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Metabolic Effects of Cryolipolysis in Overweight Individuals on a Low-Calorie Diet: A Protocol for a Randomized Controlled Trial

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ABSTRACT

Introduction: Obesity, with its associated health risks, remains a major global concern. While traditional methods of weight management have their limitations, new non-invasive options, such as Cryolipolysis, have been gaining attention. Cryolipolysis is a non-invasive procedure that selectively targets and eliminates fat cells by freezing them. This study aims to investigate the metabolic effects of cryolipolysis in combination with a low-calorie diet.

Methods: This randomized controlled clinical trial aims to investigate the effects of Cryolipolysis in combination with a standardized low-calorie diet in 50 overweight women aged 18-65 years. Participants will be randomly assigned to one of three groups: combination of cryolipolysis and low-calorie diet, or a low-calorie diet only. Metabolic parameters such as body composition, fat layer thickness, and various biochemical parameters, including fasting blood glucose levels, lipid profile, and renal and liver function tests profile will be measured at baseline and at the end of the study.

Discussion: This study will provide valuable insights into the metabolic effects of combining cryolipolysis with a low-calorie diet. Understanding how these interventions interact at a metabolic level can inform the development of more effective weight loss strategies.

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Introduction

Obesity is a primary public health concern with significant impacts on both individual health and society as a whole (1, 2). Over the past 50 years, obesity rates have increased dramatically worldwide due to an imbalance between energy intake and expenditure (3, 4). Excess body weight can lead to various chronic health conditions such as high blood pressure, cardiovascular disease, dyslipidemia, type 2 diabetes mellitus, and inflammation (5-7).

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Obesity can have significant adverse effects on mental health, including discrimination and stigma. Abdominal obesity is linked to higher risks of morbidity and mortality compared to peripheral obesity (1). Obesity, particularly abdominal obesity, increases the risk of diabetes, cardiovascular disease, and other metabolic disorders (8, 9). Current treatments for obesity include diet, exercise, medication, and surgery, but many of these options have significant side effects or complications (10). Among the emerging non-invasive interventions, Cryolipolysis has attracted increasing attention for its ability to selectively reduce subcutaneous fat through controlled cooling (11).

Surgical body contouring procedures are associated with several potential complications, including infection, bleeding, adverse reactions to anesthesia, and extended recovery periods (1, 12, 13). Many individuals prefer less invasive alternatives due to these risks.

Calorie restriction represents one of the most prevalent and evidence-based approaches to obesity management. Extensive research has shown that a reduction in daily caloric intake leads to significant improvements in body weight, fat mass, and key metabolic parameters, such as insulin sensitivity and lipid profiles (10). Nevertheless, maintaining long-term adherence to these diets poses a significant challenge, prompting investigation into combination therapies to achieve more durable results

Non-invasive body contouring procedures have become increasingly popular in recent years, providing a safer alternative to surgical methods (11). Various types of non-invasive treatments are now offered, including radiofrequency, ultrasound cavitation, Cryolipolysis, and laser treatments (3, 14-16). Non-invasive methods have garnered increasing attention in recent years (17). Cryolipolysis, specifically, utilizes controlled cooling to target and eliminate subcutaneous fat cells while preserving surrounding tissues. This method induces apoptosis, or programmed cell death, in fat cells, leading to their elimination from the body through the immune system (11, 18).

Although Cryolipolysis has demonstrated efficacy in reducing subcutaneous adipose tissue, the exact physiological mechanisms governing the systemic clearance of apoptotic adipocytes remain incompletely elucidated. Several investigations have highlighted concerns regarding potential adverse effects on serum lipid profiles, hepatic function, and glycemic control, particularly in individuals with diabetes mellitus (14, 19-22). Such metabolic alterations could therefore pose risks for cardiovascular or hepatic complications (23).

Although some findings suggest that Cryolipolysis does not adversely affect lipid profiles (19), more comprehensive research is warranted to fully ascertain its safety and efficacy, particularly when used as an adjunct to dietary interventions. Accordingly, the present study was designed to investigate the combined effects of Cryolipolysis and a dietary intervention on hepatic function and serum lipid profiles. A secondary objective was to evaluate the correlation between patient-reported satisfaction and objective changes in body composition.

Study Objectives

Primary Objective

The primary research objective was to evaluate the metabolic impact of Cryolipolysis combined with a low-calorie diet in overweight individuals.

