EFFECTS AND APPLICATIONS OF CRYOEXPOSURE ASSOCIATED WITH HIGH INTENSITY ELECTROMAGNETIC FIELD IN HEALTH AND AESTHETICS: A REVIEW

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Abstract: Obesity has emerged as a global epidemic and represents a major risk factor for numerous chronic diseases, such as type 2 diabetes, cardiovascular disease, and cancer. Although adopting a healthy diet and regular exercise remain the cornerstones of weight management, recent research has revealed that cold exposure and associated muscle contractions can substantially contribute to weight loss. This article presents a comprehensive review of the physiological mechanisms that link cold exposure to weight loss, with a particular focus on the induction of browning and irisin production through pulsed electromagnetic field (PEMF) muscle stimulation technology, which uses alternating magnetic fields based on the law of electromagnetic induction to stimulate supramaximal muscle contractions with high magnetic flux density. The growing concern and interest in physical fitness, not only from an athletic but also aesthetic point of view, has driven the search for new methods and technologies that can aid in gaining muscle mass and tone. Recent studies have reported that 81% of respondents expressed dissatisfaction with their body image, even though 56% had a normal body mass index. Pulsed electromagnetic field (PEMF) muscle stimulation technology employs alternating magnetic fields based on the law of electromagnetic induction to induce supramaximal muscle contractions. PEMF generates impulses independent of brain function and at a frequency that does not allow muscle relaxation, thus characterizing tetanic contractions. Electrical currents and electromagnetism have long been used in

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physical therapy and rehabilitation, primarily for muscle strengthening. However, PEMF technology has emerged as a more effective and less invasive alternative for the patient, primarily intended for toning and strengthening muscle groups. In this study, we performed a comprehensive review of all available scientific articles indexed in PubMed and Web of Science over the past 20 years on this technology and its effects on skeletal muscle. We discuss the scientific evidence available from clinical studies and analyze the effects and possible mechanisms of action on muscle contraction.

Keywords: cryoexposure, electromagnetic field, health, aesthetics

INTRODUCTION

Obesity is a significant public health problem, with cardiovascular disease associated with obesity causing 17 million deaths per year worldwide. In Brazil, 18.9% of adults are affected by obesity, according to data from the Ministry of Health in 2017. The prevalence of obesity and overweight is increasing, leading to subvisceral fat deposition, which is associated with an increased risk of several life-threatening diseases, such as cardiovascular disease, diabetes, metabolic syndrome, and some forms of cancer. Thus, weight loss through aesthetic treatments is essential to reduce the prevalence of cardiovascular diseases. Non-invasive fat reduction is gaining popularity as a frequent non-invasive cosmetic procedure in the United States, according to the Aesthetic Society. The reduction of adipose tissue is energy-dependent and can be achieved by reducing fat stores (lipolysis) or by permanently removing adipocytes.

Lipolysis is a metabolic process that breaks down the triglycerides present in fat cells into their constituent molecules, glycerol and free fatty acids (FFA), through hydrolysis. Glycerol serves as a carbon source for hepatic gluconeogenesis, whereas FFA are transported in the blood bound to albumin and are oxidized or converted to ketone bodies in tissues through β-oxidation. The byproducts of β-oxidation, ATP and NADH, promote gluconeogenesis. FFA is converted into ketone bodies in the liver, which serves as an energy source for the brain, thereby reducing blood sugar consumption that

has been depleted. The main enzymes involved in lipolysis are adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL).

In addition to lipolysis, adipose tissue can be permanently eliminated through various regulated cell death programs, such as pyroptosis, necroptosis, and apoptosis. Apoptosis is a complex intracellular pathway that allows the body to control the number of cells and tissue size. The most distinctive signs of apoptosis processes are cell shrinkage accompanied by nuclear alterations, such as chromatin condensation (pycnosis) and nuclear fragmentation (karyolysis).

