

# The Effect of Immobilization Stress on the Induction of Diabetes Mellitus in Rats

Manzarbanoo Shojaeifard,<sup>1</sup> Zahra Vojdani,<sup>2</sup> Saeed Karbaly-doust,<sup>2</sup> Samira Malekzadeh<sup>3</sup>

<sup>1</sup> Department of Physiology, Fasa University of Medical Sciences, Fasa, Iran

<sup>2</sup> Department of Anatomy, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>3</sup> Department of Physiology, College of Sciences, Islamic Azad University, Shiraz Branch, Shiraz, Iran

## ABSTRACT

**Purpose:** The present study aimed to investigate the effect of immobilization stress on the induction of diabetes mellitus in rats.

**Materials and Methods:** In this experimental study 30 mature male rats with an average weight of 200-220 gr were randomly divided into three groups of ten each. Group I served as the control while the experiment groups were Group II (10 days) and Groups III (30 days), which were immobilized in restraining cages twice a day for 45 minutes each time. After the last induction of stress period, blood samples were taken. Glucose was analyzed via the glucose oxidase method, and the levels of glucocorticoid and insulin were determined by radio immunoassay. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests.

**Results:** The results showed that stress could affect the induction of diabetes by increasing blood glucose, insulin, and glucocorticoid; these changes, however, were not statistically significant.

**Conclusion:** Our data showed that immobilization stress causes the induction of diabetes mellitus in rats.

**Keywords:** immobilization stress; diabetes mellitus; insulin; glucocorticoid.

AMHSR 2016;14:75-79  
www. journals. ajaums. ac. ir

## INTRODUCTION

Diabetes mellitus (DM) encompasses a group of metabolic diseases characterized by high levels of blood sugar referred to as hyperglycemia. DM results from defects in insulin production and/or insulin action, as well as impaired function in the metabolism of carbohydrates, lipids, and proteins, which leads to long-term health complications.<sup>(1)</sup>

The normal development and preservation of human life and species are dependent on a normally functioning stress system. Stress induces a chain of both emotional and physical processes. It has been considered a basic factor in the etiology of a number of diseases such as DM, cardiovascular diseases, and cancer.<sup>(2)</sup> The result of a stressful condition is the formation of diverse free radicals, which react with proteins, lipids, carbohydrates, and nucleic acids.<sup>(3)</sup> Furthermore, stress activates the

hypothalamic-pituitary-adrenal (HPA) axis and induces the release of glucocorticoid hormones, which exert widespread effects on the brain and periphery.<sup>(4)</sup> Stress via immobilization and restraint can influence many physiological aspects of an organism and is regarded as a stress condition that is followed by alterations in the body systems.<sup>(5,6)</sup> Studies have shown that chronic mild immobilization can act on the secretion of releasing hormones from the anterior pituitary, thereby influencing the function of the thyroid gland.<sup>(7,8)</sup>

There are different views on the relation between stress and DM.<sup>(9-12)</sup> Some researchers have suggested that psychological factors are potential contributors to an exacerbation in DM, particularly, in the juvenile diabetic population, whereas others have rejected the importance of stress psychopathology in diabetes.<sup>(9)</sup>

The aim of this study was to determine the possible

link between stress and DM. Immobilization was selected as the stressor, and its effects on serum glucose, insulin, and glucocorticoid level in rats were followed.

## MATERIALS AND METHODS

### Experimental Animals

In an experimental study, 30 mature male Wistar rats with a mean weight of 200-220 grams were used. The rats were provided by Razi Institute. During the study, a lighting condition of 12-hour light and 12-hour dark, at a room temperature of 22-24 °C and humidity of 55-60% was set. All of the studied animals were sacrificed in accordance with the Animal Protection Act (National Institutes of Health, 1985). The rats were randomly divided into three groups of ten. Group I served as the control group while the experiment groups were Group II (10 days) and Group III (30 days). The experiment groups were immobilized in restraining cages twice a day for 45 minutes each time.

