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The Possible Effects of Melatonin and Exercise on Adipose Tissue Browning in Obese Male Rats

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ABSTRACT

Background: Obesity is considered to be a pandemic that has increased during the last decades. The energy burning capacity of brown adipose tissue (BAT) makes it an attractive target in anti obesity therapies. Several strategies are being examined to activate and recruit BAT with no side effects. **Objective:** To evaluate the possible effects of melatonin on browning of white adipose tissue (WAT) in exercised obese Rats. **Material and methods:** Fifty adult male Wister albino rats were divided into five equal groups. Group I: Normal control, group II: High fat diet (HFD), group III: HFD plus melatonin, group IV: HFD plus exercise, group V: HFD plus melatonin with exercise training. At the end of the experiment, the body weight, the adiposity index, the serum levels of lipid profile, GSH, MDA, IL-6 and irisin hormone were determined. The abdominal adipose tissues were excised for histopathological examination and for measurement of gene expression of adipose tissue uncoupling protein -1 (UCP-1), peroxisome proliferator-activated receptor gamma co-activator-1α (PGC-1α) and CD-137. **Results:** Melatonin treatment and exercise training can significantly decrease body weight and adiposity index. Serum cholesterol, triglycerides and LDL-c significantly decreased, while HDL-c and GSH significantly increased. Furthermore, melatonin and exercise suppressed the increase in serum IL-6 and MDA significantly. As well as they increased the expression of UCP-1, PGC-1 α and CD-137 and consequently WAT browning. **Conclusion:** Melatonin supplementation and exercise may provide an effective therapeutic option for combating obesity as it possessed antioxidant and anti-inflammatory activities, as well as increased expression of UCP-1, PGC-1 α and CD-137 which was also proved histologically by increased brown fat cells in WAT.

Keywords: Melatonin, HFD, Exercise, Browning of white adipose tissue.

Introduction

Obesity represents a huge public health problem due to the associated risk with developing other diseases as diabetes, ischemic heart diseases and certain cancers (Cascio *et al*., 2012). Adipose tissue is the most important organ that contributes to these obesity-associated comorbidities. This is because adipose tissue is a secretory endocrine organ that release many cytokines, hormones and proteins. These substances can affect the functionality of cells and tissues all over the body (Rao *et al*., 2014). It is composed of white (WAT) and brown (BAT) adipose tissues. Both are different in morphology, distribution, gene expression and functions (Mathieu *et al.,* 2010). Brown adipose tissue (BAT) is a unique tissue that is able to convert chemical energy directly into heat when activated by the sympathetic nervous system. While initially believed to be found only in human newborns and infants, it was proved that human adults contain active BAT (Czech, 2017). Beige cells are cells share functional similarities with brown adipocytes and both exert a critical role in oxidizing nutrients at the high rates through non-shivering thermogenesis. The recruitment of beige cells in white adipocytes, termed browning, has been considered as a promising strategy for treating obesity and associated metabolic complications (Li e*t al*., 2018, Yoshida *et al*., 2018 and You *et al*., 2018).

Regular exercise training is an effective approach for prevention and treatment of obesity (Gamas *et al.,* 2015). Many of the molecular events responsible for the curative and protective role of exercise remain elusive (Roca-Rivada *et al.,* 2013). Skeletal muscle is the major target organ that participates in exercise training. More than 1000 genes are activated in skeletal muscle in response to

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exercise and contribute to enhanced health (Thompson *et al*., 2012). Also, it is the most important organ responsible for maintenance of posture and shivering. Together with the non-shivering thermogenesis produced by brown adipose tissue, shivering helps in body temperature regulation (Cannon and Nedergaard, 2011). Skeletal muscle communicates with other tissues through myokines released into the circulation during exercise, which includes irisin, myostatin, IL-4, IL-6, IL-7, IL-15, leukemia inhibitory factor, myonectin, fibroblast growth factor-21 (FGF-21), brain-derived neurotrophic factor, insulin-like growth factor, follistatin-related protein 1, erythropoietin, musclin and β-amino isobutyric acid (Stranska and Svačina, 2015 and Panati *et al.,* 2016).

