



Ultrasound Therapy: Modulation of Abdominal Fat and Triglyceride Levels in Rats

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ABSTRACT

Purpose of the study: This study aimed to investigate the effect of ultrasonic wave exposure with different intensity levels on abdominal circumference and blood triglyceride levels in white rats (*Rattus norvegicus*) as an experimental model for non-invasive fat reduction therapy.

Methodology: This study used an experimental in vivo pretest–posttest control group design involving 12 male Wistar rats. Ultrasonic therapy was administered using a 1 MHz ultrasound therapy device with three intensity modes for 4 minutes daily over 6 days. Abdominal circumference was measured using a measuring tape, while triglyceride levels were analyzed using a portable strip-based digital analyzer.

Main Findings: The results showed that ultrasonic wave intensity influenced abdominal circumference and blood triglyceride levels in white rats. Mode 2 intensity produced the greatest reduction in abdominal circumference by 6.50%, while Mode 3 most effectively suppressed the increase in triglyceride levels, with only a 0.02% increase. In contrast, the control group showed significant increases in both abdominal circumference and triglyceride levels.

Novelty/Originality of this study: This study provides a novel contribution by systematically comparing different ultrasonic intensity modes on both abdominal circumference and blood triglyceride levels simultaneously. The findings identify optimal intensity parameters for non-invasive lipid regulation therapy and expand current knowledge regarding the biophysical application of ultrasound technology in obesity and triglyceride management.

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1. INTRODUCTION

Obesity has emerged as a major global health concern characterized by excessive fat accumulation that adversely affects physiological function and physical appearance, contributing significantly to increased morbidity and mortality worldwide across both developed and developing countries. Recent epidemiological studies indicate that obesity prevalence continues to rise rapidly, leading to increased risks of metabolic disorders such as cardiovascular disease and diabetes [1]-[3]. Furthermore, abdominal obesity is particularly associated with higher cardiometabolic risk due to visceral fat accumulation and its metabolic activity compared to peripheral fat deposition [4]-[6]. This condition not only impacts health outcomes but also imposes a substantial economic

burden on healthcare systems globally through increased treatment costs and reduced productivity [7]-[9]. Therefore, understanding and addressing obesity-related mechanisms remains a critical priority in biomedical research and clinical practice.

Various strategies have been developed to reduce excessive body fat, including lifestyle modification, pharmacological treatments, and surgical interventions such as liposuction, each offering different levels of effectiveness and associated risks. Pharmacological approaches often produce adverse side effects, including hormonal imbalance and metabolic disturbances, which limit their long-term use in clinical practice [10]-[12]. Similarly, surgical procedures such as liposuction are invasive, costly, and require specialized expertise, increasing the risk of complications and limiting accessibility for broader populations [13]-[15]. These limitations highlight the need for safer, non-invasive, and cost-effective alternatives that can effectively reduce fat accumulation without significant adverse effects. Consequently, research efforts have increasingly focused on developing innovative therapeutic technologies that overcome these challenges.

Ultrasound technology has gained considerable attention as a non-invasive method for therapeutic applications due to its ability to penetrate biological tissues and induce localized physical effects without surgical intervention. Ultrasonic waves are mechanical waves with frequencies above 20 kHz that can interact with biological tissues, producing thermal, mechanical, and cavitation effects [16]-[18]. These effects enable ultrasound to be used in various medical applications, including imaging, tissue ablation, and fat reduction therapies in aesthetic and clinical medicine [19]-[21]. The non-invasive nature of ultrasound makes it an attractive alternative to conventional fat reduction methods that require invasive procedures or chemical interventions [22], [23]. As a result, ultrasound-based therapies have become increasingly popular in both research and clinical settings.

Among the mechanisms induced by ultrasound, cavitation plays a crucial role in mediating fat reduction through the formation and collapse of microbubbles within biological tissues. This process generates localized mechanical stress that disrupts adipocyte membranes and facilitates the release of intracellular lipids, particularly triglycerides [24]-[26]. Experimental studies have shown that ultrasound-induced cavitation significantly enhances triglyceride release and adipocyte volume reduction, supporting its effectiveness in lipolysis [27]-[29]. The released lipids are subsequently transported into the bloodstream and metabolized through physiological pathways, contributing to overall fat reduction. Thus, cavitation represents a key mechanism underlying the therapeutic potential of ultrasound in obesity management.