Recent research has uncovered new functions of adipose tissue, including its participation in energy balance, where white adipocytes store energy while brown adipocytes dissipate it as heat. Beige adipocytes have a flexible phenotype and can store or dissipate energy according to physiological circumstances. Adipose tissue also contributes to the production of pro-inflammatory molecules, such as cytokines, adipokines, and chemotactic factors, which are involved in the state of chronic low-grade systemic inflammation seen in obesity. Adipocytes play an active role in establishing this inflammatory state, either by producing inflammatory mediators (adipokines) or by cell-to-cell interaction with resident macrophages.

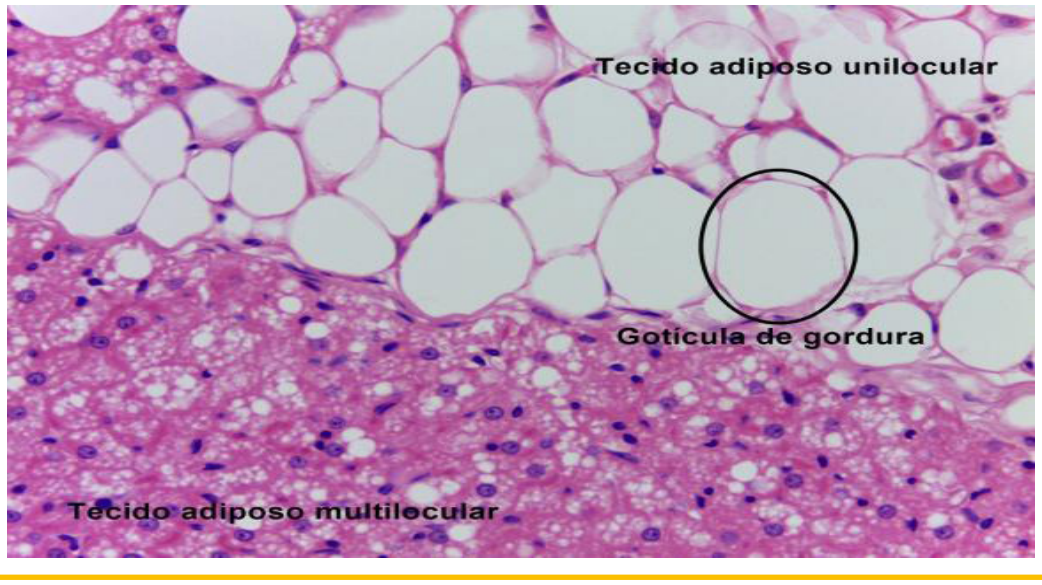
Adipokines, once considered only a passive tissue to store excess energy in the form of triacylglycerols, are now known as bioactive substances expressed and secreted by adipocytes (SILVA et al., 2020). Adipocytes are derived from mesenchymal stem cells and go through intermediate stages from adipoblasts and pre-adipocytes expressing transcription factors 21 and MyF5/BMP7 to white and brown adipocytes, respectively.

The muscular system also plays a crucial role in achieving a harmonious and aesthetically pleasing body contour. Muscle toning can be accomplished through physical activity (Gentil, 2017) or through high-intensity focused electromagnetic field (HIFEM) procedures (Kent, 2019; Kinney, 2019; 2020; Halaas, 2020). Intense muscle contractions require a significant amount of energy, which is delivered in the form of FFA from adipose tissue via lipolysis. This process not only activates beta oxidation but also leads to overall reduction of triglycerides and increased activity of the enzymes