Irisin is a novel hormone identified by Bostrom *et al.* (2012). It is predominately produced by slow-twitch type 1 muscle fibers which have high mitochondrial content (Zhou *et al.,* 2016), and considered as a proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) dependent myokine. PGC-1α promotes expression of fibronectin type III domain containing protein -5 (FNDC-5) via AMP activated protein kinase (AMPK) pathway (De Matteis *et al.,* 2013). It binds to the surface of white adipocytes, induces the expression of uncoupling protein-1(UCP-1) and triggers the transformation of white fat cells into brown fat cells (Sanchez-Delgado *et al.,* 2015). Browning of adipose tissue leads to dissipation of energy in the form of heat without ATP formation, enhances fatty acid oxidation and increased energy expenditure through non shivering thermogenesis (Seldin and Wong, 2012). The involvement of irisin in the browning of white adipocytes plays critical roles in improving insulin sensitivity, inhibiting the occurrence and development of obesity. It also promotes bone metabolism and enhances cognitive capacity during regulation of obesity (Chen *et al.,* 2016).

Melatonin (N-acetyl -5-methoxytryptamine) is a pineal gland hormone that is found in animals, humans and plants. It can enter body fluids as well as all cellular and sub-cellular compartments due to its amphiphilic nature. So it can display a wide range of physiological functions such as regulation of circadian rhythms, body weight and energy metabolism as well as its antioxidant and anti-inflammatory effects (Alzoubi *et al*., 2018).

The aim of the present study was to evaluate the possible effects by which melatonin and exercise can combat obesity-associated metabolic alterations.

Materials and Methods

Animals:

Fifty adult male albino rats of local strain (7-8-weeks old, weighing 130-180 g) were purchased from Helwan Farm (Cairo, Egypt). They were housed in standard cages (3 rats/25X30X30 cm cage), under specific pathogen-free conditions in facilities maintained at controlled room temperature (21-24°C) and under normal dark–light cycles. All animals had free access to rat chow diet and water *ad libitum* and were acclimated for two weeks prior to initiation of the experiment in the laboratory in the National Research Centre. All procedures were approved by the Animal Care Committee of the National Research Centre. The "Principles of laboratory animal care" were followed, and also the specific national laws were applicable.

Drugs:

Melatonin was purchased as capsules from Puritan´s Pride Incorporated Company, USA. It was dissolved in 0.1% ethanol solution (1 mL of absolute ethanol added to 1 L of distilled water) and prepared freshly every day according to Ewida and Al Sharaky (2016).

Diets:

Commercial rat chow diet (balanced diet), containing 65% carbohydrates, 5% crude fat, 23% crude protein, 4% vitamins and minerals and 3% fibers. The energy sources (overall calories: 3.6kcal/g). It was purchased from El Gomhorya Company (Cairo, Egypt). High fat diet (HFD), consisting of 80% of standard pellet animal diet and 20% beef tallow was prepared and used to induce hyperlipidemia. The major composition of the diets used in this study was previously characterized by Areshidze *et al*. (2015).

Experimental Design:

The rats were divided into 5 equal groups. **Group I:** Rats were assigned to normal control group and given normal balanced chow (5% fat) and supplemented orally with 1mL of 0.1% ethanol by the beginning of the 7th week till the end of experimental period. **Group II (**HFD group): Rats were given HFD (20% fat) for 14 weeks (Kubant et al., 2015**)** and orally supplemented with 1 ml of 0.1% ethanol by the beginning of the 7th week till the end of experimental period and left without exercise**. Group III (**HFD-melatonin group): Rats were kept on HFD for 6 weeks, and then were given melatonin (20 mg/kg/day) by gastric gavage (Demirtas *et al*., 2015), with HFD for 8 weeks. **Group IV (**HFD-exercise group): Rats were kept on HFD for 6 weeks. At the beginning of the 6th week of HFD intake, the rats were acclimated to exercise (5 meter/minute, for five minutes) for 1week according to Arnold and Salvatore (2014) to minimize stress. By the beginning of the 7th week, the exercise training program began at morning between 10.00 and 12.00 Am by sessions of gradually increasing speed training 40 minutes, 5 times/week (5 meter/minute for 5 minutes increased to 12 meter/minute for 5 minutes, and finally 18 meter/minute for 30 minutes, once daily, 5 times per week) for 6 weeks according to Bae *et al.* (2016) which were continued with HFD intake. **Group V (**HFD-melatonin-exercise group): Rats received HFD for 6 weeks with acclimation to the exercise (5 meter/minute, for five minutes) by the beginning of 6th week for one week. On the beginning of the 7th week, rats received melatonin orally at a daily dose of 20 mg/kg for 8 weeks**,** and exercised by the same program of group IV to the end of 14th week.

