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Efficacy and Safety of Subcutaneous Fat Reduction by Cryoadipolysis

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***Abstract*—** *Exuberant fat deposits represent one of the greatest aesthetic concerns of modern society. Furthermore, an excessive accumulation of subcutaneous adipose tissue represents a health problem that can substantially increase the risk of cardiovascular disease, diabetes or cancer. Since the most effective surgical methods to reduce fat deposit, like abdominoplasty or liposuction, imply significant risks associated with general anesthetic and long post-surgical recovery time among other issues, a non-invasive technique represents an appealing alternative to circumvent possible risks.*

One of non-invasive method to reduce adipose foci is cryoadipolysis. It is a procedure that results in elimination of adipocytes by induction of cell apoptosis after considerable temperature decrease. Cooltech® is one of the leading cryoadipolysis platforms worldwide.

Here, we address the mechanisms of action and efficacy of cryoadipolysis, as well as the temperature limits of different non-adipose tissues (skin, nerves, muscles and blood vessels) to address the safety of Cooltech® platform. The detailed revision of bibliographic data is supplemented with experimental and theoretical studies to corroborate the safety of this innovative cryoadipolytic platform.

***Keywords*—** *Cryolipolysis; Adipocytes; Cooltech®; Non-invasive; Fat reduction; Apoptosis; Body contouring.*

I. Subcutaneous Fat Reduction by Cryoadipolysis

Even though subcutaneous adipose tissue is an important part of human body with several important functions (energy store, muscle and bones protection, temperature regulation), its increased accumulation represents serious aesthetic concerns of modern society. Fat accumulation may represent also serious health problems, including heart disease and strokes, high blood pressure, type 2 diabetes, certain types of cancer, sleep apnea, fatty liver disease etc. [1,2]. Besides, obese individuals experience often social discrimination and flawed self-perception, which can lead to self-destruction [3]. Ideally, healthy life style and regular exercise should result in enough fat loss. However, this is often unfeasible to reach (health problems, lack of time, advanced age etc.) without the help of cosmetic procedures.

Even though, the most popular cosmetic fat loss procedures are abdominoplasty and liposuction, these procedures are highly invasive and implies possible complications in terms of infection, nerve damage, seroma, hematoma, and risks associated with general anesthetic and long post-surgical recovery time. To circumvent problems associated with surgical methods, non-invasive techniques for fat reduction that do not requires full anesthesia or post procedural recovery represents an appealing alternative. One of this kind of aesthetic technique is cryoadipolysis, also known as cryolysis, cryolipolysis or adipocyte lysis through cold. Cryoadipolysis is a new non-invasive method, which effectively removes or reshapes undesirable fat deposits, while do not produce any harm to adjacent tissue [4,5]. During cryoadipolysis, adipocytes are destroyed by a controlled thermal reduction. The first Cryoadipolysis device was introduced in 2007 and approved by the FDA for treatment of the focal fat deposits in the flanks in 2010 (K080521), in the abdomen in 2012 (K120023), and in the thighs in 2014 (K133212). Importantly, since the adipocytes are more sensitive to the cooling process than other cells, the surrounding tissues are exposed just to a minimal collateral damage [6–8]. However, in order to guarantee safety of the treatment, it is necessary to know the temperature ranges to which the rest of cells are susceptible and to undertake protective measures. The amount of damage caused by freezing cold temperatures depends mainly on the amount of water within the cells (cytoplasm) and in the extracellular matrix (Table 1). Of note, even though water freezes at 0ºC, above -5ºC both cells and their surrounding matrix remain unfrozen [9]. This is explained by the fact that cell membrane effectively prevents the formation of ice crystals in the cytoplasm and presence of solvents like salts and proteins decrease the freezing point of the liquid in the extracellular matrix. Below -5ºC, first extracellular matrix starts to freeze causing an osmotic imbalance with the water in the cells. Subsequently, the cells lose water and start to dehydrate. With an adequate cooling rate, the cells dehydrate before reaching the nucleation temperature (ice formation), so that the possibility of intracellular freezing and consequent cell damage is minimized [9].

