

## Biochemical Alterations Due to Overcrowding Stress Induction in Healthy Albino Rats

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**Abstract:** Exposure to stressful situations is among the most common human experiences. This study investigated changes in some lipids and biochemical parameters in male albino rats fed with an *ad libitum* diet for 7 days. Biochemical parameters studied were glucose, cholesterol, HDL, LDL, triglyceride, uric acid, urea, creatinine, total protein, globulin, albumin, GPT and GOT. The mean weight of the animals at day 7 showed an insignificant increase in the stressed group compared with the mean weight at day 1st. Uric acid, urea and creatinine levels increased significantly ( $p < 0.05$ ) over the control. There were insignificant reductions in plasma HDL cholesterol, LDL cholesterol, total protein and globulin in the animals. Blood glucose, total cholesterol, triglyceride, albumin, GPT and GOT were also insignificantly increased. In conclusion, the present data indicate that the rats which exposed to overcrowding stress on a daily basis for a period of 7 days shown an increase in some biochemical parameters including lipids related parameters while some other parameters were reduced.

**Key words:** Acute stress, overcrowding, housing conditions, lipids parameters, biochemical parameters

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### INTRODUCTION

Exposure to stressful situations is among the most common human experiences. Stress exposure has detrimental effect on several cell functions. The organism usually responds to stress with a variety of behavioral, biological and cognitive changes (Roman *et al.*, 2003). The biochemical feature which has attracted the most sustained and widespread attention in relation to etiology and prevention of these stress induced diseases is serum cholesterol and its fractions like Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL). Cholesterol and lipoprotein level correlate well with the risk of cardiovascular diseases (Nayanatara *et al.*, 2009). Stress is known to alter some of serum enzyme levels including Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) (Nagaraja and Jeganathan, 1999).

Housing conditions affect behavioral and biological responses of animals. Effects of same-sex grouped,

crowded or individually housed conditions on plasma corticosterone levels of male and female Wistar rats were documented (Brown and Grunberg, 1995). The most profound change that occurs with individual housing is an increase in aggression of males seen in both mice and rats following even relatively brief periods of individual housing (Esquifino *et al.*, 2004). Housing density is another environmental variable that could alter mouse physiology and subsequently affect scientific studies, particularly those in which behavioral analysis and immune function are involved (Laber *et al.*, 2008).

Several researchers have clearly indicated that the pattern of neuroendocrine response is dependent on the applied stress stimulus (Pacak and Palkovits, 2001; Goldsetin *et al.*, 1996; Kvetnansky *et al.*, 2002). Plasma Adrenocorticotrophic Hormone (ACTH) concentration elevates under the influence of various types of acute stressors applied. The most intense ACTH increase is provoked by 20 and 60 min heat exposure as well as by overcrowding stress being 15, 9 and 4 times as much as

that of the respective controls (Djordjevi *et al.*, 2003). On the other hand, fasting and cold stress are weaker stressors as compared to heat and overcrowding as they produce a 2, 4 times increment of ACTH concentration. The pituitary response to acute stress is rapid and so is the return to the prestress level except for Corticosterone (CORT) (Kant *et al.*, 1989). The greatest increase in CORT synthesis appears under the influence of environmental stressors such as heat and cold.

Crowding may have a detrimental effect on health. Studies indicate that crowding is associated with increases in blood pressure (D'atri *et al.*, 1981; Evans, 1979) and increased secretion of stress hormones (Lundberg, 1976) in the short term, at least. In the longer term, the picture is not as clear. Fuller *et al.* (1993) identify two reasons why crowded conditions may be detrimental to health: first, the stress associated with crowding may depress the immune system and have other direct health effects; second, overcrowded conditions may facilitate the spread of communicable diseases. A number of studies indicate that crowded conditions (measured in terms on the number of people per household) are associated with increased incidence of colds, asthma, influenza and diarrhoea, particularly in young children (Kearns *et al.*, 1992; Kearns and Smith, 1994).

The aim of the present study was to determine the effects of overcrowding stress on the various plasma health parameters (glucose, cholesterol, HDL, LDL, triglyceride, uric acid, urea, creatinine, total protein, globulin, albumin, GPT and GOT) in male rats.