The body weight of each rat was measured and recorded weekly for all groups. Hyperlipidemia was confirmed by measuring the levels of serum lipids and lipoproteins.

Adiposity index (AI) was measured as the sum of (epididymal, intraperitoneal and visceral fat pads) / final body weight × 100 (Silva *et al*., 2014).

At the end of the experiment, after overnight fasting, rats were anesthetized in the morning, and blood samples were collected from retro-orbital venous plexus by capillary tubes under light ether anesthesia. The blood was then centrifuged at 3000 rpm for 15 minute for serum collection. Serum was separated in aliquots in Eppendorf tubes and stored frozen at -20°C until analysis. The separated serum was analyzed for estimation of the levels of lipid profile, oxidative stress markers, inflammatory markers and irisin hormone. The abdominal adipose tissues were excised for the measurement of gene expression of adipose tissue uncoupling protein -1 (UCP-1), PGC-1 α and CD-137.

Biochemical analysis:

Total serum cholesterol and HDL-c were measured by quantitative enzymatic colorimetric determination using biomed diagonstic assay kits (Flegg 1973 and Lippi *et al.* 1988). Serum triglycerides were measured by quantitative enzymatic colorimetric determination using Cayman colorimetric assay kit (Mc Gowan *et al*., 1983). Serum LDL-c was calculated from the values of total cholesterol (TC), HDL-c and triglycerides using Friedewald equation: LDL-c (mg/dl) = TC - HDL-c – TG/5 (Friedewald *et al*., 1972). Serum reduced GSH was determined using Cayman's GSH assay kit (Tietze, 1969). Serum MDA has been identified by reaction of thiobarbituric acid with MDA in acidic medium at temperature of 95 °C for 30 minutes to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at wave length 534 nm (Ohkawa *et al*., 1979). Serum IL-6 was measured by commercial ELISA kits (Ray Bio® Rat, Ray Biotech, Norcross, GA, USA) (Van Snick, 1990). Serum Irisin was measured by commercial ELISA kits (Bostrom *et al.,* 2012).

Detection of uncoupling protein-1 (UCP-1), PGC-1α and CD-137 Gene expression in abdominal adipose tissue:

RNA was extracted, reversely transcribed into cDNA and amplified by PCR and then detected using agarose gel electro-phoresis (Overbergh *et al*., 2003).

Quantitative real-time reverse transcription PCR analysis:

Total RNA was extracted from the tissue using TRIzol Reagent (Invitrogen, San Diego, California, USA).The concentration of total RNA was measured by absorbance at 260/280nm. The reverse transcription reaction for the first-strand cDNA synthesis was carried out with reverse

transcriptase (Bio-Rad, Hercules, California, USA) using 2µg of total RNA. Real-time PCR was initiated on a Step One Plus Real time**.** PCR System (ABI Applied Biosystems, San Francisco, USA) using Power SYBR Green PCR Master Mix (Pfaffl, 2001).

Statistical Analysis:

All values are presented as means ± standard deviation of the means (SD). Comparisons between different groups were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni Post Hoc test for multiple comparisons. Graphpad Prism software, version 5 (Inc., San Diego, USA) was used to carry out these statistical tests. The difference was considered significant when $p < 0.05$.

Results

Effect of Melatonin and/or Exercise on body weight, adiposity index and serum lipid profile :

High fat diet for 14 weeks induced a robust increase in body weight by 44.8% and adiposity index by 85.6% in comparison to normal control rats. Body weight increase was ameliorated upon melatonin administration, exercise and their combination by 25.4%, 28.1% and 30.2% respectively, in addition to the adiposity index by 35%, 39.4% and 42.4% respectively as compared to HFD group .

Furthermore, the induced dyslipidemia in HFD group was presented by a significant elevation of TC and TAG by about 0.5 and 0.6 folds, respectively, and decrease in HDL-c by a 0.6 % as compared to normal control. Notably, melatonin administration, exercise or a combination of both successfully ameliorated the afore-mentioned deleterious effects as compared with HFD group (Table 1)**.**

Table 1: Effect of melatonin and/or exercise on body weight, adiposity index and serum lipid profile.

