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Biochemical, Hormonal, and Body Weight Changes in Chronic Stressed Young and Middle Aged Sprague Dawely Rats

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Keywords

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Abstract

Background: the literature is controversial about influence of stress on body weight. Chronic stress can activate both orexogenic and anorexigenic pathways with subsequent increase or decrease in body weight. Mechanisms behind these changes still need further evaluations. **Aim and objectives**: in the present study effect of different chronic stressors on body weight of young and middle aged rats and associated changes in leptin level have been investigated. **Results**: Chronic stress both (noise and restraint stress) resulted in a significant decrease in weight gain in young rats but significant weight loss in middle aged rats. There was significant elevation of blood glucose level and lipid profile in all stressed groups as compared with control groups. Serum level of insulin, leptin, and HOMA index were significantly elevated in noise stress group but significantly reduced in restraint stress groups. **Conclusion:** chronic stress caused significant body weight changes that differ according to age of animal associated with metabolic changes that could result in many forms of metabolic syndrome as a result of impaired lipid and glucose metabolism.

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INTRODUCTION

In the modern societies, stress is a constant event in the daily life. In order to restore homeostasis and promote behavioral adaptation during stressful situations, a primary response is set up **(1)**. This response to stress would be different according to the type, duration, and intensity of the stress **(2)**.

Altered feeding behaviors, is a common feature in humans associated with stress exposure with approximately 40% eating more or 40% eating less than usual **(3)**. Also increased consumption of highly palatable foods, has been reported by others **(4)**. This active seeking of highly palatable foods is thought to be a form of self-medication wherein stress response is down regulated in response to the rewarding properties. Previous studies have been tried to explore the mechanisms responsible for complex, stress-induced eating behaviors with resultant changes in body weight **(5)**.

These effects of stress would be differing according to the age at which the stress takes place and neuroendocrine response to stress varies as a function of age. For example, early in postnatal life, hormonal responses to some stressors may be lower than during adulthood **(6)**.

Adipose tissue is recognized as a major endocrine organ that secretes signaling molecules playing a central role in inflammation, weight regulation and metabolic function **(7)**. One possible modulator of stress eating and body weight regulation is leptin, which affect both feeding behavior and energy expenditure **(8)**.

Material and methods:

1- Experimental animals

48 male albino rats were obtained from the animal house of medical experimental research centre (MERC), faculty of medicine, Mansoura University. They were housed in plastic cages, four animals per cage, under controlled conditions of humidity (40–70%), lighting (12 h light/dark cycle), and temperature (20–22C), with free access to food and water.

All animal experimental procedures were performed according to the guidelines of our local committee of animal research ethics. The rats were of two age groups; 2 months old age with average body weight 200 – 250 gm (young) and 12months old with average body weight 350-400 gm (middle age). Rats were divided into six groups each include 8 rats;

Group I: control young group; **Group II**: young stressed rats exposed to chronic noise stress (YNS**); Group III**: young stressed rats exposed to chronic restraint stress (YRS); **Group IV**: middle age control group; Group **V**: middle age middle age stressed rats exposed chronic noise (MNS); and Group **VI**: middle age stressed rats exposed chronic restraint stress (MRS).

2- Experimental protocol

At the beginning, the animals were left in their cages for 1 week without intervention to be habituated to the surrounding environment. The non stressed adult and middle aged rats were handled in the routine way undisturbed, while the stressed groups were exposed to stress daily for 6 weeks. 2 types of stressors were used;* **physical restraint** stress where the method described by

(9) was used in order to perform chronic restraint stress model in studied animals. Rats were restrained using small cages that limit their movement. The cages used for restraining mature rats has compartments 6cm wide x 7cm high x 18 cm long, and up to 6 rats could be restrained simultaneously. Animals were exposed daily to 3h of stress in the morning (between 9:00 and 12:00), 6 days a week for 6 weeks **(10)**.