### Hormonal Analysis

Taking blood samples was repeated 2 and 12 days after the end of the induction of the stress period, and the serums were thereafter used to assay the amount of blood glucose, glucocorticoid, and insulin. The glucose was analyzed using the glucose oxidase method, and the levels of glucocorticoid and insulin were determined by the RIA method (Spectra Kits and Irma Kits, respectively; both were purchased from Kavoshyar Co. ). After the last induction of the stress period, the blood samples were obtained.

### Statistical Analysis

The results were expressed as mean  $\pm$  standard deviation (SD). The collected data in each of the groups were analyzed by Kruskal-Wallis and Mann-Whitney tests with a ( $P = .05$ ) as the level of significance.

## RESULTS

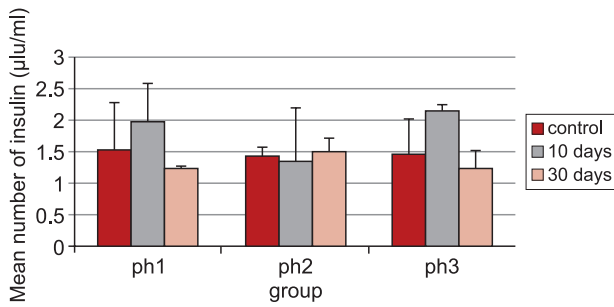
The results of the evaluation of 30 rats are depicted in **Figure 1**. There was no significant relationship between the experimental groups, exposed to immobilization stress, in terms of factors such as blood glucose, insulin, and glucocorticoid ( $P = .05$ ).

The mean value of plasma glucose in the control group was higher than that in the two groups of 10days and 30 days in three phases (**Table 1**).

Also, the mean value of blood insulin in the group of 10days in phases 1 and 3 was higher in comparison with the control group. However, that in the group of 30

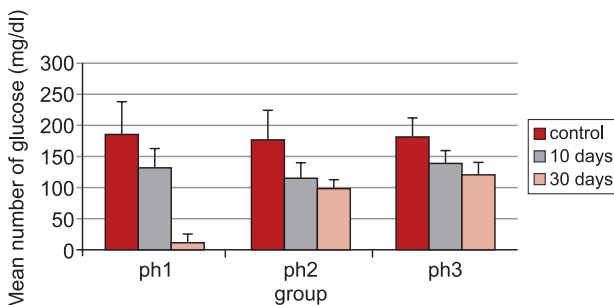
**Table 1.** Mean value of blood insulin, glucose, and glucocorticoid in three phases: Ph1: before stress induction; Ph2: a day after last stress induction; and Ph3: 12 days after last stress induction. A ( $P = .05$ ) was considered statistically significant.

	insulin ph.1 uIU/ml	insulin ph.2 uIU/ml	insulin ph.3 uIU/ml	glucose ph.1 mg/dl	glucose ph.2 mg/dl	glucose ph.3 mg/dl	glucocorticoid ph.1 mg/dl	glucocorticoid ph.2 mg/dl	glucocorticoid ph.3 mg/dl
Control	1.51 $\pm$ 0.79	1.43 $\pm$ 0.15	1.47 $\pm$ 0.55	187.40 $\pm$ 53.13	180.50 $\pm$ 47.13	183.95 $\pm$ 0.50	2.43 $\pm$ 0.18	2.38 $\pm$ 0.51	2.40 $\pm$ 0.34
10 days	1.97 $\pm$ 0.63	1.37 $\pm$ 0.84	2.13 $\pm$ 0.11	136 $\pm$ 29.37	119.66 $\pm$ 23.27	143 $\pm$ 19.84	3.23 $\pm$ 1.63	3.28 $\pm$ 0.89	2.50 $\pm$ 0.28
30 days	1.24 $\pm$ 0.4	1.50 $\pm$ 0.21	1.23 $\pm$ 0.31	114.33 $\pm$ 14.36	99.28 $\pm$ 16.68	124.50 $\pm$ 19.62	2.61 $\pm$ 1.01	2.22 $\pm$ 0.61	2.15 $\pm$ 1.23