Previous studies have demonstrated the effectiveness of ultrasound-based therapies in reducing adipose tissue thickness and body circumference in both human and animal models. Clinical trials have reported significant reductions in abdominal fat and waist circumference following ultrasound cavitation combined with exercise interventions [30], [31]. Additionally, recent experimental studies confirm that ultrasound exposure can alter lipid metabolism and influence triglyceride dynamics within biological systems [1], [32], [33]. These findings suggest that ultrasound has the potential to serve as an effective non-invasive tool for fat reduction and metabolic regulation. However, the variability in experimental parameters such as intensity and exposure duration necessitates further investigation.

Despite extensive research on ultrasound therapy, studies focusing on the specific role of varying ultrasonic intensity levels in modulating fat reduction outcomes remain limited. Most previous studies have primarily examined fixed intensity parameters without systematically evaluating their comparative effectiveness [34]-[36]. Furthermore, the combined analysis of both abdominal circumference and blood triglyceride levels under different intensity conditions is still insufficiently explored [37]-[39]. This gap limits the understanding of how ultrasonic parameters influence both physical and biochemical indicators of fat metabolism. Therefore, further investigation is required to optimize ultrasonic therapy for effective and targeted fat reduction.

Based on the identified research gap, this study aims to investigate the effect of ultrasonic wave exposure with varying intensity levels on abdominal circumference and blood triglyceride levels. The study utilizes an experimental animal model to provide controlled conditions for evaluating the biological effects of ultrasonic exposure on adipose tissue. By examining both physical and biochemical parameters, this research seeks to provide a comprehensive understanding of ultrasound-induced fat reduction mechanisms. The use of controlled intensity variations allows for a more precise evaluation of optimal therapeutic conditions. Thus, this study contributes to the refinement of ultrasound-based treatment strategies.

The findings of this study are expected to contribute to the development of safe and effective non-invasive therapies for obesity management. Ultrasound-based treatments offer advantages such as minimal side effects, ease of application, and reduced cost compared to surgical methods. Additionally, understanding the relationship between ultrasonic intensity and fat metabolism can support the optimization of clinical treatment protocols. This research also provides scientific evidence supporting the application of ultrasound technology in biomedical and therapeutic fields. Ultimately, the study contributes to advancing innovative approaches for addressing global obesity challenges.

2. RESEARCH METHOD

This study employed an experimental *in vivo* design to evaluate the effect of ultrasonic wave exposure on abdominal circumference and blood triglyceride levels. A pretest–posttest control group design was applied to compare changes before and after treatment under controlled conditions.

The experimental subjects consisted of twelve male *Rattus norvegicus* (Wistar strain), aged approximately seven weeks with an average body weight of 100 grams. All animals were obtained from a certified laboratory animal facility and maintained under standard laboratory conditions.

The rats were acclimatized for seven days prior to the experiment to allow adaptation to the environment. During this period, animals were housed individually in cages with controlled environmental conditions, including a 12-hour light/dark cycle.

The animals were randomly divided into four groups (n = 3 per group):

1. Control group (no ultrasonic exposure)
2. Treatment group 1 (low intensity)
3. Treatment group 2 (medium intensity)
4. Treatment group 3 (high intensity)

This grouping allowed the evaluation of the effect of different ultrasonic intensity levels on the measured parameters.

Ultrasonic exposure was administered using an ultrasound therapy device operating at a fixed frequency of 1 MHz. The treatment was applied to the abdominal region of the rats.

Prior to exposure, a coupling gel was applied to the abdominal surface to eliminate air gaps and ensure optimal transmission of ultrasonic waves. Each rat was gently restrained using a specially designed holder to minimize movement during treatment.

The exposure protocol consisted of:

- Duration: 4 minutes per session
- Frequency: once daily
- Total treatment period: 6 consecutive days

Different intensity modes (mode 1, mode 2, and mode 3) were applied according to the assigned treatment group.

