University of Thi-Qar Journal of Science (UTsci)
The 4th Scientific conference of science college/ University of Thi_Qar Volume (7), No.2

Relation between Heat Stress and Some Hormones in Alloxan Induced Diabetic Rats

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***Abstract*: The current study carried out to evaluate the levels of corticosterone, epinephrine, insulin and thyroxine accompanied induced diabetes mellitus in rats. Therefore, ninty six rats were divided into two main groups: control group which exposed to normal temperature (21±2) °C and second group which exposed to high temperature (40±2)°C each main group subdivided into three subgroups exposed to temperature for one, two and four hours respectively, also each subgroup composed of healthy rats and diabetic rats equally. At the end of experiment blood samples were collected for determination of corticosterone epinephrine, insulin and thyroxine.**

Results revealed significant increase in corticosterone and epinephrine and insulin in heat stressed group compared with control group while there was significant decrease in thyroxine in healthy and diabetic rats in heat stressed group compared with control group.Results showed significant increase in corticosterone in diabetic rats compared with healthy rats in both groups ,while the significant increase in epinephrine and insulin in diabetic rats was only in heat stressed group.Results reported significant decrease in corticosterone and thyroxine according to the increase in heat exposure duration,while epinephrine and insulin increased according to the duration. These findings indicated that heat stress may be increase harmful effects in alloxan induced diabetic rats.

Keywords : Diabetis mellitus,Heat stress,Hormones,Rats

I. Introduction

Heat stress refers to exposure to sudden high temperature for short a term duration or chronic exposure to heat (Emery, 2004).OSHA (2004) indicated that heat stress occurs when human body unable to loss the heat by sweating, also heat stress casued by high temperature and humidity direct exposure to sun, physical exertion and certain medications. Heat stress cause heat exhaustion , heat shock and healthy risks resulted from uncomfortable continuous tension (Riddell and Perkins, 1999).Stress is general and heat stress in particular has greatest influence on endocrine system and in particular hormones that play an important role in heat production and loss.

Collerir *et al.*, (2005) reported that heat stress has led to decline esterone level and increased progesterone in heat–stressed calves and that the exposure to high temperatures for short term led to elevate the levels of corticosterone and catecholamine, also found that there is an increase in prolactin and a decrease in aldosterone .In another study conducted by (Sinha, 2007) cortisol was elevated in rats after acute heat stress, but it did not occur any change after chronic heat stress also (Farooq *et al.*, 2009) indictated that heat stress wether acute on chronic led to increase cortisol initially but this decrease did not continue to long duration. Opaszawski *et al.*, (2001) found increase in growth hormone after sauna path.

Diabetes mellitus DM, defined as chronic clinical disease resulted from genetic and environmental factors (WHO, 2002) also it's hormonal disorder and unbalance in metabolism of saccharides, proteins, fats, water and electrolits accompanied with abnormal elevation in glucose level in blood, either because dysfunction in insulin secretion or defect in insulin action (Limaye *et al.*, 2003; King, 2004). DM accompanied by some disorders such as general weakness, polyuria (Allen, 2003) also lead to cardiovascular disease (Hermansen *et al.*, 2003) neuritis and skin disease (Votey and Peters, 2002), retinopathy (Oh *et al.*, 2005) and nephropathy (Azadbakht *et al.*, 2003).

Hormonal changes occurred in patients with diabetes mellitus, thyroid gland influenced by diabetes mellitus by defect in hypothalamus pituitary thyroid axis, also the increase in glucose level inhibits the enzyme deiodinase that responsible for conversion T4 to T3 (Snigh *et al.*, 2011). Also porjan *et al.*, (2010) found low levels of noradrinaline in patients with DM suffered from disorders which made them more effected by environmental conditions, in particular thermal changes, therefor current study aimed to evaluate hormonal changes in diabetic rats after exposure to heat stress for short – term

II. Materials and Methods

Experiment's animals:-

ninty six albino rats weighing (250–200) g with age (2-3) months were used in the current study ,rats were underwent to standard conditions with light and temperature and given water and diet ad libitum.

