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INFLAMMATORY MARKERS AS A POTENTIAL LINK BETWEEN INSOMNIA AND METABOLIC SYNDROME IN AGED FEMALE RATS: IMPACT OF SLEEP RECOVERY ON IL-6

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ABSTRACT

Background: Insufficient sleep is a significant public health problem that profoundly impacts metabolic health. The prevalence of insomnia is higher in women, and it increases with age.

Objectives: The present study aimed to evaluate the metabolic dysfunctions associated with 4 weeks of sleep restriction in aged female rats and to find out the possible mechanisms linking these metabolic ailments to such sleep disturbance. In addition, the potential metabolic recovery following the retrieval of regular sleep for one and two weeks was assessed.

Material and Methods: The study involved 52 aged female rats divided into two main groups: ***Insomnia group (I),*** subjected to mild sleep restriction program for 4 weeks, using the modified multiple platform method (MMPM), and ***Control group (C),*** which was not subjected to insomnia. At the end of the 4th week of the study, rats were further subdivided into 3 subgroups: **Group (I_a):** 4-week insomnia, **Group (I_b):** 4-week insomnia/1-week recovery, and **Group (I_c):** 4-week insomnia/2-week recovery, and their control groups: **(C_a), (C_b) and (C_c)** sacrificed at the end of the 4th, 5th and 6th weeks respectively, with their corresponding insomniac groups.

All rats were subjected to measurement of body weight, body mass index, and waist circumference (WC). Fasting blood glucose level and intraperitoneal glucose tolerance test

were assessed. Serum was subjected to determination of insulin, glucose, lipid profile, adiponectin, interleukin 6 (IL-6), and active caspase-3. In addition, insulin resistance (HOMA-IR) was calculated.

Results: Four-week insomnia caused a metabolic dysfunction presented by the four criteria of metabolic syndrome (fasting hyperglycemia, hypertriglyceridemia, increased WC, and decreased high-density lipoprotein cholesterol), together with insulin resistance. In addition, insomniac rats displayed a significant increase in IL-6 and caspase-3, and significantly low adiponectin. Moreover, IL-6 was significantly correlated with parameters of the metabolic syndrome. Reversion to regular sleep elicited partial improvement of metabolic dysfunction, apoptotic marker caspase-3, as well as the inflammatory marker IL-6 in a time-dependent manner, with normalization of serum adiponectin

Conclusion: Chronic, mild to moderate sleep restriction has serious metabolic, inflammatory, and apoptotic consequences, whose full recovery to normal values was not ensured for all the tested parameters after two weeks of reversion to normal sleep. Inflammation is suggested as the mechanistic link between insomnia and the metabolic syndrome observed in this work.

Keywords: Insomnia, Metabolic syndrome, Sleep recovery, Inflammatory markers

INTRODUCTION

Sleep is a vital physiological process essential to human health (***Irwin, 2015***). A significant portion of the population, about 30 - 40% of adults, does not get the recommended at least 7 hours of sleep each night (***Itani et al., 2017 and Kocevska et al., 2021***). A common cause of insufficient sleep is insomnia, which is generally defined as dissatisfaction with sleep either in quantity or quality (***Patel et al., 2018***). The likelihood of developing chronic insomnia tends to increase with age, and it is observed more frequently in women than in men ***(Léger et al., 2008 and Riemann et al., 2022***).

This concerning trend of insomnia has coincided with a notable increase in the development of glucose dyshomeostasis, insulin resistance, and obesity (***Antza et al., 2021 and Reutrakul & Van Cauter, 2018***). Despite the high prevalence of insomnia, its relationship with metabolic syndrome remains unclear (***Syauqy et al., 2019***). Some research linked insomnia to metabolic syndrome (***Chen et al., 2015***

and Lin et al., 2016), while others did not (***Troxel et al., 2010 and Chedraui et al., 2013***).

Metabolic syndrome is claimed to be related to chronic low-grade inflammation (***Reilly and Saltiel, 2017***). Many epidemiological studies have investigated the association between sleep and inflammation. Some demonstrated a significant association between short sleep duration and increased inflammatory markers, while others failed to demonstrate such relationship (***Besedovsky et al., 2019***). Moreover, increased expression of pro-inflammatory cytokines IL-6 and TNF-α has been demonstrated in the white adipose tissue of rats subjected to REM sleep deprivation (***Rosa Neto et al., 2010***). However, the mechanism linking insomnia with metabolic syndrome and inflammation is not fully understood (***Syauqy et al., 2019***).

Despite of abundance of literature that examined the impact of sleep restriction on glycemic homeostasis, there is a scarcity of studies investigating the metabolic consequences of chronic, mildly insufficient sleep in elderly females. To the best of our knowledge, the possible reversibility of sleep deprivation-induced metabolic changes, through returning to normal sleep, was not previously studied using elderly female experimental animals. Therefore, the present study was planned to investigate the metabolic dysfunctions associated with chronic mild insufficient sleep, and to find out the possible link of such a relationship in aged female rats using a mild sleep restriction protocol implemented over 4 weeks. Another goal of this study was to examine the potential for metabolic recovery upon restoring normal regular sleep in these rats.

MATERIALS AND METHODS

Experimental animals:

The current study involved 52 female albino rats of local strain, aged 24-26 months. Reproductive senescence was confirmed in a random sample of the female rats by assessment of serum FSH levels as well as vaginal smears, reflecting a state similar to menopause in humans. Rats were purchased from the Vacsera Experimental Animal Facility (Helwan, Egypt) and were subsequently housed at the Medical Ain Shams Research Institute (MASRI), located at Ain Shams University. Animals were maintained under standard boarding conditions, with ambient room temperature and natural light/dark cycle. Rats were kept in plastic

cages (four rats/cage) for two weeks for acclimation. The rats were provided with a regular diet consisting of bread, milk, and vegetables with free access to water. The current study received approval from the Research Ethics Committee at the Faculty of Medicine, Ain Shams University (The FMASU REC) that operates under Federal Wide Assurance No. FWA 000017585; (*Approval Number: FMASU MS 418/2021*).

Animal grouping:

The rats were divided into two main groups: ***Insomnia group*** (I, n=26) and ***Control group*** (C, n=26).

All rats in the insomnia group were exposed to the same sleep restriction program: 5 hours daily, 5 days/week for 4 weeks. At the end of the 4th week of the study, rats were further subdivided into the following 3 subgroups: **Group (I_a)**: 4-week insomnia group (n=10): This group was subjected to sleep restriction, **Group (I_b)**: 4-week insomnia/1-week recovery group (n=8): Rats were subjected to 4-week sleep restriction, followed by return to regular sleep for 1 week, and **Group (I_c)**: 4-week insomnia/2-week recovery group (n=8) in which sleep restriction was followed by return to regular sleep for 2 weeks.

Control rats were not subjected to sleep restriction. At the end of the 4th week of the study, rats were subdivided into the following 3 subgroups: **Group (C_a)**: 4-week control group (n=10), sacrificed at the end of the 4th week of the study with I_a group, **Group (C_b)**: 5-week control group (n=8), sacrificed at the end of the 5th week of the study with I_b group and **Group (C_c)**: 6-week control group (n=8), sacrificed at the end of the 6th week of the study with I_c group.

Induction of insomnia

Insomnia was induced using the modified multiple platform method (MMPM), as described by ***Machado et al. (2004)*** and ***Oh et al. (2012),*** with slight modifications. Animals were deprived of sleep 5 hours daily (08:00-13:00), 5 days weekly over a period of 4 consecutive weeks.

This method involved the use of transparent plastic tanks (40×25×25 cm^3), containing four circular pottery plates (10 cm in diameter) that were used as platforms and were placed at the four corners of the tank. The tank was filled with water to a level 1 cm below the platform surface. Rats

in the insomniac group were placed on these platforms. When rats enter the paradoxical phase of sleep, muscle atonia occurs, causing the rats to contact water, therefore the rats were abruptly awakened. The rats were monitored to avoid accidental injury. The water in the tank was changed daily throughout the period of the experiment.

Control rats were placed in similar plastic tanks on wider platforms (16 cm in diameter), present at each side of the tank, without adding water, so they could sleep well on them. After the 5-hour experimentation period, the animals were taken back to their home cages.

