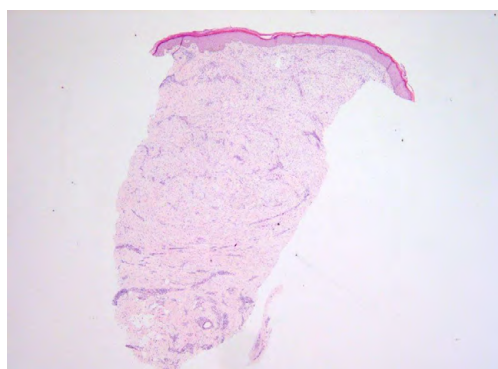


Figure 2: Histological findings in eleven subjects at different stages.

cholesterol, triglyceride, and fasting blood glucose was elucidated in the comparison of mean values at initial, 4th week and 12th week.

### Histological finding

The histological findings in eleven subjects (78.57%) showed slightly

weak to moderate inflammatory changes (perivascular dermatitis and fibrosis, panniculitis) at different histological stages. (Figures 1 and 2) No inflammatory change was noted in three cases of the subjects.

### Discussion

We did not show any difference in a measured fat amount of thigh in comparing by using between Zeltiq machine and radio frequency method. There was no statistical significant difference between data in a measured fat amount on both thighs at initial and 4th week. Paradoxically, there were increases in the fat amount in both thighs regardless of procedure at 4<sup>th</sup> week. Unexpectedly, the increase amount of fat in left thigh was higher than that of right. It was noted that all subjects gained weight as the measured fat amount increased in both thighs for 4 weeks. It is suggested that the weight gain could be partly due to localized water retention and hormonal changes related with menstruation. The reason for higher in left (3.1%) compared to right thigh (0.2%) might be the inflammation process observed within 4 weeks. It inferred that there was inflammation process in left thigh (target site) induced by Zeltiq machine. According to the recently published data, the histological change started as caspase-3 appeared within 3 days [15]. The higher increase of measured fat amount in left thigh might imply edematous change induced by lipolysis and inflammation.

In addition, there was no significant statistical difference of measured fat amount of both thighs between data at initial and 12th week. Interestingly, the difference in the measured fat amount between 4th week and 12<sup>th</sup> week was higher in left (-49.09%) than right (-30.17%) thigh, even though there were increases in the fat amount of both thighs regardless of procedure at 4<sup>th</sup> week (Tables 5 and 6).

The difference in the fat amount of left thigh was rather higher than that of right thigh for the first 4 weeks. Although there was no statistical significance, it was out of interest that the initial increase (3.1%) for former 4 weeks accompanied by greater decrease (-49.09%) for latter 8 weeks in the fat cross-sectional areas of left thigh.

We cannot find answer to the reason for the differences in the measured fat amount between 4<sup>th</sup> and 12<sup>th</sup> week by cryolipolysis were higher than those by the amplified frequency method.

		Number (percent)
pain	mild	1 (6.7)
	moderate	2 (13.3)
	severe	1 (6.7)
bruise	moderate	3 (20)
numbness	mild	2 (13.3)
	moderate	1 (6.7)
other	bloody stool	1 (6.7)

Table 5: Reported Adverse Events.

	Initial	4 <sup>th</sup> week	12 <sup>th</sup> week	p-value (0-4 <sup>th</sup> /0-12 <sup>th</sup> week)
Fasting blood glucose (mg/dl)	88.93 (5.18)	87.07 (8.09)	87.00 (15.27)	0.779/0.140
Total Cholesterol (mg/dl)	174.50 (24.59)	174.43 (25.94)	176.50 (21.71)	0.875/0.706
Triglyceride (mg/dl)	71.93 (24.60)	77.07 (27.69)	82.71 (38.72)	0.593/0.510
HDL Cholesterol (mg/dl)	64.64 (11.85)	63.36 (10.84)	62.71 (12.89)	0.285/0.362
	initial	4 <sup>th</sup> week	12 <sup>th</sup> week	p-value (0-4 <sup>th</sup> /0-12 <sup>th</sup> week)

Expressed as Mean (Standard Deviation)

Table 6: Difference of Cardio-Metabolic variables.

Lafontan et al. [16] demonstrated that isolated fat cells from the subcutaneous adipose tissue of the lateral part of the thigh exhibited resistance to adrenaline. On the other hand, the inability of adrenaline to induce lipolysis was not linked to a  $\beta$ -receptor defect since a  $\beta$ -adrenoceptor-stimulating agent (isoproterenol) exerted a clear lipid mobilizing effect. Greenway [17] suggested that the response of gluteal fat might be poorer than abdominal fat were due to lack of beta-receptors in the thigh. *According to preceding works, more than usual dose (-73 mW/cm<sup>2</sup>) might be needed to achieve the outcome from thigh that was similar to the previous result in trunk fat.*

Besides we evaluated the associated factors including socio-demographic, anthropometrical and metabolic variables related with difference of both thighs through Pearson's correlation analysis. Initial body mass index (BMI), weight, thigh girth, and measured fat amounts were positively associated with the difference of thigh circumference. *Especially, initial BMI had a very significant positive association with the difference of thigh circumference after the adjustment of age, socio-demographic variables.*

We did not find any change in metabolic variable between initial, 4<sup>th</sup> and 12<sup>th</sup> week. The investigators did not find any significant change in serum lipid values. In this study, inflammatory reaction and adipose tissue loss were closely correlated. Both proceeded for many weeks after a single, local exposure to cold, reaching an apparent maximum at 4 weeks after and resolving about 3 months after the cold exposure. In its early inflammatory phase, panniculitis might further damage adipocytes. In its later phase, however, phagocytosis appeared to account for removal of adipocytes and loss of fat tissue.

Adipocytes cooled to temperatures of 14, 21 and 28°C [18] showed no necrotic injury and all demonstrated approximately the same amount of apoptotic injury after 48 hours of recovery. The result at higher temperatures suggested that the mechanism of action responsible for adipocytes' death was based on an event that triggered apoptosis.

Whereas caspase-3 was an enzyme activated during induction of apoptosis, it might be considered as next experiment if there will be associated between caspase-3 and temperature on the thigh. The caspase-3 activity in cytoplasmic fraction [19] was determined as described.

There are some shortcomings in our study. In the beginning, because this study has some drawback for small sample size and the inappropriate design setting, the present result would not be plausible for effect on thighs and could not generalize for young overweight Korean women. Secondly, although the novel treatment process for radio frequency method included more than 6 sessions, it was performed just at once as control (sham) procedure in this study.

Only minor adverse events like pain, numbness and bruit were recognized during 12 weeks. These observed symptoms disappeared within several weeks.

The clinical efficacy of cryolipolysis on thigh might be evident through longer and larger controlled clinical trial considering the above several depicted thesis (BMI, more than usual dose, more than three months). As mentioned earlier in the beginning, lots of different therapeutic modalities including invasive as well as non-invasive tools [20] were still in evaluation. There was no significant difference in fat reduction of thigh between cryolipolysis and radio frequency method for 12 weeks in present study. Cryolipolysis might be considered as a better alternative non-invasive approach to reduce fat through further controlled investigation.

## Conclusion

In conclusion, there was no significant difference between measured fat amounts of thigh by cryolipolysis and radio frequency method on Korean overweight women for 12 weeks. Therefore, cryolipolysis showed no beneficial clinical efficacy to reduce fat on thigh for 12 weeks.

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