Induction of diabetes mellitus type: rats were prevented the food for 24 hours and injected with alloxan at dose (100 mg/kg) (katsumata and katsumata, 1990). glucose solution (5%) was given for prevention acute glucose deficiency, determination of glucose was conducted by blood glucose strips test to choice rats which infected with diabetes mellitus

Experiment design:

Rats randomly divided into tow main groups:

1- control group: 48 male rate exposed to normal temperature (21±2) °C and subdivided to three subgroups, each subgroup consist of healthy and diabetic rates equally and exposed to the normal temperature for 1, 2 and 4 hours respectively

2- Heat stressed group: 48 male rats exposed to high temperature (40± 2) °C and subdivided into healthy and diabetic rate equally, which exposed to high temerature for 1, 2 and 4 hours respectively

Determination of hormones **:**

after each duration of the exposure to normal or high temperature, healthy and diabetic rats were sacrificed and blood samples were collected for hormonal assays.Levels of corticosterone epinephrine and insulin were determinate by use Elisa and according to method in kits (ABO, Switzerland) thyroxine level was measured by radioimmunoassay method.

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III. Results

Corticosterone increased significantly(P <0.05) in heat-stressed group in comparison with control group either in healthy or diabetic rats also results showed significant increase(P <0.05) in diabetic rats compared with healthy rats in all three different duration of the heat exposure in both groups.On other hand , epinephrine also elevated significantly (P <0.05) in heat stressed subgroups compared with control group either the diabetic and healthy, rats in three different durations, epinephrine was not change significantly between healthy and diabetic rats after one hour of heat exposure, while there was significant difference(P <0.05) in epinephrine in diabetic rats compared with healthy rats in heat stressed group after two and four hours of heat exposure in addition, there was non significant difference within control group.

Results showed there was non significant difference in insulin in healthy and diabetic rats in heat stressed group compared with control group after one hour while after two and four hours there was significant increase(P <0.05) in insulin level in heat stressed group in comparison with control group Insulin decreased significantly in diabetic rats compared with healthy rats in heat stressed group for one hours while after four hours there was significant increase in diabetic rats compared with healthy rats in the same group.

On the other hand, thyroxine did not differs significantly in diabetic rats in heat stressed group for one hour compared with control group while there was significant decrease in healthy rats in heat stressed group after one hour of heat exposure but after two and four hours there was significant decrease (P<0.05) in diabetic and healthy rats in heat stressed group compared with control group.In regarding to the effect of induced diabetes mellitus there was significant increase(P <0.05) in diabetic rats compared to healthy rats in heat stressed group for one hour while there was significant decrease (P<0.05) in thyroxine in control group after one , two and four hours. Results reported significant decrease in corticosterone and thyroxine according to the increase in heat exposure duration,while epinephrine and insulin increased according to the duration.

Table (1): Levels of corticosterone in healthy and diabetic rats exposed to different temperatures

Duratin	Control group		Heat stressed group	
	Healthy	diabetic	healthy	diabetic
One hour	**2.86 ± 024** A a	***4.03 ± 0.22** A a	**4.73 ± 0.20** B b	***6.26 ± 0.35** B a
Two hours	**2.76 ± 0.16** A a	***4.16 ± 0.11** B a	**5.15 ± 0.81** C a	***6.53 ± 0.42** C a
Four hours	**2.10** ± 0.12 A b	***3.86 ± 0.21** B b	**4.80 ± 0.15** C b	**5.06 ± 0.70** C b

* indicates the presence of significant differences(P<0.05) between healthy and diabetic rats for each temperature and duratin,large different letters refer to significant difference between groups, small different letters refer to significant difference among durations.• values represent averages ± standard error. LSD=0.38 (among different durations in each group)

Table (2): Levels of epinephrine in healthy and diabetic rats exposed to different temperatures

Duratin	Control group		Heat stressed group	
	Healthy	diabetic	healthy	diabetic
One hour	**3.56 ± 0.14** A a	**3.60 ± 0.11** A a	**10.06 ± 0.16** BC c	**10.01 ± 0.35** C b
Two hours	**3.49 ± 0.11** A a	**3.53 ± 0.36** A a	**10.63 ± 0.27** B b	***12.43 ± 0.52** B a
Four hours	**3.63 ± 0.13** A a	**3.83 ± 0.13** A a	**12.43 ± 0.12** B a	***12.00 ± 0.19** B a