Experimental Studies:

All rats were subjected to estimation of zoometric and body temperature measurements as well as intraperitoneal glucose tolerance test (IPGTT) both at the outset and at the end of the 4th week of the study before randomizing the insomniac rats (I) into three insomniac rat groups (I_a, I_b, Ic), and the control rats into three control rat groups (C_a, C_b, C_c). Thereafter, at the end of the experimental period of each group, the same measurements were obtained again, and blood was collected for later biochemical assay. Afterward, visceral fat was collected.

Experimental procedure:

At the end of the experimental period of each group, rats were subjected to IPGTT one day before sacrifice. On the day of sacrifice, overnight fasted rats were weighed and anaesthetized by intraperitoneal injection of thiopental sodium, in a dose of 40 mg/kg. When the stage of surgical anaesthesia had been reached, the animal was placed on its back and fixed on the dissecting table, and body length, waist circumference, and body temperature were measured. Retro-orbital venous blood was collected and allowed to clot at room temperature, then centrifuged for serum collection. Immediately after collecting the blood samples, an abdominal incision was made to collect visceral fat. Separated serum was immediately used for determination of serum glucose, and the remaining serum was divided into small aliquots and stored frozen at -20 °C for later biochemical assay of insulin, triglycerides (TGs), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), interleukin 6 (IL-6), adiponectin and active caspase-3. In addition, serum glucose and insulin were involved in the calculation of the homeostasis model assessment for insulin resistance (HOMA-IR).

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- **Zoometric measurements:** Body weight and body length were utilized for the calculation of body mass index (BMI), according to the following equation: Body mass index (BMI) = Body weight (gm)/ length (cm^2) **(*Bernardis, 1970*)**. Waist circumference (WC) was carefully measured according to ***Panchal et al. (2011)***.

- **Body temperature measurement:** Body temperature was measured using a digital thermometer with LCD screen (**Sure Qualicare**) that was inserted into the anus until the start of pep sound.

- **Estimation of intraperitoneal glucose tolerance test (IPGTT):** The glucose tolerance testing was performed according to ***Zhang et al. (2008)*** using D-Glucose stock (Anhydrous glucose A.R) supplied by El-Nasr Pharmaceutical Chemicals. The rats were given intraperitoneal glucose (2 gm/kg) after a 12-h fasting period. Blood samples were taken from the tip of the tail at 0, 30, 60, 90, and 120 min after glucose injection. Then, samples were tested with a portable blood glucose monitoring system (GlucoDr.auto™A, All Medicus Co., LTD., Republic of Korea). The highest value of blood glucose in the IPGTT of each rat was selected to be used in the calculation of peak glycaemia (PG). Calculation of the area under the curve of IPGTT (AUC) was done by adding the areas under the graph between each pair of consecutive observations (***Matthews et al., 1990***).

- **Measurement of visceral fat weight:** Visceral fat (retroperitoneal and gonadal fat) was excised and dried with filter paper, then freshly weighed using a 5-digit precision Metler balance (AE163). The results were expressed as absolute values (gm) and relative values to the body weight (absolute tissue weight/body weight).

- **Estimation of serum glucose, insulin, and HOMA-IR:** Fasting glucose was determined in serum, based on the enzymatic colorimetric method described by ***Trinder (1969)*** using a kit supplied by **EGY-CHEM** for lab technology. Fasting serum insulin was estimated by an enzyme immunoassay (ELISA) using kits supplied by **RayBio®**, Inc., USA for the quantitative measurement of rat insulin. The homeostasis model assessment for insulin resistance (HOMA-IR), which is an indication of basal insulin sensitivity was calculated using fasting serum insulin and glucose (HOMA-IR = [fasting insulin (μU/mL) x fasting glucose (mmol/L)]/22.5) ***(Matthews et al., 1985)***.

- **Estimation of serum lipid profile:** Serum TG estimation was carried out by an enzymatic colorimetric method according to ***Fossati and Prencipe (1982)***, using BioMed-Triglycerides kits supplied by **EGY-CHEM** for lab technology. Total cholesterol was estimated by a quantitative colorimetric method, using EnzyChrom™ AF cholesterol assay kit (E2CH-100) supplied by **BioAssay Systems**, Hayward, CA, USA. Serum HDL-Cholesterol was determined by a direct enzymatic colorimetric method, using BioMed-HDL cholesterol kits supplied by **EGY-CHEM** for lab technology. LDL-Cholesterol was calculated by subtracting both levels of HDL-Cholesterol, and one fifth of triglycerides from the level of total cholesterol ***(Friedewald et al., 1972)***.

- **Determination of serum IL-6, adiponectin, and active caspase-3:** Quantitative determination of rat IL-6, adiponectin, and active caspase-3 concentrations in serum was performed, using **ELISA** kits supplied by **Quantikine®,** Inc., USA for IL-6 and **MyBioSource®,** Inc., USA for adiponectin and active caspase-3.

Statistical analysis:

Statistical analysis was performed using the Statistical Package for the Social Science (SPSS Inc., Chicago, IL, USA) version 20. Data were presented as Mean ± Standard error of the mean (SEM). Statistical significance for differences between groups was determined using Student's t-test for paired and unpaired data. Correlations were determined by linear regression analysis using the Least Square method. A p-value less than or equal to 0.05 was considered statistically significant.

RESULTS

Changes in body weight (BW), body mass index (BMI), waist circumference (WC), and body temperature (Temp) in control (C) and insomnia (I) rat groups (Table 1).

The initial values of BW, BMI, WC, and temp were insignificantly different between the control (C) and the insomnia rat groups (I).

At the end of the 4th week, the insomnia rat group (I) exhibited a significant increase in their BW, BMI, WC, and temp compared to their initial values. On the other hand, the control (C) group showed insignificant difference in these parameters compared to their initial values. Upon comparing the 4th week values in the insomnia group (I) with the control group (C), insomniac rats displayed significantly higher values of BW, BMI, and WC, with an insignificant difference in body temperature.

Table (1): Results of the initial and 4th week values of body weight (BW), body mass index (BMI), waist circumference (WC), and body temperature (Temp) in control (C) and insomnia (I) rat groups.

	BW (gm)		**BMI (gm/cm^2)**		**WC (cm)**		**Temp (ºC)**	
	Initial	**4th week**	**Initial**	**4th week**	**Initial**	**4th week**	**Initial**	**4th week**
(C) (*n=26*)	226.8 ± 3.4	242.9 ± 4.1	0.51 ± 0.08	0.51 ± 0.01	14.52 ± 0.13	15.12 ± 0.16	35.53 ± 0.24	36.22 ±0.23
P*		***NS***		***NS***		***NS***		***NS***
(I) (*n=26*)	231.08 ± 3.1	265.9 ± 5.1	0.48 ± 0.01	0.59 ± 0.01	14.81 ± 0.17	15.7 ± 0.2	36.07 ± 0.22	36.72 ± 0.14
P*		***< 0.001***		***< 0.001***		***< 0.005***		***< 0.05***
P#	***NS***	***< 0.05***	***NS***	***< 0.05***	***NS***	***< 0.05***	***NS***	***NS***

Data were expressed as Mean ± SEM

P: Significance from initial value, by Student's "t" test for paired data, at P ≤0.05, P#: Significance from control group value (C), by Student's "t" test for unpaired data, at P ≤0.05. NS: not significant.*

Changes in fasting blood glucose (FBG), intraperitoneal glucose tolerance test (IPGTT) in control (C) and insomnia (I) rat groups (Figure 1).

The initial values of FBG, blood glucose levels at 30, 60, 90, and 120 min were insignificantly different between the control (C) and the insomnia (I) rat groups. ***At the end of the 4th week of the study***, the control (C) rats group exhibited no significant changes in such parameters compared to their initial values. On the other hand, the insomnia (I) rats group displayed a significant increase in their FBG and all values of IPGTT at the end of 4thweek, when compared to their initial values. Upon comparing the 4th week values of the insomnia (I) group with those of the control (C) group, FBG and all values of IPGTT were significantly increased.

