# The effects of cold exposure on body composition: Analysis with Computerized Bioimpedance

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#### Abstract

Introduction: Weight gain and obesity are common concerns for midlife women. Obesity is characterized by an accumulation of body fat resulting in body weight at least 20% above the optimum. **Objective:** To evaluate the efficacy of cryolipolysis on body composition in midlife women. **Meth**ods: Thirty women aged 30-55 with overweight underwent three cryolipolysis sessions, (45 minutes each), spaced 15 days apart. Body composition was assessed using bioelectrical impedance before and after the intervention. Parameters measured included fat mass, lean mass, muscle mass, muscle/fat ratio, and total body water (TBW) percentage. Results: Significant reductions were observed in both absolute fat mass (from  $26 \pm 1.5$  kg to  $24.4 \pm 1.51$  kg, p<0.05) and fat mass percentage (from  $34.4 \pm 1.2\%$  to  $32.8 \pm 1\%$ , p<0.05). Lean mass remained stable ( $48.91 \pm 1.56$  kg to  $48.82 \pm 1.4$  kg), with a significant increase in lean mass percentage (65 ± 1.2% to 67 ± 1.2%, p<0.05). Muscle mass percentage rise from  $29 \pm 0.6\%$  to  $31 \pm 0.7\%$  (p<0.05). The muscle/fat ratio improved significantly from 0.85  $\pm 0.04$  to 0.95  $\pm 0.2$  (p<0.05), and TBW percentage increased from 46.5  $\pm 0.9\%$  to 48  $\pm 0.9\%$  (p<0.01). Conclusion: Cryolipolysis significantly enhances body composition by reducing fat mass, maintaining lean mass, and improving muscle/fat ratio and hydration status. These findings underscore cryolipolysis as an effective non-invasive alternative for body contouring with potential health benefits, including improved metabolic and cardiovascular health. Future research should explore long-term effects and mechanisms to optimize clinical applications.

**Keywords**: Cryolipolysis; body composition; bioelectrical impedance; fat reduction; obesity.

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# BACKGROUND

Weight gain is a common concern for midlife women and has been reported in several studies. In the Study of Women Across the Nation (SWAN), midlife women (n=3064) gained an average of 0.7 kg per year, independent of age at baseline or menopause status<sup>1</sup>. Although racial and socioeconomic disparities impacted body weight at baseline (i.e., non-white and lower socioeconomic status are associated with higher baseline weight), subsequent studies showed that weight gain occurred across all midlife women, suggesting the uniformity of this trend<sup>2</sup>. However, weight gain is not limited to midlife; numerous studies have documented an average yearly weight gain of 0.5 kg to 1 kg in US adults<sup>3,4</sup>. Obesity is a critical condition characterized by an accumulation of body fat resulting in body weight that is at least 20% more than the optimum weight<sup>5</sup>. The rate of obesity has increased more than threefold since 1975 globally due to decreased physical activity and increased consumption of high energy-dense, nutrient-poor foods with high levels of carbohydrates, sugar, and saturated fats<sup>6,7</sup>. In one specific study conducted in United States of America, women aged 36–79 experienced an increase of 5.4 kg over the previous 10 years, with the age group of 36–39 years showing the highest weight gain (9 kg)<sup>8</sup>. Persistent weight gain throughout adulthood, particularly when it does not level off during midlife, can significantly contribute to the progression or onset of overweight and obesity. The escalating global prevalence of obesity represents a complex health crisis with profound implications that extend beyond physiological effects to significant aesthetic concerns<sup>9-11</sup>.

Obesity significantly elevates the risk of various chronic diseases, including diabetes mellitus and cardiovascular diseases (CVD), particularly heart disease and stroke<sup>11</sup>. The normalization of overweight and obesity further blurs the distinctions between healthy and unhealthy body weights, perpetuating misconceptions about ideal aesthetics and reinforcing harmful lifestyle behaviors. Thus, addressing the obesity epidemic requires a comprehensive approach that targets both physiological health and the complex interplay between societal perceptions of beauty and body image<sup>12,13</sup>. Despite its popularity, alternative noninvasive body contouring techniques, such as cryolipolysis, are gaining global attention. Cryolipolysis, also known as "cold body exposure" or "body cryotherapy," is emerging as a promising technique for reducing localized fat and improving body contour. It involves the application of cold for a predetermined duration, inducing apoptosis and lipolysis in fat cells through localized and systemic mechanisms<sup>13,14</sup>. Lipolysis, the release of fatty acids from adipose tissue, is predominantly regulated by catecholamines like noradrenaline and adrenaline during energy demand and stress situations. This process relies on adrenergic receptors, particularly β-adrenoceptors, which may exhibit dysfunction in obesity, potentially resulting in reduced fatty acid release during energy-demanding situations such as exercise and other stressors controlled by the neuro-hormonal axis Hypophysis-Hypothalamus-Adrenal<sup>15</sup>.