Secondary Objectives

The secondary research objective was to assess changes in body composition, such as fat layer thickness, waist circumference, and hip circumference, after intervention and investigate the effects of Cryolipolysis on biochemical parameters, including fasting blood glucose, lipid profile, renal function, and liver function.

Materials and Methods

"This study was conducted as a randomized, controlled, parallel-group phase 3 clinical trial, with blinding of the data analyst. The protocol received ethical approval from the Research Ethics Committee of Mashhad University of Medical Sciences (Ref: IR.MUMS.REC.1399.372) and was prospectively registered with the Iranian Registry of Clinical Trials (IRCT20200927048848N1) on September 30, 2020. Following enrollment, eligible participants were randomly allocated in a 1:1 ratio to either the intervention or the control group. A detailed overview of the study protocol is provided in Table 1.

Table 1. A SPIRIT diagram of the recommended content for the schedule of study

	Enrolment	Allocation	Intervention			Close-out
Timepoint	**0 to first 24h**	**0**	**Baseline**	**end of 4th week**	**end of 8th week**	
Enrolment						
Eligibility screen	*					
Informed consent	*					
Past medical history	*					
Allocation		*				
Interventions						
Cryolipolysis			*	*	*	
Placebo			*	*	*	
Assessments						
Body composition and anthropometrics indices			*	*	*	
FBS			*		*	
Lipid profile			*		*	
Urea, Cr			*		*	
Liver enzymes			*		*	
Hs-CRP			*		*	
Thickness of fat layer millimeter			*		*	
Demographic information			*			
Beck and BSQ questionnaires			*		*	
Follow-up						
Diet check and dietician visit				*	*	*

Sample Size

This study was conducted at the nutrition clinic of Ghaem Hospital in Mashhad, Iran. The sample size was determined based on the primary outcome, which was the change in abdominal fat thickness. Given the absence of directly comparable studies, the calculation utilized a formula for comparing two means. The analysis indicated that a sample of 50 participants (n=25 per group) was required to detect an effect size of 80% with a power of 80% and a significance level of 5% ($\alpha = 0.05$).

Participants

Inclusion Criteria

The inclusion criteria were overweight women with a BMI ranging from 25 to 29.9 kg/m^2 and no underlying health conditions, as well as Women between the ages of 25 and 45 to ensure a stable metabolic rate. Participants must engage in moderate-intensity aerobic activity (150 minutes or more per week) to maintain a baseline level of physical fitness. Individuals who have maintained a stable diet without significant changes over the past 6 months were included to reduce variability in dietary outcomes. Non-smokers were included to prevent potential confounding effects of smoking on metabolic parameters and fat distribution. Women with regular menstrual cycles (24-35 days) were selected to minimize hormonal fluctuations that could impact metabolism and fat distribution. Participants should have maintained a stable weight (within ± 2 kg) for at least 3 months before the study to prevent any weight fluctuations not related to the intervention. Women who have not undergone Cryolipolysis or liposuction treatments within the past year were included to eliminate potential influence from previous interventions. Participants must be non-smokers with regular menstrual cycles, stable weight, normal liver and kidney function, and not taking medications that could affect weight, metabolism, or hormonal balance. Participants must have psychological health within normal limits, as determined by the Beck Depression and Anxiety Inventories, to avoid any psychological factors influencing the study results.

Exclusion Criteria

Individuals who did not meet the inclusion criteria, declined to participate, or had medical conditions that might interfere with the study results were excluded. Current medications that affect metabolism, body weight, or biochemical parameters were considered. Factors such as pregnancy, lactation, or recent participation in similar studies were taken into account. Contraindications for Cryolipolysis, such as cold-related conditions or skin issues in the treatment area, were carefully reviewed. Individuals who

did not meet the inclusion criteria, declined to participate, or had medical conditions that may interfere with the study results were excluded. Current medications affecting metabolism, body weight, or biochemical parameters were considered. Factors such as pregnancy, lactation, or recent participation in similar studies were taken into account. Contraindications for Cryolipolysis, such as cold-related conditions or skin issues in the treatment area, were carefully reviewed. Lifestyle factors, such as extreme physical activity or dietary habits that significantly differ from the study requirements, might impact study outcomes. Severe psychological conditions, eating disorders, or non-compliance with study protocols were determined by the Beck Depression and Anxiety Questionnaire, as well as participant willingness to adhere to study requirements. Lifestyle factors, such as extreme physical activity or dietary habits that significantly differ from the study requirements, might impact study outcomes.