Groups / Parameters	Normal control	HFD	HFD + Melatonin	HFD + Exercise	HFD + Melatonin + Exercise
Body weight(g)	112.67±6.37	163.17 ± 3.31[a]	121.67 ± 9.0[b]	117.33 ± 6.77[b]	113.83± 5.49[b]
Adiposity index(g/100g BW)	2.43±0.50	4.51±0.53[a]	2.93±0.57[b]	2.73±0.54[b]	2.60±0.74[b]
Total cholesterol (mg/dL)	141.10 ±13.01	218.35 ± 20.40 [a]	173.53 ± 12.53 [ab]	180.13 ± 11.90 [ab]	162.40 ± 7.78[b]
HDL-c (mg/dL)	61.60 ± 4.44	25.58 ± 4.84 [a]	43.15±3.47 [ab]	38.46±4.82 [ab]	51.96±3.52 [abcd]
Triglyceride (mg/dL)	75.35 ± 3.86	116.93±5.67 [a]	89.88±.6.83 [ab]	92.71±8.29 [ab]	75.91±4.69 [bcd]
LDL-c (mg/dL)	64.36 ±10.89	169.33±16.09 [a]	110.80 ±11.70 [ab]	123.23 ± 9.97 [ab]	95.20±5.86 [abd]

[a] Significantly different from normal control (ethanol) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + melatonin, [d] significantly different from HFD + exercise at *P<0.05*.

Effect of melatonin and/or exercise on oxidative stress, inflammation and serum irisin level:

MDA levels elevated after HFD supplementation by about 29 fold and GSH level showed reduction by about 0.6 fold. These effects were associated with disturbance in the pro-inflammatory markers by causing a prominent elevation in serum levels of pro-inflammatory marker IL-6, by about 4 fold as compared to normal control. On the other hand, melatonin and/or exercise administration showed a significant decline in MDA and IL-6 serum levels and elevation in GSH activity as compared with HFD group. In addition, HFD showed marked reduction in serum irisin level by a 0.8 fold from the control level. Conversely, exercise and its combination with melatonin administration, concomitantly with HFD increased serum irisin level by a 2.1 and 2.2 folds respectively, while melatonin administrated with HFD did not change serum irisin level as compared with HFD group (Table 2).

Table 2: Effect of melatonin and/or exercise on serum reduced glutathione, malondialdehyde, interleukin-6 and irisin.

Groups / parameters	Normal Control	HFD	HFD+ Melatonin	HFD+ Exercise	HFD + Melatonin+ Exercise
GSH (µM)	72.65±2.88	26.71± 4.98[a]	47.48±3.94[ab]	41.03 ±4.88 [ab]	55.56±4.01[abcd]
MDA (nmol/mL)	1.19±0.23	35.90±7.35[a]	12.93±3.49 [ab]	16.23±2.99 [ab]	7.31±1.68 [bd]
IL-6 (pg/mL)	28.28±4.23	141.48±9.57[a]	83.33±13.9 [ab]	95.40±7.86 [ab]	56.65±8.05 [abcd]
Irisin (µg/mL)	2.44±1.13	0.59±0.17[a]	1.03±0.26[a]	1.86±0.56[b]	1.87±0.70[b]

[a] Significantly different from normal control (ethanol) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + melatonin, [d] significantly different from HFD + exercise at *P<0.05*.

Effect of melatonin and/or exercise on adipose tissue UCP-1, PGC-1α and CD-137 gene expression:

HFD produced a significant reduction of the browning marker UCP-1 gene expression by 56%, PGC-1α by 76% and beige fat-specific marker CD-137 gene expression by 78% in adipose tissue compared to their respective controls. On the other hand, melatonin administration, exercise and a combination of both concomitantly with HFD increased UCP-1 expression by a 0.8, 1.06 and 1.5 folds, respectively, and PGC-1α expression by a 1.6, 1.1 and 2.4 folds respectively, as well as increased CD-137 expression by a 0.9, 1.5 and 1.9 folds, respectively, as compared with HFD group **(Figure 1).**