Cold temperatures induce physiological stress by triggering vasoconstriction, reducing blood flow to the skin and extremities, which can lead to decreased tissue oxygenation and increased risk of frostbite. Exposure to cold activates the body's thermoregulatory mechanisms, including shivering and increased metabolism, to maintain core body temperature, placing additional strain on metabolic pathways and energy reserves. Prolonged exposure to cold can disrupt hormonal balance, including increased secretion of stress hormones such as cortisol and adrenaline, contributing to heightened physiological stress responses<sup>15</sup>. In this context, cryolipolysis has become a popular treatment for noninvasively addressing localized fat accumulation but specially altering body composition, and promoting health<sup>16</sup>. The new findings suggest possible applications in metabolic syndrome, cardiovascular prevention and diabetes or insulin resistance.

Over the past few years, there has been a significant increase in interest in the use of bioelectrical impedance analysis (BIA) as an easy, accessible, and safe method of body composition analysis in various fields of medicine. BIA is a technique used primarily to assess muscle mass, fat mass, hydration, and phase angle (PhA) among many other body composition parameters. With PhA, the hydration state can be expressed, and the quality

of cell membranes can be assessed. BIA has become extremely popular for assessing body composition because it is an easy-to-use, portable, quick, relatively inexpensive, and non-invasive technology. Consequently, BIA is widely used in hospitals, clinics, and other healthcare facilities<sup>17</sup>. This study seeks to assess the effects of cold exposure on body composition in midlife women.

#### **METHODS**

## Study Design

This clinical study aimed to evaluate the efficacy of cryolipolysis in promoting lipolysis in the abdominal and flank regions of women with localized fat deposits and to assess changes in body composition. The research was conducted at the Health Technology Laboratory of Evangelical University of Goiás (UniEVANGÉLICA), Anápolis (GO), Brazil, adhering to the Guidelines and Regulatory Norms for research involving human beings established by the National Health Council, Ministry of Health in October 1996 and updated in Resolution 466/2012, Brazil. The study received approval from the Ethics Committee of the UniEVANGÉLICA, under registration number 6.574.522, on December 23rd, 2023.

#### **Population and Sample**

The study comprised 30 female participants, aged 30 to 55, who had localized adiposity in the abdominal region and flanks and were diagnosed with overweight or obesity. Participants were selected through convenience sampling and underwent three sessions of cryolipolysis.

## **Data Collection**

Participants were recruited at the university campus without distinction of activities and provided informed consent before participating in the study. Initial assessments included a detailed body history to evaluate lifestyle habits and medical history, classification of body biotype, abdominal diastasis testing, body composition analysis using bioimpedance with a BIA device (Tera Science – São José dos Campos, São Paulo -Brazil), and anthropometric measurements using a standard tape measure. Standardized photography before and after each of the three cryolipolysis sessions, spaced 15 days apart, was recorded for comparisons. For the photos, participants were positioned in anterior, posterior, right profile, and left profile positions. The distance observed for each image was 70 centimeters, and participants signed an authorization form for the use of the images. All parameters were compared before and after three sessions, with each individual being compared to themselves using the paired Student's t-test.

#### Cryolipolysis

Cryolipolysis (Body Cold Exposure) treatment was administered using four simultaneous handles for 45 minutes at a temperature of -5°C, utilizing Asgard Equipment (Adoxy Equipamentos, Votorantim, São Paulo - Brazil). Standard antifreeze blankets were employed to protect the treated areas. Each session lasted 45 minutes, with intervals of 15 to 20 days between ses S65sions. The procedure adhered to the manufacturer's guidelines, placing the four handles on the abdominal region. Participants were positioned supine during the cryolipolysis procedure, with the four device handles

placed on the abdominal region. After the procedure, a reperfusion massage was administered to facilitate the return to normal body temperature, with continuous monitoring every 5 minutes using an infrared thermography camera (FLIR – S65, Sweden).

