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Reduction of Body Fat Mass by Cryolipolysis without Changes in Clinical Biomarkers Level

Redução da Massa Gorda Corporal por Criolipólise sem Alterações nos Níveis de Biomarcadores Clínicos

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Abstract

Cryolipolysis is a non-invasive technique used to reduce localized body fat mass. The changes in fat percentage have been assessed by many analytical methods, including bioelectrical impedance analysis. The most common effects of cryolipolysis on reducing subcutaneous fat tissue have been addressed but potential changes in biochemical and hematological biomarkers have been not safely approached. This study investigates potential changes in hematological and inflammatory biomarkers following the reduction of body fat mass. A prospective longitudinal approach included a total of 20 participants divided into groups of 5 men and women in each with ages between 25 and 45 years. They were submitted to standard cooling exposure for 60 minutes at -5°C and -10°C with 360° and "shielded" equipment, respectively. The blood samples were analyzed at times T0 (baseline), T1 (2 days), T2 (14 days), T3 (30 days) and T4 (60 days) after the procedure. The percentage of body fat mass were analyzed by bioimpedance at times T0 (baseline), T1(30 days), T2 (60 days), T3 (90 days) and T4 (120 days). No changes in hematological and inflammatory biomarkers serum levels were observed in both temperatures. Bioimpedance analysis revealed a reduction in the percentage of body fat mass only in men, with a decrease of 28 (24.5/ 29.9) compared to 30 (26.2/ 33.30) when submitted to -5°C after 120 days. These findings support that cryolipolysis can be considered an effective and safe method for localized body fat reduction without significant changes in blood biochemical parameters.

Keywords: Biomarkers. Lipids. Body Fat. Bioimpedance.

Resumo

A criolipólise é uma técnica não invasiva utilizada para reduzir a gordura corporal localizada. A mudança no percentual de gordura pode ser avaliada de várias formas, incluindo a análise de impedância bioelétrica. Os efeitos mais comuns da criolipólise na redução do tecido adiposo subcutâneo são conhecidos, porém as possíveis alterações nos marcadores bioquímicos e hematológicos ainda não foram abordadas com segurança. Este estudo investiga possíveis alterações em marcadores hematológicos e inflamatórios após a redução da massa de gordura corporal. Uma abordagem prospectiva longitudinal incluiu um total de 20 participantes divididos em 2 grupos de 5 homens e mulheres com idades entre 25 e 45 anos. Os participantes foram submetidos à exposição padrão de resfriamento por 60 minutos a temperatura de -5°C e -10°C com os equipamentos "360°" e "blindado" respectivamente. As amostras de sangue foram analisadas nos tempos T0 (basal), T1 (2 dias), T2 (14 dias), T3 (30 dias) e T4 (60 dias) após o procedimento. O percentual de massa gorda corporal foi analisado por bioimpedância nos tempos T0 (basal), T1(30 dias), T2 (60 dias), T3 (90 dias) e T4 (120 dias). Não foram observadas alterações nos níveis séricos de biomarcadores hematológicos e inflamatórios em ambas as temperaturas. A análise de bioimpedância revelou redução do percentual de massa gorda apenas nos homens com diminuição de 28 (24,5/ 29,9) em relação a 30 (26,2/ 33,30) quando submetido a -5°C após 120 dias. Os resultados obtidos sustentam a criolipólise como um método eficaz e seguro para redução da gordura corporal localizada sem alterações significativas nos parâmetros bioquímicos sanguíneos.

Palavras-chave: *Biomacadores. Lipídeos. Gordura Corporal. Bioimpedância*

1 Introduction

Cryolipolysis is an adipose tissue cooling technique designed to reduce the localized adipose tissue. In fact, subcutaneous fat tissue may be considered a risk factor for cardiovascular diseases. Its principle consists of low temperatures exposure to cause intentional damage to the adipose tissue and preserve adjacent tissues. Fat tissues are more susceptible to cold as demonstrated by the decrease of children's cheeks who consumed popsicles, called "popsicle panniculitis" as a result of oral inflammation at low temperatures[1].

Zelickson et al.[2] used Yucatan and Yorkshire pigs to test a prototype cooling device designed to determine the selective destruction of fat cells with no damage to surrounding tissues. Furthermore, the analysis of serum lipids demonstrated no changes in the animal's lipid profile. *In vitro*, adipocytes underwent necrosis at temperatures ranging from -2 °C to 2 ºC and markedly increased apoptosis over 7 ºC[3].