In a cryoadipolytic device (e.g. Cooltech®; Cocoon Medical, Barcelona, Spain), cooling is done from the outside in, through the metal plates that are placed outside treated area, which is under moderate vacuum suction [7,11]. The resulting fat deposit reduction is a gradual process that can be appreciable over a 2-3 months period [11]. Ideally, the fat reduction process implies multiple sessions over the course of the 2-3 months. The subsequent reduction of fat layer thickness is estimated from 23 to 50% depending of each individual condition [11–14]. Previously, the efficacy of fat deposits reduction by cold was demonstrated in multicentre clinical trial that showed improvement in 86% of 518 patients [12].

Whole process of localize adipose tissue elimination by Cooltech® include six consecutives procedures that will be in

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detail described as follow (Figure 1). First, a cryoprotector is applied to the treated area, and the localized fat deposit is suctioned inside the applicator. Subsequently, the cooling process that leads to cell apoptosis of adipocytes is initiated. The process finishes by massage of treated area and posterior elimination of damaged adipose cells by lymphatic system (Figure. 1).

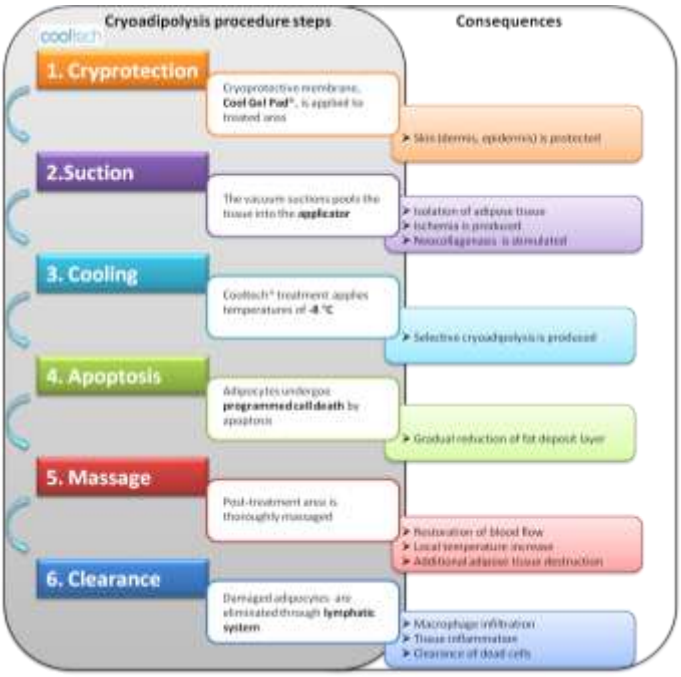

Fig. 1. Schematic description of cryoadipolysis process by Cooltech® that include six consecutive proceedings; cryoprotection, suction, cooling, apoptosis, massage and clearance of death adipocytes.

1.1. Application of cryoprotectant device membrane

To avoid any possible harm of surrounding tissue, mainly epidermis and dermis, during cooling process the applicator does not come into direct contact with the skin since the cold during cryoadipolysis is transmitted via cryoprotectant membrane, Cool Gel Pad® [product associated to the patent applications: 2018/060533 A1 and PCT/ES2018/070185].

This membrane contains glycerol, a penetrating cryoprotectant among other compounds, which has a cryopreservation effect on cells with high water content (Table 1) [15,16]. Cool Gel Pad® act as antifreeze solution that avoids the formation of ice crystals on the skin. Moreover, it protects cells by minimizing intracellular ice formation and reducing intracellular salt concentration. Thanks to low molecular weight (92 g/mol), glycerol enters by diffusion through the skin and penetrates in the cells [17]. It has two main effects: a) the decreasing of the freezing point by the cryogenic descent of water, thus avoiding the formation of ice and decreasing the threshold of thermal damage of the skin; b) the combination of glycerol with water (colligative effect) that avoid the dehydration of the cell at low temperatures and its contraction, thus minimizing the possibility of cellular damage. Moreover, the glycerol is a baro-protector, which acts as a protector for the skin from injuries that may be caused by the suction, which function is explained below.

