Commentary

Dose-dependent beneficial effect of melatonin on obesity; interaction of melatonin and leptin

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Summary

Although numerous studies have noted leptin's role in obesity, there are still important mechanistic insights that need to be elucidated. Disturbed leptin production is associated with eating disorders, leading to alter food intake and energy expenditure. Proper regulation of protein homeostasis is critical for metabolic diseases such as obesity. Thus, the purpose of the present work was to study the unfolded protein response, which is implicated in the alleviation of endoplasmic reticulum stress-dependent dysregulation of nutritional status. We studied the effect of leptin deficiency on liver, brain and skeletal muscle tissues in obese (ob/ob) mice and the actions of a daily melatonin administration, as a possible treatment. Our findings showed that the leptin-deficient mice presented tissue-specific alterations of the three adaptive unfolded protein responses. ATF6α arm is strongly activated in all of them, indicating a deregulated lipid metabolism by the lack of leptin. Likewise, melatonin also alleviates unfolded protein response in a tissue-specific manner, acting mainly in the restoration of this disturbed ATF6α pathway. These findings support the use of melatonin as a potential therapeutic treatment against leptin-associated disorders.

Keywords: melatonin, leptin, obesity, ob/ob, UPR, reticulum stress, ATF6α.

Obesity is considered as one of the most serious health concerns of the society due to the high rates of morbidity and mortality (1). Obesity is also a risk factor for a variety of diseases including hyperlipidemia, type 2 diabetes and hypertension (2), which cause different cellular lesions depending on the organ; the mechanisms of the damage are still largely unknown.

Leptin has been identified as a pleiotropic hormone mainly produced by the adipocytes of white adipose tissue. Leptin exhibits potent anorexic effects, acting to regulate the size of body fat depot (3). Leptin signaling leads to the inhibition of appetite, establishing a feedback loop that regulates feeding behavior and energy homeostasis (3). However, leptin production is usually critically

compromised in obesity, which results in hyperphagic behavior and nutrient overload. It has been found that obesity decreases the amplitude of nocturnal melatonin peak (4). Furthermore, reduced leptin production is attributed to a lack of circadian control (5-7). In fact, melatonin deficiency is associated with leptin resistance (7). Numerous publications have studied the chronobiological properties of melatonin, finding that it is broadly involved in the regulation of circadian rhythms, sleep and food intake (8-10). It is also necessary to highlight that melatonin is also secreted, at least locally, in extrapineal sites, tissues and organs, which appear to be controlled by nutritional sensing (11). Accordingly, the study of melatonin as a regulator of leptin production is gaining attention in obesity and leptin-related disorders.

Protein homeostatic disturbances constitute a currently critical area of research of metabolic diseases such as obesity, type 2 diabetes and non-alcoholic liver disease, among others (12-14). Under healthy and adequate cellular bioenergetic conditions, the complex network of protein renewal process remains stable. Proteostasis regulation is mediated by protein kinase B (AKT) and AMP-activated protein kinase (AMPK), which are considered as the two primary effectors of the mammalian target of rapamycin (mTOR). mTOR acts as a switch that determines cellular metabolism towards anabolic or catabolic responses. The endoplasmic reticulum (ER) has emerged as a metabolic checkpoint of protein homeostasis ensuring the synthesized protein quantity and quality (15, 16). The ER is most involved in coordinating the proper folding and assembling of proteins, as well as, controlling the restoration of misfolded proteins or the clearance of damaged ones (17, 18). Altered ER homeostasis leads to the accumulation of misfolded proteins in the lumen of ER, which induces an ER stress response. ER stress activates a series of intracellular transduction pathways implicated in the alleviation of cellular stress and the re-establishment of homeostasis, which are collectively designated as unfolded protein response (UPR). The three arms of UPR are mainly regulated by ER transmembrane proteins: inositol-requiring protein 1 alpha (IRE1α), 90 kDa activating transcription factor 6 alpha (ATF6α) which is proteolytically cleaved to produce the active form of 50 kDa (19) and RNA-dependent protein kinase like ER eukaryotic translation initiation factor 2 alpha kinase (PERK) which activates eukaryotic initiation factor 2 alpha (eIF2α) by its phosphorylation. Recently, some studies have found that UPR is activated by obesity, whose origin could be related to increased ER stress and/or perturbed mTOR signaling by the leptin disorders-induced obesity (20, 21).