A. * indicates the presence of significant differences(P<0.05) between healthy and diabetic rats for each temperature and duratin,large different letters refer to significant difference between groups, small different letters refer to significant difference among durations. • values represent averages ± standard error.LSD=0.45 (among different durations in each group)

Table (3): Levels of insulinein healthy and diabetic rats exposed to different temperatures

Duratin	Control group		Heat stressed group	
	Healthy	diabetic	healthy	diabetic
One hour	0.80 ± 0.02 B a	*0.66 ± 0.01 A a	0.80 ± 0.10 B b	*0.70 ± 0.05 A c
Two hours	0.82 ± 0.10 A a	*0.60 ± 0.07 A a	0.96 ± 0.07 C a	0.93 ± 0.02 B b
Four hours	0.82 ± 0.03 A a	*0.63 ± 0.06 B a	0.96 ± 0.01 C	*1.10 ± 0.10 C a

* indicates the presence of significant differences(P<0.05) between healthy and diabetic rats for each temperature and duratin,large different letters refer to significant difference between groups, small different letters refer to significant difference among durations.
• values represent averages ± standard error.

LSD=0.06 (among different durations in each group)

Table (4): Levels of thyroxine in healthy and diabetic rats exposed to different temperatures

Duratin	Control group		Heat stressed group	
	Healthy	diabetic	healthy	diabetic
One hour	11.13 ± 0.58 A a	*9.10 ± 0.91 A a	8.80 ± 0.60 B a	*9.63 ± 0.85 A a
Two hours	10.99 ± 0.75 A a	*9.23 ± 0.90 B a	8.56 ± 0.66 C	*5.26 ± 0.91 A c
Four hours	10.83 ± 0.23 A a	*8.96 ± 0.33 B a	5.86 ± 0.45 C b	6.13 ± 0.75 D b

* indicates the presence of significant differences(P<0.05) between healthy and diabetic rats for each temperature and duratin,large different letters refer to significant difference between groups, small different letters refer to significant difference among durations.
• values represent averages ± standard error.

LSD=0.81 (among different durations in each group)

IV. Discussion

Different types of stress such as thermal stress activate hypothalamus pituitary adrenal axis this response was induced by ACTH releasing (Paris *et al.*, 2010). ACTH stimulates adrenal gland to produce glucocorliods such as corticosterone and catecholamines to increase glucose level (Bhattacharya *et al.*, 2000; Silberman *et al.*, 2002) demonstrate that corticosterone increased and returned to normal level in mice exposed to heat stress. All these studies agreed with the result of the current study that corticosterone level increased after heat exposure for one two and four hours.On the other hand, induced diabetes mellitus affect in corticosterone increase, which may resulted from oxidative stress caused by DM which stimulate hypothalamus pituitary axis there by ACTH release (Djordjeric *et al.*, 2003).

Regarding to the effect of heat stress on diabetic rats, stress hormone such as (corticosterone) in particular increase in case of exposure to different types of stress, and thus heat stress affect and increase the effect of DM (Goldstein *et al.*, 1994; Radahmadi *et al.*, 2006). In previous study, cortisol increased after the exposure of diabetic rats to another type of stress (Dutour *et al.*, 1996).Heat stress play role in stimulation hypothalamus pituitary adrenal gland axis and sympathetic nervous system and releasing glucocorticoids and catecholamines (AL-Khalil, 2003), Epinephrine is on of stress hormones increase in particular acute stress (Goldstein *et al.*, 1994) also (Marco *et al.*, 2008) indicated that diabetes mellitus affect most function in body and causes defect in catecholamines metabolism.