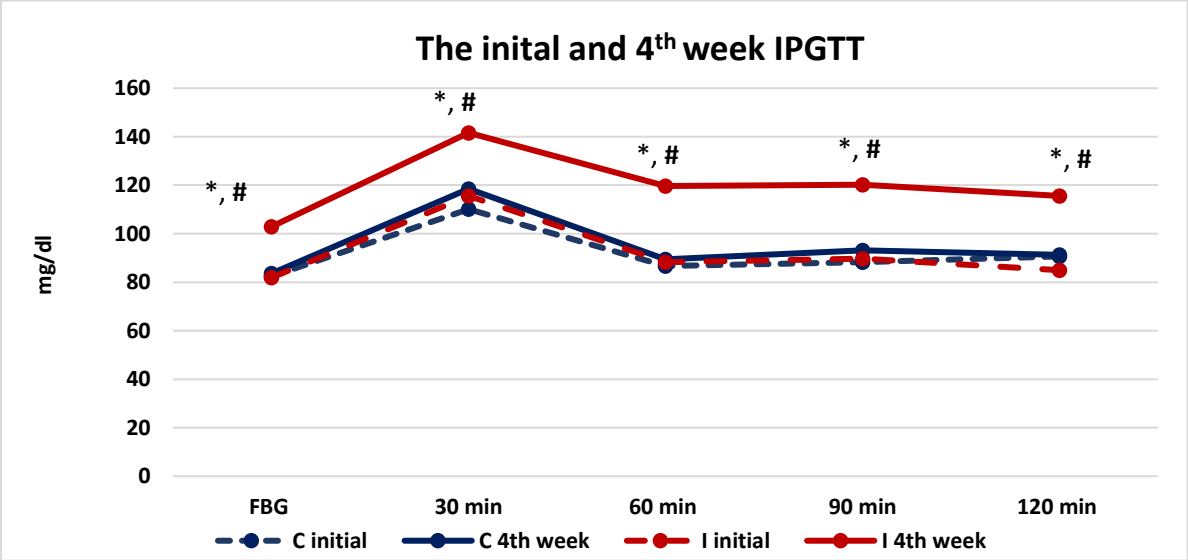

Figure (1): Graphs showing the initial and 4th week values of intraperitoneal glucose tolerance test (IPGTT) in control (C) and insomnia (I) rat groups.
**: Significance from initial value, by Student's "t" test for paired data, at P ≤0.05. #: Significance from control group value (C), by Student's "t" test for unpaired data, at P ≤0.05. NS: not significant.*

Changes in the final values of body weight (BW), body mass index (BMI), waist circumference (WC), body temperature (Temp) visceral fat weight (VFW) and visceral fat/body weight ratio (VFW/BW) in the studied rat groups (Table 2 & figure 3).

After four weeks of insomnia, the I_a rat group exhibited a significant increase in BW, BMI, WC, and body temp compared to their control rat group (C_a), with insignificant changes in VFW and VFW/BW ratio.

After 1 week retrieval to normal sleep, the 4-week insomnia/1-week recovery group (I_b) showed a significant decrease in VFW/BW ratio compared to the 4-week insomnia rat

group (I_a), with insignificant changes in BW, BMI, WC, body temp, and VFW. When the I_b group was compared to its matching 5-week control group (C_b), BW, BMI, and body temp were still significantly higher, with insignificant changes in WC, VFW, and VFW/BW ratio. Following two weeks of sleep retrieval (I_c), all these parameters were still insignificantly different compared to (I_a) 4-week insomniac rats; but when compared to the insomnia/one week recovery group (I_b), BMI was significantly higher, and body temp was significantly lower. When the I_c rats were compared to their matching 6-week control group (C_c), BW and BMI of I_c rats were significantly higher, with insignificant changes in WC, body temp, VFW, and VFW/BW.

Table (2): Results of the final values of body weight (BW), body mass index (BMI), waist circumference (WC), body temperature (Temp), visceral fat weight (VFW) and visceral fat weight/body weight ratio (VFW/BW) in the studied rat groups.

	BW (gm)	**BMI (gm/cm^2)**	**WC (cm)**	**Temp (ºC)**	**VFW (gm)**	**VFW/BW**
(C_a) (*n=10*)	228.6± 4.88	0.52± 0.01	14.6± 0.24	34.9± 0.4	13.6± 1.1	0.05± 0.0
(I_a) (*n=10*)	253.2± 7.54	0.57± 0.02	15.3± 0.28	36.6± 0.3	13.4± 1.2	0.05± 0.01
P_a	***<0.01***	***<0.03***	***<0.05***	***<0.002***	***NS***	***NS***
(C_b) (*n=8*)	*230*± 4.57	*0.50* ± 0.01	*14.4*± 0.24	*35.3*± 0.5	*11.7*± 1.2	0.05± 0.01
(I_b) (*n=8*)	*255*± 5.3	*0.57*± 0.01	*14.9*± 0.25	*36.8*± 0.2	10.2± 0.9	0.04± 0.01
P_{ins}	***NS***	***NS***	***NS***	***NS***	***NS***	***<0.05***
P_b	***<0.003***	***<0.000***	***NS***	***<0.01***	***NS***	***NS***
(C_c) (*n=8*)	*235*± 7.1	*0.53*± 0.02	*14.5*± 0.23	*34.9*± 0.4	*10.3*±0.9	*0.04*± 0.01
(I_c) (*n=8*)	*261.5*± 7.1	*0.59*± 0.02	*14.8*± 0.3	*35.7*± 0.4	12.4± 1.4	0.05± 0.0
P_{ins}	***NS***	***NS***	***NS***	***NS***	***NS***	***NS***
$P_{rec.1w}$	***NS***	***<0.04***	***NS***	***<0.05***	***NS***	***NS***
P_c	***<0.02***	***<0.05***	***NS***	***NS***	***NS***	***NS***

C_a: 4-week control group, I_a: 4-week insomnia group; C_b: 5-week control group, I_b: 4-week insomnia/1-week recovery group; C_c: 6-week control group, I_c: 4-week insomnia/2-week recovery group.
Data were expressed as Mean ± SEM.
Significance by Student's "t" test for unpaired data, at P ≤0.05, was used. NS: not significant.
P_a: Significance from C_a group, P_{ins}: Significance from I_a group, P_b: Significance from C_b group, $P_{rec.1w}$: Significance from I_b group, P_c: Significance from C_c group.

Changes in the final values of intraperitoneal glucose tolerance test (IPGTT), peak glycaemia (PG), area under the curve (AUC), fasting serum glucose and insulin levels, and HOMA-IR in the studied rat groups (Table 3 & figures 2 and 3).

After four weeks of insomnia, the 4-week insomnia rats (I_a) showed significant increase in their FBG, blood glucose levels at 30, 60, 90, 120 min, PG and AUC of IPGTT as well as fasting serum glucose, insulin levels and HOMA-IR compared to their 4-week control group **(**C_a) *(P < 0.001).*

After one week of recovery from insomnia, the I_b rats exhibited significantly lower values of FBG, AUC of IPGTT, fasting serum glucose and insulin levels, as well as HOMA-IR compared to the 4-week insomnia rats (I_a), with insignificant changes in blood glucose values at 30, 60, 90 & 120 min as well as PG of IPGTT. Upon comparison with their matching 5- week control group (C_b), I_b rats still had significantly higher blood glucose values at 30, 60, 90, 120 min, PG and AUC of IPGTT, serum fasting insulin and HOMA-IR, with maintenance of the improvement in fasting glucose levels (blood and serum glucose), which were comparable to control values.

After two-week recovery from insomnia, the I_c rats displayed significantly lower values of FBG, fasting serum glucose, insulin, and HOMA-IR with significantly higher blood glucose values at 90 min compared to the 4-week insomnia rats (I_a), however their blood glucose levels at 30 min & 60 min, PG and AUC of IPGTT remained comparable to the I_a rat values. When compared to the 4-week insomnia/1-week recovery rat group (I_b), only FBG was significantly lower (*P< 0.03*), while all IPGTT parameters and serum glucose, insulin and HOMA-IR were insignificantly different. Upon comparing I_c group with the matching 6-week control group (C_c), fasting blood and serum glucose levels and blood glucose level at 120 min of IPGTT were comparable to the control values, while the other parameters were still significantly higher.

