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The Effect of Melatonin on Leptin and Growth Hormone in Mice.

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ABSTRACT

Melatonin is a hormone secreted by the pineal gland in response to darkness, because of that it named hormone of darkness. Melatonin has generated an important deal of interest as a therapeutic modality for many diseases especially sleep disorders. Melatonin and leptin show a role in energy metabolism and circadian variation. A relationship between these hormones has been discussed. Melatonin has been appearing to be able to positively affecting the leptin in the circulation. Also, Growth hormone (GH) and melatonin belong to the group of growth factors. This research assumes to measure the effect that happened on leptin and growth hormone under the melatonin treatment by using male and female albino, laboratory-bred mice, 72 mice were divided to 3 groups, group A as control group, group B with 3mg and group C that treated with 12mg melatonin. The killing process was done in 2 stages (6 and 10 weeks). Blood samples were taken and analyzed in addition. The results were proved significantly higher levels of plasma leptin and GH in mice that treated with melatonin compared to control mice. Our discovery from this study that melatonin elevates the leptin and GH levels in blood, decrease the body weight.

Introduction

Melatonin has many physiological functions in animals, such as influencing circadian rhythms, body temperature, retina physiology, mood, sleep, food intake, locomotors activity, seasonal reproduction, sexual behavior and the body immunity [1]. The pineal gland secretes the melatonin which it is the sleeping hormone in the dark and it helps to regulates the body temperature and the “circadian rhythm” depend on the cycle of 24 h day\night [2].

One of the major health issue is obesity, it may involve in chronic diseases like type 2 diabetes, hypertension, sleep apnea, stroke, a lot of psychosocial problems and even cancer. According to World Health Organization around 30% of the population is suffering from obesity because of sedentary lifestyles and unbalanced eating habits [3]. High carbohydrate, high fat consumption and ecological factors changing all play a major role in increase fatness, cause a severe dysfunction to the white adipose tissue [4, 5].

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One of the important peril factors for lead to obesity is Sleep deprivation [6]. Owing to the fact that lake of sleep may affect hunger hormone levels, increasing ghrelin hormone, which lead to decreasing leptin levels and hunger feeling, that makes you feel full [7, 8, 9]. A hormone released in the stomach, Ghrelin that signals hunger in the brain. Its Levels are high before eating (the stomach is empty), and low after eating. Fat cells release a hormone called leptin that suppressing hunger and indicating fullness in the brain [10, 11]. Leptin is a peptide hormone that synthesis and secreted from the adipose tissue. Thus, the amount of body fat is related to the leptin levels [12, 13].

Leptin is a 160-kDa hormone “repletion hormone” that transmit feedback signals to the central nervous system (CNS) from the adipose tissue [14]. The brain receives a satiety signal that regulates food intake to maintain thermogenesis and energy homeostasis [15]. Studies state a strong positive relation between the percentage of body fat and serum leptin levels. To execute the function of leptin in the hypothalamus gland, it is necessary to pass the blood brain barrier (BBB) by binding to specific transporters. Lower leptin sensitivity in overweight body may happen owing to the Reduced BBB permeability by saturation of its transporters. [16].

Leptin is a hormone produced primarily by fat tissue. Also, it may produce by other tissue such as stomach [17]. and also, it is produced by other tissues, including the mammary gland, pituitary gland and skeletal muscles [18, 19, 20].

It is an important factor in weight regulation process [21]. The hormone was studied since its discovery in 1994 for its function in obesity and weight regulation in both humans and animals [22]. Leptin gives feedback to the brain that you have sufficient amount of fat stored in the body, which reduce your appetite, signaling the body to burn calories normally and prevents excessive consumption of food. Conversely, that mean when leptin levels are low, the brain triggers starvation, the appetite increases, then brain signaling to take in a bigger food amount and slow down calories burn rate [23].

Growth hormone is a peptide hormone secreted from somatotroph cells in the anterior pituitary gland. The Growth hormone (GH) gene cluster has five GH variants and it's located on chromosome 17.

The variants of GH are:
1. The pituitary GH-N (or GH-1) variant.
2. Four placental GH-V (or GH-2) variants.

