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The impact of intermittent and continuous training on the levels of CIDE and Perilipin-1 proteins and their effect on the size of lipid droplets in the visceral adipose tissue of obese male rats

Shaghayegh Radmehr 1, Fatemeh Dehghani2, Yan Bai3, Xiao Yang 3 and Jian Li 4,*

[1] Cheeloo College of Medicine, Shandong university, Jinan, China
[2] Assistant Professor of Pediatrics Department of Nursing, Iranshahr University Medical Sciences, Iranshahr, Iran
[3] The School of Humanities and Social Sciences, Guangzhou Civil Aviation College, Guangzhou 510000, Guangdong, China
[4] Asset Management Office, Guangzhou Sport University, Guangzhou 510500, Guangdong, China

*Correspondence: (JL): 51115@gzsport.edu.cn ORCID ID nº: 0009-0005-6568-4196

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Abstract: Intense interval training and moderate-intensity continuous exercise produce lipid droplets that change size and impact visceral adipose tissue. 50 male Wistar rats were divided into 5 groups, each consisting of 8 rats, in order to accomplish this objective. Regarding dietary intake, 2 clusters of 32 rats were subjected to a normal or elevated fat diet over 10 weeks. Post the induction of obesity, 16 animals were euthanized, with an equal number originating from both the high-fat and normal diet cohorts. The ramifications of a high-fat diet were examined through the utilization of samples. The remaining 24 rats were randomly allocated to 3 groups: a sedentary high-fat diet control group, a high-intensity interval training (HIIT) protocol group, and a moderate-intensity continuous training (MICT) protocol group. The 12-week training program had 5 sessions per week. Western blot measurement of perilipin-1, CIDE, and Oil-Red proteins assessed lipid droplet size. Research indicates that HIIT and MICT training significantly decreased CIDEc protein levels (p<0.05) but not CIDEa. CIDEc protein upregulation and perilipin-1 downregulation cause obesity in high-fat diets. HIIT and MICT training reduce fat droplet size and CIDEc protein production. Enhancing perilipin-1, which breaks down fats, may reduce obesity by lowering lipid droplets and weight.

Keywords: High intensity interval training, CIDE, Prilipin-1, Lipid droplets, Moderate intensity continuous training

1. Introduction

Lipid droplets are organelles found in all organisms that serve as specialized storage for fat (Brasaemle & Wolins, 2012). They are composed of a hydrophobic center part filled with neutral fat and surrounded by a membrane made of a single layer of phospholipids (Murphy, Martin, & Parton, 2009). The range of lipid droplet diameters varies from 0.1 μm to 100 μm, which justifies their classification as dynamic organs based

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on their morphological characteristics (Barneda & Christian, 2017). Through the process of fusion, lipid droplets can increase in size (Murphy, Martin, & Parton, 2010). The involvement of Cell death-inducing DNA Fragmentation Factor Alpha (DFFA)-like Effector (CIDE) proteins is of great significance in facilitating this particular process (Gao et al., 2017). These proteins play a crucial role on the external surface of lipid droplets, enabling the transference of triacylglycerol content from one droplet to another by establishing a trans homodimer between two droplets. Due to their accumulation, a more giant droplet is created (Xu et al., 2016).