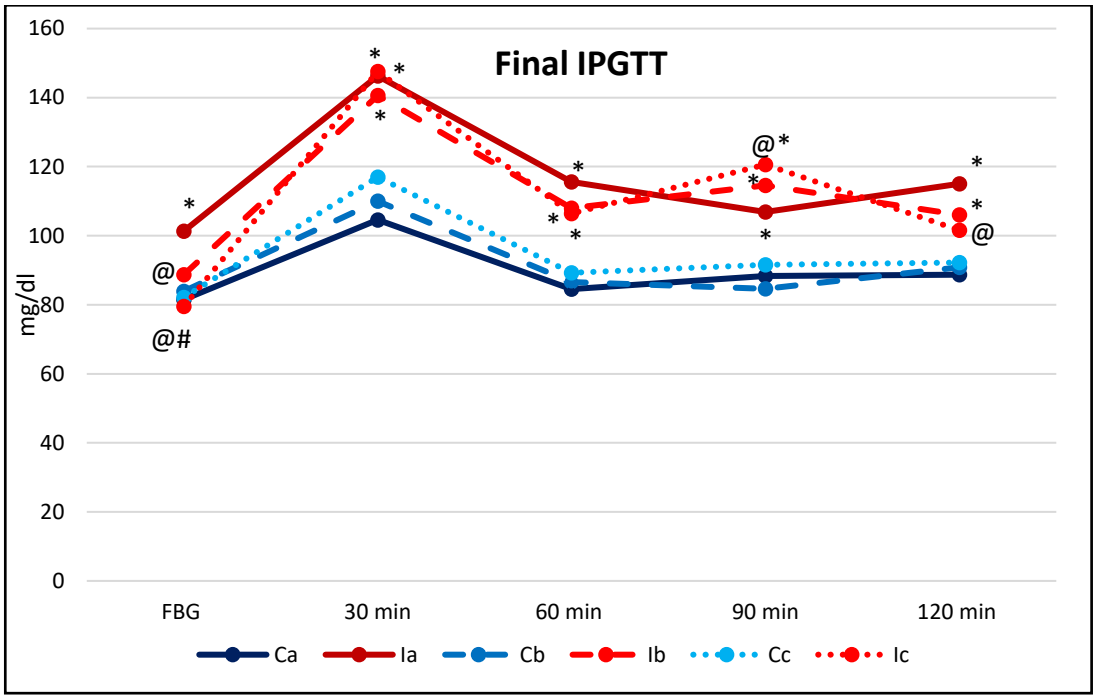

Figure (2): Final values of intraperitoneal glucose tolerance test (IPGTT) in the studied rat groups; C_a: 4-week control group, I_a: 4-week insomnia group, C_b: 5-week control group, I_b: 4-week insomnia/1-week recovery group, C_c: 6-week control group, I_c: 4-week insomnia/2-week recovery group.

Significance by Student's "t" test for unpaired data, at P ≤0.05, was used.

***:** Significance from corresponding control rat group, @: Significance from I_a rat group, #: Significance from l_b rat group

Table (3): Results of final intraperitoneal glucose tolerance test (IPGTT) showing fasting blood glucose (FBG); blood glucose levels at 30, 60, 90, and 120 minutes; peak glycaemia (PG) and area under the curve (AUC), fasting serum glucose, insulin, and (HOMA-IR) in the studied rat groups.

	FBG (mg/dl)	**30 min (mg/dl)**	**60 min (mg/dl)**	**90 min (mg/dl)**	**120min (mg/dl)**	**PG (mg/dl)**	**AUC**	**Serum glucose (mmol/ L)**	**Serum insulin (µU/mL)**	**HOMA -IR**
(C_a)	81.5 ± 2.4 (10)	104.6 ± 6.3 (10)	84.5 ± 3.4 (10)	88.3 ± 4.6 (10)	88.7 ± 3.3 (10)	108 ± 5.5 (10)	181.3 ± 5 (10)	5.2 ±0.2 (7)	6.2 ± 0.2 (7)	1.4 ±0.1 (7)
(I_a)	101.3 ± 3.4 (10)	146.3 ± 9.8 (10)	115.6 ± 4 (10)	106.9 ± 2.2 (10)	115 ± 4 (10)	148.4 ± 9 (10)	245 ± 7.4 (10)	6.3 ±0.3 (7)	18 ± 1.2 (7)	5.1 ±0.5 (7)
(P_a)	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***< 0.001***	***<0.001***	***<0.001***
(C_b)	*83.9* ± 3 (8)	*110* ± 7.8 (8)	*86.6* ± 2.8 (8)	*84.6* ± 4 (8)	*90.8* ± 4.6 (8)	112.3 ± 7 (8)	184 ± 6.8 (8)	5.1 ±0.2 (7)	6.6 ± 0.6 (7)	1.5 ±0.1 (7)
(I_b)	*88.7*± 1.1 (8)	*140.6*± 5 (8)	*108*± 2.6 (8)	*114.*± 3.5 (8)	106± 5.4 (8)	140.6 ± 5 (8)	218.5 ± 7 (8)	5.4 ±0.1 (7)	10.9± 0.5 (7)	2.6 ±0.1 (7)
(P_{ins})	***<0.003***	***NS***	***NS***	***NS***	***NS***	***NS***	***<0.004***	***<0.005***	***<0.001***	***<0.001***
P_b	***NS***	***<0.007***	***<0.001***	***<0.001***	***<0.03***	***<0.05***	***<0.003***	***NS***	***<0.004***	***<0.001***
(C_c)	*82.1*± 3.5 (8)	*117*±6. 7 (8)	*89.3*± 4.4 (8)	*91.6*± 3.4 (8)	*92.2*± 3.7 (8)	*118.2* ± 6 (8)	*192.6* ± 6.1(8)	*5.1* ±0.3 *(7)*	*6.4*± 0.3 (7)	*1.5* ±0.1 (7)
(I_c)	*79.5* ± 2.4 (8)	*147.6*± 6.1 (8)	*106.4* ± 2.8 (8)	*120.6* ± 4.4 (8)	101.6 ± 7 (8)	149.3 ± 5.7 (8)	232.7 ± 4.7 (8)	5.0 ±0.2 (7)	9.8± 0.7 (7)	2.1 ±0.1 (7)
(P_{ins})	***<0.001***	***NS***	***NS***	***<0.01***	***NS***	***NS***	***NS***	***< 0.001***	***<0.001***	***<0.001***
($P_{rec.1w}$)	***<0.03***	***NS***	***NS***	***NS***	***NS***	***NS***	***NS***	***NS***	***NS***	***NS***
(P_c)	***NS***	***<0.007***	***<0.002***	***<0.001***	***NS***	***<0.003***	***<0.01***	***NS***	***<0.001***	***< 0.05***

C_a: 4-week control group, I_a: 4-week insomnia group; C_b: 5-week control group, I_b: 4-week insomnia/1-week recovery group; C_c: 6-week control group, I_c: 4-week insomnia/2-week recovery group.

Data are expressed as Mean ± SEM. In the parenthesis is the number of observations.

Significance by Student's "t" test for unpaired data, at P ≤0.05, was used. NS: not significant.

P_a: Significance from C_a group, P_{ins}: Significance from I_a group, P_b: Significance from C_b group, $P_{rec.1w}$: Significance from I_b group, P_c: Significance from C_c group.

Changes in the final values of serum triglycerides (TGs), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), serum IL-6, adiponectin, and active caspase-3 in the studied rat groups (Table 4 & figures 3 and 4).

The 4-week insomnia (I_a) rats exhibited a significant increase in serum TGs, TC, LDL-C, IL-6, and active caspase-3 along with a significant decrease in HDL-C and adiponectin compared to their 4-week control rat group (C_a).

Following one-week reversion to regular sleep, I_b rats exhibited significantly lower values of serum TGs, IL-6, and active caspase-3 and significantly higher values of adiponectin with insignificant changes in TC, LDL-C, and HDL-C values compared to insomniac rats (I_a). When compared to their 5-week controls (C_b), serum TC, TGs, LDL-C, IL-6, and active caspase-3 were still significantly higher in I_b rats, whereas HDL-C and adiponectin were significantly lower.

