

EFFECTS OF STRESS ON DIABETES MELLITUS, SERUM GLUCOSE, SERUM CORTISOL LEVEL AND BODY WEIGHT IN MICE.

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ABSTRACT

The effects of stress on the blood glucose, serum cortisol level and body weight were investigated to clarify the possible link between the stress and diabetes. The experiments were performed on non-diabetic and streptozotocin diabetic mice divided to control, and stressed groups. Water immersion, and forced swimming was used as stressor. After the experiment, blood samples were collected. The serum glucose level (SGL) was measured by using glucometer and serum cortisol level (SCL) was determined by radioimmunoassay. Stress caused a significant increase in glucose level in both nondiabetic and diabetic mice. In diabetes mice, a significant increase in Serum Cortisol Level was observed. A significant weight loss took place in mice exposed to stress and that was much greater in diabetic control animals. The stress specially related with physical and psychic component worsen the diabetes in streptozotocin treated mice and the glucose levels increased significantly also in non-diabetic controls, but not considered as diabetic.

Keywords: Stress, Serum Cortisol, Diabetes, weight loss

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INTRODUCTION

There is no evidence that stress causes diabetes. However, stress may sometimes unmask diabetes, by causing blood glucose levels to rise [1]. In people who have diabetes, the fight-or-flight response does not work well. Insulin is not always able to let the extra energy into the cells, so glucose piles up in the blood [2]. The secretion of stress hormones (glucagon, catecholamine, cortisol and Growth Hormone) and especially, cortisol increases during the acute stress and emotional stimuli. Some of these hormones are diabetogenic and might be involved in the development of diabetes during the stress. For example, epinephrine inhibits the insulin secretion both in animals and humans, and it thus can have a diabetogenic effect [3,12]. Diabetic mice under physical or mental stress have elevated glucose levels [13]. The stress response causes even more glucose and fatty acids to be mobilized in the bloodstream. It blocks insulin production and it promotes insulin resistance [14].

When something stressful happens, many hormones are released by the brain, nervous system, and other organs. The base of the brain, the hypothalamus, secretes an array of hormones into the blood, mainly corticotropin releasing factor, which triggers the pituitary to release the hormone corticotropin (ACTH) [14]. ACTH in the bloodstream triggers the release of glucocorticoids by the adrenal gland. The sympathetic nervous system releases epinephrine (adrenaline), and norepinephrine (noradrenaline) into the bloodstream [14]. The pancreas releases a hormone called glucagon, which raises the circulating levels of glucose in the blood [14]. Cortisol might cause DM through permissive effect on catecholamines and growth hormones (GH) [15]. Hypothalamo–pituitary–adrenal (HPA) axis responds to stressful situations by elevating the cortisol metabolite levels [15-17].

Bjornthrop et al. [18] have reported that HPA axis and the sympathetic nervous system are responsible for the development of endocrine abnormalities, including Type II DM. Couch [19] has suggested that the patients with poorly controlled diabetes have a higher level of serum cortisol level. Therefore, there might be a correlation between stress, plasma cortisol level and diabetes.

Hence, the objective of this study was to determine the possible link between the stress and diabetes. Short water immersions were used as the stressor, its effects on serum glucose level (SGL), serum cortisol level (SCL) and body weight in nondiabetic and diabetic mice were observed.

MATERIALS AND METHODS

Animals

Swiss Albino Mice were purchased from the Wockhardt Ltd. Chikalthana, Aurangabad. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy Aurangabad (Approval number- CPCSEA/IAEC/P'col-20/2011-12/44).

Experiments were carried out on 32 Swiss albino mice of either sex weighing between (25-40 g) were used. They were maintained at temp. of $25 \pm 2^{\circ}\text{C}$ and relative humidity of 45 to 55% and under standard environmental conditions (12 h. light /12 h. dark cycles). The animals had free access to food and water. All the experiments were carried out between 9 to 18 hrs.

The experimental groups

Mice were randomly divided into four groups ($n = 8$ in each) as follows:

- A. Normal control(Received saline water as a vehicle control),
- B. Diabetic control(Received Streptozotocin 75 mg/kg),
- C. Diabetic under stress(Received Streptozotocin 75 mg/kg and under stress condition),
- D. Non- Diabetic under stress(Received saline water and under stress condition).