The GH variations express themselves at different times during development, and they all produce comparable 22 kDa protein products. Two isoforms, 22 and 20 kDa, are produced by alternative splicing of the pituitary GH-N mRNA; the more prevalent isoform is the one that was previously in circulation [10]. GH's main purpose is to promote postnatal longitudinal growth. It promotes the growth of bones [24]. Studies additionally demonstrate that growth hormone (GH) decreases body fat [25]. Moreover, it controls the metabolism of minerals, fats, carbohydrates, nitrogen, and electrolytes [26]. It promotes adipocyte lipolysis, which reduces body fat; it also improves muscle uptake of amino acids and nitrogen retention, which preserves muscular mass and strength. [27]. Neurogenesis, the central nervous system, the immunological system, the cardiovascular system, and aging are all impacted by GH [28].

Consequently, several tissues and organs may be affected by aberrant GH secretion. GH hypersecretion, in instance, causes gigantism in children and acromegaly in adults [29], On the other hand, congenital disruption of GH signaling results in Laron syndrome and short stature. GH deficiency syndrome is the term for the insufficiency in adults [30].

Material and Methods

Ethical statement

Informed consent according to the Declaration of Helsinki was obtained from ethics committee of College of Science, Mustansiriyah University (Ref. No.: BCSMU/0123/00033Z).

Experimental Design and Animal Treatment

Seventy-two laboratory mice (36 males and 36 females) were bought from the Iraqi center for cancer research in Al-Qadisya, Baghdad. The animals were housed in cages made of polypropylene (43 cm × 30 cm × 15 cm) Animals had access to food and water. Within a temperature controlled (23 ± 2 °C) with constant 12 h light–dark cycles. The mice were divided into three groups. Group A as a control group, group B treated with 3mg melatonin and group C which treated wuth 12mg melatonin. The juvenile mice that been chosen for this study were at postnatal days 10–24. The Mice received melatonin (puritan's pride/USA) every evening (8:00 PM) at doses of 3mg/kg and 12 mg/kg (melatonin was dissolved in Distilled water to the final Concentration of 1.06% and 4.2% sequentially).

The killing process was done through two period's durations. The first groups were killed after 6 weeks for each tree groups, collect the blood samples and then conduct the required test. While the second kill were after 10 weeks for all three groups and conduct the required test. For the detection of hormone, blood samples of each group were collected via the venous that in the eyelid. Then the serum was obtained by centrifugation at 3000 rpm for 10 min and stored at −18 °C.

Leptin in serum has been measured by the means of enzyme-linked immunosorbent assay (ELISA) kits (Feiyue/ China Shanghai).

Growth hormone levels in serum have been measured by the assay carried by immunochemistry testing using Elecsys kit.

The body weight changing rate been measured by weighting the mice before the experiment and after. Then calculate the body weight changing rate.

Statistical analysis

Results were expressed in terms of mean ± SE. The data were examined for many comparisons after one-way analysis of variance (ANOVA), Regression analysis utilizing analysis of combined variance was done (ANCOVA). Stat view 5.0 was used to conduct all of the statistical analysis of these experiments. When $p< 0.05$ was reached, the differences were considered significant.

Results and discussion

About the 6weeks groups, the GH levels were: group (A) (2.115±0.336), group B (4.031±0.632) and the group C (5.214±0.245). It observed significant increment in GH levels between group A and group B p-value=0.0038 and between group A and group C p-value<0.0001, no significant change between group B and group C. The ten weeks groups, the GH levels were: group A (3.939±0.457), group B (5.219±0.296) and group C (7.725±0.632).

The 6weeks leptin levels were: group A (1.357±0.122), group B (1.555±0.187) and group C (2.476±0.512). The results show significant increment between group A and group C, p-value 0.0051. Also, reported significant increment between group B and group C p-value 0.0180. The leptin levels after 10weeks were: group A (1.870±0.284), group B (2.549±0.183) and group C (2.638±0.153). The results show significant increment between group A and group B p-value 0.0336. No significant change between other groups.

Table 1. The melatonin effect on the Leptin and Growth hormone Depend on the duration.

Group	GH (ng/ml)		LEP (ng/ml)	
	6 weeks	10 weeks	6 weeks	10 weeks
Control (A)	2.115±0.336	3.939±0.457	1.357±0.122	1.870±0.284
3 mg (B)	4.031±0.632	5.219±0.296	1.555±0.187	2.549±0.183
12 mg (C)	5.214±0.245	7.725±0.632	2.476 ±0.512	2.638±0.153

Depending on the table above that shows mean±standard error, we noticed that Growth hormone and leptin increase with extension in duration of melatonin intake.