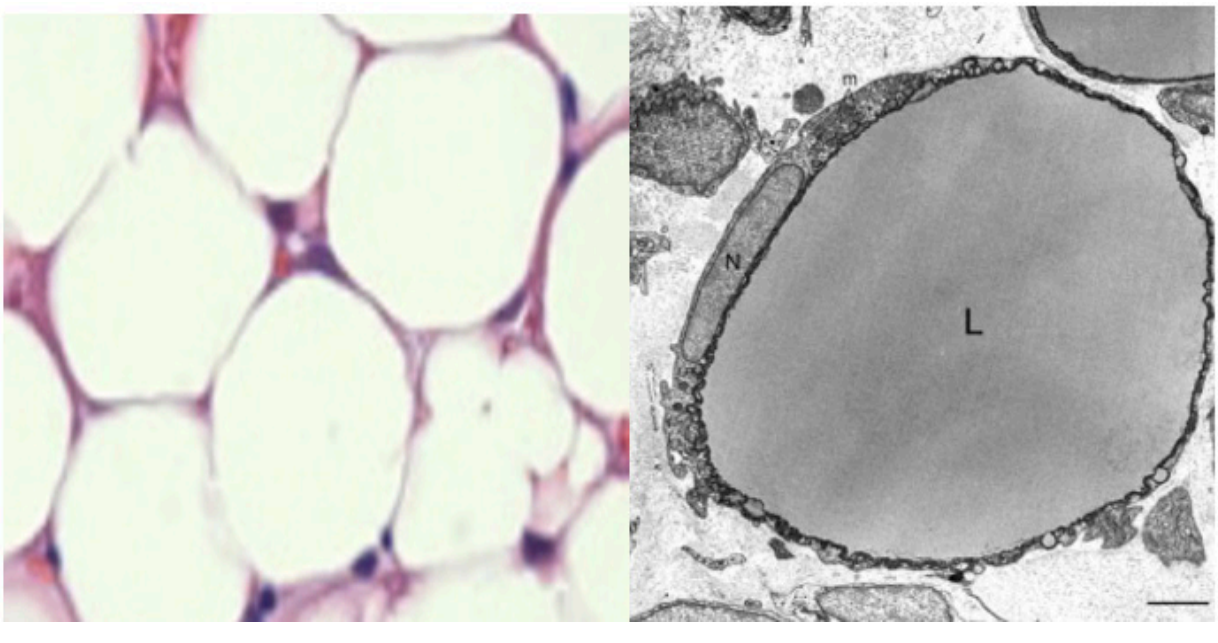
malate dehydrogenase, succinate dehydrogenase, and beta oxidation enzymes of NADH (Goldberg, 2021).

ADIPOSE TISSUE

Adipose tissue is a complex and dynamic organ composed of adipocytes, which are specialized cells that store and release lipids. These cells are highly regulated by a multitude of hormonal and environmental factors. Adipose tissue can be classified into two distinct types based on its color and function: brown adipose tissue (BAT) and white adipose tissue (WAT). Brown adipocytes contain numerous mitochondria and are highly innervated and vascularized, which makes them capable of generating heat through the uncoupling of oxidative phosphorylation. In contrast, white adipocytes are less metabolically active and primarily serve as a storage site for excess energy. The relative proportion of BAT and WAT in adipose tissue varies depending on age, diet, and ambient temperature, among other factors. In addition, adipose tissue is an important endocrine organ that secretes a variety of bioactive substances, including adipokines, which can influence metabolism, inflammation, and cardiovascular health. Therefore, the proper functioning of adipose tissue is crucial for maintaining metabolic homeostasis and overall health.