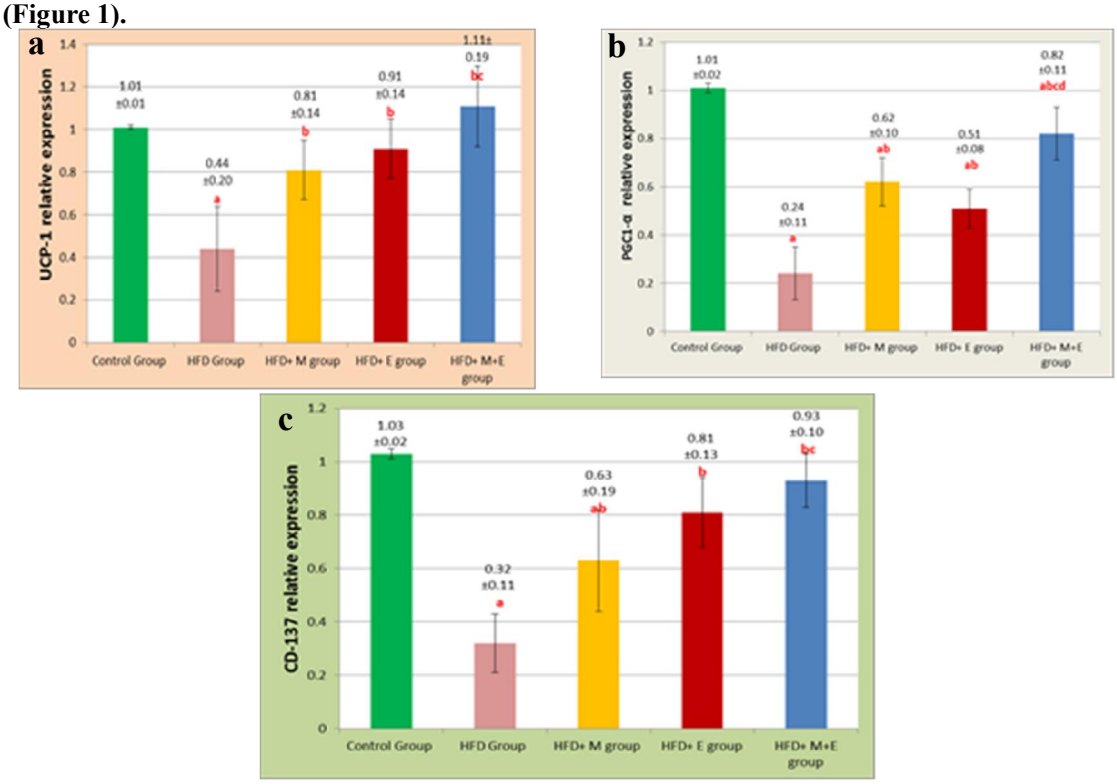

Fig. 1: Effect of melatonin and/or exercise on adipose tissue UCP-1 (a), PGC-1 (b) and CD-137 gene expression (c).

[a]Significantly different from normal control (ethanol) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD + melatonin, [d] significantly different from HFD + exercise at *P<0.05.*

Histopathologiacl results:

HFD produced a significant elevation in the adipocyte cell diameter by 64.6% compared to their respective controls. On the other hand, melatonin administration, exercise and a combination of both concomitantly with HFD significantly decreased adipocyte diameter by 29.7 %, 26.9 % and 62.1 %, respectively compared with HFD group (Table 3 & Figure 2).

Table 3: Effect of melatonin and/or exercise on epididymal adipocyte cell diameter.

Groups parameters	Normal Control	HFD	HFD+ Melatonin	HFD + Exercise	HFD + Melatonin+ Exercise
Adipocyte cell diameter (µm)	67±10.1	110.3± 6.4 [a]	77.5±9.4 [b]	80.6±5.8 [b]	41.8±5.2 [abcd]

[a] Significantly different from normal control (ethanol) at *P<0.05*. [b] Significantly different from HFD control, [c] significantly different from HFD +melatonin, [d] significantly different from HFD + exercise at *P<0.05*.

