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Effects of Diabetes on Bone Formation and Bone Resorption

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ABSTRACT

An experiment was conducted to study the effect of heat or cold stresses on bone formation and bone resorption in diabetic rats. The study included two experiments. The first experiment in summer season include 60 adult male albino rats with an average live body weight 100±10g. Rats were distributed into 4 groups (15 rats each). Group (1) control (2) Aloxan diabetic under Rome condition (3) normal rats under heat stress (the Temperature in open area and relative humidity were 40.40 ±4.63 C and 50.65 ±10.86 % respectively) (4) Aloxan diabetic rats under heat stress. The second experiment in winter season included 60 adult male albino rats with an average live body weight 100±10g. Rats were distributed into 4 groups (15 rats each). Group (1) control (2) Aloxan diabetic under Rome condition (3) normal rats under cold stress (the Temperature in open area and relative humidity were 09.44±4.28 C and 55.95 ±9.92 % respectively) (4) Aloxan diabetic rats under cold stress. Blood sample were collected after 3, 6 and 9 weeks from the start of the experiment and centrifuged at 3000 rpm for 15 min to obtained serum. Results show that serum lactic dehydrogenase (LDH), Alkaline phosphatases (ALP), Alanine transaminase (ALT), and Aspartate transaminase (AST) activities were significantly increased in diabetic rats than normal rats. Serum T3 (Triiodothyronine) and T4 (Thyroxine) were significantly decreased in diabetic rats than normal rats. Serum glucose and creatinine levels were significantly increased in diabetic rats than normal rats.

Key words: Heat, cold stresses**,** bone formation, bone resorption, Diabetes

Introduction

Diabetes Mellitus affects skeletal system and bone metabolism through multiple pathways and it recognized as a major risk factor for osteoporosis Leidig-Bruckner and Zeigler 2001.

Process of bone formation (osteogenesis) involves three main steps: 1-production of the extracellular organic matrix (osteoid); -2-mineralization of the matrix to form bone; 3-bone remodeling by resorption and reformation.

The cellular activities of osteoblasts, osteocytes, and osteoclasts are essential to the process. Osteoblasts synthesize the collagenous precursors of bone matrix and also regulate its mineralization. As the process of bone formation progresses, the osteoblasts come to lie in tiny spaces (lacunae) within the surrounding mineralized matrix and then called osteocytes. The cell processes of osteocytes occupy minute canals (canaliculi) which permit the circulation of tissue fluids. To meet the requirements of skeletal growth and mechanical function, bone undergoes dynamic remodeling by a coupling process of bone resorptionthrough osteoclasts and reformation by osteoblasts. Diabetes mellitus is accepted to be the commonest endocrine disease resulting from deficiency in the secretion or action of the pancreatic hormone insulin which, in turn produced profound abnormalities of metabolism (Celik *et al*., 2002) and alternation of neuronal and/or vascular functions (Hilton *et al*., 1983; Shalaby *et al*., 1989; and Wick *et al*., 2005). These factors may influence the thermoregulatory competence (Scott *et al*., 1987; and Tulp *et al*., 1994). Khalil (2012 and 2005a) demonstrated that thermoregulation can be accomplished by physiological or behavioral ways through the increase of heat production which is adequate with oxygen consumption and regulation of heat loss in suitable form according to heat production. Diabetes mellius was recorded to be accompanied by thermoregulatory abnormalities (Kilgour and Williams, 1996).

The present study aimed to study the relation between alloxan diabetic rats, normal rats and bone formation and bone resorption during cold or heat stress.

Materials and Methods

This study was carried out in Animal House Lab. Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt which provided standard laboratory chemicals and equipment for this study..

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Experimental Animals:

The albino rats used in the study were originally bought from El Osman Farm, Cairo, Egypt. Animals were housed in cages under ambient temperature (ranged from 29 to45 C^o) in summer or (between 5to17.5 C^o) in winter. Light dark cycle was maintained on about 12-hour. The standard laboratory chow and tap water were provided adlibitum. All animals were healthy and clinically free from diseases. Diabetes mellitus was induced by interperitoneal injection of Alloxan solution (0.1 ml/100g body weight). Alloxan solution consists of 0.12g (120 mg/kg) Alloxan hydrasin per 1 ml 1 buffer solution.). Some rats resistant to Alloxan.so were excluded.

