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## Effects of chronic restraint stress on energetic metabolism and the evolution of depression, evaluated in the open field test in the female wistar rat

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### ABSTRACT

*The present study was undertaken with an aim to elucidate the effects of chronic restraint stress on the metabolic variations of the glycemia, cholesterol, triglycerides and the evolution of depression. The chronic restraint stress was applied in rats Female Wistar albinos (1hour /day, 4 days / week for 5 weeks) in perforated plastic cylinders so that they can breathe. The results obtained show a reduction in the cholesterol level and increase in the triglycerides rate and glycemia, with appearance of a depressive state evaluated in the test of open Field at the batch stressed after the application of the pallet of the chronic stress.*

**Key words:** Rat female wistar, Chronic restraint stress, Cholesterol, Glycemia, Triglycerides, Open Field test.

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### INTRODUCTION

Many studies have been conducted to identify the role of various factors contributing to the development and maintenance of depression and anxiety [1, 2]. It has been reported that emotional and environmental stressors affect the functioning of the brain that are considered key factors in the genesis of neuropsychiatric disorders [3,4], and the restraint stress is widely used as an emotional stressor [3]. Exposure to chronic stress induces a decrease in body weight, adrenal hypertrophy and also affects neuronal plasticity [5, 1, 6]. Several protocols of stress were applied to identify the neuroendocrine mechanisms involved in the development of anxiety and depression in both animals and humans. The term of stress is conventionally defined as a nonspecific response to the action of environmental factors on the body and is accompanied by various physiological, psychological and behavioural changes, which can be related with a modifications of the HPA axis [3, 7,8,9,10,11,12,13]. Stressful events activate the hypothalamic-pituitary-adrenal axis (HPA) and increase the secretion of corticotropin-releasing hormone (CRH) from the core paraventriculaire of the hypothalamus, which causes the release of adrenocorticotrophic hormone (ACTH) from the former pituitary gland, the latter stimulates the secretion of the corticosterone from adrenal cortex using the cholesterol as mere substance [14, 15]. Glucocorticoids are able to modulate many cellular processes, such as energy metabolism, neurotransmitter metabolism [16], which are all intervening elements in response to stress and in the restoration of homeostasis. Any imbalance of brain lipid composition appears to be involved in the onset and development of some neuropathology, particularly anxiety and depression [17, 18, 19]. Cholesterol is particularly abundant in the nervous system and is important in many aspects of cellular structure and function. It ensures the fluidity and permeability of the cell membranes and the exchange process [20, 21].

The aim of this study was undertaken to investigate the effects of chronic restraint stress on hyper activation of the HPA axis and its relationship with energy metabolism and the development of depression.

## MATERIALS AND METHODS

### 2.1. Animals

Rats female coming from Pasteur Institute of Algiers were used during this study. The rats were acclimatized to the natural photoperiod standards' conditions: an average temperature of  $22 \pm 4$  ° C and a relative humidity of 50-70%. After a three weeks adaptation period, we selected 25 females according to the weight between (140-170) grams then we divided them into two experimental batches: control batch of 8 rats and stressed batch of 17 rats.

### 2.2. Restraint stress

Our model of stress is based on that of Bardin et al., (2009), at the end of the pallet of stress ,a behavioural evaluation was carried out.

### 2.3. Open field test

The apparatus based on that of Hall in 1934 [ 23 ] , it is composed of a base surrounded by parapets of Plexiglas of  $70\text{cm} \times 70\text{cm} \times 40\text{cm}$  respectively . The floor is in the form of squares of  $10\text{cm} \times 10\text{cm}$  in diameter, it has been divided into two zones: central zone and peripheral zone of each is 35cm. The animal is placed in the center of the apparatus, its displacement to measure the number of squares crossed and the time spent in each zone and the sequence was filmed for 5 minutes. Thus, this test shows the locomotor activity and anxiety behavior respectively [24]. The latter is much stronger than the rat spends more time in the peripheral zone. As for the central area, exploration represents a sign of reduced anxiety. Parameters measured include the time spent in the center of the apparatus, time spent in the periphery, distance covered.