Melatonin has been identified as a potent indolamine that can control ER stress via oxidative stress reduction, which is emphasized by its ability to regulate autophagy, among others processes, via the mTOR pathway (22, 23). Although numerous investigations have studied the modulatory effects of melatonin against ER stress, further research focused on the impact of this hormone is necessary to better understand the bidirectional crosstalk between mTOR signaling and UPR in obesity. Thus, to understand the global role of melatonin at the organism level, we have studied its effects on tissue-specific dysregulation of the three UPR induced by obesity. Sixteen six-week-old male wild type (C57BL/6J) and sixteen six leptin- deficient ob/ob (B6.V-Lepob/J) mice were housed under 12:12 h dark-light cycle. Half of the mice of each experimental group was subcutaneously injected with 500μg melatonin/kg for four weeks at ZT14.

The Oviedo University Local Animal Care and Use Committee approved the experimental protocol. All experiments were carried out according to the Spanish Government Guide and the European Community Guide for Animal Care (Real Decreto 53/2013).

Liver, brain and skeletal muscle tissues were collected for the study. Western blot analyses were performed using the following primary antibodies: ATF6α (sc-22799; Santa Cruz Biotechnology), eIF2α (5324; Cell Signaling) and IRE1α (3294; Cell Signaling). Protein levels

were analyzed and normalized to the loading control. Data were presented as semiquantitative optical density (OD) arbitrary units.

The results clearly show that leptin deficiency causes tissue-specific dysregulation of the different adaptive UPR to ER stress (Fig.1). Melatonin, in each case, alleviated the situation also in a tissue-specific role. Obesity in the liver triggered the three UPR pathways, whereas brain and skeletal muscle tissues specifically increased the ATF6α arm. Presumably, these divergent adaptive responses can be mainly understood by the diverse metabolic demands and metabolic plasticity of each tissue under both fed and fasted state (24). It has been previously noted that obesity induces a defective hepatic autophagy negatively regulated by mTOR signaling (25, 26). Thus, the altered function of proteolytic systems leads to the accumulation of damaged proteins, increasing ER stress and triggering the three UPR arms. For its part, melatonin is able to reestablish the IRE1α and p-eIF2α mediated responses in the liver of ob/ob mice. Thus, the melatonin administration not only lowers ER stress, but also reduces the risk of obesity-related disorders, such as ER stress-induced insulin resistance (27).

In brain the changes are more difficult to be interpreted since the relationship between leptin and melatonin is more complex. In brain the hypothalamic mTOR signaling has been considered as a target of leptin modulation. Cota *et al*. found that S6 kinase 1-deficient mice did not respond to leptin, implying a key role of protein synthesis in regulating the action of leptin (28). Given that ER is also involved in the neurotransmitter synthesis, the lack of leptin exhibited by ob/ob mice leads to a decreased protein folding, which, in turn, would contribute to degenerative neuronal circuitry. Curiously, the only active UPR pathway is that mediated by the cleavage of ATF6α, which is directly involved in the regulation of lipid biosynthesis (29). In the brain, melatonin's role is not as direct as was observed in the liver, but just as important. The oxidative stress, induced by obesity in the brain, translates into a clear ER stress with unfolded protein appearance. But, the subsequent UPR cascade was disrupted, with only a visible in the increase of ATF6α arm. However, it is important to note that the brain is highly sensitive to alterations at the level of oxidative stress and, thereby melatonin, as a powerful antioxidant, should be the perfect tool to mitigate it. Thus, melatonin restores the disturbed ATF6α pathway. However, since melatonin treated ob/ob mice showed a recovered behavior, without the depression and the excitability observed in non-treated ob/ob mice (30), ATF6α restoration is only the final effect. Given that melatonin reduces ER stress and improves protein synthesis, the triggers of other UPR arms were unnecessary.