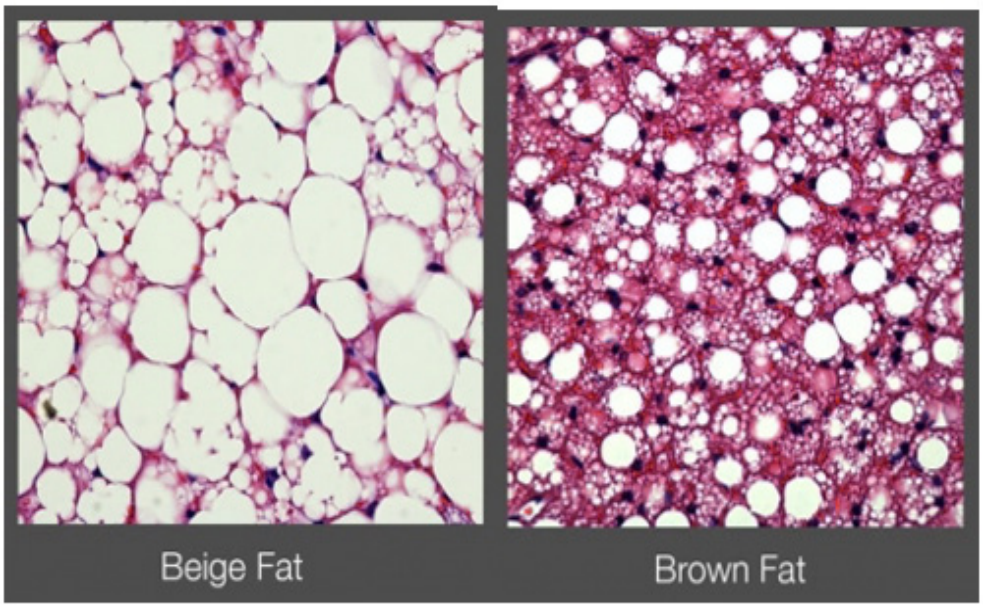
White adipocytes, which are the main energy storage site in the body, have a unique cellular structure that reflects their specialized functions. Adipocytes are characterized by a single, large drop of lipid that occupies most of the cell volume. This structure allows for efficient lipid storage and mobilization in response to energy needs. In contrast to brown adipocytes, which have abundant mitochondria and high metabolic activity, white adipocytes have poorly developed organelles and relatively low metabolic activity. In addition, the nucleus is displaced to the periphery of the cell and the cytoplasm forms a thin border, which facilitates the secretion of adipokines and other signaling molecules. The small, elongated mitochondria in white adipocytes have short, randomly arranged ridges, which reflects their relatively low oxidative capacity. Despite their apparent simplicity, white adipocytes play a crucial role in metabolic homeostasis, and their dysfunction is implicated in the pathogenesis of several metabolic disorders, including obesity and type 2 diabetes.



In addition to its thermogenic function, brown adipose tissue (BAT) also plays a role in glucose and lipid metabolism. BAT has a high rate of glucose uptake and is able to utilize glucose as a fuel source for thermogenesis. In addition, BAT activation has been shown to increase insulin sensitivity and decrease plasma triglyceride levels. This is due to BAT's ability to absorb and store

lipids, reducing the amount of circulating lipids that can contribute to metabolic disorders. In addition, recent studies have suggested that BAT-derived factors such as adiponectin and fibroblast growth factor 21 (FGF21) may act as endocrine signals to regulate systemic metabolism and insulin sensitivity in other tissues, highlighting the complex interaction between adipose tissue and the endocrine system.