Experiment Outline:

One heat stress experiment was carried out during summer (First of July to30th of August, 2014).One cold stress experiments was carried out during winter (15th un of December 2014 till 15th of February, 2015). The experiments started after one week ofAlloxan injection in summer and winter.

Heat stress experiment:

In summer, total of 60 animals were assigned into four groups each contain 15 rats

G1 Normal ratsunder room temperature (Non diabetic animals), (Control)

G2 Diabeticrats under room temperature., (Control)

G3 Normal rats under Heatstress (Non diabetic animals)

G4 Diabetic rats under Heat stress

The mean ambient temperature (AT) was 30.20 ± 3.8 $C^0$and relative humidity (RH) 71.65 ± 10.26 % for groups G1 and G3. Heat stress groups (exposed to solar radiation) where temperature in open area was 40.40 ±4.63 C^0 and relative humidity was50.65 ±10.86 % and black body temperature (BBT) was 46.8 ±4.61 % for group G2&G4. The group G2&G4 exposure to the outside every day for two hours, (from 10.00. am to 12.00 pm).

In all groups Rectal temperature (RT) was measured immediately after exposure.

Cold-stress experiments:

In winter a total of60 animals were assigned to four groups: each contain 15 rats.

G1 Normal rats under room temperature (Control).

G2 Diabeticrats under room temperature.

G3 Normal rats under cold stress.

G4 Diabetic rats under cold stress.

The mean ambient temperature (AT) was 14± 2.1 C^0 and relative humidity (RH)was65.1 ± 9.75 % for G1 and G3. Cold stress groups (G2&G4) (exposed to open area) where temperature was 09.44±4.28 C^0 and relative humidity 55.95 ±9.92 %.

In all groups rectal temperature (RT) was measured immediately after exposure. Rats exposed to cold stress under atmospheric temperatures (9.5 -12.0 C^0) every day for two hours, (from 12.00. am to 02.00 am).

Serum Collection:

Blood samples were obtained from rats by withdrawing blood from the orbital venous plexuses using a capillary tube. Samples were collected after 3, 6 and 9 weeks from the start of the experiment. Blood samples collected and taken and centrifuged at 3000 rpm for 15 min to obtain serum. Serum was transferred to Ependorff tube and stored at20C^o until subsequent analyses

Serum parameters:

The glucose concentration determined by glucose oxidase method Trinder, (1969). Activities of ALT and AST in serum were measured by colorimetric method as described by White *et al*., (1970). Alkaline phosphatase (ALP) measured by a colorimetric method as described by (Marsh)(1959)**.**Creatinine was determined by a kinetic method according to Henry,(1974). Lactic dehydrogenase was measured by LDH stabino Kit. LDH specifically catalyzes the oxidation of lactate to pyruvate with the subsequent reduction of NAD to NADH2. The rate at which NADH forms is proportional to LDH activity. The method described determines the increase in NADH absorbance per minute Henry, (1974).

Hormonal measurements:

T3 and T4 hormones in serum were measured by radio-immunoassay (RIA) Bablok 1988.

Osteocalcin hormone in serum was measured by radio-immunoassay (RIA) Osteocalcin (OC) (Garnero 1996). PTH hormone in serum was measured by radio-immunoassay (RIA) for PTH (Silverman, 1973).

Thermoregulatory parameters:

Rectal temperature was measured with clinical thermometer (Model HI98509) inserted 1.5 cm in rectum and taped to the tail for 1minute.

Statistical Analysis:

Data was subjected to analysis of variance using the General Linear Models procedure of SPSS software program package (SPSS, 2001, version 11.0). All percentages were first transformed to arcsine then analyzed to approximate normal distribution before ANOVA. In addition, significant differences among means were determined by Duncan's multiple range test (Duncan, 1955) at 5% level of significance. Data were analyzed by one- way method.