*__Noise stress__ that was produced by two loud speakers mounted 40 cm a part on opposite sides of the cage and driven by a white noise generator (range 0 – 26 KHz) installed (suspended) 30 cm above the cage. The noise level was set at an intensity of 100 dB uniformly throughout the cage and monitored by a sound level meter. Each treated animal was exposed for 3h/ day for 8weeks. Control rats were kept in the above-described cage during the corresponding period of time, without noise stimulation (**11**).

Body weight & food intake assessment

Animals' body weights have been recorded before the start of experimental protocol, weekly during the protocol and at the end before scarification. The food intake was recorded daily throughout the experiment by measuring the difference between the amount of feed put in the cage and the remaining amount after 24 h.

Collection of blood samples.

At the end of the study the rats were fasted for the overnight 12 hours. The next morning, rats were scarified by over dose of intraperitoneal thiopental at a dose of 40 mg/Kg **(12)**. By cardiac puncture blood samples were collected from the heart. At 3000 rpm for 20 minutes blood samples were centrifuged to separate the serum. At -20°C the separated serum was stored for subsequent biochemical analysis.

Biochemical parameters assessment *:*

Lipid profile including serum cholesterol, HDL, and triglyceride were estimated using commercial kit **(Spinreact**, Spain).

LDL-C= TC – VLDL – HDL.

VLDL was calculated**: VLDL = (TGs/5).**

Blood glucose level estimation using commercial kit (Human).

Insulin level was estimated using rat insulin ELISA kit Sunred biological technology company that we purchased from Biogene Company.

Determination of HOMA-IR index, the formula is: HOMA-IR= (*c*i × *c*g)/405, where *c*i is fasting insulin level (m IU/L) and *c*g is fasting glucose level (mg/dl).

Leptin level was measured by leptin ELISA kit for rat obtained from Company of Biospes, Chongqing #cat no BYEK1081.

Statistical analysis:

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 23.0. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). In the statistical comparison between the different groups, the significance of difference was tested using one way ANOVA (analysis of variance):- Used to compare between more than two groups of numerical (parametric) data followed by Tukey post-hoc test.

3. Repeated measures ANOVA (analysis of variance):- Used to compare between more than two related groups of numerical (parametric) data followed by post-hoc bonferroni

A *P* value <0.05 was considered statistically significant

Results

Table (1) shows the body weight of young rat subgroups at the start of the study before implementation of stress protocol. A comparison of body weight was done weekly and at the end of experimental protocol. There was no significant difference between young rats allocated regarding their body weight. However starting from 3rd week weight gain in young stress- restraint group was less in comparison with young non stressed and young stress- noise group. By the 4th week the weight gain in both young stress- noise and young stress- restraint groups was significantly lower when compared with young non stressed group.

As regard delta weight it was significantly reduced in young stress- noise and young stress- restraint groups when compared with young non stressed one (Δ weight = 55.31± 5.42 in YNS group and 33.36± 4.54; 20.43± 3.82 in YS-N and YS-R respectively).

Table (2): shows the body weight of middle age rat subgroup at the start of the study before implementation of stress protocol. A comparison of body weight was done weekly and at the end of experimental protocol. There was a significant weight loss in the middle age stress- restraint group starting from the 1st week till the end of the study when compared with the middle aged non stressed one. By the 2nd week there was a significant weight loss in both middle age stress- noise and middle age stress- restraint groups when compared with the middle age non stressed group.

As regard delta weight , there was a significant reduction in delta weight in middle age stress- noise and middle age stress- restraint groups when compared with middle age non stressed one (Δ weight = 23.33± 4.52 in MNS group and -26.67± 4.54 ; -45.00 ±9.36 in MS-N and MS-R respectively).

Table (3): shows that exposure of young rats to noise led to a significant increase in fasting blood glucose, serum insulin, HOMA index, and leptin level when compared with the non stressed group. Restraint stress resulted in a significant increase in blood glucose level but with a significant decrease in serum insulin, HOMA index, and serum leptin level when compared with the non stressed group. Restraint stress led to a greater increase in blood glucose level but with a significant decrease in serum insulin, HOMA index, and serum leptin level when compared with the noise stress group.