Diabetes induced by alloxan, led to decrease insulin because degradation of B- cells (Subbiah *et al.*, 2005), in addition to oxidative stress associated with diabetes cause reduction of B-cells (Szkudelski, 2001). Heat stress dosen't affect insulin level compared with control group but the decrease in insulin level continued in diabetic rats compared with healthy rats in heat stressed group.Diabetes mellitus has inhibiting effect on deiodination and then decrease T3 production (Jatwa and Kar, 2006; Hamendra and Anand, 2007), on the other hand, alloxan injection lead to effect on hypothalamus. Pituitary thyroid axis. (Moura *et al.*, 1986; Udoing *et al.*, 2007) found that insulin modified the secretion of TRH and TSH in patients with DM.The decrease in thyroid hormones in rates exposed to heat stress due to homeostasis mechanisms which aimed to decrease metabolism rats and heat production (Horowitz, 2002)

V. References

AlKhalil,M.F. (2003). Effect of heat stress in some productive and physiological characterstics in briler chickens and offspring .PhD thesis.university of Al Mosul.

Allen, D. E. 2003. The manual of diabetes education. Navigating Diabetes center. New York. USA,.

Azadbakht, L.; Shakerhosseini, R.; Atabak, S.; Jamshiclian, M.; Mehrabi, Y. and Esmaill-Zadeh, A. (2003). Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy. Ear. J. C. Nutr., 57 (10): 1292-1294.

Bhattacharya, S. K.; Bhattacharya, A. and Chakrabarti, A. (2000). Adaptogenic activity of Siotone, a polyherbal formulation of Ayurvedic rasayanas. Indian J. Exp. Biol. 38:119-128

Collier, R.J.; Baumgard, L.H.; Lock, A.L. and Bauman, D.E. (2005). Physiological limitations, nutrient partitioning. In Yield of farmed species. Constraints and

opportunities in the 21st Century (ed. R Sylvester-Bradley and J Wiseman), pp. 351–377. Nottingham University Press, Nottingham, UK. conditions. J. Anim. Sci. 78: 1458-1466.

Djordjevic, J.; Cvijic, G. and Davidovic, V. (2003). Different Activation of ACTH and Corticosterone Release in Response to Various Stressors in Rats. J. Physiol. Res. 52: 67-72.

Dutour, A.; Boiteau, V.; Dadoum, F.; Feissel, A.; Atlan, C. and Oliver, C. (1996). Hormonal response to stress in brittle diabetes. J. Psychoneuro Endocrinology 21 (6): 525–543.

Emery, J. (2004). Heat stress in poultry-Solving the acclimatization immediately prior to heat stress might be problem. Defra publications (ADAS). attributed to a reduction in feed consumption in Endocrine. J. Rev., 23: 38–89.

Farooq, U.; Samad, F.; Shen, F. and Qayyam, A. (2009). Physiological responses of cattle to heat stress. J. App., Sci., 56: 38-43.

Goldstein, R.E.; Cherrington, A.D.; Reed, G.W.; Lacy, D.B.; Wasserman, D.H. and Abumrad, N.N. (1994). Effects of chronic hypercortisolemia on carbohydrate metabolism during insulin deficiency, Am. J. Physiol. 266: 618–627.

Hamendra, S. and Anand, K. (2007). Antidiabetic potential of Citrus sinensis and Punica granatum peel extracts in alloxan treated male mice. J. Bio. Factors. 31(1): 17-24.

Hermansen, K.; Dinesen, B.; Hoie, L. H.; Morgenstem, E. and Gruenwald, J. (2003). Effects of soy and other natural products on LDL: HDL ratio and other lipid parameters: a literature review. Adv. Ther., 20(1): 50-78.

Horowitz, M. (2002). From molecular and cellular to integrative heat defence during exposure to chronic heat. J. Comparative Biochemistry and Physiology Part A 131(3): 475–483.

Jatwa, R. and Kar, A. (2006). Anti-hyperglycaemic and anti-peroxidative roles of acarbose in type II diabetes mellitus are possibly mediated through changes in thyroid function. J. Clin. and Exper. Pharmaco. and Physiol. 33: 1104–1106.

Katsumata, K. and Katsumata, Y. (1990). The potentiating effect of the simultaneous administration of tolbutamide, glibenclamide, and gliclazide on the development alloxan-induced diabetes ion rats. J. Hom. Metab. Res., 22: 51-52.

King, M.W. (2004). Medical Biochemistry. Academic Excellence, pp. 171-175.