When catecholamines induce lipolysis of lipid droplets, many proteins surrounding the surface of the lipid droplet are influenced by interactions that can impact essential factors such as size, stability, and regulation of fat storage (Lafontan & Langin, 2009). One of these proteins is perilipin-1, which, together with CIDEa and CIDEc, plays a role in determining the size of lipid droplets (Konige, Wang, & Sztalryd, 2014). Increasing the levels of CIDEa and CIDEc proteins while reducing perilipin-1 levels leads to an increase in the size of lipid droplets (Marcinkiewicz, Gauthier, Garcia, & Brasaemle, 2006; Sun et al., 2013). Exercise plays a vital role in the degradation of visceral fat tissue through lipolysis (Sahu-Osen et al., 2015). Elevated levels of catecholamines stimulate beta-adrenergic receptors, activate protein kinase A, and consequently induce the lipolysis pathway, leading to weight loss (De Farias et al., 2013). Stimulation of protein kinase A leads to phosphorylation of three regions of the N-terminal of serine in prilipin-1, including Ser517 (Ahmadian, Duncan, Jaworski, Sarkadi-Nagy, & Sook Sul, 2007). Phosphorylation initiates the release of comparative gene identification-58 (CGI-58) from perilipin-1. Subsequently, CGI-58 engages in an interaction with adipose triglyceride lipase (ATGL), thereby activating it. The process that encompasses this phenomenon is commonly referred to as lipolysis (Marcelin & Chua Jr, 2010). The primary aim of this investigation was to examine the effects of intermittent and continuous training on the concentrations of CIDE and perilipin-1 proteins, as well as their influence on the size of lipid droplets in the visceral adipose tissue of obese male rats.

2. Materials and Methods

The investigations were conducted by the Guidelines for the Care and Use of Laboratory Animals, per the US National Institutes of Health specifications. Furthermore, they obtained authorization from the Institutional Animal Care and Use Committee of Guangzhou Medical University in Guangzhou, People's Republic of China. Forty male Wistar rats, aged six weeks and weighing between 120±20 grams, were procured from the Guangdong Province Medicine Experimental Animal Center (Guangzhou, Guangdong, China). Following their transfer to Guangzhou Sport University, the animals were housed in four cages, maintaining standard conditions, which involved a 12-hour light-dark cycle and a temperature of +25°C. They were provided with unrestricted access to both food and water. After two weeks of acclimatization to their new environment and consumption of a regular diet, the rats were divided into two groups: one group

was given a normal diet (N=8), while the other group was given a high-fat diet (N=32) for ten weeks. Following the initial phase of inducing obesity, the normal diet group and

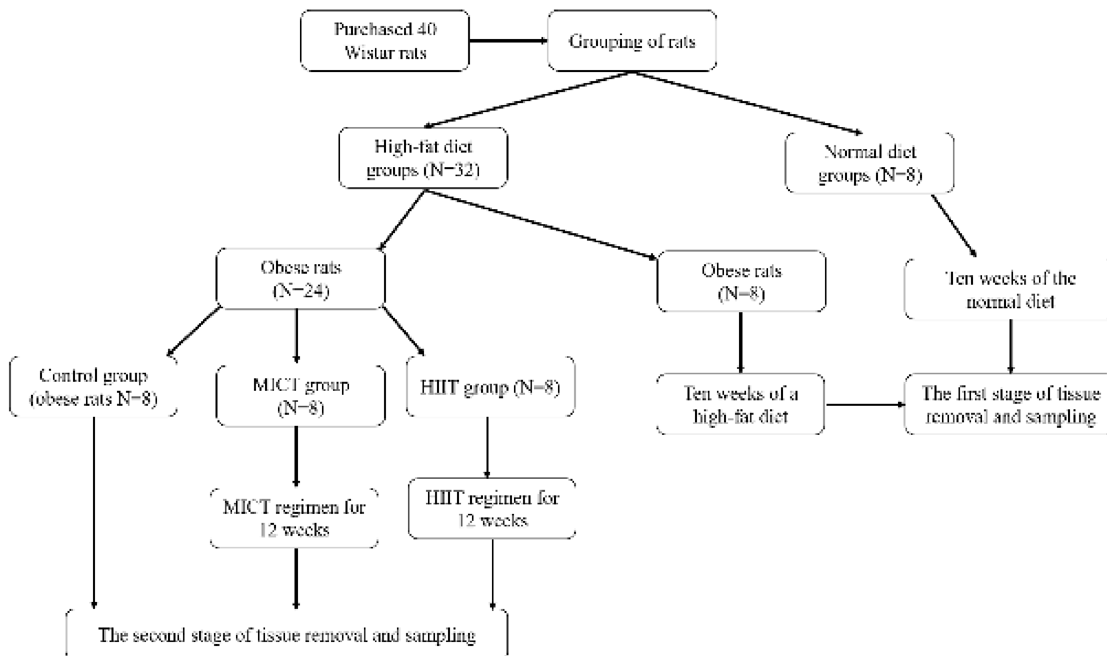

Figure 1. The study's methodology; *HIIT: high-intensity Interval Training, MICT: moderate-intensity continuous training.*