### 2.4. Collecting blood

The blood sampling is done starting from the lachrymal vein at the end of the application of chronic restraint stress. The blood sampled was collected in the heparinized tubes then centrifuged with 5000 tr/min during 15 mn, the plasma separated was used for the proportioning of the biochemical parameters.

### 2.5. Biochemical parameters

The plasma concentrations of glucose, triglycerides, cholesterol were determined by the Trinder method [25].

### 2.6. Data analysis

The results are presented as mean  $\pm$  standard error (SEM), and were analysed by using test T of Student with the program Minitab (version13). They are regarded as being significant  $p < 0.05$ .

## RESULTS

### 3.1. Body Wight

The evolution of the body weight during 5 weeks is shown in Fig.1.

No significant difference found between the two groups (control S1 : $162.6 \pm 26.1$  vs stressed S1: $174.7 \pm 32.6$  ; control S2 : $181.1 \pm 31.8$  vs stressed  $165.3 \pm 40.1$  ; control S3 : $192.7 \pm 24.3$  vs stressed  $175.2 \pm 31.2$  ; control S4 : $204.5 \pm 24.5$  vs stressed : $194.8 \pm 25.6$  ;control S5 : $216.5 \pm 18.2$  vs stressed : $213.8 \pm 23.8$ ).

### 3.2. Biochemical parameters

Figures (2.3.4) represent the effects of chronic restraint stress on biochemical parameters, in which we find a significant increase in blood glucose (Fig. 2) the fourth and fifth week of stress respectively (control :  $0.90 \pm 0.08$  vs stressed :  $1.19 \pm 0.02$  , control :  $0.86 \pm 0.14$  vs stressed :  $1.34 \pm 0.04$ ). A highly significant reduction of cholesterol (fig.3) in the stressed lot (control:  $1.49 \pm 0.08$  vs. Stressed:  $1.21 \pm 0.021$ ) with highly significant increase of triglycerides (Fig. 4) in the same batch (control:  $0.82 \pm 0.008$  vs. stressed:  $0.93 \pm 0.026$ ).

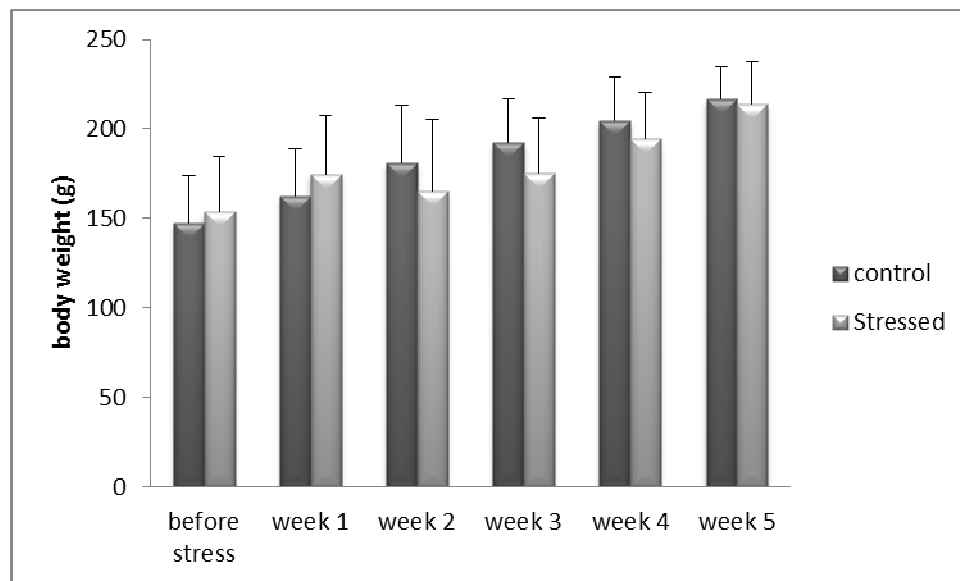


Figure 1. Effect of chronic restraint stress on the evolution of the body weight (g) of each batch, 24 after the last day of the stress  
( $m \pm s$ ;  $n$  control = 8,  $n$  stressed = 17)  
(\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