## **Computerized Bioimpedance Analysis**

BIA is a method used to assess body composition. The computerized version of this test offers a more detailed and precise analysis, allowing data to be easily interpreted and monitored over time. This is particularly useful in clinical and fitness contexts to monitor changes in body composition and adjust treatment or training plans. It is a non-invasive, quick, and relatively easy method to use, although its accuracy can be affected by factors such as hydration, food intake, and recent physical activity. Specifically, the PhA is a useful indicator of cell membrane integrity, the distribution of water between intracellular and extracellular spaces, and the prediction of body cell mass, as described by the components of electrical impedance (Z): resistance (R; a function of the volume of intracellular and extracellular fluid) and reactance (Xc; a function of the dielectric material of tissue cells). The PhA is geometrically calculated from R and Xc measured at 50 kHz. The PhA can be simply calculated as an arctangent using the raw data of R and Xc at a frequency of 50 kHz, as follows: (Xc/R) x 180°/ $\pi$ . Thus, the PhA is obtained directly from the BIA without using a regression equation.

## **Preparation for Bioimpedance Testing**

Participants received instructions to fast for 4 to 6 hours before the test. They were also advised to avoid excessive water intake immediately before the test but maintain normal hydration in the 24 hours prior. Participants were instructed to avoid strenuous exercise in the 12 hours prior to the test and to avoid alcohol and caffeine consumption 24 hours before the test. At the time of the test, participants were kept on their feet with their body relaxed and limbs slightly separated from the torso. Electrodes were typically placed on the wrists and ankles. The skin where the electrodes were applied was cleaned and dried before fixing the electrodes. Then, the electrodes were connected to wires, which, in turn, were connected to the BIA device (Tera Science, São José dos Campos, São Paulo - Brazil). Once the electrodes were correctly positioned and connected, the machine was activated. A small, typically imperceptible electric current was sent through the electrodes. This current quickly traveled through the body from the lower to the upper limbs. Results were automatically calculated by the device.

## **Inclusion Criteria**

Participants aged between 30 and 55 years old who had not undergone a cryolipolysis procedure in the past 12 months and exhibited a fat fold deemed suitable for treatment on the abdomen and flanks, as determined by the thickness of the fat layer. A minimum of 2 cm of fat in the specified regions and a body mass index (BMI) of 25 to 40 were required.

#### **Exclusion Criteria**

Exclusion criteria included pregnancy, lactation, hernia in the region, scars in the region, skin conditions, autoimmune diseases, decompensated diabetes, neoplasms, obesity, paroxysmal hemoglobinuria in the cold, post-herpetic neuralgia, and cold-related diseases, along with participants who did not meet the evaluation criteria.

#### **Statistical Analysis**

Participants were evaluated at an initial baseline moment and then again 15 days after completing three sessions of cryolipolysis. Each participant was compared with themselves before and after the treatment using the Student's t-test for paired samples. Values with  $p \leq 0.05$  were considered significant.

## RESULTS

## **Changes in Fat Mass**

Figure 01 illustrates the changes in fat mass in kilograms (kg) and the percentage of fat mass over the course of the study period. The bar graph in Panel 01A presents the mean fat mass at two distinct time points: Initial and Final. At the Initial measurement, the average fat mass was  $26 \pm 1.5$  kg. At the Final measurement, the average fat mass decreased to 24.4 ± 1.51 kg. The reduction in fat mass is statistically significant, as indicated by the asterisk (\* - Paired Student t-test), suggesting that the intervention led to a meaningful decrease in fat mass. Panel 01B showed the percentage of Fat Mass (%): Panel 01B shows the mean percentage of fat mass at the same two time points: Initial and Final. The y-axis indicates the percentage of fat mass, and the x-axis denotes the time points. Initially, the average percentage of fat mass was  $34.4 \pm 1.2\%$ . By the final measurement, this percentage had reduced to  $32.8 \pm 1\%$ . The asterisk (\*) indicates that this reduction is statistically significant (paired Student t-test), demonstrating the effectiveness of the intervention in reducing the percentage of body fat. The data presented in both panels of Figure 01 indicate a significant reduction in both the absolute fat mass and the percentage of fat mass from the Initial to the Final measurement. The significant reductions, as marked by the asterisks, suggest that the intervention or time period examined had a positive effect on reducing body fat. These findings underscore the effectiveness of the treatment or lifestyle changes implemented during the study, highlighting their potential benefits for fat loss.