The experimental procedure

Overnight fasted experimental mice from groups B and C were injected with Streptozotocin (Sigma, USA) at a dose of 75 mg/kg body weight [20]. The STZ was injected intravenously (i.v.) within 10 min after dissolving in saline water. The mice in group A were injected with saline water as a vehicle control. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia. Fasting

blood glucose (FBG) was estimated at the time of induction of diabetes and postprandial glucose (PPG) was checked regularly until stable hyperglycemia was achieved.

Experiments were performed in the morning between 08:00 and 12:00 h [21]. One minute per hour of forced swimming and water immersion program developed at our laboratory was used (4 h every day and for 14 days) to provoke the stress as it produces a sense of fear of drowning and asphyxia [22]. This water immersion stress model was developed in our laboratory. Water immersion causes the emotional stress (fear and asphyxia), and forced swimming causes the physical stress.

After the experiments, the rats were sacrificed by decapitation in the morning (8–10 h) [17] and blood samples were collected, serum was separated and stored at -20°C until analysis [10, 22]. Then, serum glucose level was measured by using Accucheck Glucometer and serum cortisol level was measured by radioimmunoassay [5, 17, and 19].

Data analysis

Results are expressed as Mean \pm SEM ($n = 6$). The data was analysed using One-way ANOVA followed by *Dunnett's- test*. ** $P < 0.01$, * $P < 0.05$ with respective Control.

RESULTS

The mean values of serum glucose, serum cortisol levels and the changes of body weight are listed in Table ;

Table The mean values of serum glucose, serum cortisol levels and the changes of body weight.

Group	N	Treatment	Serum glucose (mg/dl)	Cortisol level (µg/DL)	Body weight (g)
A	8	Control	70.83±2.72	4.64±1.09	31.00±1.31
B	8	Diabetic control	225.00±24.73**	6.97±0.09	14.92±0.59**
C	8	Non-Diabetic in stress	105.50±26.10**	5.64±1.09	21.00 ±1.79
D	8	Diabetic under Stress	263.00 ±14.55**	8.00±1.19*	7.50 ±3.77**

Results are expressed as Mean ± SEM (n = 8). The data was analysed using One-way ANOVA followed by *Dunnett's- test*. ** P<0.01, * P<0.05 vs Respective control.

Serum glucose level [SGL]

Diabetic mice in B and D groups showed very significant (P<0.01) increase in serum glucose level as compare to control animals (A) (70.83±2.725). The SGL of non diabetic stressed mice (C), was significantly higher (P < 0.001) than in controls (A) but it did not produce diabetes. (Figure 1)

Serum cortisol level [SCL]

The STZ-treated stressed mice (D) showed significant increase in cortisol level (8.00±1.19) compare to vehicle treated control group (A). Mice from Diabetic control (B), and Non-

Diabetic under stressed (C) mice showed non-significant increase in SCL when compared with vehicle treated control group (A). (Figure 1)

Body weight

Diabetic control mice showed very significant ($P < 0.01$) decrease in body weight as compare to vehicle treated control animals (31.00 ± 1.31), and diabetic under stress groups showed very significant ($P < 0.01$) decrease in body weight as compare to diabetic control animals, indicating the effect of stress on decreasing the body weight.

Non-Diabetic under stress showed non-significant decrease in body weight when compared with respective vehicle treated control group, also indicating the effect of stress on decreasing the body weight. (Figure 1)

DISCUSSION

In recent years, the complexities of the relationship between stress and diabetes have become well known but have been less well researched. During diabetes there is increased production of Oxygen Free Radicals may contribute to the oxidative stress found in hyperglycemia associated with diabetes mellitus [23].

Our experimental results showed that the stress caused by water immersion, forced swimming showed a significant increase in SGL in streptozotocin diabetic mice and also in non-diabetic animals without causing diabetes. The water immersion causes mainly psychical stress (fear of drowning and suffocation) and consequently also physical stress (vigorous activity to come out), which lasted only 1 min per session.