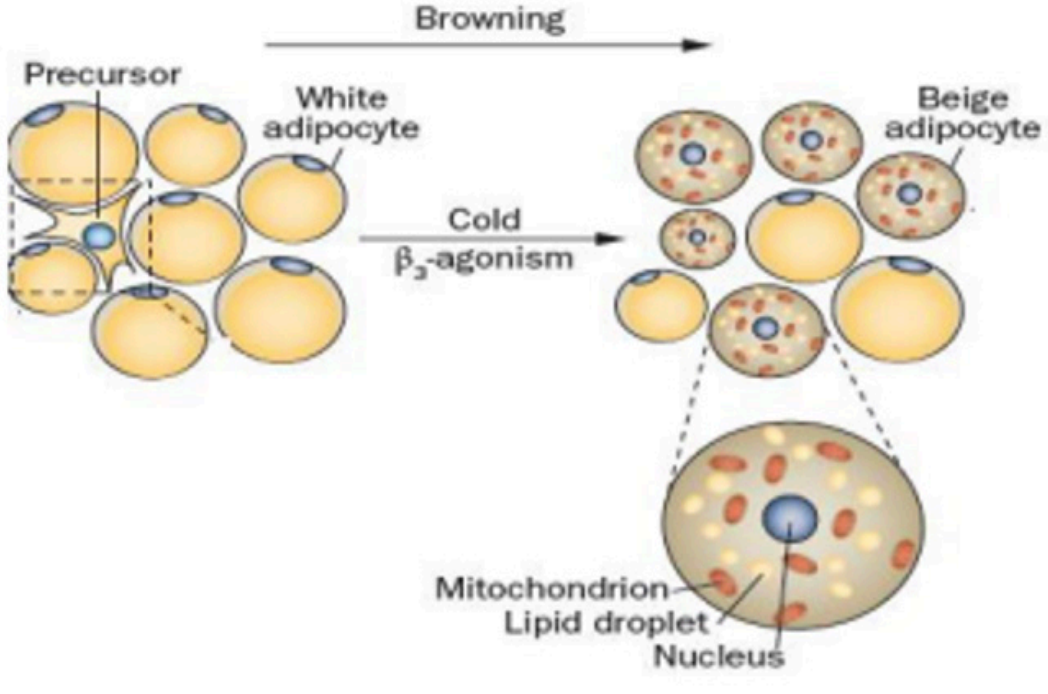
Adipose tissue is a complex organ composed of different types of cells with distinct morphologies, functions, and developmental origins. White adipocytes serve as energy storage sites, storing lipids, which are later released during fasting or increased energy demands. These cells have a spherical lipid drop that maximizes volume and minimizes the space occupied, and their cytoplasmic organelles, especially mitochondria, are poorly developed. On the other hand, brown adipocytes are smaller and contain numerous lipid droplets and large mitochondria with abundant uncoupling protein 1 (UCP1). These multilocular cells produce heat through the oxidation of fatty acids to meet the body's energy needs in response to cold temperatures or other stimuli. The recent discovery of paucilocular or beige adipocytes with an intermediate morphology between white and brown adipocytes provides new insight into the plasticity of adipose tissue and its ability to adapt to different environmental and physiological stimuli. These cells have the potential to transdifferentiate into brown adipocytes when exposed to certain stimuli, such as chronic colds or pharmacological agents, leading to the development of "dark" adipose tissue. Activation of brown adipose tissue is mediated by the sympathetic nervous system, which increases the level of cyclic adenosine monophosphate and activates lipolysis to provide fuel for thermogenesis. The molecular mechanisms underlying thermogenesis in brown and beige adipocytes involve the unique property of mitochondrial uncoupling, which separates electron transport from ATP synthesis and generates heat instead of ATP.



Human brown adipocytes possess molecular attributes such as constitutive UCP1 expression, homogeneous multilocular morphology, and myogenic origin (Myf5+), while beige adipose tissue arises from non-myogenic progenitors (Myf52) under environmental stimuli such as cold and exercise, and exhibit low levels of UCP1 expression under unstimulated conditions (Okla, 2017). Studies conducted by Dr. Lee in 2013-2016 demonstrated that exposure to cold and exercise increases levels of the hormone irisin (produced by muscle) and FGF21 (produced by brown fat), and that in the laboratory, irisin and FGF21 can differentiate cells into beige adipose tissue cells over a six-day period (Loap, 2018). These two hormones, irisin and FGF21, are the main signals to stimulate energy expenditure in the laboratory. Recent research suggests that there is an inverse association between BMI and plasma irisin concentrations in morbidly obese individuals. In mammals, UCP1 BAT provides the primary mechanism for shiver-free adaptive thermogenesis to defend body temperature in cold environments and for transcriptional activation of UCP1, several transcription and co-regulatory factors, including PPARs, PGC1α, are required (Dempersmier, 2015; Kelpert, 2017). However, the temperature range used to assess cold-induced thermogenesis represents another source of variability. The concept of cryoexposure is the use of local tissue cooling to control obesity. In cryolipolysis, apoptosis (hypoxia and reperfusions) is observed with loss recorded for a long period (Avram, 2009; Coleman, 2009;