01A



01B



Figure 01. Changes in fat mass and percentage of fat mass

**Note:** Panel 01A: Fat Mass (kg) - This panel illustrates the mean fat mass in kilograms at two time points: initial and final. The bar on the left shows the initial fat mass, while the bar on the right shows the final fat mass. The asterisk (\* p < 0.05) Panel 01B: Percentage of Fat Mass (%) - This panel displays the mean percentage of fat mass at two time points. The bar on the left represents the Initial percentage of fat mass, and the bar on the right represents the Final percentage of fat mass. The asterisk (\*) indicates a statistically significant decrease in the percentage of fat mass from Initial to Final.

#### **Changes in Lean Mass**

Figure 02 depicts the changes in lean mass in kilograms (kg) and the percentage of lean mass over the course of the study period. The bar graph in Panel 02A presents the mean lean mass at two distinct time points: Initial and Final. At the Initial measurement, the average lean mass was  $48.91 \pm 1.56$  kg. At the Final measurement, the average lean mass remained  $48.82 \pm 1.4$ , indicating no significant change in lean mass over the study period. Panel 02B shows the mean percentage of lean mass was  $65 \pm 1.2\%$ . By the final measurement, this percentage had increased to approximately  $67 \pm 1.2\%$ . The asterisk (\*) indicates that this increase is statistically significant (paired Student t-test, demonstrating the effectiveness of the intervention in increasing the percentage of lean body mass.

The data presented in both panels of Figure 02 indicate no significant change in absolute lean mass from the Initial to the Final measurement. However, there is a significant increase in the percentage of lean mass, as marked by the asterisk, suggesting an improvement in body composition. These findings highlight that while the absolute lean mass remained stable, the proportion of lean mass relative to total body mass increased, indicating a positive shift in body composition.



Figure 02. Changes in Lean Mass and Percentage of Lean Mass

**Note:** Panel 02A: Lean Mass (kg) - This panel illustrates the mean lean mass in kilograms at two time points: initial and final. There is no significant change in lean mass from initial to final. Panel 02B: Percentage of Lean Mass (%). This panel displays the mean percentage of lean mass at two time points: Initial and Final. The y-axis indicates the percentage of lean mass. The asterisk (\*) indicates a statistically significant increase in the percentage of lean mass from Initial to Final.

#### **Changes in Muscle Mass**

Figure 03 illustrates the changes in muscle mass in kilograms (kg) and the percentage of muscle mass over the course of the study period. Panel 03A: Muscle Mass (kg) - The bar graph in Panel 03A presents the mean muscle mass at two distinct time points: Initial and Final. The y-axis represents muscle mass in kilograms, while the x-axis displays the time points. At the Initial measurement, the average muscle mass was  $22.3 \pm 0.9$  kg. At the Final measurement, the average muscle mass remained 22.5 ± 0.8 kg, indicating no significant change in muscle mass over the study period. Panel 03B shows the mean percentage of muscle mass at the same two time points: Initial and Final. The y-axis indicates the percentage of muscle mass, and the x-axis denotes the time points. Initially, the average percentage of muscle mass was  $29 \pm 0.6\%$ . By the Final measurement, this percentage had increased to 31 ± 0.7%. The asterisk (\*) indicates that this increase is statistically significant, demonstrating the effectiveness of the intervention in increasing the percentage of muscle body mass. The data presented in both panels of Figure 03 indicate no significant change in absolute muscle mass from the Initial to the Final measurement. However, there is a significant increase in the percentage of muscle mass, as marked by the asterisk, suggesting an improvement in body composition. These findings highlight that while the absolute muscle mass remained stable, the proportion of muscle mass relative to total body mass increased, indicating a positive shift in body composition.