Participants were randomly allocated to Group I (Cryolipolysis + diet) and Group II (diet-only).

Baseline Data Collection

At baseline, a comprehensive set of data was collected from all participants. This included demographic information, socioeconomic status, occupation, lifestyle, and medication history, gathered via standardized questionnaires and interviews. Psychological status was evaluated using the Beck Anxiety and Depression Inventories and the Body Shape Questionnaire (BSQ). Body composition analysis was conducted using a body analyzer (770 BIA, South Korea), in addition to anthropometric measurements such as waist circumference, hip circumference, and abdominal circumference (measured with a non-elastic meter). The fat layer thickness in the abdomen and flanks was measured. Blood tests were conducted to assess serum fasting blood glucose, blood urea nitrogen, creatinine, lipid profile, liver function tests, and Hs-CRP levels.

Procedures

I) Low-Calorie Diet Implementation

A. Dietary Recommendations

Basal metabolic rate (BMR) was calculated using the Harris-Benedict Principle and adjusted for activity level. About 500 kcal was subtracted from daily calorie needs, and participants were instructed to follow this diet plan throughout the study.

B. Diet Adherence Monitoring

Dietitian visits were scheduled at the beginning of the study (week 0), during the fourth week (week 4), and at the end of the study (week 8) to monitor adherence to the diet plan.

II) Cryolipolysis Procedure

Participants eligible for Group I underwent Cryolipolysis using the BIOTEC device (Fusiomed, Italy) for 60 minutes at weeks 0, 4, and 6. The treatment area was the central abdomen, with the device positioned 5 cm below and 5 cm above the navel.

Pre- and post-session vital signs were assessed, and the procedure was conducted with the participant lying in a 45-degree dorsal decubitus position.

III) Data Collection

Participants underwent diet assessments and met with a dietitian during week 4 and at the end of the study (week 8). Additionally, anthropometric measurements and ultrasound examinations were conducted.

Following the study (week 8), participants underwent biochemical tests and completed the Beck Depression and Anxiety Questionnaire to evaluate both physical and psychological well-being.

Safety Measures

Participants were closely monitored throughout the study for any adverse events, and their vital signs were regularly assessed.

Any reported side effects were assessed and managed by a trained medical specialist, either directly or by referring participants to the appropriate specialist or primary healthcare provider.

Data Collection and Analysis

Participants were required to attend three study visits: at baseline (week 0), week 4, and the final assessment at week 8. Following the provision of informed consent, baseline data, including demographic information and anthropometric measurements (waist, hip, and abdominal circumference measured with a non-elastic tape), were collected. Body composition was assessed using bioelectrical impedance analysis (770 BIA, South Korea), and ultrasound examinations were performed to evaluate abdominal and flank fat layers. At both the beginning and end of the study, 10 mL of venous

blood was collected from each participant for the assessment of biochemical markers. These markers included fasting blood glucose, blood urea nitrogen, creatinine, lipid profile (LDL-C, HDL-C, triglycerides, and total cholesterol), liver function tests, and high-sensitivity C-reactive protein (Hs-CRP).

Data Collection and Storage

During each study visit, the research team completed specialized forms to be scanned, reviewed, and imported into a local site database within 48 hours.

Data were regularly checked for completeness and accuracy to ensure their reliability.

All forms were de-identified using a unique code and securely stored in a locked folder until the end of the study, and then were destroyed.

Data Analysis

All statistical analyses were performed using SPSS software, version 18. The normality of data distribution was evaluated using the Kolmogorov–Smirnov test. The chi-square test was employed to compare categorical variables between the two groups. Normally distributed variables were expressed as mean ± standard deviation, while non-normally distributed variables were reported as median and interquartile range. Group comparisons were conducted using the Student's t-test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. Correlation analyses were performed using Pearson or Spearman correlation coefficients, as appropriate. Linear regression analysis was conducted to adjust for potential confounding variables, if necessary. A p-value of <0.05 was considered statistically significant.

Discussion

The global prevalence of obesity increased, leading to significant public health challenges due to its association with various health conditions (3-7). Traditional methods of managing obesity included dietary interventions, physical activity, medications, and surgery, but each had its limitations (10). As a result, non-invasive body contouring procedures gained attention as alternatives for reducing fat and contouring the body (1, 11-13).