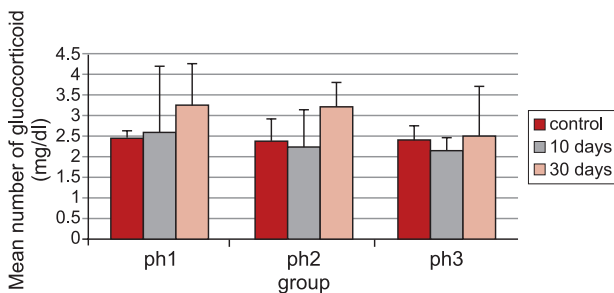
**Figure 1.** Mean value of blood insulin in three phase that ph1: before stress induction, ph2: a day after stress induction, ph3: 12 days after last stress induction.

A ( $P = .05$ ) was considered statistically significant.



**Figure 2.** Mean value of blood glucose in three phase that ph1: before stress induction, ph2: a day after stress induction, ph3: 12 days after last stress induction.

A ( $P = .05$ ) was considered statistically significant.



**Figure 3.** Mean value of blood glucocorticoid in three phase that ph1: before stress induction, ph2: a day after stress induction, ph3: 12 days after last stress induction.

A ( $P = .05$ ) was considered statistically significant.

days was lower than that in the control group in phases 1 and 3 (**Table 1**).

In another way, the mean value of glucocorticoid hormone in the group of 10 days in three phases was higher than that in the control group (**Table 1**). But, in the group of 30 days it was lower than that in the control group in phases 2 and 3 (**Table 1**).

## DISCUSSION

In the present study, the mean values of blood glucose, insulin, and glucocorticoid in the experimental groups

(10 and 30 days) were not different from those of the control group ( $P = .05$ ).

Forced immobilization is one of the best explored models of stress in rats. This model combines emotional stress (escape reaction) and physical stress (muscle work), resulting in both restricted mobility and aggression. Furthermore, painful stimuli are not directly involved in restraint stress and hence this form of stress is probably more akin to physiological stress.<sup>(13)</sup> Immobilization limits the movements of the body, which can affect the body systems and can produce various pathological states. A number of diseases are related to immobilization under conditions of chronic stress that can cause degenerative diseases such as aging and many other brain dysfunctions.<sup>(14)</sup>

There is no evidence expressing that stress causes DM. However, stress may sometimes unmask diabetes, by causing blood glucose levels to rise.<sup>(15)</sup> This is often seen after a heart attack or stroke, where raised blood sugar levels may be encountered for the first time. Dutour and colleagues in 1996 reported that acute psychological stress may play a role in the glycemic instability of some patients with type I diabetes. It has also been proposed that stress makes one more prone to diabetes. If one is in a pre-diabetic phase or possesses hereditary background, psychical stress would be able to provoke diabetic state.<sup>(16)</sup> In support to this hypothesis, it has been reported that an emotional stress in entirely normal non-diabetic subjects induces a delay in the disposal of a carbohydrate load administered to patients or produced within the body. This delay in the disposal of the carbohydrate load results in an undue elevation of the blood glucose.<sup>(17)</sup> In another investigation, the children of patients who were previously exposed to high stresses were affected by diabetes more often than the children of the controls.<sup>(18)</sup> Stress can have an adverse effect on the control of glucose level. Barglow in 1985 supported the hypothesis that psychical stressors diminish the diabetic control and increase the blood glucose.<sup>(9)</sup> Some studies have reported that emotional stress cannot affect the blood glucose level,<sup>(11,19,20)</sup> although hyperglycemia may ensue after exceptionally stressful situations.<sup>(11)</sup>