Blood triglyceride levels were measured using a portable digital analyzer with strip-based detection.

Blood samples were collected from the tail vein using sterile lancets. Prior to sampling, the tail was immersed in warm water to facilitate blood flow. The collected blood was applied directly onto the test strip inserted into the analyzer.

Triglyceride levels were recorded in mg/dL and measured at:

- Pretest (baseline)
- Posttest (after treatment)

To ensure experimental consistency, several variables were controlled throughout the study:

- Type, age, and sex of experimental animals
- Daily food intake (10 g corn + 10 g commercial feed)
- Water intake (100 mL/day)
- Environmental conditions (lighting and housing)

Data were analyzed descriptively and comparatively. Mean values and standard deviations were calculated for each group. Changes in abdominal circumference and triglyceride levels were evaluated by comparing pretest and posttest measurements. The results were presented in graphical form to illustrate the relationship between ultrasonic intensity and the observed effects.

3. RESULTS AND DISCUSSION

This study is an *in vivo* experimental research using laboratory animals, specifically rats (*Rattus norvegicus*), as the subjects. The purpose of this study was to investigate the effect of ultrasonic wave exposure on abdominal circumference and blood triglyceride levels.

The instrument used to generate ultrasonic waves was an ultrasound therapy device operating at a fixed frequency of 1 MHz. This device provides a constant current with adjustable power output. The intensity of the ultrasonic waves is defined as power per unit area. In this study, three variations of intensity were applied, namely mode 1, mode 2, and mode 3.

3.1. Effect of Ultrasonic Wave Intensity on Abdominal Circumference of Rats (*Rattus norvegicus*)

This study was conducted using white rats (*Rattus norvegicus*) of the Wistar strain. The experimental animals were male rats, totaling 12 individuals, with an average age of 7 weeks and an average body weight of 100 grams. Before treatment, the rats were acclimatized to adapt to the new environment. The acclimatization

period lasted for one week. During this period, the rats were exposed to a 12-hour light cycle consisting of natural sunlight and a 12-hour artificial light cycle using incandescent lamps.

The diet and water intake of the rats were kept consistent from the acclimatization period until the end of the experiment. The diet and water intake of the rats were kept consistent until the completion of the treatment, consisting of corn and BR-1 feed as food, and water as drinking supply. The quantity of food and water was standardized, with each rat receiving 10 grams of corn, 10 grams of BR-1 feed, and 100 mL of water per day.

After the acclimatization period, the rats were confirmed to be in good health by observing their physical condition and behavior. To facilitate the observation process, the rats were then divided into groups. The animals were grouped into four groups, each consisting of three rats. One group served as the control group, while the remaining three groups were assigned as treatment groups.

Abdominal circumference was measured using a measuring tape. The pre-treatment measurements were compared with the post-treatment measurements. The duration of ultrasonic exposure was consistent across all treatment groups, which was 4 minutes per session. To facilitate the procedure, the rats' limbs were gently restrained using an aluminum holder designed to fit the body size of the rats. Prior to treatment, a coupling gel was applied to the probe to eliminate air gaps between the transducer and the skin. This ensured optimal transmission and absorption of ultrasonic waves on the abdominal surface.

In addition, the gel functioned as a lubricant to facilitate smooth application during the therapy. After six consecutive days of treatment, the final abdominal circumference measurements were recorded. Table 4.1 presents the results of abdominal circumference measurements before and after treatment.

Table 1. Abdominal Circumference Measurements Before and After Treatment

No	Intensity Mode	Abdominal Circumference Before (cm)	Std Dev	Abdominal Circumference After (cm)	Std Dev
1	Control	10.47	0.82	11.40	0.71
2	Mode 1	10.53	0.05	11.43	0.12
3	Mode 2	12.30	0.73	11.50	0.36
4	Mode 3	11.17	0.78	11.03	0.39

Based on Table 1, it can be observed that the control group experienced an increase in abdominal circumference. The first treatment group also showed an increase, although not as significant as the control group. In contrast, the second and third treatment groups exhibited a decrease in abdominal circumference. The data in Table 4.1 also indicate relatively small standard deviation values, suggesting that the variation among sample measurements is low and the data are closely distributed around the mean values.