eight individuals from the high-fat diet group were anaesthetized using a combination of ketamine-xylazine. Blood samples were then taken in tubes without EDTA solution. The blood samples underwent centrifugation to isolate the serum, subsequently being relocated to a freezer set at a temperature of -80°C to conduct additional biochemical analysis. Additional samples were placed in a 10% formalin solution to facilitate measurement using the Oil-Red technique. The rats with obesity stemming from the high-fat diet group were randomly distributed into three groups, comprising eight rats each: control, moderate-intensity continuous training (MICT), and high-intensity interval training (HIIT). Moreover, the rats had unrestricted access to a normal diet throughout the experiment. The normal diet comprised 10% fat, 70% carbohydrates, and 20% protein, while the high-fat diet comprised 60% fat, 20% carbohydrates, and 20% protein. Both the HIIT and MICT groups of rats underwent the prescribed training regimen for 12 weeks. In addition, the control group did not get an exercise program over these 12 weeks. After 12 weeks of training procedures, as previously reported, the rats from the MICT, HIIT, and control groups were knocked unconscious, and the samples were collected similarly. Additionally, the rats' body weights were regularly checked weekly throughout the intervention (Figure 1).

Training protocols — Following 10 weeks of ingesting a diet high in fat, the rats in the MICT and HIIT training groups were given a week to adapt to running on a treadmill before engaging in 12 weeks of exercise training. The study encompassed the implementation of both HIIT and MICT protocols for 12 weeks. The subjects engaged

in five training sessions each week, with a slope of 25 degrees, using modified training protocols described by Hafstad et al. (Anne Dragøy Hafstad et al., 2011; Anne D Hafstad et al., 2013). The HIIT regimen comprises 10 repetitions, each lasting 4 min, with an effort level varying from 85–90% of VO_{2max}. These phases are followed by active rest periods of 2 min, with an intensity level ranging from 45–50% of VO_{2max}. The treadmill's speed progressively increased until the 10th week, with a consistent level maintained during the final two weeks (the 11th and 12th). The speed of the treadmill gradually rose from 17 meters per minute in the initial week to 26 meters per minute by the 10th week and subsequently remained constant for the last two weeks. The MICT program was executed at an intensity corresponding to 65-70% of the VO_{2max}, while the distance covered was the same as in the HIIT protocol. The velocity of the treadmill steadily increased until the tenth week and remained constant during the final two weeks. The speed of the treadmill escalated from 12 meters per minute in the initial week to 16 meters per minute by the tenth week and sustained a consistent speed during the concluding two weeks (11th and 12th). Additionally, the participants engaged in a 10-minute warm-up and a 5-minute cool-down at the commencement and conclusion of each training session, respectively, at a low intensity.