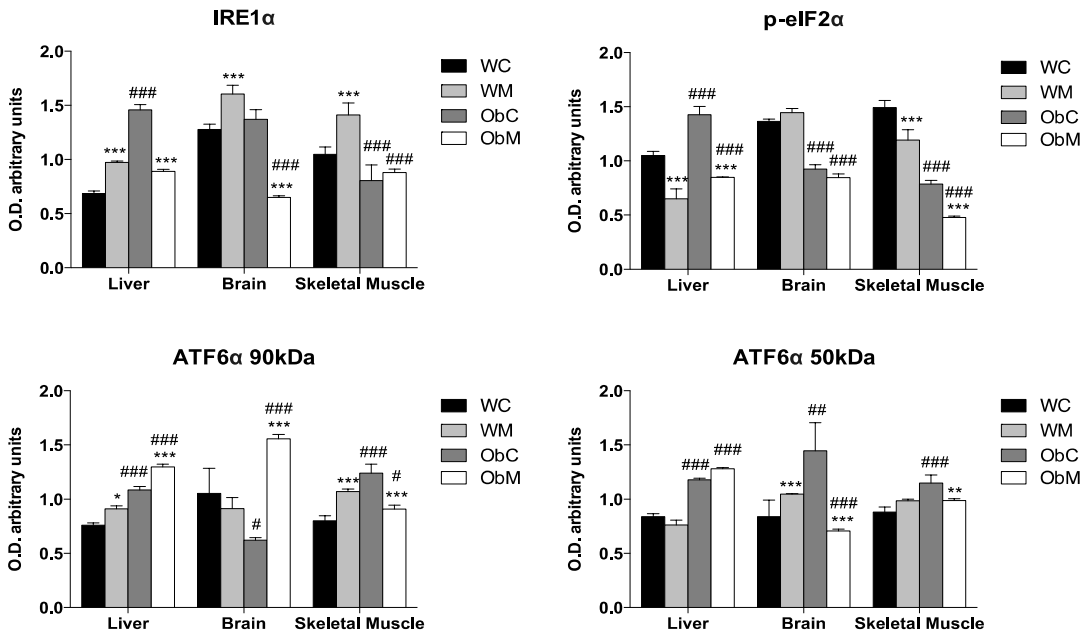

Figure 1. Determination of the main unfolded protein response markers from the liver, brain and skeletal muscle tissues in mice.

*WC: wild-type control; ObC: ob/ob control; WM: wild-type melatonin-treated group and ObM: ob/ob melatonin-treated group. The bar charts show the semiquantitative optical density (OD) arbitrary units of inositol-requiring protein 1 alpha (IRE1α), the phosphorylation of eukaryotic initiation factor 2 alpha (p-eIF2α) and the activating transcription factor 6 alpha (ATF6α). Data are represented as the mean ± SD from three independent experiments. #wild-type vs. ob/ob; *Control vs. Melatonin. The number of symbols marks the level of statistical significance: one for P < 0.05,two for P < 0.01 and three for P < 0.001*

Finally, skeletal muscle tissue plays an essential role in eating disorders, especially under the situation of altered leptin signaling. The high energetic demand required by leptin-deficient obese mice has a clear effect on skeletal muscle tissue, since it is considered as a master regulator of energy homeostasis (31). Skeletal muscle proteostasis is regulated according to the cellular requirements, increasing protein degradation systems in response to a negative energy balance (32). Thus, the metabolic reprogramming drives the skeletal muscle metabolism switch towards catabolic responses. Consequently, the reduced protein synthesis leads to diminished protein folding and lower ER stress. As in the brain, the only active UPR arm is the one regulated by ATF6α. Collectively, ATF6α is triggered in the three tissues, indicating that leptin deficiency strongly enhances ER-stress-dependent deregulated lipid metabolism (Fig. 2). Melatonin in skeletal muscle tissue has the same effect as observed in brain. ATF6α pathway in ob/ob mice is re-established by melatonin, suggesting the reduction of the lipid anabolic response. Indeed, it has been previously reported that melatonin reduces adipocyte hypertrophy in obese animals due to the reestablishment of adipokines secretion (33, 34). Moreover, melatonin also drives the differentiation of white adipose tissue into beige fat, contributing to the control of body weight (35). Broadly, our study also indicates that melatonin seems to modulate the disturbed nutrient

signaling elicited by leptin-deficiency-induced obesity, by improving protein homeostasis in skeletal muscle fibers.