The change in abdominal circumference of the rats was calculated by subtracting the mean value after treatment (l_2) from the mean value before treatment (l_1). If the abdominal circumference increases, the result is positive, whereas a decrease results in a negative value. The percentage change in the mean abdominal circumference was calculated using the following equation:

$$\% \text{ change in abdominal circumference} = \frac{l_1 - l_2}{l_1} \times 100\% \quad \dots(1)$$

Table 2. Average Change in Abdominal Circumference Before and After Treatment

No	Intensity Mode	Mean Change (cm)	Percentage Change (%)
1	Control	0.93	8.88
2	Mode 1	0.90	8.55
3	Mode 2	-0.80	-6.50
4	Mode 3	-0.14	-0.01

Note: Negative values indicate a reduction in abdominal circumference of the rats.

Based on Table 2, the control group showed the highest increase in abdominal circumference, with an average change of 0.93 cm or 8.88%. The first treatment group also showed an increase of 0.90 cm or 8.55%. Meanwhile, the second treatment group showed a decrease in abdominal circumference of 0.80 cm or 6.50%, indicating the most effective reduction among all groups. The third treatment group also experienced a slight decrease of 0.14 cm or 0.01%.

The graph illustrating changes in the abdominal circumference of white rats before and after treatment, based on the measurement data presented in Table 4.1, is shown in Figure 1 below.

Abdominal Circumference of White Rats Under Different Intensity Variations:

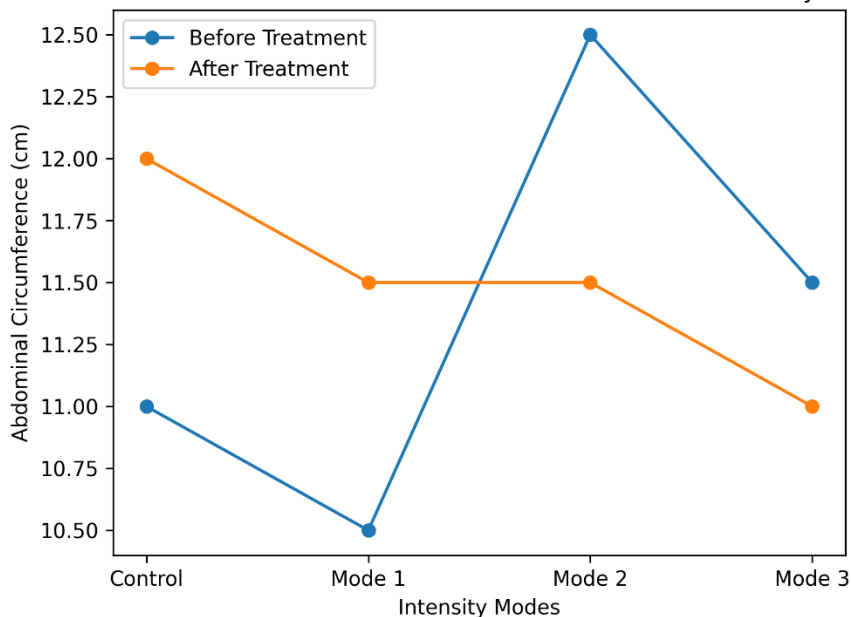
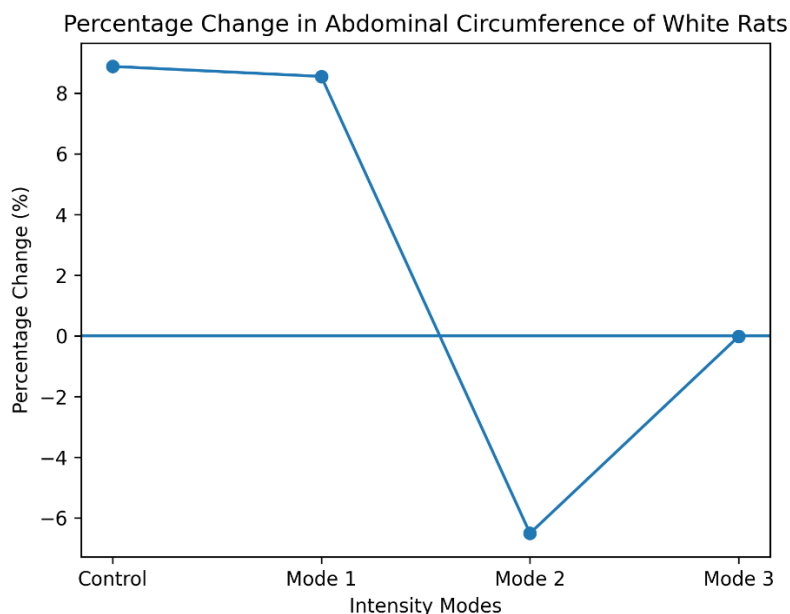