Results and Discussion

Rectal Temperature (RT):

Table (1) shows that during summer after 1,3,5,7 and 9 weeks of start of the experiment had no significant changes in rectal temperature between the control and non-stressed diabetic rats. Meanwhile rectal temperature was significantly increased in normal and diabetic rats under heat stress than normal rat under room temperature. Table (1) also show that under heat stress rectal temperature was significantly decreased in diabetic rats under heat stress than normal rats under heat stress.

Table (2) shows that during winter after 1,3,5 and 7 weeks of start of the experiment had no significant changes in rectal temperature between the control rats under room temperature and normal rat under cold stress. Meanwhile rectal temperature was significantly decreased in diabetic rats under room temperature and diabetic rats under cold stress than the control rats while after 9 weeks rectal temperature significantly decreased in cold stress diabetic rats than other rat groups.

During summer or winter seasons diabetic rats fail to maintain the balance between heat production and heat loss

The above results show that heat stress during summer significantly increased rectal temperature in normal and diabetic rats. Exposure to heat stress had been previously noted to increased rectal temperature (Abdel Hamid, 2004; Lawrow, 1950 and Yousef, 1971). The data also showed that under heat stress rectal temperature was significantly decreased in diabetic rats than normal rats. These results are in contrast with those found by Ibrahim and Abdellatif, (2012) who reported that during summer the diabetic rabbit group had higher rectal temperature compared to non-diabetic groups.

The above results also show that during winter rectal temperature significantly decreased in diabetic rats than normal rats. These results may be due to that the heat production was decreases in diabetic rats than normal rats.

Table 1: Mean ±S.E for the effect of Diabetes Rectal Temperature C^{o} (RT) at week (1,3,5,7 and 9) in Summer

Groups	Summer									
	Week 1		Week 3		Week 5		Week 7		Week 9	
	Mea±SE	dt1	Mean±SE	dt1	Mean±SE	dt1	Mean±SE	dt1	Mean±SE	dt1
G1	37.82±0.11	C	37.82±0.12	C	37.82±0.11	C	37.82±0.11	C	37.82±0.11	C
G2	37.45±0.22	C	37.45±0.22	C	37.55±0.19	C	37.45±0.23	C	37.45±0.23	C
G3	41.87±0.50	A	42.37±0.21	A	41.87±0.50	A	41.62±0.38	A	42.12±0.29	A
G4	38.90±0.31	B	39.05±0.18	B	39.00±0.35	B	38.90±0.32	B	39.15±0.44	B

G1 = Normal Rat under Room temperature (control), G2 = Diabetic rat under Room temperature, G3 = Normal control rat under heat stress, G4 = Diabetic rat under heat stress, S.E = stander error, dt1= Duncan's Multiple Range test between group, dt2= Duncan's Multiple Range test between times (1, 3, 5, 7& 9 weeks) on each week.
Mean within each row with similar letters are not significant different at p ≥0.05.

Table 2: Mean ±S.E for the effect of Diabetes Rectal Temperature ^{o}C (RT) at week (1,3,5,7&9) in Winter

Groups	Winter									
	Week 1		Week 3		Week 5		Week 7		Week 9	
	Mean±SE	dt1	Mean±SE	dt1	Mean±SE	dt1	Mean±SE	dt1	Mean±SE	dt1
G1	37.10±0.15	A	37.10±0.15	A	37.35±0.13	A	37.35±0.14	A	37.10±0.14	A
G2	36.55±0.18	B	36.30±0.15	B	36.30±0.41	B	36.30±0.15	B	36.55±0.18	A
G3	36.85±0.18	AB	37.10±0.37	A	37.10±0.07	AB	36.85±0.18	A	36.85±0.18	A
G4	34.77±0.13	C	34.77±0.13	C	35.02±0.34	C	34.52±0.21	C	35.02±0.34	B

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature, G3 = Normal control rat under cold stress, G4 = Diabetic rat under cold stress, S.E = stander error, Dt1= Duncan's Multiple Range test between group, Dt2= Duncan's Multiple Range test between times (1, 3, 5, 7& 9 weeks) on each week.
Mean within each row with similar letters are not significant different at p ≥0.05.