Some studies show that melatonin effect the GH level in mice, the GH level increase with the intake of melatonin [24, 31]. There is some evidence that chemically, melatonin can behave somewhat like a growth hormone-releasing peptide and trigger the hypothalamus to tell the pituitary gland to make and release more GH. Also, by influencing two enzymes that regulate lipolysis—the breakdown of stored triglycerides into free fatty acids—and lipogenesis—the accumulation of fat—growth hormone decreases fatness. These are hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) hormones [32].

Since the other studies demonstrate that melatonin appears to triggers leptin synthesis [15, 33]. The Leptin has increased with the melatonin treatment. Elevated leptin levels are associated with decrease weight and inhibit food intake [34]. Thus, both increment of leptin and GH cause decrease the body weight, When the body doesn't need calories, leptin helps regulate energy balance and inhibit (avoid) hunger. This prevents the body from producing the hunger response. More recently, it has been shown that melatonin effectively synchronizes leptin secretion in Syrian hamsters and rats [35]. Melatonin also drives the daily rhythmicity of plasma leptin in accordance with the metabolic state based on photoperiod and controls glycemic homeostasis.

The levels of GH in females were: group A (2.448±0.484), group B (3.671±0.561) and group C (5.537±0.342). These results record significant increment between group A and group C, pvalue<0.0001. Also, significant increase was observed between group C and group B p-value 0.0118. No significant change between A and B. In males, GH results were: group A (3.575±0.413), group B (5.579±0.247) and group C (7.366±0.723). These results

show significant increment between group A and group B, group A and group C and between group B and C, with p-value 0.0041, 0.0001 and 0.0132 sequential.

The results of leptin levels in males were: A (1.157±0.083), B (1.792±0.221) and C group (2.175±0.138). From this result we noticed significant increment between group A and B group p-value 0.0062. Also, significant increment observed between group A and C the p-value<0.0001. The results of leptin levels in females were: group A (2.088±0.242), B group (2.313±0.236) and C group (2.990±0.534). These results show no significant change between the groups.

Table 2. The effect of Melatonin on Leptin and Growth hormone Depend on the gender

Groups	GH (ng/ml)		LEP (ng/ml)	
	Female	Male	Female	Male
Control	2.448±0.484	3.575±0.413	2.088±0.242	1.157± 0.083
3 mg	3.671±0.561	5.579±0.247	2.313±0.236	1.792± 0.221
12 mg	5.537±0.342	7.366±0.723	2.990±0.534	2.175± 0.138

It implies that melatonin affects the release of somatostatin or GHRH, which both have an impact on central hypothalamic control. The effects of melatonin administration have been demonstrated in the past by Valcavi et al [36]. Who examined the GH response to GHRH. Since the melatonin effect vanished after pyridostigmine injection, it was hypothesized that the enhanced GH response was aided by a suppression of endogenous somatostatin release.

The results show increment in leptin levels. Normally females have higher levels of leptin than males [37] and that due to two distinct systems a greater percentage of adipose tissue in the body and a faster rate of leptin synthesis per mass of adipose tissue.

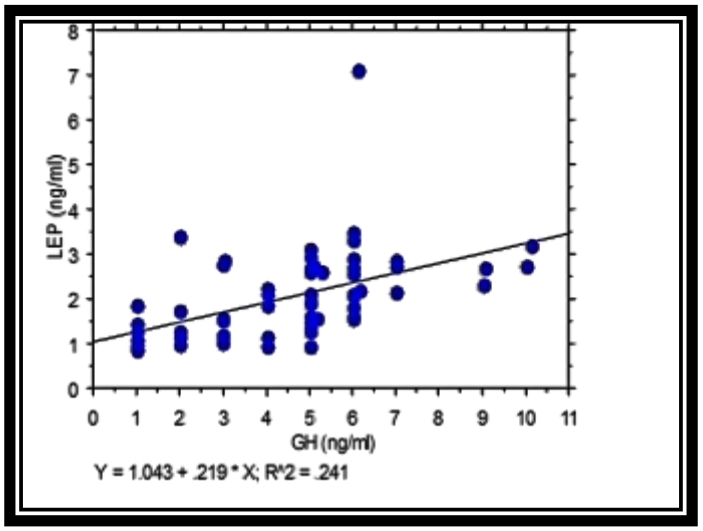

Figure 1. the correlation between GH and leptin.

The figure 1 above shows positive relation between LEP and GH under the treatment of melatonin. With p-value <0.0001 and R=0.49.