Supporting findings of Bjorntop [24] has explained the physiological links between the stress and diabetes. At the same time, increase in visceral adiposity is seen, which plays important role in diabetes by contributing insulin resistance [25].

With regard to the effects of stress on the neuroendocrine system, the Hypothalamo-Pituitary-Adrenal axis is of considerable importance [26]. Upon encountering a threat or a stressor, the hypothalamus secretes corticotropin-releasing factor, which causes the release of adrenocorticotropin. This in turn travels to the adrenal cortex, where it leads to the secretion of glucocorticoid hormones, in particular cortisol. Where stressful conditions have been involved in the onset of Type 1 diabetes, Stress also affects on autoimmune system [27]. One study hypothesized that environmental factors trigger the autoimmune destruction of the β -cells [28].

Our study showed that serum cortisol level was higher in diabetic than in non-diabetic rats. This confirms some previous studies. Cortisol influences a wide range of processes,

including the breakdown of carbohydrates, lipids, and proteins to provide the body with energy. Cortisol has an immunosuppressive effect and therefore plays a role in the regulation of immune and inflammatory processes. Stress involves complex biochemical, neural and immunological mechanisms and plays a crucial role in the genesis and progression of variety of diseased states. [29]

The stress in the present study caused a much higher weight loss in diabetic than nondiabetic mice due to their metabolic disorder and greater sensitivity. The weight was decreased most probably due to the psychical stress associated with the physical stress (vigorous activity, although it lasted only 1 min per exposure) or both.

CONCLUSION

Our findings correlates to earlier researchers viz., Couch [19] and Bjorntop [24, 25] and the stressor used in this study caused a significant increase in blood glucose level in both, nondiabetic and diabetic mice. It exacerbated the disease in the diabetic mice indicated by their greater weight loss due to the stress program, but it not produce diabetes in nondiabetic under stress mice. Also stress caused a significant increase in serum cortisol level. Moreover our finding underlines effect of stress on increase in SGL, SCL and decrease in body weight in both i.e. Diabetic and nondiabetic under stress.