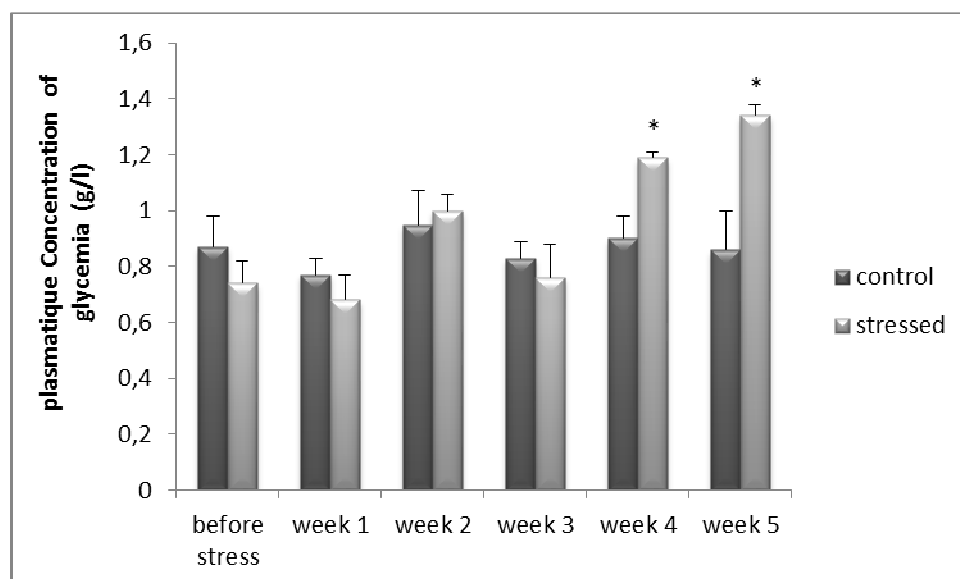


Figure 2. variation of plasmatic concentration of glucose (g/l) 24 hours after the last day of the application of restraint stress of each week  
( $m \pm s$ ;  $n$  control = 8,  $n$  stressed = 17)  
(\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

### 3.3. Results of the open field test

The results show a highly significant decrease of the distance traveled in stressed rats (control:  $1928 \pm 199$  vs. stressed:  $1400 \pm 170$ ) and significant increase of the time spent in the peripheral zone (control:  $260, 0 \pm 26, 7$  vs. stressed:  $189, 8 \pm 37, 7$ ) and a highly significant reduction in the time spent in the central area (control  $7.40 \pm 1.52$  vs. stressed:  $3.40 \pm 1.14$ ).

Table 1: parameters of the open Field test in rats 24 hours after the last day of the last week of restraint stress  
( $m \pm s$ ,  $n = 8$  control,  $n = 17$  stressed).

Paramètres / lots	témoïn	Stressé
Distance traveled (cm)	1928 ±199	1400 ±170**
Time in the peripheral zone	189,8 ±37,7	260,0±26,7 *
Time spent in the central area	7.40±1.52	3.40±1.14**

\* Indicates a significant difference between the 2 groups (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ).