Table (4): shows that exposure of the middle aged rats to noise resulted in a significant increase in fasting blood glucose, HOMA index, serum leptin level, but with a non significant increase in serum insulin when compared with the non stressed group. Restraint stress resulted in a significant increase in blood glucose level but a significant decrease in serum insulin, HOMA index, and serum leptin level when compared with both noise stress group and the non stressed one.

Graph (1): shows that chronic exposure of young rats to noise resulted in a significant increase in serum triglycerides, cholesterol, VLDL, and LDL but a significant decrease in Serum HDL when compared with non stressed group.

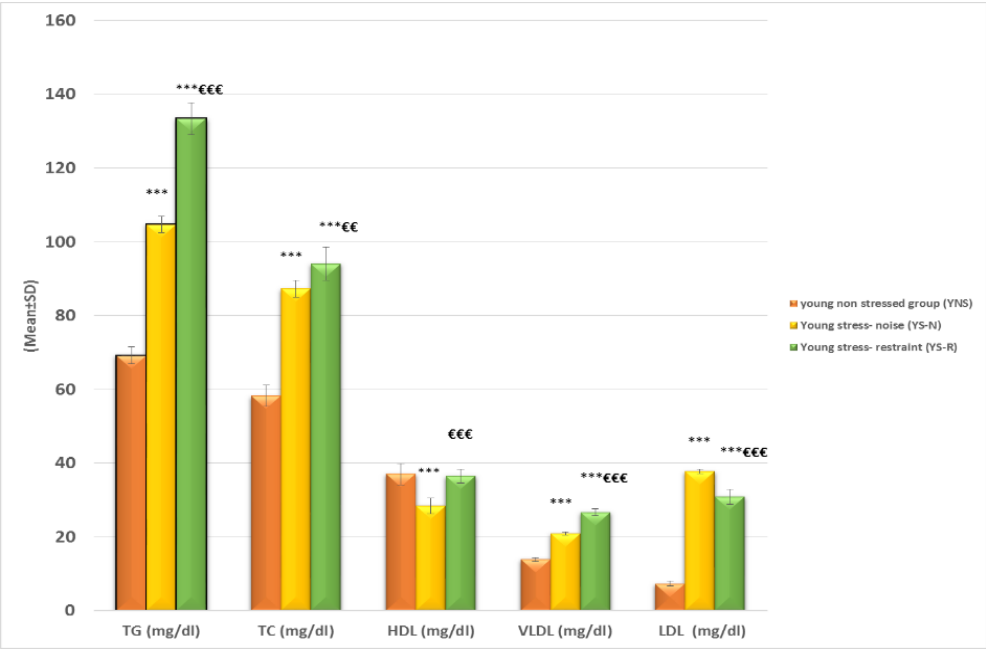
Restraint stress caused a significant increase in serum triglycerides, cholesterol, VLDL, and LDL with a non significant decrease in Serum HDL when compared with non stressed group.

Chronic restraint resulted in a significant increase in serum triglycerides, cholesterol, VLDL, and LDL with significant decrease in Serum HDL when compared with chronic noise group.

Table (1): Comparison of body weight and delta weight (final weight-initial weight) in young non-stressed (YNS), young stress-noise (YS-N), and young stress-restraint (YS-R) rats