The results of the body weight changing rate in female group during six weeks were: group A (0.937±0.017), group B (0.994±0.059) and group C (0.927±0.057). No significant change in body weight in female groups during the first six weeks. And the female group during ten weeks: group A (0.963±0.12), group B (0.926±0.116) and group C (0.585±0.36). After ten weeks significant decrement between group A and group C with p-value 0.0033 and significant decrement between group B and group C with p-value 0.0066.

In the male groups, during the first six weeks the result: group (A) (1.013±0.058), group B (0.818±0.030) and group (C) (0.752±0.036). Significant decrement between group A and group B with p-value 0.0060. Significant decrement between group A and group C with p-value 0.0007. No significant change between group B and group C.

After ten weeks the male group results were: group A (0.970±0.068), group B (0.945±0.003) and group C (0.686±0.031). Significant decrement recorded between group A and group C with p-value 0.0012 and significant decrement between group B and group C with p-value 0.0023. No significant change between group A and group B.

Table 3. Body weight changing rate.

body weight changing rate				
groups	female		male	
	6w	10w	6w	10w
control	0.937±0.017	0.963±0.12	1.013±0.058	0.970±0.068
3mg	0.994±0.059	0.926±0.116	0.818±0.030	0.945±0.003
12mg	0.927±0.057	0.585±0.36	0.752±0.036	0.686±0.031

The decrement that happened to the body weight may because of melatonin treatment. Many studies proved that melatonin causes losing weight [38]. Studies suggest that melatonin, a cheap medicinal drug, may help with diseases associated with obesity. To assess whether melatonin can effectively improve the inflammatory profile brought on by obesity and delay or perhaps prevent the harm that excessive consumption of a high-fat diet (HFD) causes in mice [39]. The leptin has major role in the body weight losing process [40]. Also, GH decrease the body fat that led to weight loss [41, 42]. And those studies compatible with our result.

Conclusion

In conclusion, through the result of this study it's noticed that melatonin has an effect on leptin leading to increase the leptin levels and as it mentioned leptin has important role in weight loss process. Growth hormone levels also seem to be increased under the influence of the melatonin. This relation also effects the body weight changing rate led to decrease the body weight of the mice.

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Notes

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Abbreviations

GH, growth hormone; ELISA, enzyme-linked immunosorbent assay; HSL, hormone sensitive lipase; LPL lipoprotein lipase; HFD, high-fat diet.

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تأثير الملاتونين على هرمون اللبتين و هرمون النمو في الفئران

رسل مهدي جباره، لمى قاسم علي

قسم علوم الحياة ، كلية العلوم ، الجامعة المستنصريه ،بغداد، العراق.

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الخلاصة:

الميلاتونين هو هرمون تفرزه الغدة الصنوبرية استجابةً للظلام، ولهذا سمي بهرمون الظلام. لقد ولّد الميلاتونين قدرًا كبيرًا من الاهتمام كطريقة علاجية للعديد من الأمراض وخاصة اضطرابات النوم. يُظهر الميلاتونين و هرمون اللبتين دورًا في ايض الطاقة والاختلاف في الساعة البيولوجية. وقد تمت مناقشة العلاقة بين هذه الهرمونات. يبدو أن الميلاتونين قادر على التأثير بشكل إيجابي على هرمون الليبتين في الدورة الدموية. كما ينتمي هرمون النمو (GH) والميلاتونين إلى مجموعة عوامل النمو. يفترض هذا البحث قياس التأثير الذي حدث على هرمون اللبتين وهرمون النمو تحت معاملة الميلاتونين باستخدام فئران ألبينو ذكور وإناث، تم تربيتها مختبرياً، وتم تقسيم 72 فأراً إلى 3 مجموعات، المجموعة أ كمجموعة سيطرة، والمجموعة ب بجرعة 3 ملغ والمجموعة C المعالج بـــ 12 ملجم من الميلاتونين . تمت عملية القتل على مرحلتين (6 و 10 أسابيع). بالإضافة إلى ذلك تم أخذ عينات الدم وتحليلها. وقد أثبتت النتائج وجود مستويات أعلى من هرمون الليبتين وهرمون النمو في الفئران التي عولجت بالميلاتونين مقارنة بفئران السيطرة. اكتشفنا من هذه الدراسة أن الميلاتونين يرفع مستويات اللبتين و هرمون النمو في الدم، ويقلل من وزن الجسم.

الكلمات المفتاحية: *ميلاتونين، هرمون النمو، لبتين، وزن الجسم.*