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REFERENCES

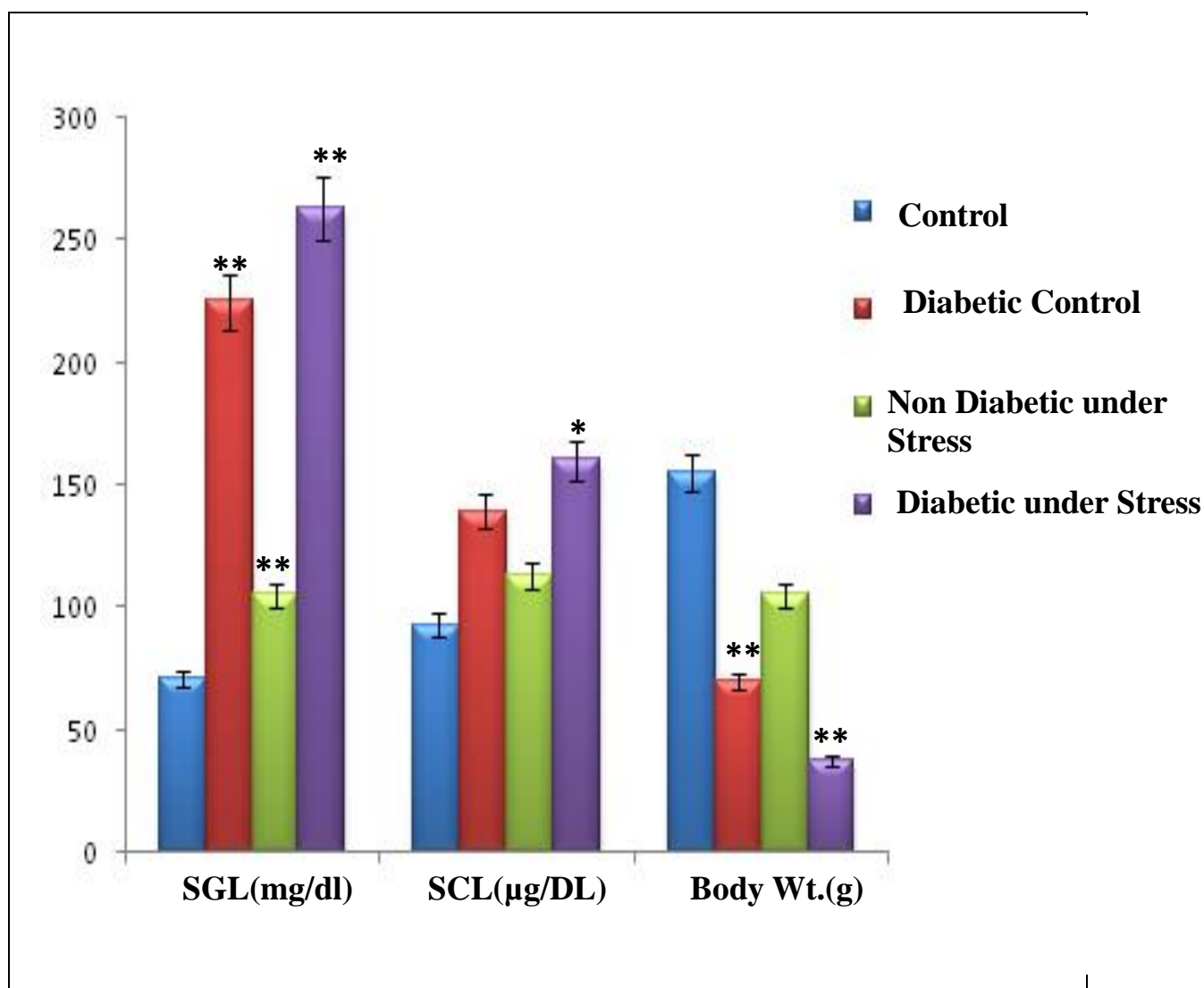
1. Kahn, C. R. and Weir. G. C. (Eds.). *Joslin's Diabetes Mellitus*. New Delhi: B.I. Waverly Pvt. Ltd. 1996.
2. American Diabetes Association. Official Home Page of American Diabetes Association. 2007. How Stress Affects Diabetes. Retrieved on 14. 2. 2007
3. Barglow P., Hatcher R., Berndt D., Phelps R., Psychosocial childbearing stress and metabolic control in pregnant diabetics, *J. Nervous Mental Dis.*;173 (10):615–620, 1985.
4. Mac Gillivray M.H., Bruck E., Voortess M.L., Acute diabetic ketoacidosis in children: role of the stress hormones, *Pediatr. Res.*; 15: 99–106, 1981.
5. Goldstein R.E., Cherrington A.D., Reed G.W., Lacy D.B., Wasserman D.H., Abumrad N.N., Effects of chronic hypercortisolemia on carbohydrate metabolism during insulin deficiency, *Am. J. Physiol.*; 266: E618–E627, 1994.
6. Schade D.S., Eaton R.P., The temporal relationship between endogenously secreted stress hormones and metabolic decompensation in diabetic man, *J. Clin. Endocrinol. Metabol.*;50 (1):131–136, 1980.
7. Wortsman J., Frank S., Cryer P.E., Adrenomedullary response to maximal stress in human, *Am. J. Med.*; 77: 779–784, 1984.
8. Garces L.Y., Kenny F.M., Drash A., Preeyasombat C., Cortisol secretion in acidotic and non-acidotic juvenile diabetes mellitus, *J. Pediatr.*; 74 (4): 517–522, 1969.
9. Barnes R.F., Raskind M., Gumbrecht G., Halter J.B., The effects of age on the plasma catecholamine response to mental stress in man, *J. Clin. Endocrinol. Metab.*;54 (1):64–69, 1982.