Groups(n=8) / parameters	**Young non stressed group (YNS)**	**Stressed groups**	
		Young stress- noise (YS-N)	**Young stress- restraint (YS-R)**
Initial weight (before start of stress protocol)	359.17± 21.16	367.50± 19.64	358.33± 23.68
1st week	390.50± 18.77	364.50± 17.07	352.00**± 17.18
2nd week	393.83± 13.29	360.33**± 16.80	350.00***± 11.64
3rd week	398.83± 17.87	357.50**± 26.90	344.17***± 16.25
4th week	402.33± 11.82	353.00***± 13.20	336.67*** ± 20.30
5th week	405.17± 15.81	349.17***± 17.90	330.00***± 12.87
6th week	403.67± 14.46	346.83***± 13.18	329.17***± 17.03
Delta weight= (final weight-initial weight)	23.33 ± 4.52	-26.67***± 7.80	-45.00***€€± 9.36

All results expressed as mean ± SD, One way ANOVA with Tukey post hoc test (significance at p≤0.05).*; p≤ 0.05,**; p ≤ 0.01, and***; p≤ 0.001) stressed group vs non stressed group.**€**; p≤ 0.05, **€€;** p ≤ 0.01, and **€€€;** p≤ 0.001) restraint stress vs noise stress group.

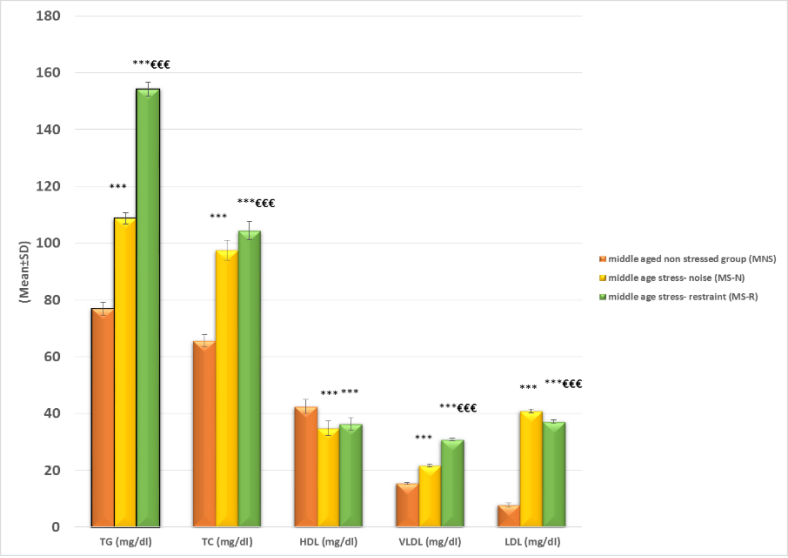


Graph (1): Serum level of triglycerides (TG), cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) in young non stressed (YNS), young stress- noise (YS-N), and young stress- restraint (YS-R) rats). *; p≤ 0.05,**; p ≤ 0.01, and***; p≤ 0.001) stressed group vs non stressed group. **€**; p≤ 0.05, **€€;** p ≤ 0.01, and **€€€;** p≤ 0.001) restraint stress vs noise stress group.

Table (2): Comparison of body weight and delta weight (final weight- initial weight) in middle aged non stressed (MNS), middle age stress- noise (MS-N), and middle age stress- restraint (MS-R) rats

Groups(n=8) / parameters	Middle non stressed group (MNS)	Stressed groups	
		Middle stress- noise (MS-N)	Middle stress- restraint (MS-R)
Initial weight (before start of stress protocol)	223.33 ± 19.83	240.00 ±15.24	239.33±9.21
1st week	255.00 ±21.68	255.83 ±20.83	236.83±9.83
2nd week	262.50±20.19	260.50 ±12.43	240.31± 11.64
3rd week	272.32±16.05	265.00 ±14.6 1	244.76**€ ±8.45
4th week	285.83±14.29	267.50* ±10.40	249.83*** ±11.34
5th week	300.83±18.55	269.17**±11.63	250.33*** ±12.87
6th week	304.17 ±22.89	270.50**±14.40	255.61***±14.03
Delta weight= (final weight- initial weight)	55.31± 5.42	33.36*** ±4.54	20.43***€€€ ±3.82

All results expressed as mean ± SD, One way ANOVA with Tukey post hoc test (significance at p≤0.05).*; p≤ 0.05,**; p ≤ 0.01, and***; p≤ 0.001) stressed group vs non stressed group.**€**; p≤ 0.05, **€€;** p ≤ 0.01, and **€€€;** p≤ 0.001) restraint stress vs noise stress group.