Figure 1. Graph of abdominal circumference of white rats under different intensity variations.

Figure 1 shows that the control group experienced an increase in abdominal circumference. This is likely because the white rats were still approximately 8 weeks old at the initial measurement, indicating that they were still in a growth phase. Therefore, the increase in abdominal circumference is associated with normal physiological growth processes.

In contrast, treatment with ultrasound at intensity mode 1 was able to inhibit the increase in abdominal circumference. Furthermore, at intensity modes 2 and 3, ultrasound therapy was observed to reduce the abdominal circumference of the rats.

Figure 2. Percentage Change in Abdominal Circumference of White Rats (*Rattus norvegicus*)

The percentage change in abdominal circumference of white rats based on the data in Table 4.2 is presented in Figure 2. The graph shows varying trends across different intensity modes of ultrasound treatment.

In the control group, the abdominal circumference increased by 8.88%, indicating normal growth without any intervention. A similar trend is observed in Mode 1, where the increase reached 8.55%. This suggests that

ultrasound treatment at intensity Mode 1 has not been effective in reducing abdominal circumference, as the rats still experienced a considerable increase comparable to the control group.

In contrast, Mode 2 shows a significant decrease of -6.5% in abdominal circumference. This indicates that ultrasound treatment at this intensity level is effective in reducing abdominal size, likely due to its influence on fat metabolism or tissue structure.

Meanwhile, in Mode 3, the decrease is very minimal at only -0.01% . Although this technically indicates a reduction, the effect is negligible. Additionally, it was observed that the experimental rats in this group died after the final treatment during blood triglyceride testing, suggesting that this intensity level may have adverse effects.

Overall, the results indicate that ultrasound treatment at Mode 2 provides the most optimal effect in reducing abdominal circumference, while Mode 1 is ineffective and Mode 3 may pose potential risks.

3.2. Effect of Ultrasonic Wave Intensity on Blood Triglyceride Levels in White Rats (*Rattus norvegicus*)

Blood triglyceride levels were measured using the strip method, in which blood samples were collected from *Rattus norvegicus*. Prior to measurement, the rats were fasted for 10 hours to stabilize triglyceride levels in the bloodstream.

Blood sampling was carried out by first placing the rats in a restrainer to minimize movement and ensure that the blood vessels were clearly visible. The tail area was then cleaned with alcohol before being punctured using a sterile lancet. Gentle pressure was applied to the tail to facilitate blood flow. The blood droplets obtained were placed onto a prepared test strip and inserted into the measuring device. After a few moments, the triglyceride levels were displayed on the device. Table 4. presents the results of blood triglyceride measurements in *Rattus norvegicus* before and after treatment.

Table 4. Blood Triglyceride Levels in *Rattus norvegicus* Before and After Treatment

No	Intensity Mode	Before Treatment (mg/dL)		After Treatment (mg/dL)	
		Mean	Std. Dev	Mean	Std. Dev
1	Control	85.00	16.97	143.33	53.11
2	Mode 1	94.67	4.03	100.00	2.45
3	Mode 2	114.00	6.38	118.67	35.46
4	Mode 3	83.00	4.08	84.33	15.92

The diagram illustrating blood triglyceride levels in white rats (*Rattus norvegicus*) before and after treatment, based on the data in Table 4.3, is presented in Figure 3.