Macho and colleagues in 1992 demonstrated that short-term hypokinesia, as a stressful situation in rats, increased the plasma levels of corticosterone, epinephrine, and norepinephrine; nonetheless, if rats were exposed to hypokinesia for a long time, the plasma corticosterone level was similar to that of the control animals. Also, it was shown that repeated stress eventually leads to the absence of an adrenal response to the stress.<sup>(21)</sup> There are,

however, reports showing that long-term stress increases the level of serum corticosterone.<sup>(22,23)</sup> Nevertheless, it is deserving of note that the response highly depends on the type, intensity, and duration of the stress. For instance, tail snipping as a mild stress did not markedly change the corticosterone level immediately after stress exposure.<sup>(24)</sup> On the other hand, similar to our results, exposure to repeated stress decreased corticosterone response immediately after the stress exposure as compared to the first stress exposure, reflecting adaptation to stress.<sup>(25,26)</sup>

The results of the present study demonstrated changes in parameters that were expected; however, the differences did not constitute statistical significance. The changes in the amount of insulin or glucocorticoid hormones in the two experimental groups (10 or 30 days) in phases 1 and 2 by the end of the stress induction period do not indicate a strong effect in comparison with that before receiving stress. However, the changes in the level of the blood glucose in the two experimental groups (10 or 30 days) in phases 1 and 2 in comparison to the values before receiving stress suggest that despite the decrease in the blood sugar in the first phase, it rose in the second phase. Moreover, if there had been no time and financial constraints in conducting the third phase, it would have demonstrated a stable increase in the blood glucose level. In another study, results derived from Psychic stress were similar to our results which observed no diabetes in non-diabetic rats as the urine samples remained glucose free.<sup>(27)</sup>

## CONCLUSIONS

Our data have not shown that immobilization stress causes the induction of diabetes mellitus in rats. It reflects the probable adaptation of animal to the stress via stimulation of insulin resistance which may be affecting of glucocorticoid hormone secretion.

## ACKNOWLEDGMENT

This work was derived from the project, entitled “The effect of Immobilization Stress on the Induction of Diabetes Mellitus” and was supported by Fasa University of Medical Sciences, Iran.

## CONFLICT OF INTEREST

None declared.

## REFERENCES

- Gezginci-Oktayoglu S, Basaraner H, Yanardag R, Bolkent S. The effects of combined treatment of antioxidants on the liver injury in STZ diabetic rats. *Dig Dis Sci*. 2009;54:538-46.