Graph (2): Serum level of triglycerides (TG), cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) in middle age non stressed (MNS), middle age stress- noise (MS-N), and middle age stress- restraint (MS-R) rats. *; p≤ 0.05,**; p ≤ 0.01, and***; p≤ 0.001) stressed group vs non stressed group.**€**; p≤ 0.05, **€€;** p ≤ 0.01, and **€€€;** p≤ 0.001) restraint stress vs noise stress group.

Graph (2): Shows that chronic exposure of the middle aged rats to noise resulted in a significant increase in serum triglycerides, cholesterol, VLDL, and LDL but a significant decrease in Serum HDL when compared with the non stressed group. Restraint stress caused a significant increase in serum triglycerides, cholesterol, VLDL, and LDL with a significant decrease in Serum HDL when compared with the non stressed group. Chronic restraint resulted in a significant increase in serum triglycerides, cholesterol, VLDL, and LDL respectively) and a non

significant decrease in Serum HDL when compared with the noise stress group

Table (3): Serum level of blood glucose, insulin, HOMA index, and leptin in young non stressed (YNS), young stress- noise (YS-N), and young stress- restraint (YS-R) rats

Groups (n=8) / parameters	Young non stressed group (YNS)	Stressed groups	
		Young stress- noise (YS-N)	Young stress-restraint (YS-R)
Fasting blood glucose (mg/dl)	**84.83± 4.17**	**104.33***± 3.98**	**109.17***€± 2.86**
Serum insulin level (mIU/L)	**12.28±.74**	**13.67*±1.18**	**7.70***€€€± .60**
HOMA index	**2.54± .06**	**3.51***± .22**	**2.08***€€€± .19**
Serum leptin (ng/mL)	**6.22±.37**	**8.62***± .81**	**4.18 ***€€€± .26**

All results are expressed as mean ± SD, One way ANOVA with Turkey post hoc test (significance at p≤0.05). *; p≤ 0.05,**; p ≤ 0.01, and***; p≤ 0.001) stressed group vs non stressed group. **€**; p≤ 0.05, **€€;** p ≤ 0.01, and **€€€;** p≤ 0.001) restraint stress vs noise stress group.

Table (3): Serum level of blood glucose, insulin, HOMA index, and leptin in Middle non stressed (MNS), Middle stress- noise (MS-N), and Middle stress- restraint (MS-R) rats

Groups (n=8) / parameters	Middle non stressed group (MNS)	Stressed groups	
		Middle stress- noise (MS-N)	Middle stress-restraint (MS-R)
Fasting blood glucose (mg/dl)	**86.83± 2.32**	**109.50***± 3.73**	**115.83***€€± 4.36**
Serum insulin level (mIU/L)	**11.30± 1.41**	**12.57 ± .83**	**6.78***€€€ ± 1.24**
HOMA index	**2.43± .33**	**3.40***± .26**	**1.93**€€€ ± .30**
Serum leptin (ng/mL)	**8.28± .34**	**9.28***± .43**	**7.23***€€€± .28**

All results are expressed as mean ± SD, One way ANOVA with Turkey post hoc test (significance at p≤0.05). *; p≤ 0.05,**; p ≤ 0.01, and***; p≤ 0.001) stressed group vs non stressed group. **€**; p≤ 0.05, **€€;** p ≤ 0.01, and **€€€;** p≤ 0.001) restraint stress vs noise stress group.

Discussion:

Finding novel methods to limit the stress-linked cardio-metabolic diseases occurrence requires explanations why, in some situations, there was an increase in food intake and obesity that outbalance stress anorexigenic effects. In this study the effect of chronic stress on body weight was examined in two different age groups using two different chronic stressors.