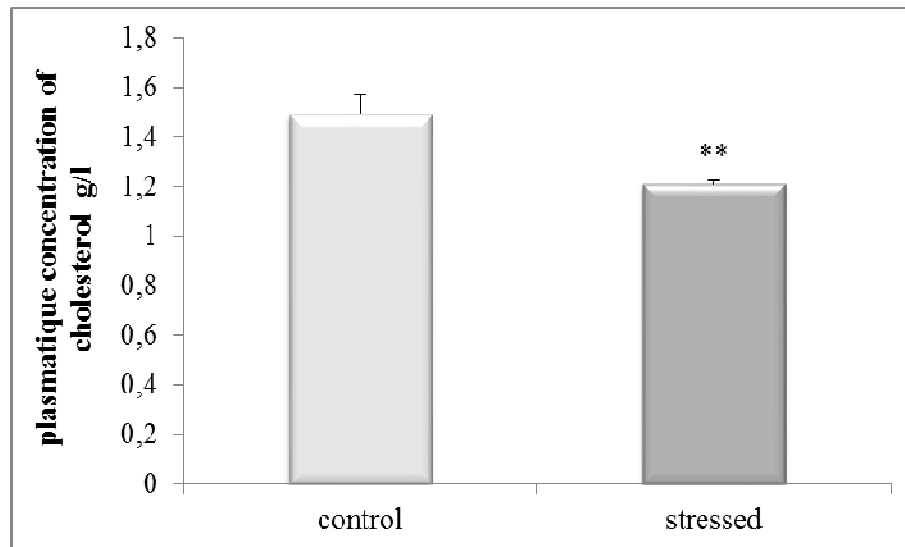


Figure 3. Variation of plasmatic concentration of cholesterol (g / l) 24 hours after the last day of the last week of restraint stress  
( $m \pm s$  ;  $n$  control =8, $n$  stressed=17)  
(\*  $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

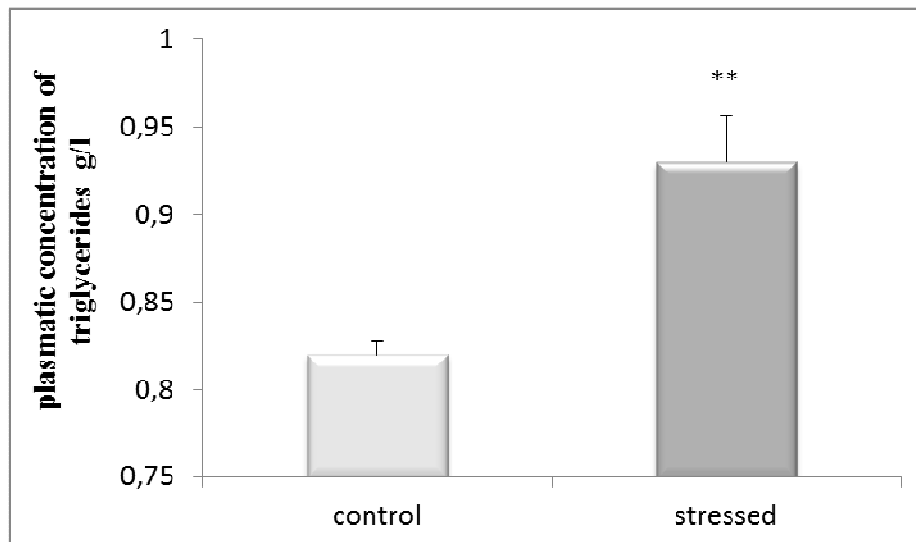


Figure 4. Variation rate triglycerides (g / l) 24 h 24 hours after the last day of the last week of restraint stress  
( $m \pm s$  ;  $n$  control=8, $n$  stressed=17). (\*  $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ).

## DISCUSSION

In case of chronic stress, morphological changes can occur and body weight can be an interesting index response capacity of the animal [26]. Chronic or repeated application of a stressor causes a decrease in weight gain [27, 28] the same result was mentioned by L. Bardin in 2009 [22] using the same type of stress. During the implementation of our protocol we found a disruption of food intake in the stressed lot compared to control group [29, 30, 31], decreased body weight is one of the depressive symptoms. [32] The differences in weight observed in this experiment may raise the hypothesis addressed by Strekalova et al. [33] which highlights a lower food intake consistent with depression. Stressed batches are anhedonia showing the installation of anxiety in response to stress. Stress also causes behavioral changes, and it would, in humans, many psychiatric disorders such as depression or anxiety disorders [34] Recent studies have noted that the restraint stress-induced glycemia, behavior anxiety and depression in rodents [35,36,37,38,39] . In contrast, other studies have not found such changes after restraint stress [40,41]. This difference in results could be attributed to the duration of chronic restraint stress [41], and the experimental procedures (eg, day-night phase of the application of stress and sex of the animal and also age) may affect the behavior [37] and the physiology of the animal. Stress can also cause aggressiveness [42, 43]. It induces in particular an increase in the level of anxiety evaluated in the open Field test [44,45] .The reduction of the distance covered in the device of open Field at stressed spleens indicates a reduction in the exploring activity