Figure 03. Changes in Muscle Mass (Kg) and % of Muscle Mass

Note: Panel 03A: Muscle Mass (kg) - This panel illustrates the mean muscle mass in kilograms at two time points: initial and final. There is no significant change in muscle mass from initial to final. Panel 03B: Percentage of muscle mass (%). This panel displays the mean percentage of muscle mass at two time points: Initial and Final. The y-axis indicates the percentage of muscle mass. The asterisk (\*) indicates a statistically significant increase in the percentage of muscle mass from Initial to Final

## Changes in Muscle/Fat Ratio and Total Body Water

Figure 04 illustrates the changes in the muscle/fat ratio and the percentage of total body water (TBW) over the course of the study period. The bar graph in Panel 04A presents the mean muscle/fat ratio at two distinct time points: Initial and Final. At the Initial measurement, the average muscle/fat ratio was  $0.85 \pm 0.04$ . At the Final measurement, the average muscle/fat ratio increased  $0.95 \pm 0.2$ . The asterisk (\*) denotes a statistically significant increase in the muscle/fat ratio from Initial to Final, indicating an improvement in body composition. Panel 04B shows the mean percentage of TBW at the same two time points: Initial and Final. Initially, the average percentage of TBW was 46.5  $\pm$  0.9%. By the Final measurement, this percentage had increased to 48  $\pm$  0.9%. The double asterisks (\*\*) indicate that this increase is statistically significant (p  $\leq$ 0.01), demonstrating the effectiveness of the intervention in increasing TBW percentage. The data presented in both panels of Figure 04 indicate significant improvements in body composition from the Initial to the Final measurement. The significant increase in the muscle/fat ratio, suggests a positive shift towards a healthier balance of muscle to fat. Similarly, the significant increase in TBW percentage, highlights an improvement in hydration status. These findings underscore the overall effectiveness of the intervention in enhancing body composition and hydration.



Figure 04: Muscle/Fat Ratio and Total Body Water (%)

**Note:** Panel 04A: Muscle/Fat Ratio - This panel illustrates the mean muscle/fat ratio at two time points: initial and final. There is a significant increase in muscle/fat ratio from initial to final. Panel 04B: Percentage TBW (%). This panel displays the mean percentage TBW at two time points: initial and final. The y-axis indicates the percentage of TBW (%). The asterisk (\*\*) indicates a very significant increase in the percentage of TBW from initial to final.

#### DISCUSION

The present study investigated the impact of cryolipolysis, a technique involving prolonged exposure to cold, on body composition in 30 participants. Participants underwent three sessions of cryolipolysis, each lasting 45 minutes, with 15-day intervals between sessions. The results, as illustrated in Figures 01 to 04, indicate significant alterations in body composition, suggesting the efficacy of cryolipolysis in improving various health aspects. The results of this clinical investigation affirm the efficacy of cryolipolysis in reducing body fat, particularly, showcasing its potential as a non-invasive alternative to surgical interventions such as liposuction<sup>14</sup>.

The significant reduction in both absolute fat mass and percentage of fat mass (Figure 01) underscores the effectiveness of cryolipolysis in targeted fat reduction. Specifically, the mean fat mass decreased from approximately 25 kg to 23 kg, and the percentage of fat mass decreased from 35% to 31%. Besides the well known lipolytic effect of cold exposure, Cryolipolysis induces apoptosis of adipocytes through cold exposure, leading to a gradual reduction in fat tissue without damaging surrounding structures<sup>18,19</sup>. This non-invasive approach provides a promising alternative to surgical fat reduction methods such as liposuction, with minimal downtime and fewer associated risks. Figure 02 demonstrates that while the absolute lean mass remained stable around 50 kg, the percentage of lean mass significantly increased from 66% to 68%. This stability in lean mass, coupled with a reduction in fat mass, suggests an improvement in body composition. The preservation of lean mass during fat loss is crucial as it is associated with better metabolic health, higher basal metabolic rate, and improved functional capacity<sup>20</sup>. Maintaining lean mass is particularly important in aging populations, where sarcopenia (loss of muscle mass) can lead to decreased mobility and increased risk of falls and fractures.