1.2. Suction: vacuum effect

Cryoadipolysis treatment is performed using vacuum applicators. The vacuum suctions and pools the area to be treated into the applicator and helps thus to isolate adipose tissue from the rest of internal tissues in order to make the treatment more effective and safer (Fig 2). An adequate suction is essential to assure that tissue is in contact with the inner cooling surface of the applicator. The effect of vacuum also produces the ischemia of the tissues, since it produces vasoconstriction of blood vessels and as a consequence reduces blood flow. Since the blood flow is a mechanism of temperature regulation, by reducing it, lower temperatures are achieved in the zone with fat content. As a result, abrupt temperature changes in the adipose cells producing greater death by apoptosis [18]. Besides, vacuum suction, which pulls the tissue bulge into the applicator, contributes to neocollagenesis [19]. This is important in order to preserve natural firmness of the skin and its good adherence to new body contours after the treatment [20,21]. Vacuum suction stretches the fibroblasts, what lead to collagen production [19].

The availability of applicators with different shapes and sizes permits cooling fat deposits of different shapes and volumes. Larger and deeper applicators allow for higher-volume fat reduction. Smaller and fatter applicators target small areas more precisely.

However, a non-adequate vacuum has negative consequences on the results of a Cryoadipolysis treatment: a) defective heat transfer to the tissue and consequently less treatment efficacy, b) more pain and c) higher risk of lesions on the skin [22,23].

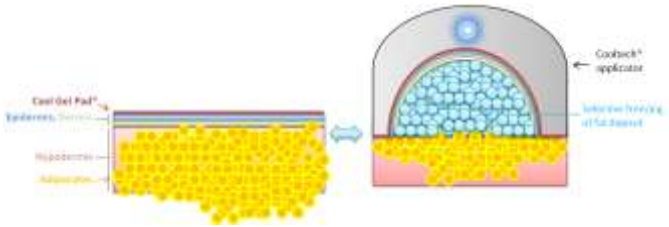

Fig. 2. Schematic description of Cooltech® applicator suction and cooling process.

1.3. Cooling process

Cryoadipolysis takes advantage of differences in cold-sensitivity of different cell types, being adipocytes the most susceptible cells. This is because adipocytes contain excess of triglycerides that crystallize under temperature around 10 °C [14,24]. On the other hand, the surrounding cells contain more water (Table 1), which freezes below -5°C [8]. These facts make the adipose tissue more susceptible to cold than other tissues such as muscle, nerves and skin [4,5].

Cooltech® treatments use minimum temperatures between -5 and -10 °C. As mentioned before, to avoid any possible harm during cryoadipolysis due to the formation of ice crystals in the skin, the cold of the applicator is transmitted via cryoprotectant membrane, Cool Gel Pad®. Besides, the cold is

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transmitted by conduction through the dermis and epidermis towards subcutaneous adipocytes and is limited by the mechanisms of thermal regulation of the organism during cooling process. As a result, the deeper layers are less cold than the outer layers of the skin. The layers of the skin should not be considered as a homogeneous block of temperature, but rather a temperature gradient due to thermal conduction. When the skin is at room temperature and the Cooltech® applicator cools, there is a temperature difference between the two parts.

TABLE 1. Composition of water in tissues and organs by weight [10]

Tissue	Water content
Brain	75 %
Skin	72 %
Blood	83 %
Heart	79 %
Lungs	79 %
Liver	68 %
Intestine	75 %
Kidney	83 %
Spleen	76 %
Adipose tissue	10 %
Muscle	76 %
Bone	22 %

The Fourier law establishes that there will be a flow of energy between the two objects until equilibrium is achieved [25,26]. As a consequence, a temperature gradient is generated, in which the closer the tissue to the applicator, the colder it will be, and the further away, the higher the temperature will be reached. Based on the thickness of the different skin layers, we can predict an approximate temperature of the respective skin layer, and which cell type will be affected by temperature drop. Since the deeper layer (adipose tissue) is the most sensitive to cold, it results more affected even at higher temperature and almost despicable damage will be cause to surrounding cells (muscles, blood vessels, nerves etc) [25,26].