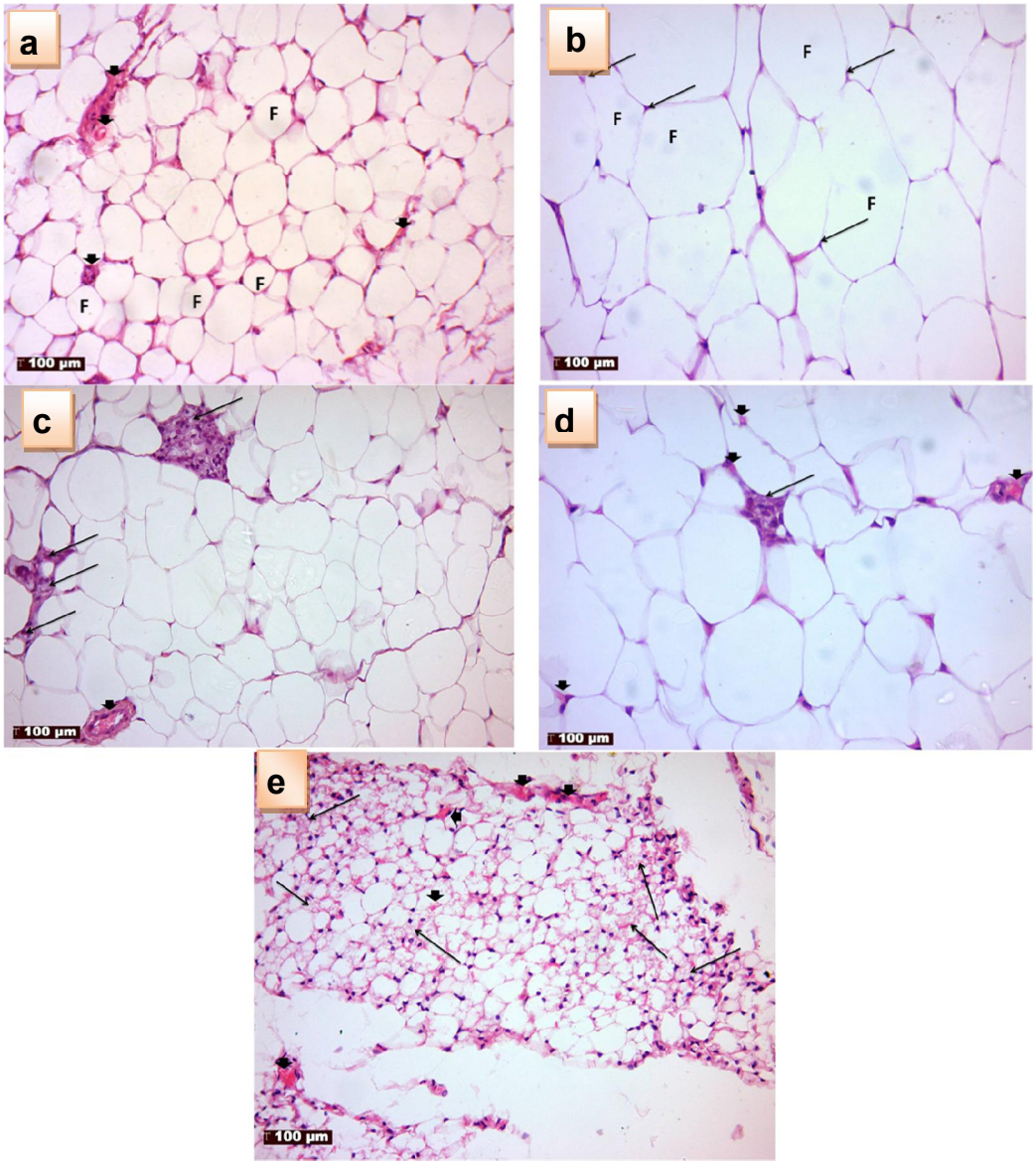

Fig. (2): Histological and morphometric illustration of epididymal adipose tissue in:
(a) Normal control group showing the showing the empty signet ring appearance of the adipocytes (F) with peripheral flat nuclei (arrows). **(b) HFD group**, the adipocytes (F) appeared polyhedral in shape and apparently larger than that of the control with the peripheral flat nuclei (arrows).
(c) HFD/Melatonin group, the adipocytes are smaller with appearance of some multilocular adipocytes (arrows) among them.
(d) HFD/Exercise group, notice the apparently decrease in the size of the adipocytes than the HFD group with multiple blood vessels (arrow head) and appearance of multilocular cells (arrows).
(e) HFD/ Melatonin /Exercise group, notice the many multilocular adipocytes (arrows) and the high vascularity (arrow head) in between the adipocytes of WAT (H&E stain. All ×200).

Discussion

Current drug treatments for obesity produce small and usually unsustainable decrease in body weight with the risk of major adverse effects. Because all medications have more risks than diet and exercise, pharmacological therapy should be used only in patients in whom the benefits justify the risk (de Melo *et al*., 2017). These have aroused interest in studying natural hormones as melatonin, as a potential irisin inducer, and its combination with chronic treadmill exercise as an agent that could stimulate the adipose tissue browning for correction of obesity.