Clinical studies also showed that cryolipolysis did not affect serum lipids and liver function as revealed by no significant changes in the lipid profile of liver biomarkers[4]. However, the local injury after the freezing application may

cause systemic changes in the body by affecting body thermal balance. Tissue response includes the increase in energy metabolic rate to maintain its thermoregulation[5].

Increased mitochondrial energy metabolism generates reactive oxygen species, which may activate proteolytic caspases enzymes in the apoptosis cascade signaling[6]. Other intracellular disturbances caused by ice-induced tissue ischemia affect the osmoregulation by changing Na-K-ATPase activity, adenosine triphosphate (ATP) levels and lactic acid acidosis[7].

Despite the fact that more than 650,000 procedures have been performed worldwide since its inception, cryolipolysis remains unstandardized, with few physio pathological approaches[8]. The current scientific basis outlines the equipment used, as well as the location, exposure period and temperature. Inflammation that occurs as a result of adipose tissue cooling injury has not been addressed. In this context, the use of peripheral blood biomarkers might be useful in ensuring patient safety when applying the technique. These findings contribute to maximize the safe use of cryolipolysis technique while achieving the most desirable results.

2 Material and Methods

2.1 Study design

In this prospective and open label clinical study, the participants were selected according to the inclusion criteria that included healthy individuals, with adipose tissue deposits in the abdomen greater than 2.0 mm as measured by the Lafayette Adipometer and a body mass index between 21.0 and 33.6 in men and 22.9 and 45.8 kg/m in women. A total of 20 participants were divided by computer-generated simple randomization into two groups, A and B, each group consisting of 10 participants (5 men and 5 women). Body fat mass was measured and blood samples were collected at time zero (baseline), time one (2 days), time two (30 days), time three (60 days), and time four (90 days) after the procedure. This work was conducted in accordance with human study rights and approved by the Research Ethics Committee of Tuiuti University of Paraná (CAAE: 17004919.3.0000.8040).

2.2 Cryolipolysis

Cryolipolysis technique was applied to the lower and upper abdomen. During the 60-minute experiment, the temperature was -5 ºC for group A and -10 ºC for group B. Applications were performed in the upper abdomen (above the navel) and lower abdomen (below the navel) in a single application. The Dermasul company supplied the Adoxy Medical, Asgard VC10 model equipment to carry out the procedure. Two types of handles, 360º conventional "shielded" models were used. Procedure steps included the pieces of equipment installation, room acclimatization at 17 °C, skin protect of participants with anti-freeze membrane and vacuum application for 60 minutes. Diuretic drugs were not taken for at least 24 hours before the tests, food and beverages were not consumed up to 4 hours before tests, and the patient was kept at rest. Body fat mass was measured using the Bioelectrical Impedance Analysis (BIA), following the Brazilian Medical Association (AMB) and the Federal Council of Medicine (CFM) guidelines (2009).

2.3 Hematological and inflammatory biomarkers

Each study participant had 3 mL of venous blood drawn into an EDTA tube via venin puncture. A blood liquate was extracted from the whole blood sample for hematological analysis. Hematological parameters such as white blood cells (WBC) and red blood cells (RBC), hematimetric indices and platelets, were determined using an automated hematology analyzer, the ADVIA 120 (Siemens Healthcare, Berlin, Germany), a hematology analyzer that performs a complete blood count and provides results in printouts. An experienced Senior Laboratory Technologist who had special training and was certified on the automated hematology analyzer performed hematological analysis in accordance with the standard protocol and manufacturer instructions of the hematology analyzer machine and the Clinical and Laboratory Standards Institute (CLSI) guideline. Inflammatory biomarkers such as mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR) and the immune inflammation index (SII) were calculated as described previously[9-11].

2.4 Statistical analysis

Quantitative variables were expressed as mean ± standard deviation. Gaussian distribution (i.e., normality) were analyzed by the Shapiro-Wilk test. The levels in biomarkers and percentage of body fat mass among the groups were performed using analysis of variance (ANOVA) followed by Holm-Sidak post hoc test or Friedman´s test followed by Dunn´s pos hoc test for multiple comparisons. All the tests were two-tailed, with a value of $p<0.05$ considered statistically significant. All the statistical analyzes were performed using GraphPad Prism 9.2.0 software (GraphPad Software, Inc., La Jolla, California, USA).

3 Results and Discussion

3.1Subject demographics

In this study, 20 participants were included and allocated into two experimental groups, namely: group A (n=10) and group B (n=10), according to the protocol of intervention described early. Their age ranged from 25 to 45 years (man 35 ± 5 and women 35 ± 8 years old). The mean age was 36 ± 8 for group A and 35 ± 5 for group B. Within group A, the mean age for women was 35 ± 10, and for men it was 36 ± 7. Considering group B, women had a mean age of 36 ± 7, and men had a mean age of 34 ± 5.