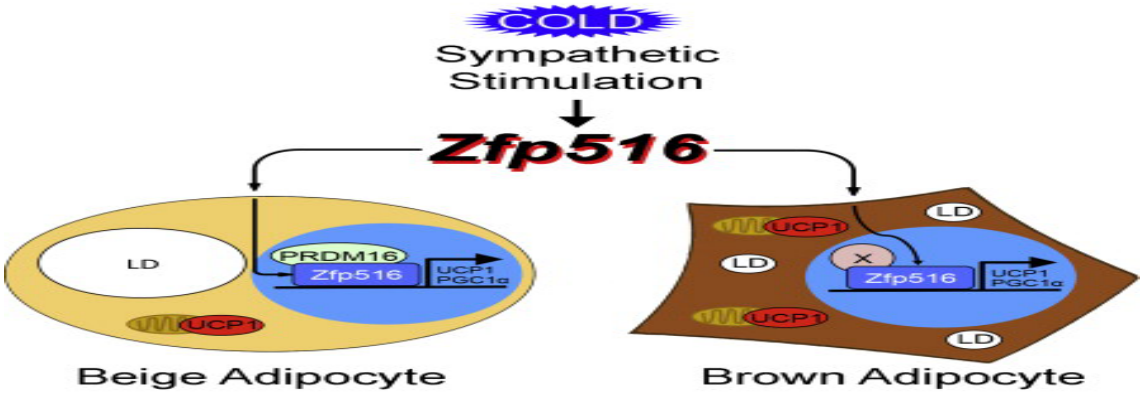
González, 2019; Jaliman, 2013; Manstein, 2008; 2009; Nelson, 2015; Pugliese, 2020; Zelickson, 2009). Cryoexposure is a controlled method that uses cooling to induce lipolysis without an inflammatory process (controlled by biochemical tests), a result generated by adaptive cryothermygenesis (Loap, 2018; 2022; Pinto H, 2012; Rauen, 1999, 2000; Velrand, 1999). Exposure to environmental cold leads to a decrease in adipose tissue.

When exposed to cold temperatures, homeothermic mechanisms in humans include increased vasoconstriction of the skin to reduce heat loss and an increase in endogenous heat production, which is triggered by an increase in basal metabolic rate. Exposure to cold also causes skeletal muscles to produce heat through muscle shivering-dependent thermogenesis, while muscle shivering-independent thermogenesis occurs in brown adipose tissue (BAT). Although the hypothesis of using cold exposure as a weight loss strategy was abandoned due to the belief that humans lacked brown adipose tissue, studies have since shown that humans possess significant amounts of brown fat deposits, and the browning process can be stimulated by β3-AR receptor agonists. (Ikeda, 2020; Lee, 2013).



The physiological response of brown adipose tissue (BAT) to cold stimulation during physical

activity or cold exposure induces a greater thermogenic response, characterized by an increase in uncoupling protein 1 (UCP1) expression and uncoupled respiration relative to canonical brown cells (DEMPERSMIER, 2015). The differentiation process of BAT is driven by specific transcription factors such as peroxisome proliferator-activated receptor (PPAR) when activated by non-inflammatories, Zinc Finger Protein 516 (ZFP516), and CCAAT/beta binding protein.



Loap's recent publication brought to light the concept of weight loss with cryoexposure, based on the hypothesis of transformation of white fat into brown tissue that dates back more than a century (Loap, 2018). The use of cold exposure increases energy and caloric expenditure via thermogenesis and fat metabolism (Loap, 2018). Despite previous beliefs that brown adipose tissue was absent in humans, fluorodeoxyglucose imaging showed the presence of large expanses of brown fat deposits (Loap, 2018). Evidence suggests that the conversion of white tissue to brown tissue is possible through the activation of BAT genes by transcription factors (Dempersmier, 2015).

Daily serial cooling of abdominal adipose tissue results in a progressive loss of adipose tissue, confirmed by dual-energy X-ray absorptiometry, with no evidence of systemic inflammation, abnormal fat mobilization, or disruption of cellular integrity and necrosis of death (Loap, 2018). Cryoexposure has been shown to reduce circumference and BMI without affecting inflammatory biomarkers, enabling daily applications (Loap, 2018). Lipid profile markers, including total cholesterol

(TC), high-density lipoprotein (HDL), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), triglycerides (TG), and their relevant ratios, as well as thyroid hormone (TSH, total T4, and free T4), were also analyzed (Loap, 2018).

Each application of cryoexposure results in the extraction of 1330 Kcal via thermogenesis, as reported by Yoneshiro, Lans, and Le in 2013 (Loap, 2018). Cold-induced upregulation of UCP-1 factors ZFP 516 in low-temperature fat cells generates thermogenesis, allowing mitochondria to metabolize fat via beta-oxidation and generate heat (Dempersmier, 2015). Exposure to cold also increases the expression of metabolic activity in beige tissue, leading to increased expression of the UCP-1 protein, which promotes thermogenesis and heat generation (Ikeda, 2020).