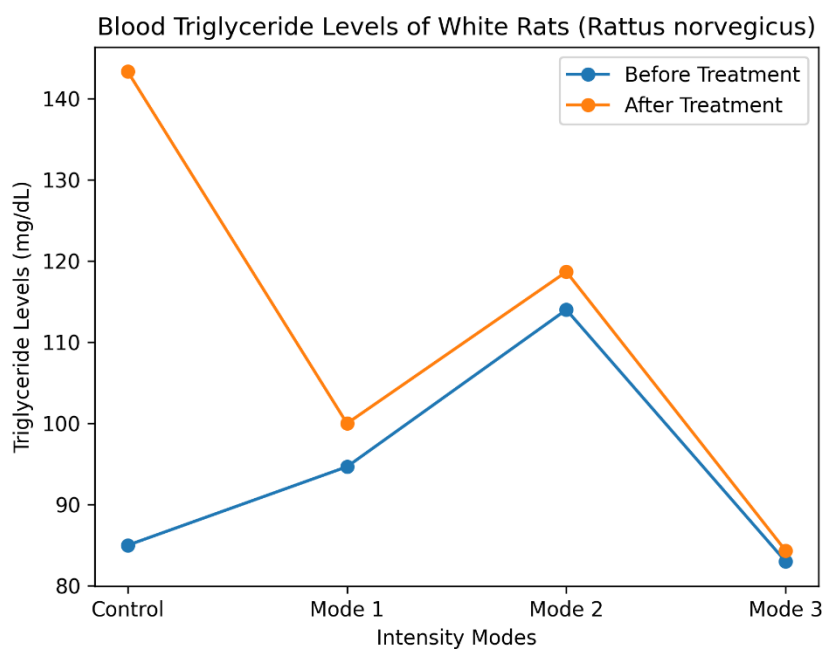


Figure 3. Graph of Changes in Blood Triglyceride Levels in White Rats Under Different Intensity Variations

Figure 3 shows that there was an increase in blood triglyceride levels after 6 days in all experimental groups, including the control group. The increase observed in the control group indicates a normal physiological change that is not influenced by external treatment.

However, the increase in triglyceride levels in the treatment groups is relatively smaller compared to the control group. This suggests that ultrasound therapy has an effect in inhibiting the rise of triglyceride levels in the blood.

Overall, the results indicate that ultrasound treatment contributes to suppressing the increase in blood triglyceride levels, although the effect varies depending on the intensity applied. This finding highlights the potential of ultrasound as a therapeutic approach in regulating lipid metabolism.