After two weeks of recovery from insomnia, I_c rats showed significantly lower values of TC, TGs, LDL-C, IL-6 and active caspase-3, and a significantly higher value of adiponectin when compared to 4-week insomnia rats (I_a), with insignificant change in HDL-C values. When compared to the insomnia/1-week recovery group (I_b), the I_c rats showed significantly lower values of TC, LDL-C, IL-6 and active caspase-3 and significant increase in adiponectin, with insignificant changes in TGs and HDL-C. However, serum TGs, IL-6 and active caspase-3 were still higher in I_c rats compared to their 6-week control rat group (C_c), whereas serum HDL-C was significantly lower with insignificant change in the values of TC, LDL-C and adiponectin.

Table (4): Results of serum total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), interleukin-6 (IL-6), adiponectin, and active caspase 3 in the studied groups.

	TC (mg/dl)	**TGs (mg/dl)**	**HDL-C (mg/dl)**	**LDL-C (mg/dl)**	**IL-6 (pg/ml)**	**Adipo-nectin (ug/ml)**	**Active caspase 3 (ng/ml)**
(C_a) (*n=7*)	151.3 ± 4.6	102.6 ± 4.2	60.4 ± 2.4	70.3 ± 4.5	65.8 ± 3.5	39.4 ± 1.4	1.4 ± 0.15
(I_a) (*n=7*)	198.4 ± 7.1	173 ± 6.8	40.0 ± 1.9	123.7 ± 8.5	219.6 ± 7.5	8.0 ± 0.6	9.0 ± 0.5
(P_a)	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
(C_b) (*n=7*)	*152.0* ± 3.7	*102.7* ± 2.8	*59.7* ± 2.2	*71.7* ± 5.0	*58.0* ± 2.0	40.6 ± 3.7	1.4 ± 0.1
(I_b) (7)	*183.4* ± 3.6	*125.0* ± 2.6	*46.0* ± 2.4	*112.7* ± 5.7	173.0 ± 6.7	29.0 ± 2.4	6.2 ± 0.5
(P_{ins})	***NS***	***<0.001***	***NS***	***NS***	***<0.001***	***<0.001***	***<0.001***
P_b	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.01***	***<0.001***
(C_c) (*n=7*)	*149.6* ± 4	*101.0* ± 4.1	*61.6* ± 2.2	*67.8* ± 3.3	*63* ± 3.8	*40.0* ± 4.3	*1.5* ± 0.2
(I_c) (7)	*149.5* ± 7.5	*124.3* ± 4	*46.0* ± 3.1	*78.7* ± 7.7	133.0 ± 10.0	37.8 ± 3.4	3.6 ± 0.4
(P_{ins})	***<0.001***	***<0.001***	***NS***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
($P_{rec.1w}$)	***<0.001***	***NS***	***NS***	***<0.001***	***<0.001***	***<0.04***	***<0.001***
(P_c)	***NS***	***<0.001***	***<0.001***	***NS***	***<0.001***	***NS***	***<0.001***

C_a: 4-week control group, I_a: 4-week insomnia group; C_b: 5-week control group, I_b: 4-week insomnia/1-week recovery group; C_c: 6-week control group, I_c: 4-week insomnia/2-week recovery group. Data were expressed as Mean ± SEM.

Significance by Student's "t" test for unpaired data, at P ≤0.05, was used. NS: not significant.

P_a: Significance from C_a group, P_{ins}: Significance from I_a group, P_b: Significance from C_b group, $P_{rec.1w}$: Significance from I_b group, P_c: Significance from C_c group.

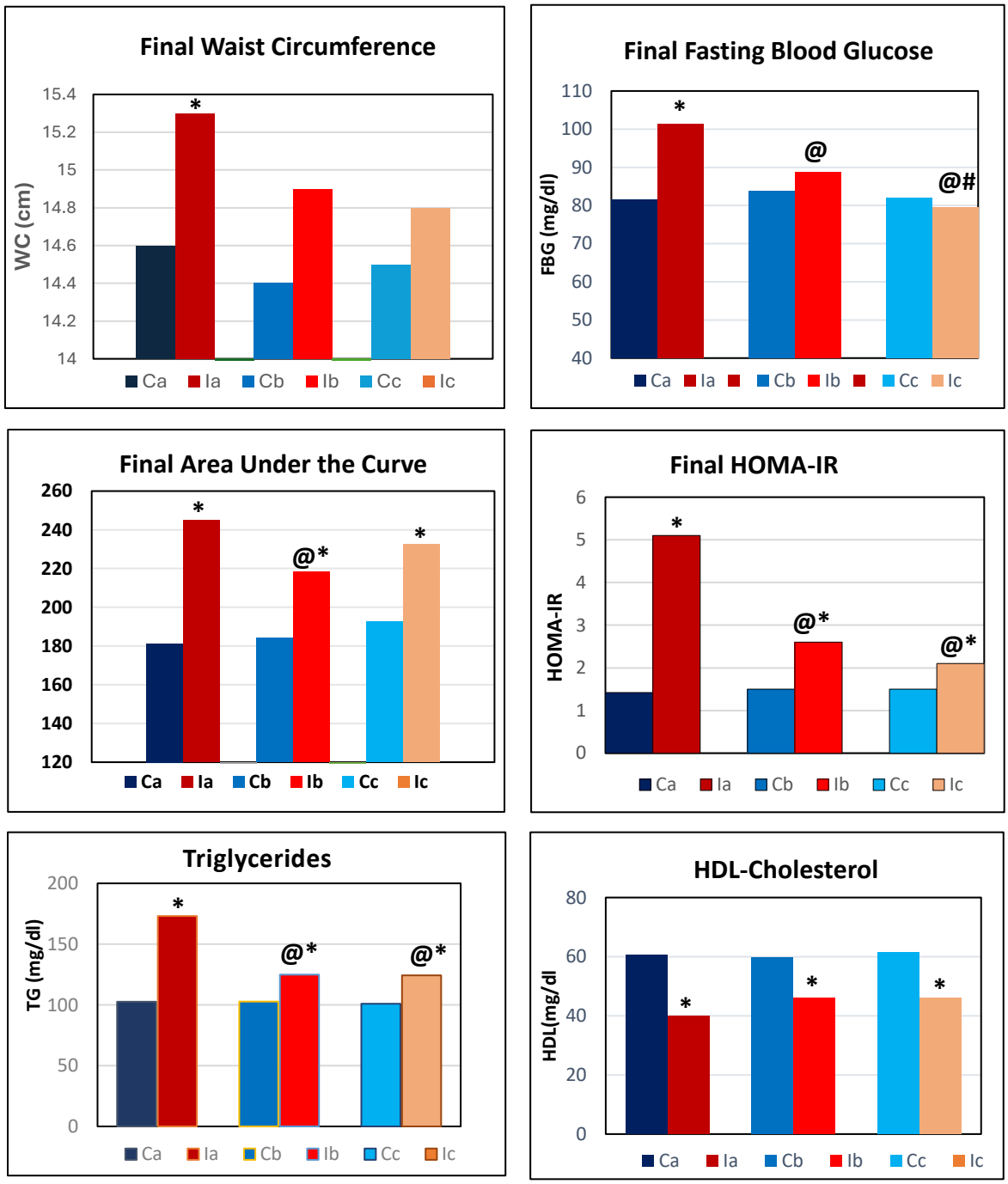

Figure (3): Graphs showing final values of waist circumference, fasting blood glucose, area under the curve, HOMA-IR, serum triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) in the studied groups. C_a: 4-week control group, I_a: 4-week insomnia group; C_b: 5-week control group, I_b: 4-week insomnia/1-week recovery group; C_c: 6-week control group, I_c: 4-week insomnia/2-week recovery group.

Significance by Student's "t" test for unpaired data, at P ≤0.05, was used.