The key to maximizing the effects of cryoexposure is to freeze as many areas as possible simultaneously and stimulate sympathetic nervous system receptors through electrolipolysis or electrostimulation to induce Zfp516 expression through cold, sympathetic stimulation (contraction). Zfp516 binds to the promoter regions PGC1a and Cox, promoting transcriptional activation (Dempersmier, 2015). Intense contractions increase the expression of signaling proteins, such as PPAR PGC-1 alpha, which regulates mitochondrial biogenesis. Supramaximal contractions above 10 Hz and high magnetic flux density are the most effective at activating PGC1-alpha and inducing dimming. These contractions stimulate the anaerobic muscle fibers to undergo repetitive contractions, leading to a high energy demand and a subsequent shift from aerobic to anaerobic metabolism.

Peroxisome proliferator-activated gamma-1 receptor alpha (PGC1-alpha) is a transcriptional coactivator that plays a critical role in the regulation of energy metabolism and thermogenesis. One of its main functions is to induce the browning of white adipose tissue (WAT) by promoting the conversion of white adipocytes into brown adipocytes. This transformation involves the activation of uncoupling protein 1 (UCP1)-mediated thermogenesis, which generates heat and increases metabolic activity in brown adipocytes.

Several physiological factors, such as temperature and exercise, have been shown to regulate

the browning process. High-intensity supramaximal contractions, a type of muscle contraction that involves rapid and repetitive stimulation of muscle fibers, have been shown to activate PGC1-alpha, leading to increased mitochondrial biogenesis and oxidative metabolism. PGC1-alpha activation occurs through two mechanisms: first, supramaximal contractions cause a rapid and sustained increase in intracellular calcium levels, which activates calcium-dependent calmodulin kinase (CaMK); CaMK, in turn, phosphorylates PGC1-alpha. Second, supramaximal contractions promote the release of lactate and other metabolic intermediates that can activate AMP-activated protein kinase (AMPK), a key regulator of energy homeostasis that also activates PGC1-alpha.

Interestingly, performing high-intensity supramaximal contractions in environments with temperatures close to 0°C has been shown to be more effective in inducing dimming compared to performing these contractions at room temperature. This is because exposure to cold can activate the sympathetic nervous system and increase the secretion of norepinephrine, a hormone that stimulates lipolysis and activates mitochondrial thermogenesis through the activation of β3-adrenergic receptors. Activation of β3-adrenergic receptors leads to activation of AMPK, which in turn phosphorylates and activates PGC1-alpha. PGC1-alpha induces the expression of genes involved in mitochondrial biogenesis and oxidative metabolism, leading to an increase in mitochondrial thermogenesis and energy expenditure.

In addition to its effects on thermogenesis, cold exposure can also activate the transient receptor potential of the cationic channel member V subfamily 1 (TRPV1), which is sensitive to cold temperatures and is expressed in sensory neurons and adipose tissue. Activation of TRPV1 leads to the secretion of neuropeptide Y (NPY) and agouti-related protein (AgRP), two neuropeptides that stimulate food intake and reduce energy expenditure. However, activation of TRPV1 in adipose tissue can also lead to activation of mitochondrial thermogenesis through activation of sympathetic nerves and norepinephrine secretion.

In summary, the performance of sub- and supramaximal contractions, with high magnetic flux density, in environments with temperatures close to 0°C, is more efficient in inducing browning

than at room temperature, due to the activation of the sympathetic nervous system and an increase in norepinephrine strength, which promotes the activation of MTD thermogenesis through the activation of β3-adrenergic receptors and the activation of PGC-1α. In addition, an exposure to cold can also activate the cation channel of subfamily V member 1 receptor transient potential (TRPV1), leading to the cause of neuropeptides that stimulated food intake and produced energy expenditure. However, activation of TRPV1 in adipose tissue can also lead to activation of MTD thermogenesis through activation of sympathetic nerves and inhibition of norepinephrine.

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