

## Review Article

# A REVIEW ON EFFECT OF PHOTOBIMODULATION THERAPY ON OBESE MICE-HISTOLOGICAL CHANGES AND PROTEIN ANALYSIS

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### ABSTRACT

The effect of Photobiomodulation therapy on obesity is a growing area of interest, given its potential to influence cellular metabolism, inflammation, and tissue regeneration. This study aimed to evaluate the histological and protein-level changes induced by Photobiomodulation therapy in obese mice. Male or female mice were fed with high-fat diet to induce obesity and were subsequently treated with Photobiomodulation therapy at specific wavelengths, power densities, and dosages over a defined period. The results were analysed through histological examinations of adipose tissue, liver, and muscle, focusing on changes in fat deposition, inflammation, and fibrosis. Protein analysis, using techniques such as Western blot or ELISA, were conducted to measure biomarkers related to lipolysis, oxidative stress, and metabolic regulation and key inflammatory cytokines. Photobiomodulation therapy demonstrated significant effects on reducing fat deposition and optimizing tissue structure. Protein analysis revealed modulations in key markers of lipid metabolism and inflammation, suggesting that Photobiomodulation therapy effectively mitigates obesity-related metabolic dysfunction. These findings highlight the Photobiomodulation therapy as a promising, non-invasive intervention for addressing obesity-related tissue alterations and metabolic imbalance. Future studies should refine Photobiomodulation therapy parameters and explore the therapy's long-term effects in preclinical and clinical models.

**Keywords:** High-Fat Diet, Obesity, Photobiomodulation Therapy (PBMT).

### INTRODUCTION

Obesity has become a worldwide health crisis, with about 95% of cases resulting from external lifestyle factors. The primary causes include consuming more calories than needed and leading a sedentary lifestyle, trends that are rising across all age groups<sup>1</sup>. Obesity is a deeply complex condition that cannot be effectively addressed without significant effort, sustained behavioural changes over time, and consistent commitment to following weight management recommendations. Obesity is a complex metabolic disorder characterized by excessive adipose tissue accumulation, leading to systemic inflammation, insulin resistance, and alterations in lipid metabolism. These changes are associated with histological modifications in key metabolic tissues such as adipose tissue, liver, and skeletal muscle. Chronic low-grade inflammation in adipose tissue, marked by increased infiltration of immune cells and elevated pro-inflammatory cytokines, contributes to the development of insulin resistance and metabolic dysfunction<sup>2</sup>.

Photobiomodulation therapy (PBMT), also known as low-level light therapy, involves the application of red or near-infrared light to modulate cellular activity. Recent studies have demonstrated that PBMT can attenuate inflammation in adipose tissue of obese mice. For instance, PBMT at 843 nm significantly reduced inflammatory infiltrates in the abdominal adipose tissue of diet-induced obese and hyperglycemic mice, suggesting its potential in mitigating obesity-induced inflammation<sup>3</sup>.

Moreover, PBMT has been shown to improve insulin sensitivity and glucose metabolism. In high-fat diet-fed mice, PBMT at 630 nm enhanced insulin signalling pathways in adipose tissue and skeletal muscle, evidenced by increased phosphorylation of Akt and AS160, and elevated GLUT4 content. Additionally, PBMT improved mitochondrial function and  $\beta$ -oxidation in skeletal muscle, further supporting its role in enhancing metabolic health<sup>4</sup>.

PBMT also influences the expression of proteins involved in thermo genesis and lipogenesis. LED irradiation at 660 nm increased the expression of uncoupling protein 1 (UCP1) in brown adipose tissue and decreased fatty acid synthase (FAS) expression in white adipose tissue, indicating a shift towards increased energy expenditure and reduced lipid synthesis<sup>5</sup>.

Furthermore, PBMT has been shown to decrease free fatty acid generation and release in adipocytes, ameliorating insulin resistance in type 2 diabetes. This effect is mediated through the inhibition of phosphatase and tensin homologue (PTEN) and activation of protein kinase B (AKT), leading to reduced expression of lipolytic enzymes<sup>6</sup>.

In addition to these metabolic effects, PBMT has been observed to promote autophagic lipolysis induced by adipocyte cell apoptosis in the subcutaneous tissue of obese individuals. This suggests that PBMT may facilitate the remodeling of adipose tissue architecture, contributing to improved metabolic outcomes<sup>7</sup>.