***: Significance from corresponding control rat group, @: Significance from I_a rat group, #: Significance from I_b rat group.*

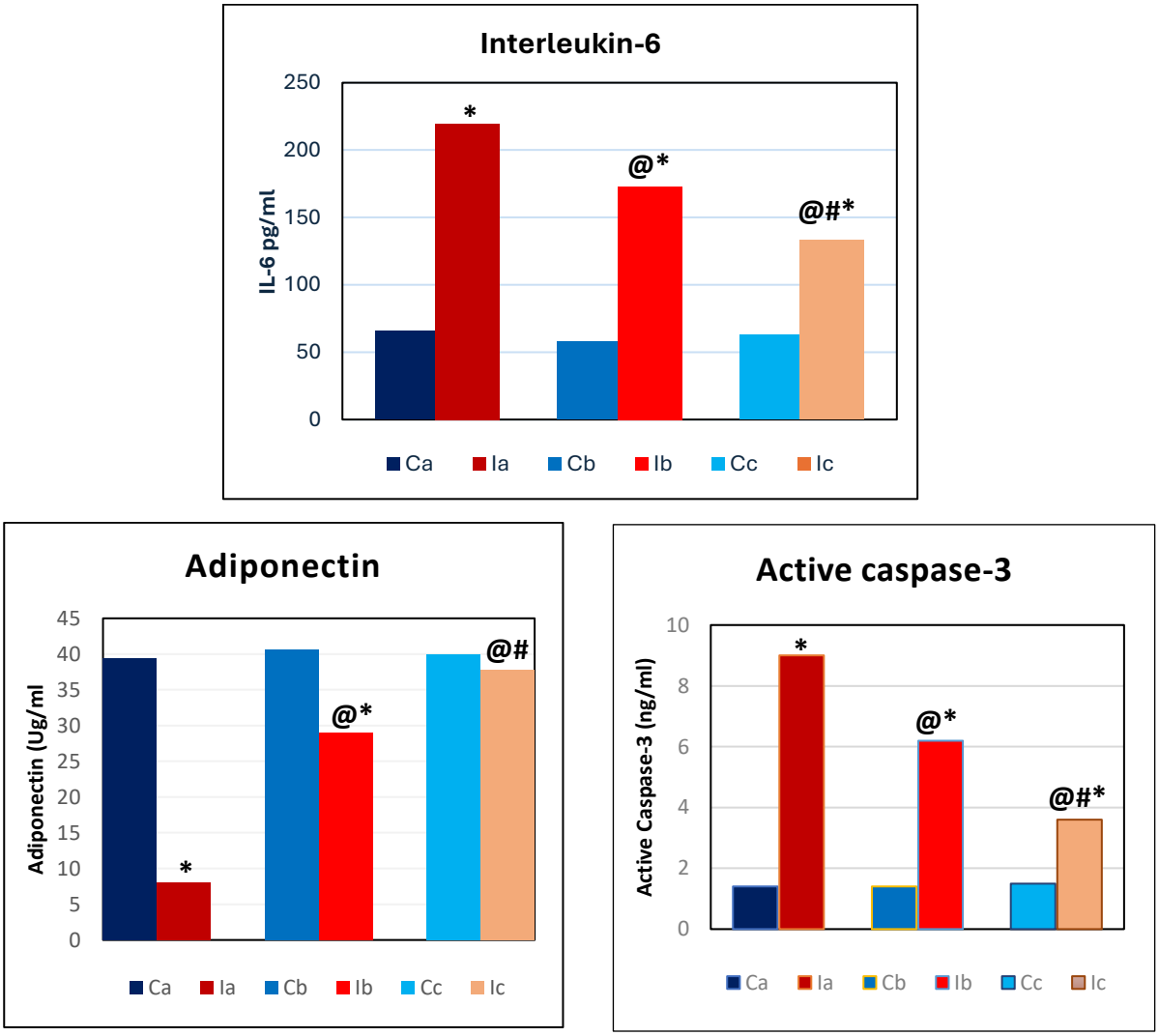

Figure (4): Graphs showing interleukin 6 (IL-6), adiponectin, and active caspase-3 in the studied groups. C_a: 4-week control group, I_a: 4-week insomnia group; C_b: 5-week control group, I_b: 4-week insomnia/1-week recovery group; C_c: 6-week control group, I_c: 4-week insomnia/2-week recovery group.

Significance by Student's "t" test for unpaired data, at P ≤0.05, was used.
***: Significance from corresponding control rat group, @: Significance from I_a rat group, #: Significance from l_b rat group.*

Correlation studies among the experimental groups:

Correlations of serum IL-6 versus other parameters (Figure 5):

When serum levels of the inflammatory marker IL-6 were plotted against the other parameters among all the studied rats, significant positive correlations were observed with BMI, WC, FBG, HOMA-IR, serum TGs, and active caspase-3. On the other hand, serum IL-6 showed inverse relationships with HDL-C and adiponectin.

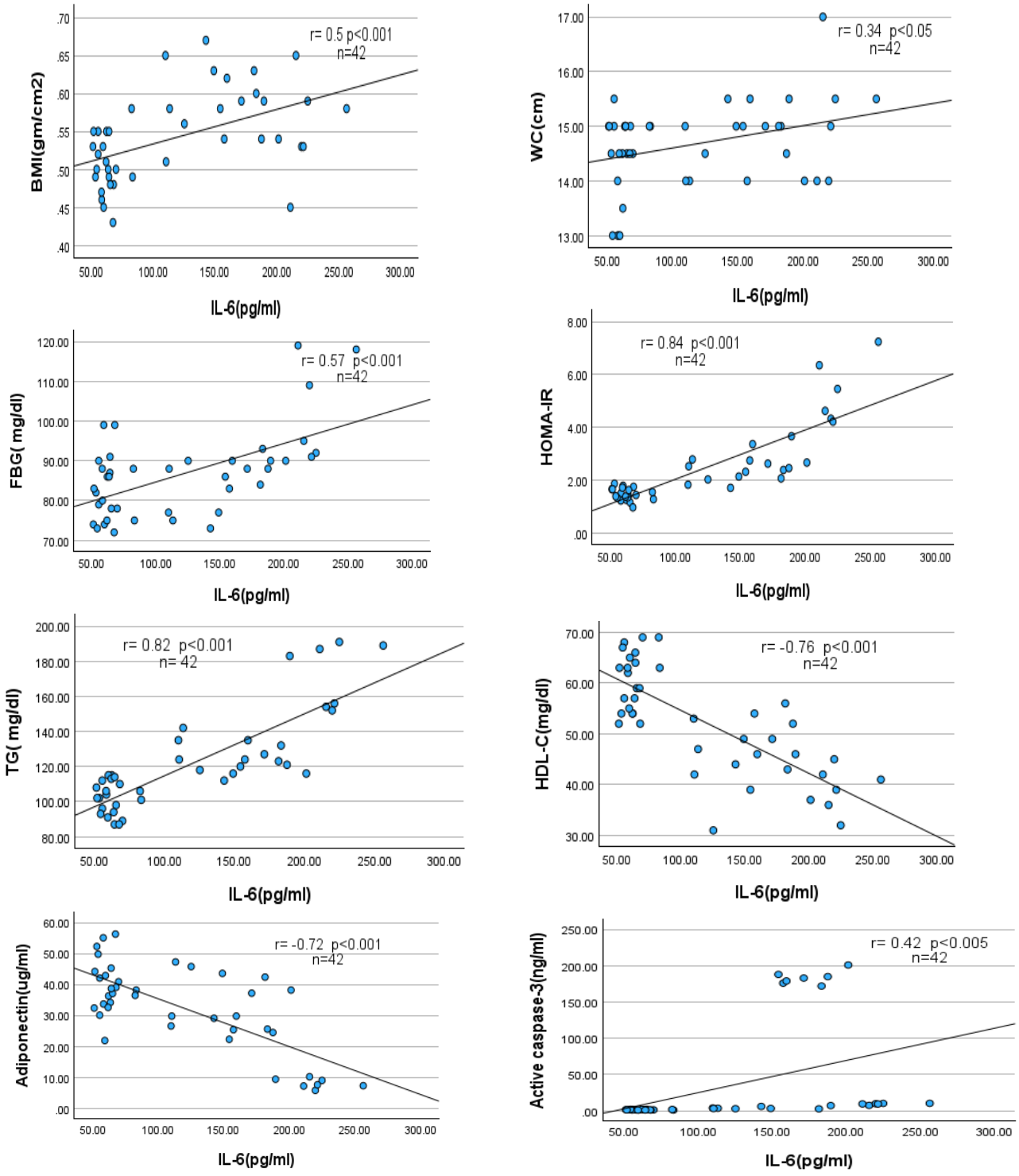

Figure (5): Graphs showing relationships between the inflammatory marker IL-6 and the different studied parameters among the experimental groups.

