Effects of IL-6 and Melatonin on adipogenesis, mitochondria and gene expression in 3T3-L1 cells

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Abstract

Obesity is associated with chronic inflammation and mitochondrial dysfunction, both of which contribute to metabolic disease. This study investigated the effects of interleukin-6 (IL-6) and melatonin on adipogenesis, mitochondrial morphology, and gene expression in 3T3-L1 adipocytes.

3T3-L1 pre-adipocytes were cultured and induced to differentiate into adipocytes. Cells were treated with IL-6 or melatonin at specific time points during differentiation. Mitochondrial morphology was evaluated using MitoTracker staining and confocal microscopy. Gene expression was analyzed using quantitative PCR to assess markers associated with mitochondrial dynamics, adipocyte differentiation, inflammatory signaling, and glucose metabolism.

IL-6-treated cells showed mitochondrial morphology consistent with increased fragmentation compared with untreated controls. Gene-expression analysis showed increased STAT3 expression following IL-6 treatment, consistent with activation of IL-6-associated inflammatory signaling. In contrast, GLUT4 expression increased with melatonin treatment and decreased with IL-6 treatment. Because GLUT4 is an insulin responsive glucose transporter important for glucose uptake in adipocytes, these findings suggest that melatonin and IL-6 may exert different effects on pathways associated with adipocyte glucose metabolism. Oil Red O staining also demonstrated differences in lipid accumulation and lipid droplet morphology across IL-6 concentrations. Melatonin treated cells displayed altered lipid droplet morphology, including smaller and more condensed lipid droplets depending on concentration.

Overall, these findings suggest that IL-6 influences mitochondrial morphology, inflammatory signaling, glucose metabolism, and lipid accumulation in 3T3-L1 adipocytes, while melatonin alters lipid-droplet morphology and may support pathways associated with glucose metabolism through increased GLUT4 expression. Further quantitative studies are needed to determine the mechanisms underlying these effects.

Results (Adipogenesis)

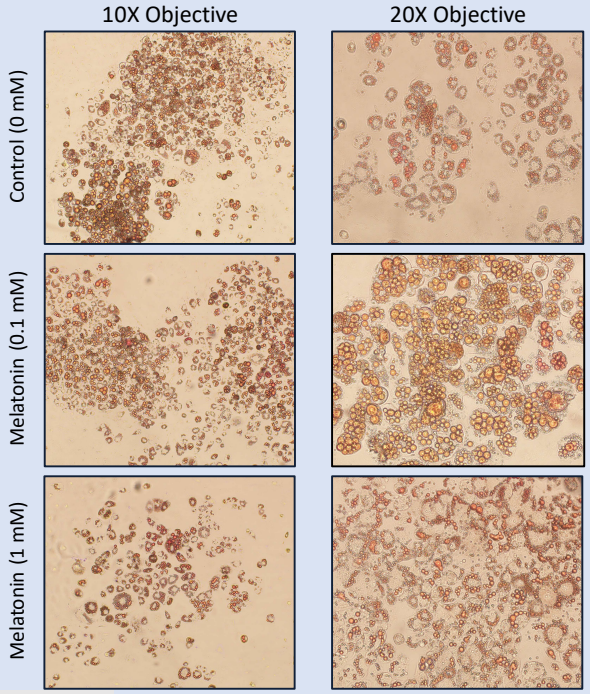


Fig: 1B *Results of Melatonin Oil Red staining of flask 4, 5 and 6, at 10X and 20X magnification.*

Results (Gene Expression)

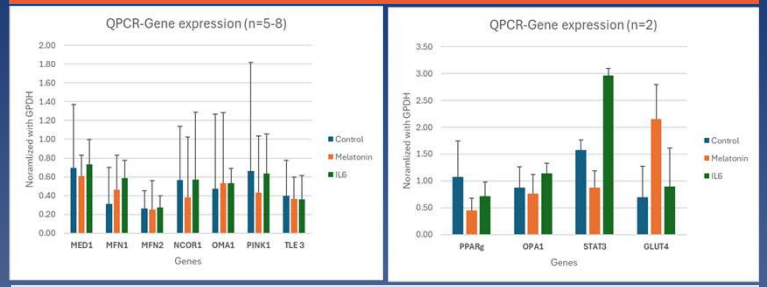


Figure 2. Effect of Melatonin (n=5) and IL-6 (n=8) on mitochondrial and brown/white fat gene expression

Results (Mitochondria)

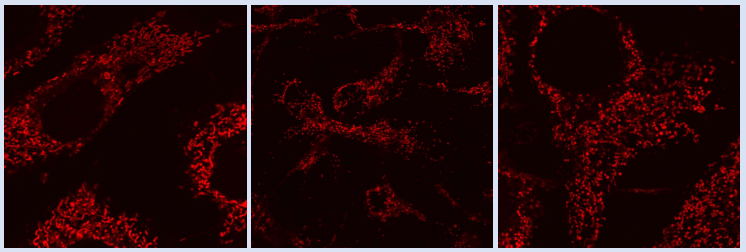


Figure 3. Effect of IL-6 on mitochondrial dynamics of 3T3-L1 preadipocytes**.** Confocal microscopy images of live non-treated 3T3-L1 pre-adipocytes cells and live 3T3-L1 pre-adipocytes treated with 200 ng/mL of Interliukin-6 (IL-6) for 48 and 96 hours stained with Mito Tracker red.