Measurement of protein quantity— The Western blot method evaluated the contents of CIDEc, CIDEa, and perilipin-1. The proteins from visceral adipose tissue were extracted using RIPA (RadioImmunoPrecipitation Assay) buffer (Sigma-Aldrich), which consisted of 0.05 mM Tris buffer (pH = 150), 8 mM sodium chloride), 1% sodium dodecyl sulfate, 0.01% EGTA (ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and 0.1% antiprotease cocktail (Roche, Basel, CH). A manual homogenizer was used to homogenize 100 mg of tissue in 500 μL of buffer with antiprotease. Subsequently, the homogenate was cooled at 4°C for 30 min and then placed under centrifugation at 12000 rpm and 4°C using a refrigerated centrifuge (Bo, SW14rfroil). The centrifugation continued for 10 min. The liquid portion that remained after the solid particles had settled was gathered, and the amount of protein present was determined utilizing a kit for quantifying proteins (Bio-Rad) at a specific wavelength of 595 nm. The liquid portion was then preserved for an extended period at a temperature of -20°C within a storage unit designed for maintaining low temperatures. Following this, the mixture of substances produced by grinding the biological material together was combined with a solution that assists in loading samples. This solution consisted of 50 mM tris-hydrogen chloride, 2% sodium dodecyl sulfate, 10% glycerol, 5% beta-mercaptoethanol, and an aqueous bromophenol solution with a concentration of 0.005%. The mixture was combined in equal proportions, resulting in a 1:1 ratio. Subsequently, the combined substances were heated for 5 minutes, ensuring that all proteins underwent complete denaturation. The proteins were then separated through a process called acrylamide gel electrophoresis, which involved the presence of sodium dodecyl sulfate. Following this, the proteins were deposited onto a membrane made of nitrocellulose. The membrane was treated with a solution containing a 5% bovine serum albumin

compound in Tris-buffered Saline and 0.1% Tween 20 (TBST) for 1 hour (Sigma-Aldrich) to prevent undesired interactions. Following this, the membrane was exposed to an incubation process with a primary antibody at a dilution ratio of 500:1. The next day, incubation with secondary antibodies was carried out at room temperature in a 4% TBST solution for 1 hour. Protein quantification was performed using an electrochemiluminescence (ECL) reaction and densitometry analysis with Image J software. The CIDEa and CIDEc antibodies employed were mouse antibodies (both at a dilution 1:200; Santa Cruz Biotechnology (Santa Cruz, CA), catalog numbers sc-293289 and sc-517232). Finally, the perilipin-1 antibody used was a rabbit antibody (Abcam, Cambridge, UK, catalog number ab3526), diluted at a ratio of 1:500.

Measurement of lipid droplet size— The Oil-Red technique was employed to quantify the size of lipid droplets. The procedure involved cutting visceral fat tissue samples into 3mm x 3mm lengths and placing them in a 24-hour incubation period in a solution containing 10% formalin, followed by another 24-hour incubation period in a solution containing 30% sucrose. Afterward, the cryopreserved tissue is coated with OCT glue and cut into slices measuring six microns in thickness using the cryostat machine. Subsequently, the tissues were initially subjected to propylene glycol treatment for 2 minutes. After this, the slides were placed in an Oil-Red solution for 6 minutes. The slides were then immersed in a solution comprising 85% propylene glycol for 1 minute. After two rounds of rinsing with distilled water, the slides were subjected to hematoxylin staining for 2 minutes. The slides were then mounted with lamellae and glue. The size of lipid droplets was determined using Image J densitometry software after the necessary preparations. The total amount of lipid was measured using the arbitrary unit approach.

Statistical Analysis— The normality of the data was confirmed using the Shapiro-Wilk test. ANOVA and least significant difference (LSD) post hoc testing were employed to assess the significance of the difference in average variables (perilipin-1, CIDEc, CIDEa, and lipid droplet size) between the test groups. The weight of rats was compared using these two tests at the end of a 10-week diet and training protocol. Upon collecting the necessary data, we analyzed using SPSS version 20 statistical software, employing a significance level of $P \leq 0.05$.

3. Results

Figure 2 shows the mean weight of rats in different groups during obesity and physical activity. The data analysis indicated that the group that followed a high-fat diet for 10 weeks experienced a considerably higher weight gain in comparison to the group following a normal diet (ND) ($P<0.05$). The body weight of rats in the ND group ranged from 168.13 ± 13.30 to 274 ± 12.80, while in the high-fat diet (HFD) group, it extended from 193.53 ± 18.44 to 362.25 ± 21.14. Following the study's conclusion, the HIIT group exhibited a 5% reduction in body weight, while the MICT group experienced a 6% decrease. The percentages observed were markedly lower compared to those with a high-fat diet and a sedentary lifestyle (HFD+SED). Moreover, there was no discernible difference between the HIIT and MICT groups until the experiment's conclusion.