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DISCUSSION

The present study was conducted to evaluate the metabolic dysfunctions associated with insomnia and to find out the possible link of such relationship in aged female rats. Additionally, the potential reversibility of these changes with the retrieval of regular sleep for one and two weeks was assessed. The experimental model of insomnia adopted in the present study was in the form of decreased sleeping hours (i.e., sleep restriction) for five hours/day (from 8:00 to 13:00), 5 days weekly for four weeks.

The experimental method of sleep restriction used was the modified multiple platform method (MMPM), which primarily deprives the animal from rapid eye movement (REM) sleep, while the non-REM sleep unaffected without the need for monitoring the EEG, according to ***Vogel (1975)***, although in a later study by ***Machado et al. (2004)*****,** they reported that slow wave sleep (SWS) was said to be also fragmented. Therefore, the experimental model of insomnia implemented in the present study was considered to be chronic, mild to moderate REM and slow wave sleep restriction in the aging period of female rats.

In the present study, by the end of the 4th week, all the insomniac rats exhibited a significant increase in their body weight (BW), body mass index (BMI), and waist circumference (WC). Their intraperitoneal glucose tolerance test (IPGTT) indicated a prediabetic state as evidenced by the significantly high fasting blood glucose level. Upon sacrifice of the 4-week insomniac rats (I_a), insulin resistance was demonstrated as evidenced by the significant increase in HOMA-IR, and fasting TGs >150 mg/dl, according to the criteria described by ***Freeman et al. (2023)*****.** There was also a significant decrease in HDL-C and a significant increase in LDL-C, together with hypertriglyceridemia, which are indicative of dyslipidemia, in accordance with ***Lee et al. (2017)*****.** Moreover, these rats showed a significant increase in the inflammatory and apoptotic markers (IL-6 and active caspase-3, respectively) as well as a significant decrease of adiponectin. The above-cited results indicate that aged female rats subjected to chronic, mild to moderate sleep restriction became prediabetic and insulin resistant, together with the development of dyslipidemia, increased WC, and enhanced inflammation.

Analysis of the results of the insomniac rats confirmed inclusion of 4 out of 5 criteria of the metabolic syndrome according to NCEP ATPIII (2005 revision) guidelines **(*Grundy et al., 2004*)**. The four criteria of metabolic syndrome developed by the 4-week insomniac rats (I_a) in the present study were the fasting hypertriglyceridemia (173 mg %), which is > 150 mg/dl; the low fasting HDL-C (40 mg %), which is < 50 mg/dl (women); the fasting hyperglycemia (101.3 mg/dl) which is > 100 mg/dl and the significant increase in waist circumference compared to control rats. Despite the variability in the duration of insomnia and the experimental periods, the present results agree with the results of ***Van Leeuwen et al. (2010); Gangwisch et al. (2010),*** **and** ***Broussard et al. (2015 & 2016)*****.**

The aforementioned data and the results of this work suggest insulin resistance as the link between insomnia and the development of metabolic syndrome in insomniac rats. Moreover, the significantly increased level of the inflammatory markers IL-6, as well as the significant positive correlation between HOMA-IR and IL-6, raised the possibility that the insomniac inflammatory process, presented by IL-6, might be considered as another possible pathophysiological link in the development of metabolic syndrome. ***Vgontzas et al. (2004)*** reported that short sleep duration has been linked to increased levels of the inflammatory markers IL-6 and TNF-alpha. Moreover, the intracellular expression of the inflammatory transcription factor nuclear factor κB (NF-κB) was found to be enhanced in the morning following partial sleep deprivation as reported by ***Irwin et al. (2008)***. Therefore, inflammation is considered another possible pathophysiological mechanism for metabolic syndrome in the current study.

Recently, ***Engert and Besedovsky (2025)*** reported that sleep deficiency has been demonstrated to increase inflammatory molecules and trigger pro-inflammatory signaling cascades, which, when chronically activated, may elicit immunopathological changes. Moreover, it was shown that inflammatory activation impacts sleep through pro-inflammatory mediators, such as prostaglandins and cytokines, that act on the central nervous system ***(Engert and Besedovsky, 2025)***. Therefore, sleep and inflammation are concluded to be bidirectionally linked ***(Besedovsky et al., 2019 and Irwin, 2019)*****.**

Chen et al. (2025) suggested that insomnia is associated with increased risk of poor glycemic control. Moreover, ***Zuraikat et al. (2024)*** suggested that chronic sleep restriction, even if mild, can induce insulin resistance in healthy women, being more noticeable in postmenopausal women. Suggested mechanisms of insulin resistance include the changes in inflammatory markers, possibly due to circadian misalignment, which means misalignment of circadian rhythm for behavioral, hormonal, and metabolic changes ***(Mesarwi et al., 2013 and Yuan et al., 2021).***

The current study revealed the development of dyslipidemia in I_a rat group, in the form of hypertriglyceridemia, high LDL-C, and low HDL-C. Moreover, a significant positive correlation was demonstrated between IL-6 and each of HOMA-IR and triglycerides. Considering that insulin resistance had been suggested to be the first impairment in insomnia, it would be plausible to suggest dyslipidemia as a serious consequence of insomnia, and it might be accounted for by activation of inflammation. Moreover, ***Zheng et al. (2017)*** concluded that dyslipidemia induces insulin resistance and causes impaired β-cell response to insulin in normoglycemic individuals and diminishes β-cell function in individuals with glucose intolerance. Thus, it can be assumed that insulin resistance and dyslipidemia have a mutual causal relationship that would establish a vicious cycle, aggravating both insulin resistance, glucose dysregulation, and dyslipidemia.

Rodents are reported to be nocturnal animals and to sleep mostly in the light phase of the day ***(Campos-Beltrán and Marshall, 2021).*** Throughout the study period, insomniac rats were observed to be unexpectedly awake in the morning compared to their matched controls, who were sleepy. Therefore, circadian misalignment in the form of change in the period of light exposure can be suggested as a mechanism underlying the metabolic changes of insomnia with the induction of insulin resistance, because insomniac rats were exposed to extra periods of light compared to controls ***(Yuan et al., 2021).***

Insomniac rats in the present study were obese and had significantly higher levels of IL-6 compared to their controls. The significant positive correlations demonstrated between IL-6 with BMI and WC, together with HOMA-IR agree with the results of ***Xu et al. (2003)*** that chronic inflammation plays a key role in the

pathogenesis of obesity-related insulin resistance. Central obesity, a key component of metabolic syndrome, is commonly observed among individuals with insomnia. The high BMI and WC of insomniac rats in the present study agree with the findings of ***Calvin et al. (2013)*** **and** ***Covassin et al. (2022)*****,** who reported that sleep restriction is associated with obesity and central accumulation of fat. Recently, ***Takahashi et al. (2025)*** reported short sleep duration as a significant risk factor for obesity.

Four-week insomniac rats (I_a) in the present study had a high level of IL-6, whose value increased 333.7 % compared to control values; these results agree with the data of ***Suarez (2008)*** **and** ***Irwin et al. (2016)*****.** IL-6 is not only an immune molecule synthesized in local lesions in the initial stage of inflammation ***(Tanaka et al., 2014)*** but is also a hypothalamic factor expressed in the median eminence and is released into the hypophyseal portal vessels to modulate the ACTH stress response ***(Jankord et al., 2006)*****.** Therefore, it could be postulated that the expression of IL-6 in the hypothalamus of I_a rat group was increased to modulate the ACTH stress response, and because the time of induction of insomnia was in the morning, IL-6 showed a high morning level.