Cryolipolysis is a non-invasive body contouring procedure that utilizes controlled cooling to induce apoptosis in adipocytes, leading to their gradual elimination from the body without the need for surgery or significant downtime (11, 18). Previous studies have demonstrated its effectiveness in reducing fat deposits in various areas of the body. However, concerns remain regarding its potential effects on metabolic parameters, such as lipid profile and liver function (19, 20).

Various studies have investigated the effectiveness of cryolipolysis in reducing adipose tissue and its impact on metabolic parameters. Coleman et al. reported a significant reduction in abdominal fat in 10 participants treated with a prototype cooling device (24). Meyer et al. also demonstrated positive effects of cryolipolysis on fat reduction in a cohort of 15 women aged 25–50 years. Both ultrasound and histological analyses confirmed a significant decrease in adipocyte volume after a single 60-minute session (23).

Consistent with the studies mentioned above, Klein et al. conducted a 2-month study involving 35 adults (both males and females) who underwent cryolipolysis treatment for the abdomen and flanks, with a maximum treatment duration of 30 minutes per session. The study found no significant changes in liver function tests or lipid profiles compared to baseline, suggesting that cryolipolysis is safe concerning these metabolic parameters (14).

Savacini et al. investigated a modified cryolipolysis technique, termed Contrast Cryolipolysis, in 21 subjects treated on the abdomen. Their protocol involved initially heating the subcutaneous fat layer, followed by cooling with the cryolipolysis device, and concluding with a final heating cycle. Significant reductions in the abdominal and flank areas were observed at the 90-day follow-up, with no significant changes in laboratory outcomes compared to baseline (21).

Garibyan et al. assessed changes in fat volume following cryolipolysis treatment in 11 participants and observed significant reductions in flank fat. These findings support the efficacy of cryolipolysis for targeted fat reduction (22).

The proposed study aimed to evaluate the metabolic effects of Cryolipolysis combined with a low-calorie diet in individuals with excess weight. The study was expected to observe significant reductions in fat tissue and improvements in metabolic parameters. These

results could potentially enhance non-invasive treatment options for weight management.
This study had certain limitations. Individual variability, including genetic predisposition, hormonal profiles, and differences in fat distribution and metabolism, might influence participants' responses to cryolipolysis and dietary interventions. Despite the use of stringent inclusion criteria and randomization, these factors could affect the generalizability of the findings. Future studies with larger sample sizes and genetic profiling might help clarify these individual differences

Conclusion

In conclusion, our study protocol presents a promising approach for investigating the effects of cryolipolysis combined with a low-calorie diet on weight reduction and metabolic parameters in individuals with excess weight. Based on previous research, we expect to observe significant improvements in body composition and metabolic health, with minimal adverse effects. If successful, this study could provide a novel, non-invasive approach to weight management.

Abbreviations

BIA: Bioimpedance analysis
BMI: Body mass index
BMR: Basal metabolic rate
BSQ: Body Shape Questionnaire
HS-CRP: High-sensitivity-C reactive protein
HDL-c: High-density lipoprotein-cholesterol
LDL-c: Low-density lipoprotein-cholesterol
TG: Triglycerides

Declaration

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Declaration of Conflicting Interests

The authors of this study declare that they do not have any competing interests that could impact the objectivity or impartiality of their work.

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Ethics Considerations

The data collection process will only begin after receiving local ethical approval. Participants or their legal representatives will be asked for consent before random assignment. The study proposal has been approved by the Research Ethics Committees of Mashhad University of Medical Sciences (reference approval no. IR.MUMS.REC.1399.372), and the study is registered on Iran's clinical trial website with the IRCT code ID IRCT20200927048848N1.

Trial registration

Present trial has been registered with the Mashhad University of Medical Sciences Research Ethics Committees (ref approval no. IR.MUMS.REC.1399.372). Trial Registration Number (TRN): IRCT20200927048848N1, Registration Date: 30.09.2020.

Authors' Contributions

HH, SNK, MSK, MAN, and MGM developed the study protocol and will conduct the study. FF, AM, FR, MMB, and GF wrote the study protocol. HE created the statistical plan. The final protocol was reviewed and approved by all authors.

Availability of data and materials

The study dataset will be securely stored locally at the Nutrition Department, School of Medicine, Mashhad University of Medical Sciences, Iran, for long-term data storage and access. The datasets will be made available to all principal investigators upon written request to the corresponding author.

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