However, a possible harm of peripheral nervous system during cryoadipolysis represents an important issue to be considered. It is important to know the distribution of the nerves and their types throughout the body, in order to evaluate their different thermal sensitivities and take them into account when positioning Cooltech® applicators. The cold produces a decrease in the conduction velocity of the peripheral nerves and a reduction or blockage of their synaptic activity. The nerve fibers vary in sensitivity to cold according to their diameter and degree of myelination. It has been shown that the most sensitive nerves are the most myelinated and of small diameter, since the unmyelinated ones need lower temperatures to be blocked. Since, the temperature of action of cryoadipolysis is not lower than -10 °C, no injuries greater than those of the first degree will be produced during cryoadipolysis. First degree lesion, Neuropraxia (+10 to -20 °C), causes just temporal disorder of the peripheral nervous system that includes loss of motor and sensory function due to blockage of nerve conduction, which usually lasts 3.6 weeks before full recovery [27]. This effect is also commonly associated with process of dysesthesias and paresthesias in the treated area as a transient reduction in sensation.

The Cooltech® treatment is preventively contraindicated in some pathologies that affect the myelin of the nerves. Repetitive cryotherapy may result in further sympathetic nerve dysfunction causing blotching of the skin. Cryotherapy is contraindicated in such cases [28]. The above phenomenon clinically differentiates the intolerable regional neuropathic (sympathetic) pain from the somatic focalized pain. So, if the pain gets worse and the patient adamantly refuses cryotherapy, the procedure should be discontinued, and never be applied again (Figure 3). This neuropathic cold sensitivity is also called "cold sensitive syndrome" [29].

Besides skin and nerves, also muscles and blood vessels safety have to be taken into account during cryoadipolysis. Muscle tissue and blood vessels have similar cold tolerances, since the inside of the vessels is composed of smooth muscle. Apoptosis of smooth muscle cells and blood vessels occurs between -5 °C and -15 °C [30–32]. Of note, the muscle function is governed by nervous activity. Since during the Cooltech® treatments, the nervous activity (motor and sensorial) is anesthetized due to the reduction of the speed of conduction of the nerves by the cold, muscle activity in the area will also be temporarily paralysed. Of note, the blood vessels, which are situated close to the surface of the skin, where the temperature applied by the applicator can be lower than -5 °C, expected to be protected by the cryoprotectant membrane. The blood vessels situated in the hypodermis are not affected since the temperature is not lower than -5 ºC in the skin layer and the muscles situated under the adipose tissue are completely absent of any harm caused by temperature decrease.

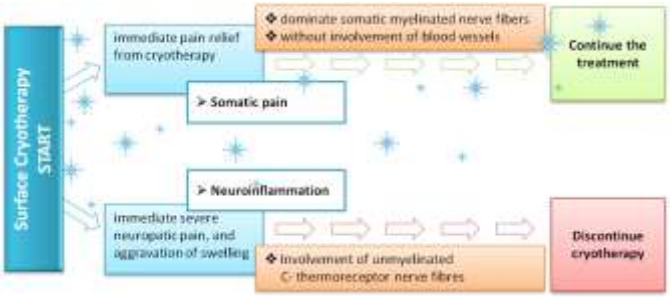

Fig. 3. Algorithm for treatment of somatic versus neuropathic pain [28].