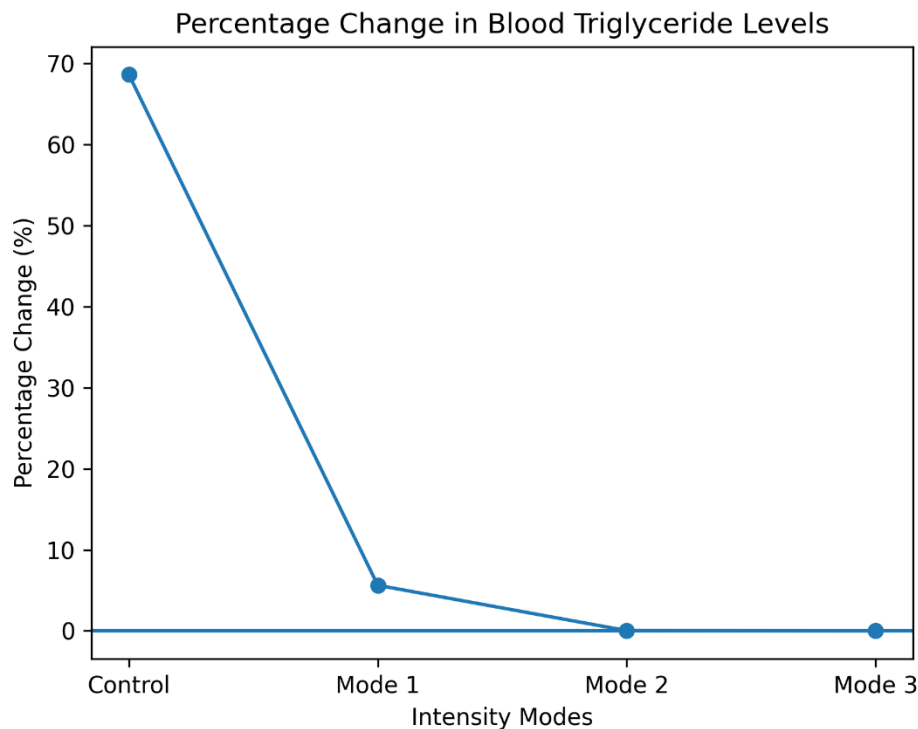


Figure 4. Graph of Percentage Change in Blood Triglyceride Levels

Figure 4, shows that rats in the control group experienced an increase in blood triglyceride levels of 68.62%. This increase is likely influenced by the age factor of the rats, which were still in a growth phase during the 6-day experimental period. In contrast, rats exposed to ultrasonic waves showed much smaller increases in triglyceride levels. In Mode 1, the increase was recorded at 5.63%, indicating that ultrasound exposure at this intensity begins to suppress the rise in triglyceride levels.

At Mode 2, the increase was extremely minimal, with an average of only 0.04%. Similarly, Mode 3 showed a very slight increase of 0.02%. These results indicate that higher ultrasound intensities are more effective in inhibiting the increase of blood triglyceride levels. Overall, the findings suggest that ultrasonic wave exposure plays a significant role in controlling the increase of triglyceride levels in rats, with higher intensity levels providing a more optimal effect.

The descriptive explanation of the research results indicates that ultrasonic wave intensity has an effect on the abdominal circumference of white rats (*Rattus norvegicus*). The first treatment group shows that ultrasound therapy at intensity Mode 1 is able to inhibit the increase in abdominal circumference in rats that are still in their growth phase. The control group experienced an increase in abdominal circumference of 8.88%, whereas the treatment group with intensity Mode 1 showed an increase of 8.85%. This difference is relatively small, indicating that Mode 1 intensity is less effective in reducing abdominal circumference.

At intensity Modes 2 and 3, ultrasound therapy was able to reduce abdominal circumference, resulting in smaller measurements compared to before treatment. Ultrasound therapy at Mode 2 reduced abdominal circumference by 6.50%, while Mode 3 resulted in a reduction of 0.01%. Thus, Mode 2 intensity is considered the most effective in reducing fat accumulation in the abdominal area, leading to a decrease in abdominal circumference.

Ultrasound waves focused on the subcutaneous area can lead to the thinning of adipose tissue. According to Zhou et al. [40], this effect is closely related to the cavitation phenomenon generated by ultrasonic energy, which results in the destruction of fat cells. Cavitation refers to the formation of small cavities or microbubbles

within a medium due to low pressure conditions. These molecules then form small, spherical cavities known as *cavities*, which can cause biological cell damage [41].

The mechanism of fat cell disruption due to cavitation begins with the formation of microbubbles in adipose tissue as a result of fat metabolism. These microbubbles absorb and release energy alternately, causing them to expand and contract. The increase in bubble size during expansion is greater than the decrease during contraction. This occurs because the absorbed energy is higher than the released energy, leading to continuous bubble growth until a threshold is reached where the bubbles can no longer absorb additional energy from the ultrasonic waves.

At this stage, the microbubbles undergo collapse (inertial cavitation), producing intense mechanical forces such as shock waves, microjets, and shear stress, which can disrupt and damage adipose tissue structures [42]. This process ultimately contributes to the reduction of fat deposits in the subcutaneous layer, which is reflected in the decrease in abdominal circumference observed in this study. Fat in the human body is stored in the form of triglycerides and functions as an energy reserve. When energy is required, triglycerides are hydrolyzed into glycerol and fatty acids, which are then released into the bloodstream. These components are transported to cells that require energy, where they are utilized to produce energy. Carbohydrates (CO₂) and water (H₂O) are produced as end products.

The findings of this study indicate an increase in blood triglyceride levels in the group that did not receive treatment. These results are consistent with the theory proposed by Spitler and Davies [43], age is a significant determinant of lipid metabolism, including triglyceride levels, as aging is associated with alterations in triglyceride metabolism and an increase in plasma triglyceride concentrations. As individuals age, triglyceride levels in the blood tend to increase. In this study, the control group showed an average increase in triglyceride levels of 68.62%.