While the HFD group demonstrated a higher quantity of CIDEa protein in comparison to the ND group, and the HIIT and MICT groups exhibited a decreased amount of this protein in comparison to the HFD+SED group, these differences did not reach statistical significance (P>0.05) (Figure 3A). After ten weeks of a HFD, there was a significant rise in CIDEc protein levels compared to the ND group (P≤0.05). Furthermore, both MICT and HIIT protocols substantially reduced CIDEc protein levels compared to the HFD+SED group (P<0.05). However, no noteworthy difference was observed among the training groups (P>0.05) (Figure B3). Following 10 weeks of a HFD, there was a remarkable decline in the level of perilipin-1 in the ND group (P≤0.05). The MICT and HIIT interventions resulted in a significant increase in perilipin-1 protein levels when compared to the HFD+SED group (P≤0.05). However, no notable difference was seen among the training groups (P>0.05) (Figure 3C).

Figure 2. Weight of rats in different groups (**A**); ND: normal diet, HFD: high-fat diet, (**B**) HFD+SED: high-fat diet+sedentary (control group), HFD+HIIT: high-fat diet+ High-Intensity Interval Training, HFD+MICT: high-fat diet+Moderate-Intensity Continuous Training

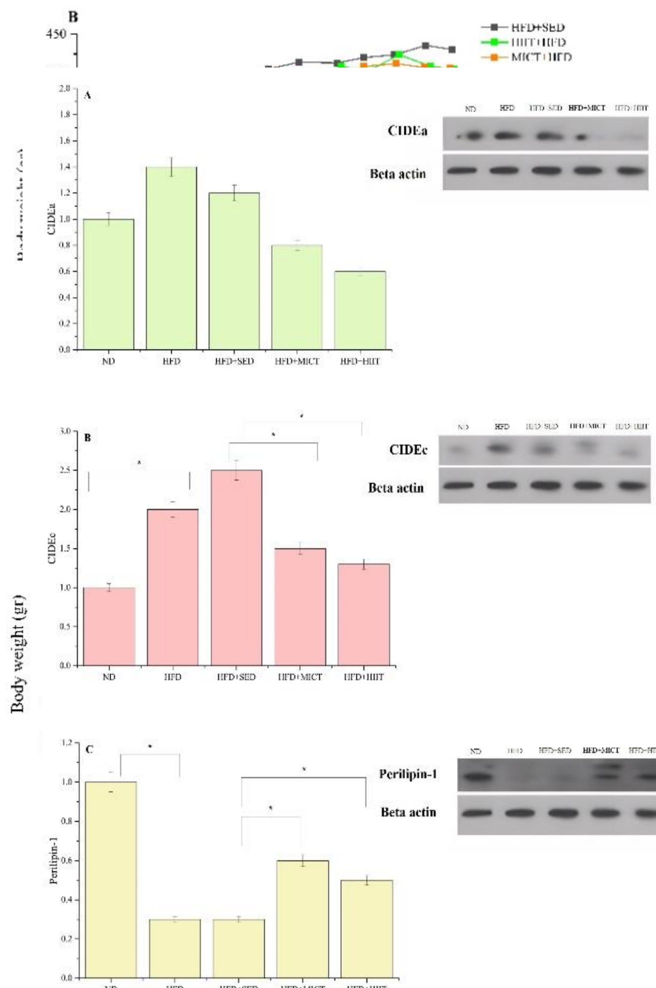

Figure 3. The amount of (**A**) CIDEa, (**B**) CIDEc and (**C**) Perilipin-1 proteins in the study groups.

The results indicate that a 10-week HFD resulted in a notable enlargement of lipid droplets in comparison to the group on a normal diet (P≤0.05) (Figure 4). In addition, the interventions of both MICT and HIIT led to a noteworthy reduction in the dimensions of lipid droplets when compared to the group that followed the HFD+SED regimen, with a significance level of P≤0.05. Furthermore, there was no observable difference between the MICT and HIIT protocols concerning the diameters of lipid droplets, with a p-value greater than 0.05.