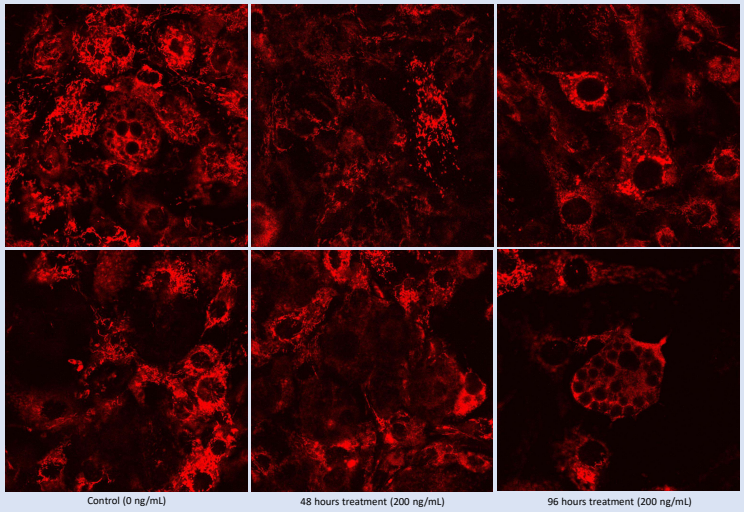


Figure 4. Effect of IL-6 on mitochondrial dynamics of differentiated 3T3-L1 adipocytes. Confocal microscopy images of live non-treated 3T3-L1 differentiated adipocytes and live 3T3-L1 differentiated adipocytes treated with 200 ng/mL of Interliukin-6 (IL-6) for 48 and 96 hours stained with Mito Tracker red.

Results (Adipogenesis)

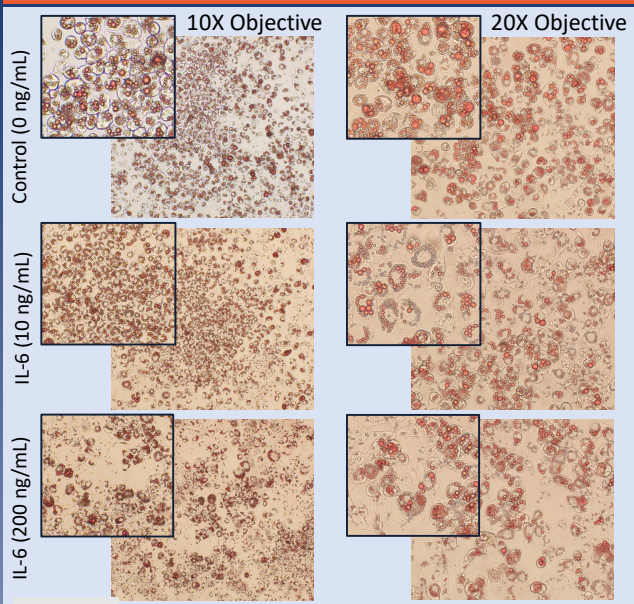


Figure 5: *Effect of IL-6 on adipocyte morphology. Oil Red Staining on flasks 4, 5, and 6, fully differentiated 3T3-L1 adipocytes. Treatment of 10 ng/mL of IL-6 for flask 5, and 200 ng/mL for flask 6 are observed with comparison of Flask 1 control.*

Conclusions

- 1 mM Melatonin treatment results in overall decreased adipogenesis and smaller more condensed lipid droplets compared to controls or 0.1 mM Melatonin treated cells.
- 3T3-L1 pre-adipocytes and adipocytes treated with 200 ng/mL of IL-6 for 48h or 96 h, show increasingly fragmented (almost punctate) mitochondrial morphology compared to the control, suggesting that under these treatment conditions, IL-6 promotes fission rather than fusion state of the mitochondria.
- IL-6-treated cells were assessed and compared across different concentrations, showing a pattern. Lipolysis increased and lipid droplet size decreased at 200 ng/mL compared to 0 ng/mL and 10 ng/mL.
- However, we could not identify mitochondrial or brown fat adipogenesis related gene expression differences by QPCR, to explain the morphological changes we observed.
- Preliminary data suggests that Melatonin treatment may upregulate Glut4 gene expression, related to a better glucose homeostasis profile.
- Preliminary data suggests that IL6 may upregulate STAT 3 gene expression, related to an inflammatory response in 3T3-L1 adipocytes.

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Acknowledgments

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