**MATERIALS AND METHODS**

**Animal maintenance and experimental design:** A total of 10 male albino rats aged 6 months were obtained and housed in the animal house of the Department of Histological Sciences, Faculty of Medicine, University of Jordan. The rats were kept at room temperature (22-26°C) in a controlled room with a 12 h light: 12 h dark cycle. The normal rat chow and tap water were provided *ad libitum* during the experiment. The duration of the experiment was 7 days. Animals were acclimatized to animal house environment for 1 week before the beginning of the experiment. Also, rats body weight were recorded at day 1st and at the end of the experiment (day 7). The study was approved by the Institutional Review Board of the University of Jordan. Rats were divided into two groups: control group (N = 5) and experimental group (N = 5). Rats in the control group were kept under standard laboratory conditions without any stress exposure. The size of the control cage was 41×28×19 cm which allowed the animals to move freely.

**Stress protocol:** The stress procedure (based on that described by Nagaraja and Jeganathan (2003) involved placing group of five male rats in cage of size 31×18×10 cm in such a way that only minimum mobility was possible inside the cages. The rats were kept in this overcrowded condition continuously for 4 h day<sup>-1</sup> then they were shifted to the control cages. All experimental stress procedures started between 9:00 am and end at 1:00 pm to minimize the effect of circadian rhythm of hormones.

**Blood collection:** Rats were anesthetized through a slight diethyl ether exposure. Blood samples were collected by heart puncture at the end of the experiment. Heparin was used as anticoagulant. The blood was centrifuged at 3000 rpm for 5 min.

**Biochemical analyses:** Laboratory analyses were performed at a private Laboratory in Amman, Jordan. Fasting blood glucose, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, total protein, albumin, globulins, uric acid, urea, creatinine, GPT and GOT of rats were measured using the commercially available kits, according to the manufacturer's instructions.

**Statistical analyses:** Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) Version 15.0 (Chicago, IL, USA). Data are presented as means±SEM. Students' t-test was used for determination of the level of significance of difference between the two groups. The difference is considered significant at p<0.05.

**RESULTS AND DISCUSSION**

Table 1 shows the values of body weight reached by the control and experimental animals. There was no significant difference observed in the weight between control and experimental groups. After 7 days, the control groups fed *ad libitum* had a mean weight of 276 g which represented an increase of ≈105.1% over their mean weight at the beginning of the experiment (≈263 g).

In the experimental group, the mean weight at day 7 was increased about 3.9% over the mean weight at day 1st. As shown in Table 2, uric acid, urea and

Table 1: Effect of acute overcrowding stress on body weight

Weights	Control (n = 5)		Experiment (n = 5)	
	Day 1	Day 7	Day 1	Day 7
Body weight (g)	263±13.5	276±15.1	248±4.6	257±7.5
Mean body weight (%)	13.4±2.5		9.6±4.6	
changes (g) (experiment vs. control)	(5.1)		(3.9)	

Values are Mean±SEM (Standard Error Mean); n = Number of animals

**Table 2: Effect of overcrowding stress on levels of some selected lipids and biochemical parameters in healthy Albino rats**

Parameters	Control (n = 5)	Experiment (n = 5)	Mean of change (experiment vs. control) (%)
Random blood sugar (mg dL <sup>-1</sup> )	131.50±11.63	138.20±19.06	6.7 (5.1)
Cholesterol total (mg dL <sup>-1</sup> )	73.80±3.02	79.00±4.62	5.2
HDL cholesterol (mg dL <sup>-1</sup> )	41.00±2.47	40.40±6.65	-0.6
LDL cholesterol (mg dL <sup>-1</sup> )	14.50±0.22	14.40±4.71	-0.1
Triglyceride (mg dL <sup>-1</sup> )	95.60±4.93	119.2±11.41	23.6
Uric acid (mg dL <sup>-1</sup> )	1.60±0.50	3.52±0.54*	1.92
Urea (mg dL <sup>-1</sup> )	32.00±0.71	39.00±1.22*	7.0
Creatinine (mg dL <sup>-1</sup> )	0.57±0.01	0.68±0.30*	0.11
Protein total (g dL <sup>-1</sup> )	7.10±0.20	6.62±0.52	-0.48
Albumin (g dL <sup>-1</sup> )	2.24±0.05	2.38±0.09	0.14
Globulin (g dL <sup>-1</sup> )	4.82±0.21	4.24±0.49	-0.58
A/G ratio	0.465	0.561	0.096
GPT (ALAT) (U L <sup>-1</sup> )	31.00±1.58	34.60±8.87	3.6
GOT (ASAT) (U L <sup>-1</sup> )	125.40±12.97	149.00±14.31	23.6

Values are Mean±SEM (Standard Error Mean); -: Decrease; n = Number of rats; \*p<0.05 vs. control

creatinine levels increased significantly (p<0.05) over the control. There were insignificant reductions in plasma HDL cholesterol, LDL cholesterol, total protein and globulin in the animals. Blood glucose, total cholesterol, triglyceride, albumin, GPT and GOT were also insignificantly increased.