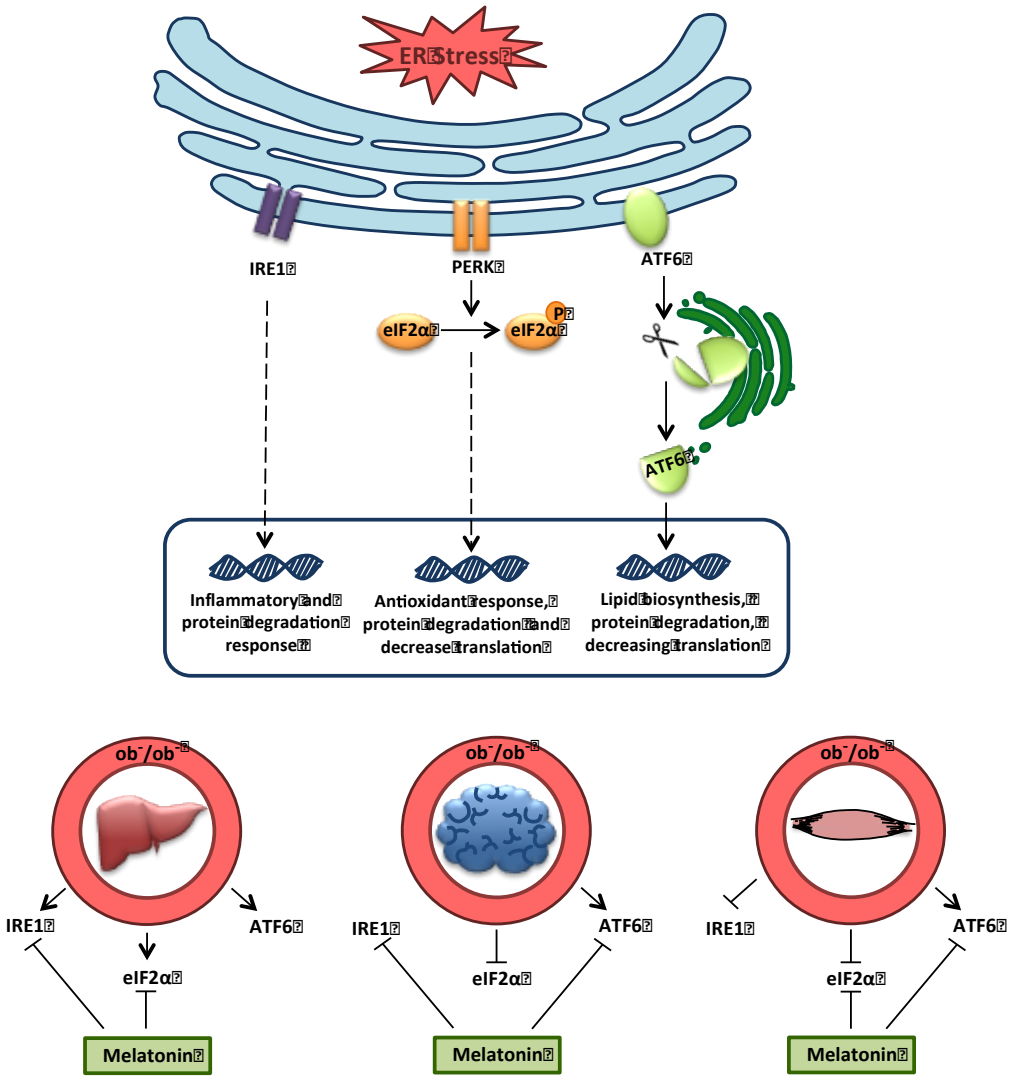

Figure 2. Illustration of the effect of leptin deficiency and melatonin treatment in the liver, brain and skeletal muscle tissues of ob/ob mice.

Leptin deficiency (in red) triggers IRE1, eIF2 α, and ATF6 arms of the unfolded protein response (UPR) in the liver and the ATF6 pathway in the brain and skeletal muscle tissues. Moreover, eIF2α in the brain and IRE1 and eIF2α pathways in the skeletal muscle are inhibited by the lack of leptin. Melatonin (in green) downregulates IRE1 and eIF2 α in the liver, IRE1 and ATF6 in the brain and eIF2 and ATF6 in the skeletal muscle.

IRE1, inositol-requiring protein 1; eIF2α, α subunit of the eukaryotic initiation factor; RNA-dependent protein kinase like ER eukaryotic translation initiation factor 2 alpha kinase (PERK); p-eIF2 α, eIF2 α phosphorylated activated form; ATF6, activating transcription factor 6 (90-kDa ATF6 inactive form and 50-kDa ATF6 active form).

Although the mechanisms are not completely understood, the synthesis and actions of melatonin and leptin seem to regulate each other. Moreover, the role of melatonin in protein synthesis regulation is well documented (36, 37). We conclude that melatonin drives dose-dependent effects in tissues and strongly suggests the use of melatonin in obesity management since it is implicated in the regulation of adipokine secretion.

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AUTHORSHIP

Y.P, B. de L-D and A. R-G. performed the experiments. Y.P. performed data analysis. A. C-M. conceptualized, designed and supervised the study. A. C-M. and R.J.R. provided suggestions and revised the manuscript. Y.P. wrote the manuscript.