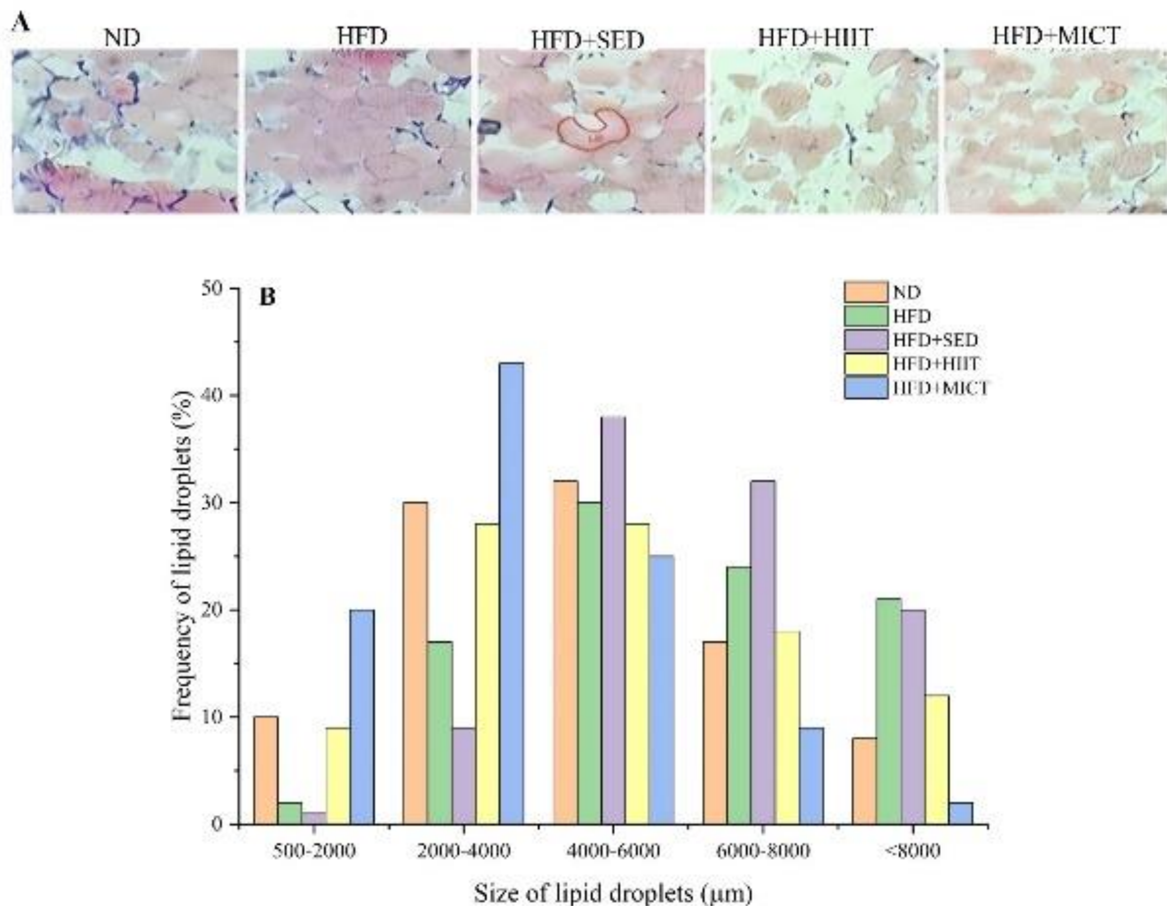

Figure 4. Comparison of the size and number of lipid droplets in different research groups. (**A**) measurement of Oil-Red and (**B**) the amount of lipid droplet along with their frequency percentage in the research group.