characteristic of a higher level of anxiety in rodents [45]. The increase in the time spent in the external zone and the reduction in the time spent in the internal zone of the open Field indicates a reduction in the motivation to explore a new environment due to an increase in the level of anxiety of spleens stressed [46, 47]. In stressful situations the hypothalamus receives direct stimulation of the limbic system and noradrenergic stimulation from the locus coeruleus and the nucleus of the solitary tract [48, 49]. In response to these stimuli, the hypothalamus releases the CRH, which activates the secretion of ACTH from the adenohypophysis, and the latter induces glucocorticoid synthesis from cholesterol in the adrenal glands, these hormones are involved in the stress within many functional regulations in the metabolism and the central nervous system [50].

The hyper activation of the HPA axis is accompanied by hyper-secretion of cortisol therefore hyper use cholesterol as parent substance of glucocorticoid synthesis which leads to a depletion of the plasma by the redirection for the synthesis of hormones derived or by the use of acetyl CoA synthesis in other substrates as cholesterol [51, 52, 53]. The relationship between cholesterol and the decrease in the development of depression has been suggested [54]. Another mechanism may be involved in the increase of triglycerides and cholesterol lowering and causes the depression and suicidal tendencies; it comes to the decrease in interleukin-2 [55]. The relationship between cholesterol, triglycerides and aggression in the human population has been proved by [56]. Increased triglycerides are an indicator of metabolic syndrome [57]. So triglycerides have a strong relationship with depression [58]. The biochemical profile of the depression is often characterized by low levels of cholesterol, and elevated triglycerides. Hypertriglyceridemia is considered metabolic cause of depression, it has also been demonstrated in controlled clinical trials a decrease triglycerides improved symptoms of depression. The link between hypertriglyceridemia and the depression entail insulin resistance [59]. Numerous studies have shown that the reduction of plasma cholesterol concentration is associated with depression, suicide and violent death [60, 61, 62, 63, 64]. Cholesterol is the main constituent of the membrane, which has an effect on cell growth and the function of membrane proteins. The operation of a receiver or a carrier may be modulated directly by specific molecular interactions [65] or indirectly affected by the induced change in the cholesterol in the membrane micro viscosity and permeability [60] modified by [66] it has been reported that the cholesterol in blood are used as markers in anxiety and depression [67].

glucose metabolism is a factor resulting from the activation of organ systems after a stressful situation [68]. Numerous studies have shown an increase in glucose concentrations after restraint as well as swimming in ad libitum fed rats [67]. Corticosterone is a real initiator and metabolic regulator, this hormone stimulates increased blood glucose, so it helps to release energy from body reserves, Carbohydrates are the main energy source necessary metabolism and functioning of the brain and nervous system. Glucocorticoids have a hyperglycemic action due to an increase in glucose production by stimulating gluconeogenesis and by a decrease in the consumption of glucose by peripheral tissues. Instead adrenalectomy causes a tendency to hypoglycemia and a high sensitivity to the effect of insulin. Experiencing adrenalectomy, the body is unable to mobilize its reserves to maintain a normal- glycemia in response to stress [69]. The metabolic actions relayed by the glucocorticoid receptors cause an increase in blood glucose to provide vital organs a readily available energy source. [70] Against by the intrahepatic transport increased to gluconeogenesis. Glucocorticoids exert different peripheral actions involved in lipid metabolism by increasing or stimulating lipolysis. Adrenalectomy leads to a decrease in fat mass in rats and administration of low-dose corticosterone restores lipid reserves. [69] and that diets rich in lipids act as a chronic stress by inducing an increase in the basal plasma concentration of glucocorticoids [71].

## CONCLUSION

We can therefore conclude that the results of this study provide additional evidence on how chronic restraint stress affects the physiology of the organism, and emphasize the importance of biochemical, hormonal and behavioral adaptations, so it seems that glucocorticoids act directly on energy homeostasis and our results demonstrate the relationship between the three biochemical parameters (triglycerides, cholesterol, glucose), the reduction of the body weight and the development of depression, these results were confirmed by using the open Field test, which is a validated test since 1934 by Hall. Finally we can say that this type of stress is depressinogene.

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