Adiponectin contributes to glucose homeostasis and fat metabolism by increasing insulin secretion and sensitivity (***Nguyen 2020*****).** The present study showed a significant reduction in adiponectin levels in 4-week insomniac rats, which agrees with the results of a study that involved sleep restriction in women, with no effect in men, suggesting a sex-specific effect for sleep loss on adiponectin ***(Simpson et al., 2010)*****.**

Active caspase-3 was found to be markedly increased in our 4-week insomniac rats, and it displayed a significant positive correlation with IL-6. Such findings, together with the results of IL-6 correlation with parameters of the metabolic syndrome and adiponectin, would point to the detrimental effect of inflammation, contributing to more glucose dyshomeostasis

An attempt was done for reversion from the insomniac state into regular sleep, as a trial to improve the observed metabolic dysfunction, and to halt the stress response that involves the rise in IL-6. Reports in literature about the effects of recovery from insomnia were few, with variable durations of sleep restriction and

recovery that were not conforming with the durations implemented in the present study.

The intervention adopted in the present study was to revert the insomniac rats to their regular sleep for one and two weeks. The inflammatory state was improved as proved by the significant decrease in the level of IL-6 after one and two weeks, compared to the insomniac group in a time-dependent manner, but still not normalized. The results of the current work indicated that the inflammatory state induced by insomnia, for four weeks, might need a prolonged duration to be completely abolished.

The significant decrease in the inflammatory marker IL-6 after one week of sleep recovery was associated with a significant decrease in FBG, AUC, serum insulin, HOMA-IR, serum TG, total cholesterol, and caspase-3. On the other hand, such reduction in IL-6 was associated with a significant increase in serum adiponectin. These observed responses achieved by the end of one week of regular sleep, displayed further improvement in a time-dependent manner towards the control values after two weeks of regular sleep, with normalization of serum adiponectin. The apoptotic activity, presented by caspase-3, was partially improved, but still not normalized.

The improvement in IL-6, insulin resistance, as well as adiponectin, might contribute to the gradual decrease in each of WC and LDL-C towards the control values by the end of the two weeks of regular sleep. IPGTT and HDL-C were improved only partially after one week of returning to regular sleep, with no further improvement noted by the second week. The partial improvement in the mentioned parameters might be promising of complete recovery with a longer period of reversion to regular sleep. Verification of these assumptions needs more studies with variable durations to find out if complete recovery might occur with more prolonged durations of normal sleep retrieval.

CONCLUSION

Based on the above-cited findings, it could be concluded that chronic, mild to moderate sleep restriction has serious inflammatory, metabolic, and apoptotic consequences in the aging period of female rats, whose full recovery to normal values was not ensured for all tested parameters after two weeks of reversion to normal sleep. We may recommend that elderly females complaining of chronic

insomnia should be assessed for IL-6 to ameliorate the metabolic dysfunction associated with chronic insufficient sleep.

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دلالات الالتهابات كرابط محتمل بين الأرق ومتلازمة الأيض في اناث الجرذان المسنة: تأثير العودة للنوم الطبيعي على الإنترلوكين-6

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قسم الفسيولوجي، كلية الطب، جامعة عين شمس، القاهرة، جمهورية مصر العربية

الملخص العربي

خلفية البحث: تعتبرمشكلة قلة النوم واحدة من أهم المشاكل الصحية ذات التأثير البالغ على الصحة الأيضية. وقد أظهرت الدراسات ان معدل انتشار الأرق أعلى في النساء، ويزيد مع التقدم في العمر.

هدف البحث: تهدف الدراسة إلى تقييم الإختلال الأيضي الناتج عن تقليل ساعات النوم لمدة 4 أسابيع في اناث الجرذان المسنة، كذلك لاستكشاف الآلية التي تربط بين الاضطرابات الحادثة في كلاً من الأيض والنوم، بالإضافة لدراسة التحسن الأيضي المحتمل حدوثه مع استعادة النوم الطبيعي لمدة أسبوع وأسبوعين.

منهج البحث: تم أجراء هذا البحث على 52 من اناث جرذان التجارب المسنة والتي تم تقسيمها إلى مجموعتين رئيسيتين:

1) مجموعة الجرذان المعرضة للأرق: والتي تعرضت للحرمان من النوم لمدة 4 أسابيع باستخدام طريقة المنصة المتعددة المعدلة(MMPM) ، وفي نهاية الأسبوع الرابع من الدراسة، تم تقسيم االجرذان إلى 3 مجموعات فرعية:

المجموعة (I_a): المعرضة للأرق لمدة 4 أسابيع و المجموعة(I_b) : المعرضة للأرق لمدة 4 أسابيع ثم التعافي لمدة أسبوع واحد والمجموعة (I_c) :المعرضة للأرق لمدة 4 أسابيع ثم التعافي لمدة أسبوعين.

2) المجموعة الضابطة: والتي لم تتعرض للأرق، وفي نهاية الأسبوع الرابع من الدراسة تم تقسيمها الى 3 مجموعات فرعية كالتالي:

المجموعة (C_a):مجموعة ضابطة لمدة 4 أسابيع (ضابطة للمجموعةI_a) والمجموعة : (C_b)مجموعة ضابطة لمدة 5 أسابيع (ضابطة للمجموعة I_b) و المجموعة (C_c): مجموعة ضابطة لمدة 6 أسابيع (ضابطة للمجموعة I_c).

- في نهاية الدراسة أخضعت الجرذان لقياس الوزن و حساب مؤشر كتلة الجسم، وقياس محيط الخصروكذلك تقدير مستوى الجلوكوز الصائم في الدم واختبار تحمل الجلوكوز داخل الصفاق (IPGTT) ، بالإضافة الى قياس نسبة الأنسولين، الجلوكوز، الدهون والأديبونيكتين والإنترلوكين-6 (IL-6) والكاسباز3 النشط (3-caspase) في عينات المصل, كما تم استخدام نسبة الجلوكوز والأنسولين لحساب تقييم النموذج المتوازن لمقاومة الأنسولين (HOMA-IR)

النتائج: أظهرت الدراسة أن تقليل النوم لدى الجرذان المصابة بالأرق لمدة 4 أسابيع (I_a) قد أدى الى حدوث خلل وظيفي للأيض متمثلاً في وجود أربعة من مؤشرات متلازمة الأيض [زيادة سكر الدم الصائم والدهون الثلاثية ،ومحيط الخصر مع نقص الكوليسترول عالي الكثافة]، مع وجود مقاومة عالية للأنسولين. بالإضافة الى ذلك فقد أظهرت تلك الجرذان زيادة ذات دلالة أحصائية في كل من مؤشر الالتهاب6-IL و مؤشر موت الخلايا المبرمج (الكاسباز3 النشط) بالإضافة إلى انخفاض في الأديبونيكتين. وقد صاحب ذلك وجود ارتباط ذو دلالة إحصائية بين مؤشر الالتهاب IL-6 مع مؤشرات متلازمة الأيض المذكورة. وجدير بالذكر ان استعادة النوم الطبيعي قد أدى الى حدوث تحسن جزئي في الإختلال الأيضي، كما تحسنت الحالة الالتهابية (IL-6) وموت الخلايا المبرمج (3-caspase) جزئيا مع زيادة مدة استعادة النوم الطبيعي الى أسبوعين -مقارنة بالقيم الملاحظة في الجرذانI_a - لكنها ظلت غير طبيعية، مع استعادة الأديبونيكتين مستوياته الطبيعية بعد أسبوعين من العودة الى النوم المنتظم.

الاستنتاج: يستخلص من هذه الدراسة أن تقليل ساعات النوم -الخفيف الى المتوسط- بشكل مزمن له تداعيات بالغة الخطورة على كلٍ من الأيض والالتهابات وكذلك موت الخلايا المبرمج. كما يمكن استنتاج انه بالرغم من استعادة النوم الطبيعي إلا انه لم يتم استعادة القيم الطبيعية مع كل القياسات المستخدمة. وجدير بالذكر أنه يمكن اعتبار زيادة مستوى الالتهابات بالجسم هي الآلية التي تربط بين الأرق ومتلازمة الأيض.

الكلمات الدالة: الأرق، متلازمة الأيض، استعادة النوم الطبيعي، دلالات الالتهابات