3.2 Determination of changes in hemogram after cryolipolysis

The effects of cryolipolysis application on hemogram showed no differences over time at -5 °C (Table 1). Similar results were observed at -10 °C (data not shown). All measure values were kept stable with means not exceeding the reference value established by the National Quality Control Program guidelines.

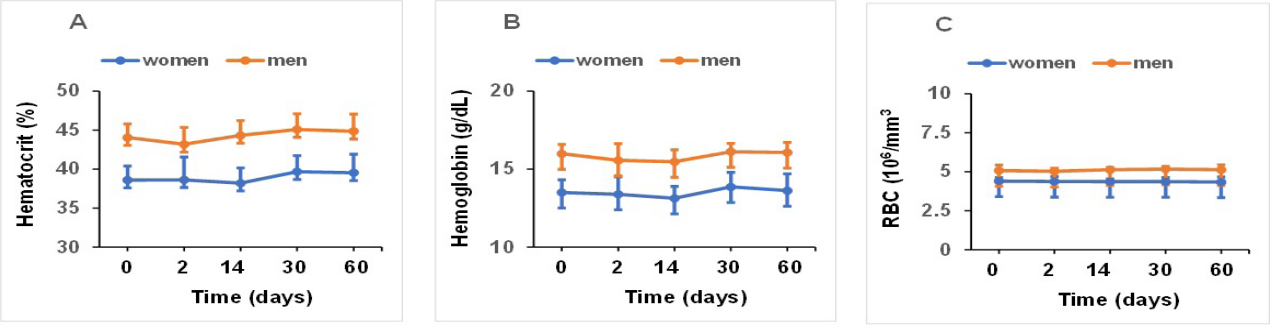
Table 1 – Analysis of hemogram in patient´s group submitted to cryolipolysis at -5 °C

Cryolipolisys AT -5° C					
Parameter	***Baseline***	**2 days**	**14 days**	**30 days**	**60 days**
HT (%)	41.2 (3.2)	40.9 (3.3)	41.4 (3.6)	42.4 (3.3)	42.2 (3.5)
HB (g/dL)	14.7 (1.4)	14.4 (1.5)	14.3 (1.4)	14.9 (1.3)	14.8 (1.5)
MCV (fL)	86.5 (3.8)	86.6 (2.9)	87.0 (3.8)	89.6 (3.7)	89.4 (4.0)
MCH (pg)	30.8 (1.4)	30.5 (0.9)	30.1 (1.2)	31.7 (1.4)	31.4 (1.3)
MCHC (%)	35.6 (0.9)	35.3 (0.8)	34.6 (0.6)	35.3 (0.8)	35.1 (1.1)
RBC (mil/µL)	4.7 (0.4)	4.6 (0.4)	4.7 (0.4)	4.7 (0.4)	4.7 (0.5)
RDW (%)	10.9 (0.5)	10.9 (0.4)	11.2 (0.2)	11.0 (0.3)	11.0 (0.3)
LINF (mm³)	3145 (1378)	2759 (745.7)	2978 (1083)	3156 (837.1)	2575 (843.0)
MONO (mm³)	550.3 (153.9)	615.1 (170.8)	489.3 (176.4)	333.3 (238.3)	597.3 (164.7)
NEU (mm³)	4614 (1422)	4380 (1941)	4095 (1499)	4619 (1130)	4742 (1421)
PLAT (mm³)	27250 (44733)	279930 (62278)	274288 (56847)	276920 (48913)	261620 (58986)
LEU (mm³)	8806 (2381)	7782 (2157)	7967 (2157)	8365 (1969)	8350 (1732)
EOS (mm³)[1]	147 (71.2/311.3)	128 (79.7/189.5)	115 (0/200.5)	218 (0/431)	128 (66.7/292.3)

[1] Data are expressed as median and interquartile interval. Friedman´s test and Dunn´s pos test are used in multiple comparisons. Legend: HT= Hematocrit; HB = Hemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC= Mean Corpuscular Hemoglogin Concentration; RBC= Red Blood Cells; RDW= Red Cell Distribution Width; LINF= Linfocytes; MONO= Monocytes; NEU= Neutrophils; ROD= Rod Cells; PLAT = Platelets; LEU= Leucocytes; EOS=Eosinophils.
Source: Resource data.