- Halliwell B, Gutteridge JM. Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet*. 1984;1:1396-7.
- Yu BP. Cellular defenses against damage from reactive oxygen species. *Physiol Rev*. 1994 Jan;74(1):139-62. Review. Erratum in: *Physiol Rev* 1995;75:preceding 1.
- McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol*. 2008;583:174-85.
- Maccari S, Morley-Fletcher S. Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. *Psychoneuroendocrinology*. 2007;32:S10-5.
- Murthy NV, Wray SR, Melville GN, Wynter HH, Ram NV, Haran NV. Testicular function in rats following immobilization stress. *Int J Gynaecol Obstet*. 1988;26:297-9.
- Kioukia N, Bekris S, Antoniou K, Papadopoulou-Daifoti Z, Christofidis I. Effects of chronic mild stress (CMS) on thyroid hormone function in two rat strains. *Psychoneuroendocrinology*. 2000;25:247-57.
- Langer P, Vigas M, Kvetnanský R, Földes O, Culman J. Immediate increase of thyroid hormone release during acute stress in rats: effect of biogenic amines rather than that of TSH? *Acta Endocrinol (Copenh)*. 1983;104:443-9.
- Barglow P, Hatcher R, Berndt D, Phelps R. Psychosocial childbearing stress and metabolic control in pregnant diabetics. *J Nerv Ment Dis*. 1985;173:615-20.
- Delamater AM, Bubb J, Kurtz SM, et al. Physiologic responses to acute psychological stress in adolescents with type 1 diabetes mellitus. *J Pediatr Psychol*. 1988;13:69-86.
- Kemmer FW, Bisping R, Steingrüber HJ, et al. Psychological stress and metabolic control in patients with type I diabetes mellitus. *N Engl J Med*. 1986;314:1078-84.
- MacGillivray MH, Bruck E, Voorhess ML. Acute diabetic ketoacidosis in children: role of the stress hormones. *Pediatr Res*. 1981;15:99-106.
- Selye H. *Stress In Health and Disease*. Butterworth's. Boston and neoplasia Science 212: 1100, 1976.
- Liu J, Wang X, Shigenaga MK, Yeo HC, Mori A, Ames BN. Immobilization stress causes oxidative damage to lipid, protein, and DNA in the brain of rats. *FASEB J*. 1996;10:1532-8.
- Kahn CR, Weir GC. *Joslin's Diabetes Mellitus*. The Endocrinologist 1995;5:157-8.
- Dutour A, Boiteau V, Dadoun F, Feissel A, Atlan C, Oliver C. Hormonal response to stress in brittle diabetes. *Psychoneuroendocrinology*. 1996;21:525-43.
- DANOWSKI TS. Emotional stress as a cause of diabetes mellitus. *Diabetes*. 1963;12:183-4.
- Hauenstein EJ, Marvin RS, Snyder AL, Clarke WL. Stress in parents of children with diabetes mellitus. *Diabetes Care*. 1989;12:18-23.

19. Berger M, Berchtold P, Cüppers HJ, et al. Metabolic and hormonal effects of muscular exercise in juvenile type diabetics. *Diabetologia*. 1977;13:355-65.
20. VandenBergh RL, Sussman KE, Vaughan GD. Effects of combined physical-anticipatory stress on carbohydrate-lipid metabolism in patients with diabetes mellitus. *Psychosomatics*. 1967;8:16-9.
21. Macho L, Kvetnansky R, Fickova M, Jezova D, Lichardus B, Carey RM. Plasma levels of catecholamines, aldosterone, atrial natriuretic peptide, and renin activity during immobilization stress in rats. *Stress: Neuroendocrine and Molecular Approaches* R Kvetnansky, R McCarty, and J Axelrod, editors Gordon and Breach Science Publishers Inc, New York: 1992;187-95.
22. Kanitz E, Tuchscherer M, Puppe B, Tuchscherer A, Stabenow B. Consequences of repeated early isolation in domestic piglets (*Sus scrofa*) on their behavioural, neuroendocrine, and immunological responses. *Brain Behav Immun*. 2004;18:35-45.
23. Sher L. Daily hassles, cortisol, and the pathogenesis of depression. *Med Hypotheses*. 2004;62:198-202.
24. Pitman DL, Ottenweller JE, Natelson BH. Plasma corticosterone levels during repeated presentation of two intensities of restraint stress: chronic stress and habituation. *Physiol Behav*. 1988;43:47-55.
25. De Boer SF, Koopmans SJ, Slangen JL, Van der Gugten J. Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. *Physiol Behav*. 1990;47:1117-24.
26. Zardooz H, Zahedi Asl S, Gharib Naseri MK, Hedayati M. Effect of chronic restraint stress on carbohydrate metabolism in rat. *Physiol Behav*. 2006;89:373-8.
27. Radahmadi M, Shadan F, Karimian SM, Sadr SS, Nasimi A. Effects of stress on exacerbation of diabetes mellitus, serum glucose and cortisol levels and body weight in rats. *Pathophysiology*. 2006;13:51-5.

---

Corresponding Author:

Shojaeifard, MB  
 Department of Physiology, Fasa University of Medical Sciences,  
 Fasa, Iran  
 Tel: +98 7132308113  
 E-mail: shojaeim@sums.ac.ir

Received April 2016

Accepted May 2016