1.4. Apoptotic cell death of adipocytes

As a result of cooling process and subsequent crystallization of the triglycerides, adipocytes undergo a programmed cell death, apoptosis [33,34], which results in gradual reduction of accumulated fat deposit layer [6,11,35,36]. The apoptosis however does not damage the surrounding cells and tissue [37]. This is due to the fact that adipocytes begin a process of apoptosis at 10 °C which is a

higher temperature threshold than the rest of cells [4,8,12]. Of note, weight loss induced by diet changes or sports activities does not reduce the number of adipocytes, but only reduces their size [38]. Contrary, reduction of adipocyte number by apoptosis, induced by cold, warrants fat deposit reduction in long-term [39].

Apoptosis is a physiological process that allows eliminating specific cells in an efficient and harmless way [40]. This is vital during early development of organism, tissue differentiation and/or after tissue injury. Apoptotic death is characterized by sequence of morphological events such as initial cell membrane budding, posterior cell shrinkage, chromatin aggregation and the appearance of apoptotic bodies and their fast phagocytosis by neighbouring cells [40–42]. Regarding the molecular level, apoptosis extrinsic pathway starts with the activation of so called "death receptors" (TNF-receptors). Subsequently, group of cysteine proteases, called caspases, are activated. These proteases selectively cleave vital cellular substrates, which results in apoptotic morphology and characteristic internucleosomal fragmentation of DNA [43]. In order to control cell death balance, different caspases either work as effectors or initiators of apoptosis. In order to proceed with apoptosis, activated caspase 8 is cleaved and thus activates caspase 3 that triggers cytochrome c release from mitochondria [43]. Mitochondria plays central role in the apoptotic cascade, generally by stopping energy metabolism and electron transport [44,45]. Release of cytochrome c initiates further caspase cascade and commits the cell to die in controlled manner. Meanwhile, members of the Bcl-2 oncoprotein family control mitochondrial events and are able to prevent or induce cell death [46]. Even though, the apoptotic pathways have been extensively studied in various tissues, the knowledge about apoptotic pathways in adipocytes is surprisingly scarce. There are different hypotheses and experimental studies that suggest that the death of adipocytes is not instantaneous, but that it occurs with a delayed effect that is not completely understood, as it may be due to internal mechanisms of adipocytes, such as mitochondrial signalling, by specific enzymatic processes or inflammatory processes in response to cell damage [11,13,47].

Importantly, the destruction of adipocytes does not affect serum lipid levels or liver function tests significantly [13]. A total of 35 patients were enrolled in the study of three independent centers. Different lipids including triglycerides and VLDL, HDL, LDL, and total cholesterol, were measured. Liver tests were proceeded by total bilirubin, alkaline phosphatase, ALT, and AST. Mean values of all measured liver and lipid tests were found to be within the reference range at every tested time point, and were never clinically meaningfully different from the baseline values. In addition, different studies show that blood lipid levels during the 3 months post-treatment are normal [13,48].

1.5. Massage of treated area

After the cooling process the treated zone is thoroughly massage in order to restore the normal blood flow and to raise the temperature of the tissue back to normal.

Besides, post-treatment massage increases additional adipose tissue destruction, more probably by mechanical harm. Boey and Wasilenchuk performed cryoadipolysis on 17 patients with the objective to evaluate the importance of the post-treatment massage and its effect on the efficacy of cryoadipolysis [49]. They found that standard cryolipolysis without massage resulted in a mean subcutaneous tissue reduction of 12.9% at 2 months and that the massaged side of the abdomen presented a 21% reduction. Subsequent histological analysis up to 120 days showed no signs of necrosis or fibrosis in either the massaged or unmassaged side [49].

1.6 Elimination of destroyed adipocytes

In the end of cryoadipolytic procedure, destroyed adipocytes need to be effectively eliminated from the body by lymphatic system. In this sense, apoptotic death of adipocytes is sufficient to initiate macrophage infiltration and tissue inflammation (Figure 4). In turn, macrophage accumulation and local inflammation stimulates secretion of pro-apoptotic molecules from macrophages, and thus further potentiate apoptosis [50]. Accordingly, close correlation was found in adipose tissue explants between CD11c expression (marker of macrophage infiltration) and the amount of apoptotic adipocytes [50]. However, since some adipocytes could die by necrotic process during cryoadipolysis, macrophage infiltration is partly mediated by necrosis and concomitant secretion of chemotactic molecules [51].