CONFLICT OF INTERESTS

The authors do not have any conflicts of interest.

REFERENCES

1. Roth J, Qiang X, Marban SL, Redelt H, Lowell BC (2004) The obesity pandemic: where have we been and where are we going? *Obes. Res.* **12:** Suppl 2, 88S-101S.
2. Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, Robinson TN, Scott BJ, Jeor St, Williams CL (2005) Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. *Circulation* **111**: 1999-2012.
3. Zieba DA, Szczesna M, Klocek-Gorka B, Williams GL (2008) Leptin as a nutritional signal regulating appetite and reproductive processes in seasonally-breeding ruminants. *J. Physiol. Pharmacol.* **59**: Suppl 9, 7-18.
4. Cano P, Jimenez-Ortega V, Larrad A, Reyes Toso CF, Cardinali DP, Esquifino AI (2008) Effect of a high-fat diet on 24-h pattern of circulating levels of prolactin, luteinizing hormone, testosterone, corticosterone, thyroid-stimulating hormone and glucose, and pineal melatonin content, in rats. *Endocrine* **33**: 118-125.
5. Mantele S, Otway DT, Middleton B, Bretschneider S, Wright J, Robertson MD, Skene J, Johnston JD (2012) Daily rhythms of plasma melatonin, but not plasma leptin or leptin mRNA, vary between lean, obese and type 2 diabetic men. *PloS one* **7**: e37123.
6. Nguyen J, Wright KP, Jr (2010) Influence of weeks of circadian misalignment on leptin levels. *Nat. Sci. Sleep* **2**: 9-18.
7. Buonfiglio D, Parthimos R, Dantas R, Cerqueira Silva R, Gomes G, Andrade-Silva J, Ramos-Lobo A, Amaral FG, Matos R, Sinesio j, Jr, Motta-Teixeira LJ, Donato J, Jr, Reiter RJ, Cipolla-Neto J (2018) Melatonin Absence Leads to Long-Term Leptin Resistance and Overweight in Rats. *Front Endocrinol*. **9**: 122.

8. Sharafati-Chaleshtori R, Shirzad H, Rafieian-Kopaei M, Soltani A (2017) Melatonin and human mitochondrial diseases. *J. Res. Med. Sci.* **2**: 2.
9. Chakir I, Dumont S, Pevet P, Ouarour A, Challet E, Vuillez P (2015) Pineal melatonin is a circadian time-giver for leptin rhythm in Syrian hamsters. *Front Neurosci.* **9**: 190.
10. Saper CB, Scammell TE, Lu J (2005) Hypothalamic regulation of sleep and circadian rhythms. *Nature* **437**: 1257-1263.
11. Acuna-Castroviejo D, Escames G, Venegas C, Diaz-Casado ME, Lima-Cabello E, Lopez LC, Rosales-Corral S, Tan DX, Reiter J (2014) Extrapineal melatonin: sources, regulation, and potential functions. *Cell Mol. Life Sci.* **71**: 2997-3025.
12. Catania C, Binder E, Cota D (2011) mTORC1 signaling in energy balance and metabolic disease. *Int. J. Obes.* **35**: 751-761.
13. Ali M, Bukhari SA, Ali M, Lee HW (2017) Upstream signalling of mTORC1 and its hyperactivation in type 2 diabetes (T2D). *BMB Rep.* **50**: 601-609.
14. Kubrusly MS, Correa-Giannella ML, Bellodi-Privato M, de Sa SV, de Oliveira CP, Soares IC, Wakamatsu A, Alves VA, Giannella-Neto D, Bacchella T, Machado MC, D'Albuquerque LA (2010) A role for mammalian target of rapamycin (mTOR) pathway in non alcoholic steatohepatitis related-cirrhosis. *Histol. Histopathol.* **25**: 1123-1131.
15. Bukau B, Weissman J, Horwich A (2006) Molecular chaperones and protein quality control. *Cell* **125**: 443-451.
16. Yalcin A, Hotamisligil GS (2013) Impact of ER protein homeostasis on metabolism. *Diabetes* **62**: 691-693.
17. Schroder M, Kaufman RJ (2005) ER stress and the unfolded protein response. *Mutat. Res.* **569**: 29-63.
18. Gidalevitz T, Prahlad V, Morimoto RI (2011) The stress of protein misfolding: from single cells to multicellular organisms. *Cold Spring Harb Perspect Biol*. **3**: 6.
19. Gotoh T, Endo M, Oike Y (2011) Endoplasmic reticulum stress-related inflammation and cardiovascular diseases. *Int. J. Inflam*. **2011**: 259462.
20. Thon M, Hosoi T, Yoshii M, Ozawa K (2014) Leptin induced GRP78 expression through the PI3K-mTOR pathway in neuronal cells. *Sci. Rep.* **4**: 7096.
21. Pagliassotti MJ, Kim PY, Estrada AL, Stewart CM, Gentile CL (2016) Endoplasmic reticulum stress in obesity and obesity-related disorders: An expanded view. *Metabolism* **65**: 1238-1246.
22. Choi SI, Kim KS, Oh JY, Jin JY, Lee GH, Kim EK (2013) Melatonin induces autophagy via an mTOR-dependent pathway and enhances clearance of mutant-TGFBIp. *J. Pineal Res.* **54**: 361-372.
23. Koh PO (2008) Melatonin prevents ischemic brain injury through activation of the mTOR/p70S6 kinase signaling pathway. *Neurosci. Lett.*. **444**: 74-78.
24. Argiles JM, Campos N, Lopez-Pedrosa JM, Rueda R, Rodriguez-Manas L (2016) Skeletal Muscle Regulates Metabolism via Interorgan Crosstalk: Roles in Health and Disease. *J. Am. Med. Dir. Assoc.* **17**: 789-796.
25. Yang L, Li P, Fu S, Calay ES, Hotamisligil GS (2010) Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. *Cell Metab*. **11**: 467-478.
26. de Luxán-Delgado B, Potes Y, Rubio-González A, Caballero B, Solano jj, Fernández-Fernández M, Bermúdez M, Guimaraes MRM, Vega-Naredo I, Boga JA, Coto-Montes A (2016). *J. Pineal. Res.* **61**: 108-123.
27. Flamment M, Hajduch E, Ferre P, Foufelle F (2012) New insights into ER stress-induced insulin resistance. *Trends Endocrinol. Metab.* **23**: 381-390.