Further hemogram analysis was stratified by gender. Figure 1 shows RBC elements by sex submitted to -5 °C. Men group showed a slight drop in hematocrit 2 days after the procedure as compared to baseline. An increase could also be seen from after 14 days. Changes in hemoglobin were minimal with a small decrease in hemoglobin in 14 days in men and no changes in RBC cells in both sexes.

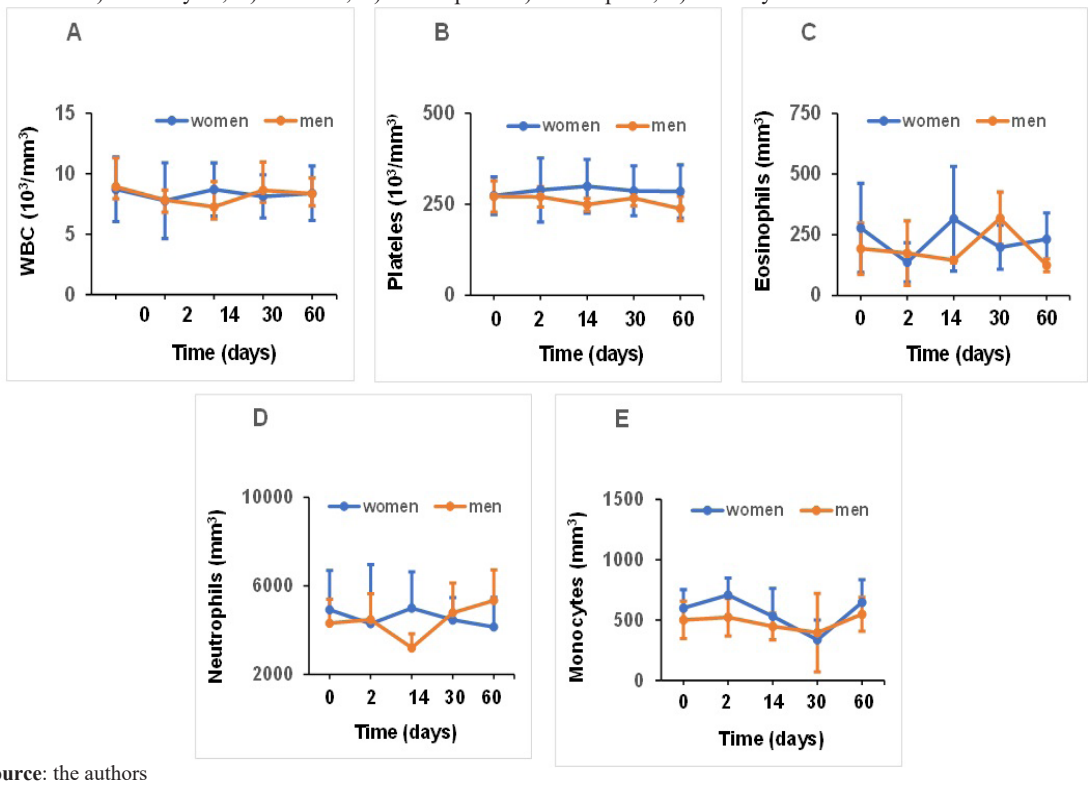
Figure 1 - Analysis of hematological parameters in men and women after cryolipolysis at -5 °C. Data are expressed as mean ± standard deviation. A) Hematocrit; B) Hemoglobin; C) Red blood cells



Source: the authors.

Figure 2 depicts the levels of leukocytes, eosinophils, and platelets levels stratified by sex. Leukocytes and platelets remained stable with very similar levels in both sexes after 60 days after cryolipolysis (Figures 2A and 2B). In women, eosinophils tended to increase after 2 days (Figure 2C). Men showed a gradual increase in neutrophils when submitted to application, which could not be seen in women (Figure 2D). As part of the immune response as precursors of tissue macrophages, monocytes had a mild decrease in both sex 30 days after criolipolysis as compared to baseline (Figure 2E).

Figure 2 - Analysis of white blood cells in men and women after cryolipolysis at -5 °C. Data are expressed as mean ± standard deviation. A) Leukocytes; B) Platelets; C) Eosinophils D) Neutrophils; E) Monocytes



Source: the authors

3.3 Body fat mass measurement

The changes in body fat mass were monitored during 120 days after the cryolipolysis at different temperatures. There were significant differences in man as revealed by BIA. The results showed a decrease in the percentage of fat mass after 120 days (28 – 24.45/29.85 *vs* 30 – 26.20/33.30) at -5 °C. Surprisingly, the same effect could not be seen in women group with statistical significance (Table 2). Tissue fat mass reduction by cryolipolysis stands for until 6 months after application, reaching up to 25% loss in total period[12]. Dierickx et al.[13] also demonstrated the fat layer reduces up to 23% in 3 months.