4. Discussion

Lipid droplets are highly active organelles that serve a crucial function in energy provision. However, the excessive accumulation of these droplets can lead to obesity and metabolic disorders (Watt & Steinberg, 2008). Visceral fat is recognized as a significant contributor to the development of metabolic disorders (Lam et al., 2011). During fat tissue production, the body can accumulate up to 20% of its weight as fat. This fat plays a vital function in controlling fat stores and facilitating the degradation of lipid droplets, a process known as lipolysis (Oliveros, Somers, Sochor, Goel, & Lopez-Jimenez, 2014). This study examined changes in the size of lipid droplets resulting from a high-fat diet and physical activity. The findings of our study indicate that 10 weeks of consuming a diet high in fat increased body weight. While HIIT and MICT led to significant weight loss, HIIT training showed a more obvious impact. The size of lipid droplets is dependent on a multitude of factors. The interaction between CIDE and perilipin-1 proteins leads to the growth of lipid droplets, which is an important step in developing adipose tissue (Bouchez et al., 2015). The fusion mechanism is catalyzed by two types of CIDEa and CIDEc, which collectively constitute the contact site of lipid droplets. A lipid droplet acts as a donor and transfers its contents to a bigger droplet through a gradient mechanism driven by internal pressure (Schneider, Zhang, & Li, 2016). CIDE proteins stabilize the paired lipid droplets by forming a trans homodimer at the junction where two lipid droplets combine. This also causes one of the lipid droplets to increase in size (Boschi, Rizzatti, Zamboni, & Sbarbati, 2015).

The study revealed that consuming a diet high in fat results in a substantial elevation of CIDEc proteins and has no impact on CIDEa proteins. Additionally, it causes an increase in the size of lipid droplets. Consequently, consuming a diet high in fat leads to an accumulation of triglycerides within lipid droplets, promoting obesity, as these proteins are essential for the expansion of lipid droplets. However, both HIIT and MICT reduced CIDEc protein levels, but there were no significant changes in CIDEa proteins. Both training protocols decreased the size of lipid droplets, suggesting that the lowering of CIDEc proteins has a more significant impact on this process. The increased activity of the sites where two lipid droplets combine via CIDEc proteins may be responsible for this effect. Simultaneously, there was no significant difference between the two training protocols.

The activation of the lipolysis pathway proceeds by beta-adrenergic stimulation, which leads to an increase in adenylate cyclase and cAMP levels (Gong et al., 2011). Additionally, phosphorylation of perilipin-1 is involved in this process. Perilipin-1, a protein located on the outer layer of lipid droplets, interacts with CIDE proteins to facilitate the movement of lipids and stimulate the enlargement of droplets (Kimmel & Sztalryd, 2016). Perilipin-1, a crucial controller of lipolysis in adipocytes, promotes the transportation of lipids. The extent of this increase is dependent on the interaction between CIDE protein domains within the core domain of perilipin-1. Removal of this specific region of perilipin-1 alters the interaction between CIDE and perilipin-1, particularly with CIDEa, resulting in accelerated growth of fat droplets (Tansey, Sztalryd, Hlavin, Kimmel, & Londos, 2004). When two lipid droplets bind, they form a pore that specifically promotes the exchange of lipids in both directions. The pore size mostly determines the amount of lipid exchange, while the lipid droplet size determines the lipid transfer rate. Thus, perilipin-1 can influence the function of CIDE proteins by acting on the pore (Sun et al., 2013). Research has shown that perilipin-1 promotes the degradation of fats, resulting in larger lipid droplets when it is not present. Conversely, the presence of perilipin-1 promotes the development of smaller lipid droplets (Skinner, Harris, Shew, Abumrad, & Wolins, 2013; Sztalryd & Brasaemle, 2017).