28. Cota D, Matter EK, Woods SC, Seeley RJ (2008) The role of hypothalamic mammalian target of rapamycin complex 1 signaling in diet-induced obesity. *J. Neurosci.* **28**:7202-7208.
29. Bommiasamy H, Back SH, Fagone P, Lee K, Meshinchi S, Vink E, Sriburi R, Frank M, Jackowski S, Kaufman RJ, Brewer JW (2009) ATF6alpha induces XBP1-independent expansion of the endoplasmic reticulum. *J. Cell Sci.* **122**: 1626-1636.
30. Rubio-Gonzalez A, Bermejo-Millo JC, de Luxan-Delgado B, Potes Y, Perez-Martinez Z, Boga JA, Vega-Naredo I, Caballero B, Solano JJ, Coto-Montes A (2018) Melatonin Prevents the Harmful Effects of Obesity on the Brain, Including at the Behavioral Level. *Mol. Neurobiol.* **55**: 5830-5846.
31. Lipina C, Hundal HS (2017) Lipid modulation of skeletal muscle mass and function. *J. Cachexia Sarcopenia Muscle*. **8**: 190-201.
32. Carbone JW, McClung JP, Pasiakos SM (2012) Skeletal muscle responses to negative energy balance: effects of dietary protein. *Ad. Nutr.* **3**: 119-126.
33. Stacchiotti A, Favero G, Giugno L, Golic I, Korac A, Rezzani R. (2017) Melatonin Efficacy in Obese Leptin-Deficient Mice Heart. *Nutrients* **9**: 1323.
34. Szewczyk-Golec K, Wozniak A, Reiter RJ. (2015) Inter-relationships of the chronobiotic, melatonin, with leptin and adiponectin: implications for obesity. *J. Pineal Res.* **59**: 277-291.
35. Jimenez-Aranda A, Fernández-Vázquez G, Campos D, Tassi M, Velasco-Pérez L, Tan DX, Reiter RJ, Agil A. (2013) Melatonin induces browning of inguinal white adipose tissue in Zucker diabetic fatty rats. *J. Pineal Res.* **55**: 416-423.
36. Coto-Montes A, Boga JA, Tan DX, Reiter RJ. (2016) I Melatonin as a Potential Agent in the Treatment of Sarcopenia. *Int. J. Mol. Sci.* **17**: 1771.
37. Brodsky VY, Zvezdina ND. (2010) Melatonin as the most effective organizer of the rhythm of protein synthesis in hepatocytes in vitro and *in vivo*. *Cell Biol. Int.* **34**: 1199-1204.

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