Table 2 – Analysis of percentage of fat mass by sex submitted to cryolipolysis at -5 °C and -10 °C

	Men					Women				
DAYS	0	30	60	90	120	0	30	60	90	120
CRYO AT -5° C	30 (26.2/ 33.30)	30 (22/ 31.95)	28 (25/ 30.50)	28 (24.6/ 29.95)	28 (24.5/ 29.9)*	43 (29.8/ 45.3)	43 (28.9/ 44.3)	42 (26.3/ 43.9)	42 (25.9/ 43.5)	42 (25.4/ 43.1)

Data are expressed as median and interquartile interval. Friedman´s test following Dunn post test for multiple comparison.
Source: the authors.

3.4 Determination of inflammatory biomarkers

Additional analysis were performed to assess potential changes in plasma inflammatory biomarkers during 60 days. The results of MPV, NLR, PLR, SSI as well as CRP showed no changes at the plasma levels. PLR and LMR means had a slight increase after 2 and 30 days respectively at -5 °C (Table 3).

Table 3 – Analysis of immune cells and inflammatory biomarker after cryolipolysis at -5°C

Cryolipolisys AT -5° C					
Parameter	***Baseline***	**2 days**	**14 days**	**30 days**	**60 days**
MPV (fL)	7.2 (0.9)	7.1 (0.8)	6.8 (0.7)	6.9 (0.6)	7.4 (1.3)
NLR (n/µL)	1.6 (0.7)	1.6 (0.7)	1.5 (0.6)	1.5 (0.2)	2.1 (1.2)
PLR (n/µL)	99.5 (35.7)	104.4 (18.1)	102.2(35.5)	92.1 (22.6)	109.2 (35.0)
LMR (n/µL)	5.9 (2.4)	4.8 (1.9)	7.1 (3.8)	13.9 (9.1)	4.4 (1.4)
RCP (mg/L)[1]	1.9 (0.65/3.55)	1.5 (1.02/5.35)	1.5 (0.87/3.67)	2.1 (0.92/4.20)	1.3 (0.92/6.27)

Data are expressed as mean ± standard deviation using ANOVA followed by Holm-Sidak post test. [1] Data are expressed as median and interquartile interval. Friedman´s test and Dunn´s pos test are used in multiple comparisons.Legend: MPV = Mean Platelet Volume; NLR=Neutrophil Lymphocyte Ratio; PLR=Platelet Lymphocyte Ratio; LMR=Lymphocyte Monocyte Ratio; CRP= C Reactive Protein.
Source: the authors.

4 Conclusion

The present work monitored body fat mass reduction and the potential changes in hematological and inflammatory parameters following cryolipolysis. No effects were found on circulating inflammatory markers in the individuals submitted to the technique application. Also, for the first time, a clinical study systematically investigated the relationship between the reduction in fat body mass at different temperatures by gender.

The effects of cryolipolysis on body fat mass reduction remain controversial. Recent clinical studies examined the single-session effect of unilateral cryolipolysis on visceral and subcutaneous over a period of 12 weeks[14]. On the other hand, the fat percentage analyzed by BIA showed a decrease of 25% and 27% in abdomen and flanks after 6 months[15]. In our study, cryolipolysis reduced fat mass percentage significantly only in men group. By the methods employed, it was not possible to see any significant change in these biochemical parameters, what reinforces cryolipolisys as safe technique. BIA measures the effect of electric current on body tissues. The method is precise and allows to determine body composition parameters.

The selective reduction in fat mass after cryolipolysis is ascribed to adipocyte cell death by apoptosis after cold exposure, which is then eliminated by macrophage engulfment as part of the inflammatory process[16,17].

The use of biomarkers has provided an additional safety tool for clinical practice. In humans, studies have shown that the procedure preserves liver function [2]. Inflammatory activity is evident after 3 days as demonstrated by the increase in inflammation biomarkers and migration of immune cells to the injury site, lasting up to 30 days before start declining[4]. Despite LMR ratio shows no statistical significance, a peak in patients submitted to either -5 or -10°C has been observed after 30 and 14 days respectively. Nevertheless, the levels returned close to baseline after 60 days. Scientifically, cryolipolysis still can be on debate as an effective method for localized fat reduction, nevertheless, these findings support the technique as a safe by employing biochemical biomarkers to predict the potential systemic effects on patients' health.

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