In contrast, the results also demonstrate that mice treated with ultrasound therapy exhibited a smaller increase in blood triglyceride levels compared to the control group. This suggests that ultrasound therapy may reduce the risk of elevated triglyceride levels in the blood. Based on the intensity of ultrasound exposure applied to white rats (*Rattus norvegicus*), there was a noticeable difference in the change of triglyceride levels, although the increase still occurred. Ultrasound therapy with mode 3 intensity was considered more effective in reducing the rise in blood triglyceride levels, with an average increase of only 0.02%.

In adults, diseases caused by elevated blood lipid levels continue to increase, one of which is atherosclerosis. This condition is associated with high triglyceride levels that exceed normal limits. Therefore, preventive measures and proper management are essential before more severe complications occur. Controlling blood triglyceride levels can be achieved through two main approaches: pharmacological therapy (using medications) and non-pharmacological therapy, such as regular exercise, dietary regulation, and lifestyle modification. Ultrasound therapy is categorized as a non-pharmacological intervention because it is non-invasive (minimizing side effects) and relatively easy to administer.

This study offers a novel contribution by demonstrating the potential of ultrasound therapy as a non-pharmacological intervention to control blood triglyceride levels. While previous studies have primarily focused on pharmacological treatments or general lifestyle modifications, this research specifically examines the effect of different ultrasound intensities on triglyceride levels in *Rattus norvegicus*. The identification of mode 3 intensity as the most effective parameter in minimizing triglyceride elevation represents a new insight, particularly in the context of optimizing therapeutic protocols. Furthermore, this study integrates a biophysical approach into lipid metabolism management, which remains relatively underexplored in current literature.

The findings of this study have important implications for both clinical practice and future research. Clinically, ultrasound therapy may serve as a complementary or alternative strategy for managing elevated triglyceride levels, especially for individuals who prefer non-invasive treatments or have limitations in using pharmacological interventions. This approach could contribute to the prevention of lipid-related diseases such as atherosclerosis. From a research perspective, these results open opportunities for further investigation into the mechanisms underlying ultrasound-induced metabolic changes, as well as its application in human subjects. Additionally, the variation in intensity levels highlights the importance of dosage optimization in achieving effective therapeutic outcomes.

Despite its contributions, this study has several limitations. First, the use of animal models (*Rattus norvegicus*) may limit the direct generalization of the findings to humans, as physiological responses can differ between species. Second, the study focuses primarily on short-term changes in triglyceride levels, without examining long-term effects or potential side effects of repeated ultrasound exposure. Third, external factors such as diet, activity level, and metabolic variability among subjects may influence the results but were not fully controlled or explored in depth. Lastly, the study does not investigate the underlying biological mechanisms responsible for the observed effects, which warrants further experimental and molecular-level research.

4. CONCLUSION

This study demonstrates that ultrasound therapy has the potential to influence blood triglyceride levels in experimental animals (*Rattus norvegicus*). Although an increase in triglyceride levels was observed in all groups, the group receiving ultrasound therapy showed a significantly smaller increase compared to the control group. Among the tested parameters, ultrasound with mode 3 intensity was identified as the most effective in minimizing the rise in triglyceride levels. These findings suggest that ultrasound therapy can be considered a promising non-pharmacological approach for managing lipid levels, particularly triglycerides, and may contribute to the prevention of lipid-related diseases such as atherosclerosis. Future studies are recommended to expand this research by involving human subjects to evaluate the clinical applicability and safety of ultrasound therapy in controlling triglyceride levels. In addition, long-term studies are needed to assess the sustained effects and potential side effects of repeated ultrasound exposure. Further investigation into the underlying biological and molecular mechanisms is also essential to better understand how ultrasound influences lipid metabolism. Moreover, future research should explore variations in ultrasound parameters, such as frequency, duration, and intensity, to determine the most optimal and standardized therapeutic protocol. Controlling external factors such as diet, physical activity, and metabolic conditions is also important to obtain more accurate and comprehensive results.

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