In animal models, it is well known that stress could change food intake and body weight. In the present study one of the numerous models of the stress suitable for the stress effects determination is the restraint stress model that is widely utilized, as it efficiently simulate effective physical and psychological stress **(13)**. It has also been used as an animal model of depression and anorexia nervosa **(14)**. Another type of stress is the noise

pollution which associated with modern life style and implicated in various illness and increased morbidity of human. Since age is another essential element that could impact stress response. During this study the available age groups that were used include; young one of 2 months age and middle age of 12 months age. Old rats were difficult to be obtained.

In this study, young rats exposed to either noise or restraint stress (YS-N and YS-R group) demonstrated significant reduction in their body weight gain when compared with the non stressed group. Starting from 3rd week the weight gain in young stress- restraint group was less in comparison with young non stressed and young stress- noise group. By the 4th week the weight gain in both young stress- noise and young stress-restraint groups was significantly lower when compared with young non stressed group (**table1**).

Associating delta weight (final weight – initial weight) changes between the stressed and non stressed one **(table 1)** confirmed the finding that the weight gain was less in stressed groups by the end of the 6th week. Similarly, comparing delta weight in young stress-noise and young stress-restraint showed that young stress- restraint had less weight gain by the end of 6th week.

Concerning the effect of stress on body weight of the middle aged rats, exposure of these rats to either noise or restraint stress resulted in a significant loss of weight (middle age stress- noise and middle age stress- restraint) along the period of the study when compared with the non stressed group **(table 2)**. Comparing delta weight in middle age stress- noise and middle age stress-restraint showed that middle aged stress- restraint had more loss of weight by the end of the 6th week **(table 2)**. From observed changes in delta weight, the effect of chronic stress exposure produced a decrease in weight gain in the young age group but weight loss in middle aged one when exposed to either types of stressors.

These results were in agreement with **(15)** who demonstrated that rats exposed to noise stress had significantly lower body weights when compared with the non stressed group. Also **(16)** found that in spite of the increase in the mean body weight of both control and chronic noise exposed rats throughout the duration the study, the noise stress group had significantly lower body weight gain in relation to the control group.

As regard restraint stress the studies of **(17,18)** showed that rats exposed to chronic restraint stress had a significant lower body weight gain that was explained to be due primarily to an initial reduction in food intake however the increases in body temperature and energy expenditure during restraint might explain maintained reduction in body weight. This reduced weight gain observed in stressed rats have been attributed to the decrease in lean body mass, such as muscle or bone mass.

Mechanisms behind these changes in body weight and food intake could be elucidated by either increased secretion of corticotrophin releasing hormone (CRH), as an anorexigenic neuropeptide after exposure to stress **(19),** or may be a result of stress activation of the rich sympathetic innervations of brown adipose tissue,

which is possibly increased in stressed rats **(20)**. Also weight loss associated with stress exposure could be explained by stress prompted increase adrenal steroid secretion, increased metabolic demands, and reduced digestion **(21)**.

To explain the difference of response between young and middle age groups upon the exposure to stress regarding the body weight the study of **(22)** examined the effect of heat stress on body weight in three age groups (weaning, young, and adult rats). He found that reduction in body weight due to chronic exposure to hot environment started early in weaning and young groups of rats, even though the adult rats showed non significant change in body weight as in relation to the non stressed group. The controverse of results of the present study with **(22)** study may be due different strain of rats or different stress protocol.

The results of the current research demonstrated a significant increase in the lipid profile of both young and middle age stressed rats as compared with the non stressed one. There was a significant increase in serum triglycerides, cholesterol, LDL, and VLDL but a significant reduction in serum HDL **(graph1, 2)**. In previous study of **(15)** they reported that chronic noise stress led to significant increase in total cholesterol, triglyceride, LDL cholesterol, and a significant reduction in HDL-cholesterol concentrations in comparison with the non stressed group. This indicating a definite association between stress and lipid concentrations. These results were in consistent with **(21)**.