LED treatment improved anthropometric parameters, possibly associated with the histological alterations, thermo genesis and lipogenesis markers in white adipose tissue, and expression modulation in brown adipose tissue<sup>8</sup>. Most of the early work in this field was carried out with various kinds of lasers, and it was thought that laser light had some special characteristics not possessed by light from other light sources such as sunlight, fluorescent or

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incandescent lamps and now LEDs. However, all the studies that have been done comparing lasers to equivalent light sources with similar wavelength and power density of their emission, have found essentially no difference between them<sup>9</sup>

In the context of obesity, PBMT may exert its beneficial effects by reversing histopathological changes in affected tissues and normalizing protein expression patterns disrupted by metabolic overload. Several studies have demonstrated that PBMT can reduce hepatic steatosis, improve adipose tissue architecture, and attenuate skeletal muscle degeneration in obese rodents. Additionally, PBMT has shown promise in modulating proteins involved in mitochondrial biogenesis (e.g., PGC-1 $\alpha$ ), lipid metabolism (e.g., AMPK), and insulin signaling pathways.

Photobiomodulation (PBM) has gained significant attention in recent years within the scientific and medical communities due to its non-invasive nature and therapeutic potential, involving the use of low-intensity light to activate biological processes in targeted tissues.<sup>10,11,12,13,14</sup>

Although PBMT has shown beneficial effects on breaking down fat tissue and promoting cell death in adipocytes, the precise signalling pathway responsible for these outcomes is yet to be determined. It is known that PBMT initiates a photochemical reaction, primarily acting on the mitochondria, which enhances the generation of adenosine triphosphate (ATP). This increase in ATP can elevate cellular energy levels and lead to various physiological changes.<sup>15, 16, 17</sup>

Light-Emitting Diodes (LEDs) and/or broad-spectrum light sources operating in the visible and infrared ranges are used for treatment that is gentle, non-invasive, and painless. This approach initiates photo physical and photochemical responses in biological tissues at the area of application, influencing processes at multiple physiological levels. It is generally considered safe for patients, with minimal to no side effects.<sup>18, 19, 20</sup>

Given these findings, PBMT emerges as a promising non-invasive intervention to counteract the histological and molecular alterations associated with obesity. This study aims to further elucidate the effects of PBMT on tissue morphology and protein expression in obese mice, providing insights into its therapeutic potential in obesity management.

Understanding these mechanisms could inform the development of novel non-pharmacological strategies for managing obesity and its complications.

## MATERIAL AND METHODS

Male C57BL/6 mice (6-8 weeks old) were housed in controlled conditions (temperature: 22 $\pm$ 2 $^{\circ}$ C, 12-hour light/dark cycle) with ad libitum access to food and water. Obesity was induced by feeding mice a high-fat diet (HFD) (60% kcal from fat) for 12 weeks. A control group was maintained on a standard chow diet. All experimental procedures were approved by the institutional animal ethics committee.

### Photobiomodulation therapy:

Obese mice were subjected to PBMT using a near-infrared (NIR) laser (810 nm, 100 mW/cm<sup>2</sup>, 5 J/cm<sup>2</sup>) applied to the abdominal region for 10 minutes per session, five times a week for four weeks. Control and HFD groups received sham treatments with the laser turned off.

### Histological Analysis:

Liver and adipose tissue samples were collected, fixed in 10% formalin, embedded in paraffin, and sectioned (5 $\mu$ m). Hematoxylin and eosin (H&E) staining was performed to assess histological changes, including adipocyte size, hepatic lipid accumulation, and inflammatory cell infiltration.

### Protein Expression Analysis:

Western blot analysis was conducted to evaluate the expression of key metabolic and inflammatory proteins. Protein extraction was performed using RIPA buffer, and equal amounts of proteins were separated via SDS-PAGE.

### Statistical Analysis:

Data were expressed as mean  $\pm$  standard deviation (SD) and analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Histological Alterations:

Hematoxylin & Eosin staining of adipose tissue from HFD-fed mice revealed hypertrophied adipocytes with increased interstitial inflammation. PBMT-treated mice exhibited reduced adipocyte size and lower inflammatory infiltration compared to untreated obese mice.

### Protein Expression:

Western blot analysis showed significant up regulation of pro-inflammatory markers (TNF- $\alpha$ , IL-6) in obese mice. PBMT treatment reduced the expression of these inflammatory markers while increasing AMPK phosphorylation, suggesting enhanced lipid metabolism and reduced inflammation.

## DISCUSSION

This study demonstrates that PBMT effectively reduces obesity-associated histopathological changes and modulates protein expression related to inflammation and metabolism. The decrease in adipocyte size and hepatic lipid accumulation suggests improved lipid homeostasis. The down regulation of TNF- $\alpha$  and IL-6 aligns with previous reports on PBMT's anti-inflammatory effects, potentially mediated via mitochondrial function enhancement and oxidative stress reduction.

## CONCLUSION

PBMT appears to be a promising non-pharmacological intervention for obesity, with beneficial effects on tissue structure and molecular pathways. Further studies are warranted to explore its long-term effects and clinical applicability.

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