10. Miyabo S., Hisada T., Asato T., Mizushima N., Ueno K., Growth hormone and cortisol responses to psychological stress: comparison of normal and neurotic subjects, *J. Clin. Endocrinol. Metab.*; 42 (6):1158–1162, 1976.
11. Schade D.S., Eaton R.P., The pathogenesis of diabetic ketoacidosis: a reappraisal, *Diab. Care* 2;296, 1979.
12. Morrow L.A., Morganroth G.S., Herman W.H., Bergman R.N., Halter J.B., Effects of epinephrine on Insulin secretion and action in humans, *Diabetes* 42; 307–315., 1993
13. Analava Mitra, Kamla-Raj , Diabetes and Stress: A Review, *Ethno-Med*; 2(2): 131-135,2008.
14. Sapolsky, R.M., Freeman W.H., *Why Zebras Don't Get Ulcers*, and Co., New York, ISBN 0-7167-3210-6, 1998.
15. Vandenberg R.L., Sussmen K.E., Vaughan G.D., Effects of combined physical – anticipatory stress on carbohydrate – lipid metabolism in patient with diabetes mellitus, *Psychosomatics*;8: 16–19, 1967.
16. Macho L., Kvetnansky R., Fickova N., Jezova D., Lichardus B., Carry R.M., Plasma levels of catecholamins, aldosteron, atrial natriuretic peptide and renin activity during immobilization stress in rats. Stress: neuroendocrine and molecular approaches, in: R. Kvetnansky, R. McCarty, J. Axelrod (Eds.), *Proceeding of the Fifth International Symposium on Catecholamines and Other Neurotransmitters in Stress Smolenice Castle*, vol. 1, Czechoslovakia, June 24–29, 1991, Gordon and Breach Science Publishers, S.A., New York, U.S.A.; 187–195, 1992.

17. Parker L.N., Levin E.R., Lifrak E.T., Evidence for adrenocortical adaptation to sever illness, *J. Clin. Endocrinol. Metab.*; 60 (5):947–952, 1985.
18. Bjorntrop P., Holm G., Rsmond R., Hypothalamic arousal, insulin resistance and type 2 diabetes mellitus, *Diab. Med.*;16 (5): 373–383, 1999.
19. Couch R.M., Dissociation of cortisol and adrenal androgen secretion in poorly controlled insulin-dependent diabetes mellitus, *Acta Endocrinol.*;127:115–117, 1992.
20. Senthilvel G., Jegadeesan M., Austin A., et al. Effect of a Polyherbal Formulation (Diarum plus) on STZ induced experimental diabetes. *International journal of tropical medicine.*; 1(2):88-92, 2006.
21. Sakellaris P.C., Vernikos-Danellis J., Increased rate of response of the pituitary adrenal system in rats adapted to chronic stress, *Endocrinology.*;97 (3):597–602, 1975.
22. Radahmadi M., Shadan F., Karimian SE., Sadr SS., Nasimi A., *Pathophysiology.*; 13: 51–5, 2006.
23. JW Baynes. Perspectives in diabetes: role of oxidative stress in development of complications in diabetes. *Diabetes.*;40, 1991.
24. Bjorntop P: Body fat distribution, insulin resistance, and metabolic diseases. *Nutrition.*; 13:795 –803, 1997.
25. Bjorntop P: Visceral fat accumulation: the missing link between psychological factors and cardiovascular disease? *J Intern Med*230 : 195–201,1999.

26. Willemsen G, Lloyd C: The physiology of stressful life experiences. In Working for Health. Heller T, Muston R, Sidell M, Lloyd C, Eds. London, Open University/Sage, 2001
27. Thernlund GM, Dahlquist G, Hansson K, Ivarsson SA, Kudvigsson, Sjoblad S, Hagglof B: Psychological stress and the onset of IDDM in children. Diabetes Care.; 18:1323 –1329, 1995.
28. Bottazo GF, Pujol-Borrell R, Gale E: Etiology of diabetes: the role of autoimmune mechanisms. In The Diabetes Annual. Alberti KG, Krall LP, Eds. Amsterdam, Elsevier/North Holland,;16 –52, 1985.
29. Patave T. R., Une H. D. , Effect Of Ethanolic Extract Of *Moringa Oleifera* lam Pods On Learning And Memory In Streptozotocin Induced Diabetic Mice, International Journal of Pharmacy & Technology, Vol. 8, Issue No.1, 10510-10519, March-2016.

Figure 1 Effect of stress on serum glucose level (SGL), serum cortisol level (SCL) and body weight (B.W.)



Results are expressed as Mean \pm SEM (n = 8). The data was analysed using One-way ANOVA followed by *Dunnnett's-test*. ** P<0.01, * P<0.05 vs Respective control.