Population density of laboratory rodents per cage has been considered as one of the important factors which may influence the physiological, behavioural and immunological responses (Peng *et al.*, 1989).

In the present study a decrease in the body weight was observed after crowding stress. Similar results were obtained by Nagaraja and Jeganathan (2003) who found that there was a significant decrease in the body weight after 1 and 7 days of overcrowding acute stress. Decreased body weight might be attributed to lower food intake induced by the high competition for food among these animals in a crowded ambience.

In the present study, an increase in the blood glucose, total cholesterol, triglyceride, albumin, GPT and GOT levels was observed after crowding stress. These were in agreement with some researchers (Nayanatara *et al.*, 2009) who explained that crowding stress led to increased SGOT, SGPT, blood sugar, cholesterol, triglyceride and LDL were significantly increased in the stressed group when compared to the non stressed group while HDL level did not show any statistically significant changes. Elevated activities in SGOT and SGPT demonstrated liver injury in the rats exposed to stress. The observed increase in these transaminases might be as the result of cortisol induced gluconeogenesis in the liver (Rosen *et al.*, 1958). The alterations in the membrane permeability which may occur in the cells during stress, might also contribute to the release of transaminases (Nelson, 1966; Nayanatara *et al.*, 2009). Bernatova *et al.* (2007) studied the effect of chronic crowding on the cardiovascular system of Wistar rats. Rats were randomly divided into the control (480 cm<sup>2</sup> per

rat) or crowded (200 cm<sup>2</sup> per rat) group for 8 weeks. They found that body weight, blood pressure, heart rate and plasma nitrate/nitrite levels of the crowded rats were not different from controls at the end of the experiment. Plasma corticosterone exhibited an increasing trend (5.7±1.8 vs. 12.6±3.7 ng mL<sup>-1</sup>, p = 0.08) while blood glucose was significantly reduced in the crowded rats in comparison with the controls.

Dronjak and Ljubica (2005) have studied changes that occur in plasma levels of Adrenocorticotrophic Hormone (ACTH) and Corticosterone (COTR) of adult male rats exposed to two chronic types of psychosocial stress: isolation and crowding as well as physical stress. These stress measures included forced swimming and combined psychosocial and physical stress, isolation accompanied by forced swimming, both under basal conditions and in response to immobilization and cold as short-term stressors. They found that chronic isolation produced a significant elevation of basal plasma ACTH and CORT but chronic isolation accompanied by forced swimming produced a significant elevation only in basal plasma ACTH but not CORT while chronic crowding and forced swimming did not elevate the basal plasma levels of these hormones.

Observations of chronically stressed rats have indicated that the psychological aspects of stressful situations are important in determining the magnitude of the physiological response. Recently, it has been reported that physical and emotional stress differed with regard to long-term effects on behaviour because the former resulted in the inactivity in a small open field and the later led to hyperactivity of experimental animals (Pijlman *et al.*, 2002). Later, the same researchers found that physical stress induced a long-term decrease of both preference for saccharin and open field activity but emotional stress showed an increase in open field activity and a slight increase in saccharine preference. It has been concluded that response to a novel stress depends on stress modality (Pijlman *et al.*, 2003).

The effects of five levels of population density on various organs, the neuroendocrine system, skin function, skin blood perfusion and blood parameters were studied in the hairless mouse (Ishida *et al.*, 2003). The results of this study indicate that a 7 days crowding (10, 15, 20 mice/cage) significantly increased the levels of corticosterone, catecholamines (norepinephrine, epinephrine and dopamine), glucose and serum lactate dehydrogenase activity in circulating blood induced atrophy of kidney, ovary and thymus and hypertrophy of adrenal glands and decreased body weight gain in comparison with the control (5 mice/cage) (Ishida *et al.*, 2003).

Elender *et al.* (1998) studied risk factors for tuberculosis in England and Wales and found that rates were significantly higher in households with more than one person per room. However, the view that crowding per se is detrimental to health has been challenged on the basis that people living in overcrowded conditions may be affected by a range of other variables including the presence of damp and mould and poor access to and use of healthcare facilities.

### CONCLUSION

The study revealed that overcrowding had an insignificant effect on the hematological indices except for Uric acid, urea and creatinine concentration, on acute exposure. The present data indicate that rats exposed to overcrowding stress on a daily basis for a period of 7 days had a significant increases in most of the biochemical and lipids parameters. Further research may be needed to better understand the relationship between overcrowding stress and lipid and biochemical parameters included in this study. Such studies may focus on long-term effects of overcrowding stress.

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