In the present study, fourteen weeks supplementation of rats with high fat diet (20% fat) induced a significant elevation of body weight and adiposity index. This elevation was in agreement with previous studies of Ahn & Go (2017) and Marics *et al.* (2017) who showed that HFD increase the body weight significantly. de Melo *et al. (2017)* explained this elevation to activation of an inflammatory response in the hypothalamus, disturbing the anorexigenic and thermogenic signals generated by the hormones especially leptin. Another explanation was reported by Vaughn *et al.* (2015) who stated that HFD changes the composition of gut microbiota by decreasing the abundance of Bifidobacterium and Lactobacillus gasseri, which are known to have beneficial effects through suppression of dietary fat absorption.

Chronic regular treadmill training exercise five times/week for eight weeks to rats fed on HFD significantly reduced the body weight and adiposity index. These results were in consistence with the study of Zhao *et al.* (2011) who observed that treadmill exercise significantly reduce the body weight, and explained this reduction to that exercise training could improve appetite control and reduce leptin resistance by different mechanisms includes; improvements in hypothalamic leptin signaling and reduction on fat pad mass and increase in leptin receptors on the liver and the vascular smooth muscle. Melatonin supplementation induced significant decrease of body weight and adiposity index in the present study. This reduction was supported by the study of Rios-Lugo *et al.* (2015) who attributed this decrease to that concomitant administration of melatonin with the HFD counteracted the augmented expression of hypothalamic genes encoding for feeding behavior regulation such as orexigenic pathway neuropeptide Y. In addition, our work reported a decrease of body weight in exercise and melatonin combination group.

The current study showed a significant increase in the serum lipid profile level (total cholesterol, triglycerides and LDL) in HFD group, while serum HDL level significantly decreased. These results were consistent with the other studies indicating altered lipid metabolism associated with HFD intake (Tuzcu *et al.,* 2017 and Xie *et al.,* 2017). Schweiger *et al.* (2017) attributed these changes to increased FFAs and insulin resistance, as insulin resistance leads to reduction in lipoprotein lipase activity in adipocytes with increased FFAs mobilization from adipose tissue. Hyperinsulinemia increased cholesterol ester transfer protein (important in regulating lipoprotein lipid composition) and 3-hydroxy-3-methylglutaryl-coenzymeA reductase (key enzyme in cholesterol biosynthesis). Hyperinsulinemia would also increase liver production of VLDL to increase serum TAG, while at the same time reduce HDL-c. Chronic exercise for eight weeks significantly reduced the serum levels of lipid profile in exercised obese rats compared with normal sedentary rats, which is supported by the study of Wang and Xu (2017). This improvement may be attributed to increased serum levels of irisin as reported by Xiong *et al.* (2015) and Yang *et al.* (2016) that irisin increases hormone-sensitive lipase expression and phosphorylation and reduced perilipin level and adipocyte diameter in adipose tissues thus attenuates hyperlipidemia and enhances lipolysis via cAMP–PKA–HSL/perilipin pathway.

Melatonin treatment induced improvement of altered lipid profile. In agreement with these results, Agil *et al.* (2011) reported that, melatonin might inhibit the synthesis of cholesterol by modulating the macrophage activity and regulating the secretion of cytokines, such as IL-2, besides increasing the cholesterol metabolism either via a decrease of its absorption across the intestinal epithelium or by increase the conversion of cholesterol to bile acids. Another mechanism was reported by Mozaffari *et al.* (2012) stated that melatonin could lower LDL-c oxidation through its antioxidant and anti-inflammatory effects. It could also increase HDL-c by elevating cholesterol esterification and activating lecithin-cholesterol acyl-transferase. They also attributed the enhanced activity of lipoprotein lipase to suppression of visceral fat accumulation and consequently improving the insulin sensitivity. Moreover, our results showed improved lipid profile in exercise and melatonin

combination group that could be due to the synergistic effects of both melatonin and exercise especially in elevating HDL-c and decreasing TAG levels compared to each alone.