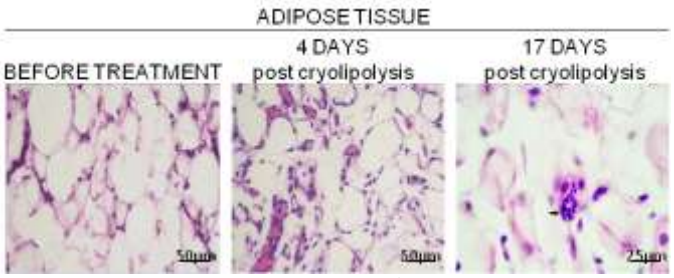

Fig. 4. Changes in adipose tissue after cryoadipolilysis.
Day 4 post-treatment, there is an increased amount of mononuclear cells (Histiocytes) of reticuloendothelial system, suggesting inflammation. Day 17 post treatment, we can appreciate structural changes of adipose tissue and the presence of adipocytes with fragmented nuclei (apoptotic sign marked with arrow).

Inflammation response is crucial in order to promote clearance of damaged adipocytes through the lymphatic system. Inflammatory response begins in general on day 3 and peaks around day 30 [34]. Of note, the resolution of inflammation and complete restoration of lipid metabolism is completed by 3 months after treatment [13,48].

II. POSSIBLE ADVERSE EFFECTS OF CRYOADIPOLYSIS

In section 1.4 it has been discussed that cryoadipolysis has no effect on blood lipid levels or liver function. However, as for any medical application, the appearance of adverse effects after cryoadipolysis is inevitable. Cryoadipolysis is a generally well-tolerated treatment with mild transient side effects. Although side effects occur, they usually resolve without intervention within 4 weeks. Most patients have no complaints

about these side effects and feel that it presents no disruption to their daily life [6,8,14,52–54]. Anyway, the possibility of manifestation of side effects of the treatment is of utmost importance.

In Table 2 there are listed the different types of adverse effects of cryolipolysis as well as their manifestation frequency. In order to establish the frequency, a scale based on the agreement of the international organization CIOMS23 has been used. The frequency categories range from: Very frequent (>1/10); Frequent (>1/100, <1/10); Infrequent (>1/1000, <1/100)

TABLE 2. Potential side effects of cryoadipolysis and its manifestation frequency.

Potential side effects of cryoadipolysis	Very usual	Common	Frequent	Infrequent	Very unusual	Extremely unusual
During the session and immediately afterwards						
Deep pulling or pinching sensation		x				
Severe itching or stinging, tingling, pain or spasm			x			
Paresthesias, dysesthesias. Temporary skin sensitivity disorders			x			
Temporary stages of mild inflammation			x			
Allergic reaction to any product	x					
Temporary localised pain			x			
Transient local erythema			x			
Thermal injuries	x					
Nausea, dizziness, or vasovagal symptoms	x					
After the session (from 24 hours onwards)						
Transient skin sensitivity disorders (sensation of stiffness, pins and needles, and itchiness)		x				
Temporary stages of mild inflammation			x			
Haematomas				x		
Temporary localised pain			x			
Local erythema or transient whitening of the skin				x		
Hyperplasia or enlargement of the treated area					x	
Nausea, dizziness, or vasovagal symptoms (absent after normal body temperature has stabilized)					x	
Hyper or hypopigmentation, hardness, discrete nodules					x	
Intensification of pre-existing hernias in the treated area					x	
In the submental area: motor nerve disorders in the perioral area; Submaxillary salivary gland disorders						x

III. Bulleted Lists of Conclusions

- In spite of being cryoadipolysis a new technology, promising results have been confirmed in clinical studies of efficacy.
- Cryoadipolysis is an excellent non-invasive alternative for localized fat reduction
- Cryoadipolysis is a generally well-tolerated treatment with mild transient side effects

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