Figure 03 indicates no significant change in absolute muscle mass, which remained around 20 kg, but shows a significant increase in the percentage of muscle mass from 30% to 32%. This relative improvement in muscle mass percentage, despite stable absolute muscle mass, reflects a healthier body composition. Increased muscle mass percentage correlates with improved strength, endurance, and metabolic health, reducing the risk of metabolic syndrome and other chronic diseases<sup>21</sup>. Additionally, a higher muscle mass percentage supports better insulin sensitivity, aiding in the prevention and management of type 2 diabetes. Figure 04 reveals a significant increase in the muscle/fat ratio from 0.85 to 0.95 and in TBW percentage from 46% to 48%. The improved muscle/fat ratio underscores a healthier balance between muscle and fat tissues, reflecting a positive shift in body composition. A higher muscle/fat ratio is indicative of better overall health and lower risk of cardiovascular diseases<sup>22</sup>. The increase in TBW percentage suggests improved hydration status, which is essential for cellular functions, nutrient transport, and waste elimination<sup>23</sup>. Proper hydration also enhances physical performance, recovery, and overall well-being.

The alterations in body composition observed in this study have several potential health benefits, such as in Cardiovascular Health. The reduction in fat mass and an improved muscle/fat ratio can lower the risk of cardiovascular diseases. Excessive fat, particularly visceral fat, is a major risk factor for conditions such as hypertension, atherosclerosis, and heart disease<sup>22</sup>. Abdominal fat is metabolically active and contributes to elevated levels of circulating lipids, which are pivotal in the development of atherosclerosis. By reducing total cholesterol, cryolipolysis may reduce the burden of cholesterol plaques within the arteries, thereby potentially lowering the risk of heart attacks and strokes. This is of paramount importance in a clinical setting, as cardiovascular diseases remain the leading cause of mortality globally. Besides, in metabolic Health, enhanced lean mass percentage and muscle mass percentage improve metabolic health by increasing basal metabolic rate and insulin sensitivity. Concerning on another important problem related do body fat, the epidemic proportions of type 2 diabetes worldwide, and implications of reducing central adiposity extend beyond cardiovascular diseases. Abdominal fat is a known risk factor for insulin resistance, the hallmark of type 2 diabetes. By diminishing abdominal fat, cryolipolysis could play a role in improving insulin sensitivity, thereby contributing to diabetes management and prevention. This can aid in the prevention and management of metabolic syndrome and type 2 diabetes<sup>20</sup>.

Concerning on physical function, increased muscle mass percentage and lean mass percentage contribute to better physical strength, endurance, and functional capacity. This is particularly beneficial for aging populations, reducing the risk of sarcopenia and associated complications<sup>21</sup>. In addition, hydration and recovery can also be improved. The changes in TBW percentage indicates better hydration status, which supports cellular functions, physical performance, and recovery. Proper hydration is crucial for maintaining homeostasis and overall health<sup>22</sup>. The results demonstrating a non-Invasive fat reduction after Cryolipolysis offers a non-invasive alternative to surgical fat reduction methods, providing a safer option with minimal downtime and fewer risks. This makes it an attractive option for individuals seeking body contouring without the complications associated with surgery (Manstein et al., 2008). The possible mechanism of fat reduction remains unclear. Besides de classical pathway driven by the sympathetic innervation and catecholamines release leading to  $\beta$ -receptor stimulation and lipolysis, local and direct mechanisms have been proposed. Physiological stimulation of brown fat  $\beta$ 3-adrenergic receptors (β3-AR) through cold stress, or through direct pharmacological activation, swiftly triggers non-shivering thermogenesis. This process is facilitated by the action of mobilized fatty acids, which serve as allosteric activators of uncoupling protein 1 (UCP1), the key molecular mechanism responsible for heat production in brown fat<sup>23-25</sup>. However, it's possible indeed that at least two different mechanisms may be involved in the observed fat reduction.

#### CONCLUSION

The three sessions of cryolipolysis significantly altered body composition in the study participants, reducing fat mass, maintaining lean mass, and improving muscle/fat ratio and hydration. These findings suggest that cryolipolysis is an effective non-invasive intervention for enhancing body composition and potentially improving various health parameters. Future studies should explore the long-term effects and underlying mechanisms of cryolipolysis to further substantiate these benefits. Integrating cryolipolysis with broader lifestyle and dietary interventions could potentially enhance its efficacy and contribute to comprehensive metabolic health management.

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