Limaye, P.V.; Raghuram, N. and sivakami, S. (2003). Oxidative stress and gene expression of an enzymes in the renal cortex of streptozoto induced diabetic rats. J. Mol., and cellu, Bioch, 243: 147-152.

II. Marco, G.S.; Colucci, J.A.; Fernandes, F.B.; Vio, C.P.; Schor, N. and Casarini, D.E. (2008). Diabetes induces changes of catecholamines in primary mesangial cells. Int. J. Biochem. Cell. Biol.40(4): 747-54.

Moran, M.R. and Romero, F.G. (1999). Increased levels of C-Reactive protein in noncontrolled type II Diabetic subjects. J. Diabet. Comp, 13: 211-215.

Moura, E.G.; Pazos,C.C.and Rosenthal, D.(1986). Insulin deficiency impaires thyroid peroxidase activity. Plenum Medical book,627-630.

Oh, S.W.; Lee, S.; Park, C. and Kim, D.J. (2005). Elevated intraocular pressure is associated with insulin resistance and metabolic syndrome. J. Diab. Metab. Res. Rev. 21(5): 434-440.

Opaszowski, B.H.; Busko, K. and Blachnio, D. (2001). Effect of dehydration to Oryx (Oryx leucoryx). J. of Experim. Biolo. 206: 1471-1478.

OSHA. **(**Occupational Safety and Health Administration) Heat Stress (2004). Home U.S. Department of Labor www.osha.gov (800) 321-OSHA.

Paris, J.J.; Franco, C.; Sodano, R.; Freidenberg, B.; Gordis, E. and Anderson, D.**A.** (2010). Sex differences in salivary cortisol in response to acute stressors among healthy participants, in recreational or pathological gamblers, and in those with posttraumatic stress disorder. J. Horm., Behav., 57: 35-45.

Peter, S. (2002). Thermoregulation . The Australian Naturopathic Met Work. 1998-2002.

Porojan, M.; Costin, S.; Poanta, L.; Cerghizan, A.; Pop, D. and Dumitrascu, D. L. (2010). Autonomic Neuropathy and Plasma Catecholamine in Patients with Diabetes Mellitus. J. Inter. Med., 48(4): 341–345.

Radahmadi, M.; Shadan, F.; Karimian, S.; Sadr, S. and Nasimi, A. (2006). Effects of stress on exacerbation of diabetes mellitus, serum glucose and cortisol levels and body weight in rats. J. Pathophy., 13: 51–55.

Riddell, M. C. and Perkins, B. A. (2009). Exercise and glucose metabolism in persons with diabetes mellitus: perspectives on the role for continuous glucose monitoring. J. of Dia. Sci. and Tech. 3(4): 914-923.

Silberman, D.M.; Wald, M. and Genaro, A.M. (2002). Effects of chronic mild stress on lymphocyte proliferative response. Participation of plasma thyroid hormones and corticosterone. J. Int. Immunopharmacol. 2: 487–497.

Singh, G.; Gupta , V.; Kumar, A.; Sharma, F .and Gupta, A. (2011). Evaluation of Thyroid Dysfunction Among type 2 diabetic Punjabi Population Adv. Biores. 2(2): 3-8.

Sinha, R.K. (2007). Study of changes in some pathophysiological stress markers in, different age groups of an animal model of acute and chronic heat stress. J. Iranian Biom., 11(2): 101-111.

Subbiah, R.; Karuran, S. and Sorimuthu, S. (2005). Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacological Reports by Institute of pharmacology. J. Polish. Aca. of Sci. 54: 90-96.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in β cells of the rats pancrease. J. Physiol. Res., 50: 536-546.

Uding,C.E.; Udoh, A.C.and Etukudoh, M.E.(2007).Evaluation of thyroid function in diabetes mellitus in calabr,Nigeria.Indian J.Clin.Bioch.22 (2):74-78.

Votey, S.R. and Peters, A.L. (2002). Diabetes Mellitus. Type 2:A Review. Los Angeles County. University of Southern California. Medical Center. USA.

WHO,World health organization. (2002). Diabetes mellitus. Saudi Medical J. 23:612-615.

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