These findings could be explained by increased hypothalamic hypophyseal axis activity with subsequent increase in corticosteroids and catecholamines secretion which stimulate lipolysis in adipose tissue with increased release of free fatty acid **(23)**. Increased transport of free fatty acids to the liver where they utilized for triglyceride synthesis could be a reason for the observed higher triglycerides in stressed rats.

The results of **(24)** reported that there was a significant increase in cholesterol level in stressed groups in relation to control non stressed one which is in agreement with our results. A well-known risk factor in development of arteriosclerosis is the LDL but HDL is considered as a protective one **(25)**. The increased level of VLDL, total cholesterol, and LDL is an indication for increased lipid peroxidation which has a detrimental effect on health that could occur following prolonged stress exposure **(21)**.

The results of the current research showed a significant increase in serum glucose, insulin, and HOMA-IR in both young and middle age rats exposed to noise stress (YS-N and MS-N) in comparison with their non stressed groups **(table 3, 4)**. Denoting that chronic stress has a strong relation towards the development of diabetes mellitus. These results were in agreement with ***(26)***.

These finding could be explained by the following mechanisms, first; increased the transcription of key enzymes of involved

gluconeogenesis for instance phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6P), second; increased lipolysis and proteolysis, thereby increasing glycerol and amino acids levels which are essential substrates for gluconeogenesis, third; suppression of glucose transporter-4 (GLUT-4) which is involved in the uptake of glucose into peripheral tissues (the hyperglycemic effect of both catecholamines and glucocorticoids, released by the activation of the sympatho-adreno-medullary and pituitary-adreno-cortical systems, respectively)*,(15)*. It appeared that noise stress impairs glucose metabolism possibly through impairment of insulin action that could be explained by higher insulin level reflecting a degree of insulin resistance and stress-induced corticosteroid secretion has a negative consequence on insulin sensitivity.

As regard restraint stress, the result of this work demonstrated a significant elevation in blood glucose level but significant reduction in insulin level, leptin, and HOMA-IR in both young and middle age rats (YS-R and MS-R) when compared with the non stressed **(table 3,4)**. High blood glucose level detected in the restraint-stress rats could be explained by the reduction of insulin secretion from β cells as a result of the inhibitory effect of sympathetic nervous system that overcome the stimulatory effect of corticosterone on the insulin secretion *(27)*.

It was also reported that increased insulin removal by the liver could be a possible cause of decreased insulin level that follow the exposure to stress. This occurs as a result of stimulation of the expression of peroxisome proliferator-activated receptor (PPAR) alpha (a nuclear hormone receptor) gene in the rat liver via secreted corticosterone *(28)*. Enhancement of insulin-degrading enzyme IDE (present within hepatic peroxisomes) is also attained by corticosterone with subsequent increase in insulin degradation *(29)***.**

The result of this study showed a significant increase in leptin hormone in the noise exposed groups both young and middle age one but significantly reduced in young and middle age restrained rats in relation to the control group **(table3, 4)**. Difference in levels of this hormone may be due to changes in weight of fat tissue between studied groups. Reduction in leptin levels demonstrated in rats exposed to restraint stress may be a result of decreased fatty tissues weight especially visceral fat accumulation as leptin is produced mainly in these tissues *(30)*.

Many molecules that have essential role in control of body weight and metabolic function are secreted from adipose tissue. As an adipocyte hormone, leptin signals the brain about the state of energy stores in the peripheral tissues *(31),* affecting feeding, behavior, and metabolism *(32)*. This peptide plays a key role in the food intake control, glucose metabolism, the immune system, energy consumption, the cardiovascular system, the secretion of the pituitary hormone, and insulin *(33)*. According to the total amount of the adipose tissue mass the circulating leptin levels are proportionate. In specific hypothalamic nuclei, leptin binds to its receptors in order to regulate energy balance through appetite reduction *(34)*.

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