The current work assessed the major compounds involved in the down-regulation of substances formed during oxidative stress MDA, up-regulation of GSH scavenging activity (antioxidant activity), and reduction of pro-inflammatory cytokines, like IL-6, in order to investigate the effect of melatonin and exercise in obese rats. Significant elevation of serum levels of MDA and IL-6 as well as decreased serum GSH activity after fourteen weeks of HFD supplementation was observed in the present work. These results were consistent with the study of Serra *et al.* (2013) who reported that hyper-cholesterolemia diminished the antioxidant defense system, decreased the activities of antioxidant enzymes and elevated the lipid peroxide content, while the elevated serum TAG increased free fatty acids bioavailability that can increase lipid peroxidation. Moreover, intracellular TAG inhibits adenosine nucleotide translocator, leading to ATP accumulation in mitochondria. As a result of ATP accumulation, the mitochondrial ADP drop and the speed of oxidative phosphorylation is reduced. This mitochondrial uncoupling promotes electron leakage and free radical release (Savini *et al.,* 2013).

On the other hand, de Melo *et al.* (2017) observed that HFD induced marked elevation of the inflammatory markers in mice and explained this elevation by the greater number of epididymal fat cells with an increase in their size and subsequent macrophage accumulation as evidenced by increased the mRNA expression of macrophage surface markers CD-68 and F4/80 in adipose tissue of HFD fed mice. Chronic exercise to HFD supplemented rats in the current study induced correction of oxidative stress markers and inflammatory marker (IL-6). This was in agreement with Mardare *et al. (2016)* who stated that exercise-induced elevation in adipocyte PGC-1α gene expression, which in turn enhances β-oxidation and mitochondrial biogenesis, allowing for greater lipid oxidation per mitochondrion. Improved mitochondrial β-oxidation reduces oxidative stress. Melatonin supplementation reduced oxidative stress and adipose inflammation. These results were explained by Najafi *et al.* (2017) who stated that melatonin could suppress the inducible NO synthase enzyme expression, through inhibition of signal transducer and activator of transcription-1 (STAT-1) signaling, with subsequent decreases in NO production. A concomitant administration of melatonin with exercise could produce a synergestic effect especially in improving GSH and IL-6 serum levels compared to each alone.

In the present work, the irisin serum levels significantly reduced in HFD treated rats. This reduction was corrected significantly by exercise. The study of Schaalan *et al.* (2018) showed that irisin levels decreased with obesity. Yang *et al.* (2015) explained this reduction by down-regulation of FNDC5/irisin expression in adipose tissues observed in HFD-induced obese mice. The elevated serum levels with exercise could be attributed to exercise induced improvement of lipid profile and oxidative stress as reported by Hou *et al.* (2016). Tsuchiya *et al.* (2015) observed that the effect of exercise on irisin level depends on exercise intensity and type. High-intensity exercise has greater improvement on irisin level than low-intensity exercise. In addition, resistance exercise causes more elevation in circulating irisin level compared to continuous moderate- intensity exercise (Huh *et al*., 2015). The adipocyte expression of the browning markers UCP-1, PGC-1α and the beige fat-specific marker CD-137 in the present study significantly reduced in obese rats, and elevated by exercise and melatonin interventions, either individually or together. The present findings were supported by those of Tanaka-Yachi *et al.* (2017) who reported that the gene expression levels of UCP-1 and CD-137 increased with agents that promote thermogenic adipocyte differentiation in mammalian white adipose tissues as the α-tocopherol. Exercise causes an increase in energy expenditure through augmentation in brown fat and the browning of white fat (Boström *et al.,* 2012). Melatonin supplementation to obese rats couldn't significantly increase the serum irisin level which might be explained Jimenez-Aranda *et al.* (2013) who proved the inability of melatonin to increase the spontaneous locomotor activity of animals. Although, melatonin could increase expression of the beige fat-specific marker CD-137 and the browning marker UCP-1 and PGC-1α, which was explained Venegas *et al.* (2012) who reported that melatonin increased the activity of type 2 thyroxin 5′-deiodinase to increase the intracellular triiodothyronine. Melatonin supplementation to obese exercised rat induced a synergetic elevation the genes expression, especially PGC-1α, but not in serum irisin level. These findings were supported by that of During *et al*. (2015) who revealed that the beiging and thermogenic gene expression by

exercise training are through increasing hypothalamic brain-derived neurotrophic factor secretion which increases of WAT sympathetic output.

Conclusion

Browning of WAT and thermogenesis promoted in rats with diet-induced obesity by melatonin supplementation and exercise through increased expression of the brown fat marker UCP-1 and PGC-1α in all types of adipose tissues, together with activation of CD-137-positive beige fat formation of WATs which promoted additional browning in BAT. This suggested that targeting a white adipose tissue in obesity by melatonin supplementation provide an effective therapeutic option for combating obesity and its related metabolic disorders.

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