This study demonstrated that a high-fat diet reduced perilipin-1 levels, suggesting that this decline results in the inactivation of the lipolysis pathway. As mentioned before, decreasing perilipin-1 levels enhances the activity of CIDE proteins, affecting the size of lipid droplets. According to the data obtained from this research (Figure 4A), there was a significant difference in the size of lipid droplets between the groups having a normal diet and those on a high-fat diet. This study demonstrated that both intermittent and continuous training protocols resulted in an increase in the levels of perilipin-1 and a decrease in the levels of CIDE proteins. These proteins opposed each other and were associated with a significant reduction in the size of lipid droplets in the experimental groups. The elevation of perilipin-1 leads to a reduction in the functionality of CIDE proteins, and the absence of their formation into complexes can be inferred. Figure 4B displays the frequency distribution of fat droplets according to their size, measured in specified arbitrary unit dimensions. It illustrates the variations in the size and quantity of fat droplets across different groups in the research, as influenced by obesity. Also, after implementing two training protocols, HIIT and MICT, what changes have occurred in the frequency of fat droplets with which size more? In the ND group, lipid droplets ranging from 2000 to 6000 μm in size exhibit the largest frequency percentage. This suggests that following a

normal diet leads to a greater prevalence of lipid droplets in visceral fat in the 2000–6000 µm range. Approximately 30% of the overall quantity (100%) is related to sizes ranging from 2000 to 4000 µm, while roughly 35% is associated with sizes ranging from 4000 to 6000 µm.

Consequently, the largest size group, representing about 65% of the total percentage of lipid droplet numbers, encompasses most of the total quantity. A high-fat diet causes an increase in the size of fat droplets from 4000 to 8000 µm. Approximately 33% of the droplets range from 4000 to 6000 µm, while about 30% are 6000–8000 µm. Although the percentage of larger droplets in this group is lower than that in the ND group, the size of the droplets has increased. Conversely, among the total frequencies, the percentage of lipid droplets in the HFD group is the greatest, with a range of 4,000 to 8,000. This difference indicates a reduction in the frequency of bigger droplets in the HFD group. However, the enlarged size of the fat droplets suggests their combination. In the HFD+SED group, the fat droplets had the largest size of 4000 to 8000 µm. The frequency of droplets measuring between 4000 and 6000 µm is approximately 40%, while the frequency measuring between 6000 and 8000 µm is around 35%.

The larger droplets, which range from 4000 to 8000 µm, constitute about 75% of the total frequency of lipid droplets. An equal percentage of lipid droplets smaller than 4000 µm and larger than 8000 µm represent a lower percentage. The reduction in the quantity of lipid droplets in the HFD+SED group suggests the integration of lipid droplets. The HIIT training group exhibits the highest frequency of fat droplets, ranging from 2000 to 6000 µm in size. To clarify, around 30% of the total frequency corresponds to sizes ranging from 000 to 4000 µm, while another 30% corresponds to sizes ranging from 4000 to 6000 µm. Together, these size ranges account for 60% of the frequency of large fat droplets in this group, compared to the HFD+SED group. The percentage of large lipid droplets among this group has markedly decreased.

Similarly, the frequency of larger droplets decreased, indicating that the HIIT protocol has effectively reduced the size of lipid droplets measuring 6000 to 8000 µm and greater. Within the MICT group, the most prevalent frequency is observed in the 2000–4000 µm range, constituting around 45% of the overall frequency. This is the largest frequency percentage compared to other groups. Conversely, the frequency percentage in other size categories particularly drops measuring 6000 to 8000 and larger, has significantly declined among this group. These findings demonstrate that the MICT protocol has a stronger effect on reducing extremely large droplets. Compared to the HIIT protocol, it has a better influence on reducing larger droplets. The HIIT protocol has nearly the same impact on reducing all sizes, as shown in Figure 4.

5. Practical Applications

The impact of intermittent and continuous training on CIDE and Perilipin-1 proteins and their effect on lipid droplet size in visceral adipose tissue provides valuable insights for various practical applications. From designing exercise regimens and customized training programs to monitoring treatment efficacy and identifying therapeutic targets, this research can significantly contribute to developing effective strategies for combating obesity and improving metabolic health in obese individuals. Addressing these challenges and limitations requires a multidisciplinary approach involving healthcare professionals, exercise specialists, psychologists, and nutritionists. By considering these factors and implementing appropriate strategies, intermittent and continuous training can be effectively tailored to meet the needs of obese

individuals and improve their overall health and well-being.

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