Body Weight:

Tables (3and 4) shows that during summer or winter seasons there was no significant differences in body weight between the control and other treatment groups. Meanwhile after 5 or 9 weeks of treatments body weight

was significantly decreased in all treatment groups than control group. The results also showed that body weight was significantly decreased in diabetic rats than the control and normal rats under heat or cold stress. Moreover body weight was significantly decreased in diabetic rats under heat or cold stresses than diabetic rats at room temperature.

The above results indicate that exposure of normal or diabetic rats to heat or cold stress significantly decreased body weight as compared to control group.

The obtained results also show that decreases in body weight after exposure of rats to heat or cold stress was more pronounced in diabetic rats under heat or cold stresses than diabetic rats under normal condition. These results are in agreement with Johan el at, (1990) who observed that after 12 weeks of diabetes, the adult diabetic body weight rats had lost considerable weight; their final weight was only 60% of that of control rats. Bernard, *et al.* (1970) observed that animals respond to the severe alloxan-induced diabetes. Approximately half of all the examined animals either became "obese" or strikingly emaciated and moribund.

Table 3: Mean ±S.E for the effect of Diabetes Body Weight (g) at week (1,5 and 9) in summer.

Groups	Week 1		Week 5		Week 9	
	Mea± SE	dt1	Mean± SE	dt1	Mean± SE	dt1
G1	96.25±2.393	A	192.5±1.443	A	280±3.535	A
G2	96.25±3.145	A	115±2.041	C	116.2±2.393	C
G3	100±3.535	A	185±2.041	B	265±2.89	B
G4	100±2.041	A	97.50±1.443	D	97.50±3.227	D

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature,G3 = Normal control rat under heat stress, G4 = Diabetic rat under heat stress, S.E = stander error, dt1= Duncan's Multiple Range test between group, dt2= Duncan's Multiple Range test between times (1, 5, 9 weeks) on each week.
Mean within each row with similar letters are not significant different at p ≥0.05.

Table 4: Mean ±S.E for the effect of Diabetes Body Weight (g) at week (1,5 and 9) in winter.

Groups	Week 1		Week 5		Week 9	
	Mean±SE	dt1	Mean±SE	dt1	Mean±SE	dt1
G1	103.7±4.269	A	185±2.041	A	265±2.886	A
G2	100±3.535	A	105±2.041	C	107.5±3.227	C
G3	96.25±3.145	A	178.7±1.250	B	245±2.886	B
G4	100±2.041	A	88.75±2.393	D	67.50±1.443	D

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature, G3 = Normal control rat under cold stress,G4 = Diabetic rat under cold stress,S.E = stander error, dt1= Duncan's Multiple Range test between group, dt2= Duncan's Multiple Range test between times (1,5&9 weeks) on each week
Mean within each row with similar letters are not significant different at p ≥0.05.

Mortality rate:

Table (5) shows that during summer the percentage of mortality rate were 13.3, 26.66, 20 and 53.33 % for the normal group, diabetic rats at room temperature, normal rats under heat stress and diabetic rats under heat stress respectively. These results reveal that in normal rats heat stress increased the mortality rate by 6.67 % than the control group. Meanwhile mortality rate was increased by 13.33 % and 40 % in diabetic rats at room temperature and under heat stress respectively than the control group.

The above results indicate that the highest mortality rats occurred in heat stress diabetic group and decreased in diabetic rats at room temperature by 32.77 % than it.

Table 5: Mortality rate at week (1,2,3,4,5,6,7 and 9) in summer.

Groups	Summer									%
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	
G1		1			1					13.33 %
G2	1				1		1		1	26.66 %
G3		1			1			1		20 %
G4	2	1	1				2	1	1	53.33 %

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature, G3 = Normal control rat under heat stress ,G4 = Diabetic rat under heat stress

Table (6) shows that during winter the percentages of mortality rate were 20, 40, 26.6 and 73.33 % for the normal group, diabetic rats at room temperature, normal rats under cold stress and diabetic rats under cold stress respectively. These results reveals that in normal rats cold stress increased the mortality rate by 6.66 % than the control group. Meanwhile mortality rate increased by 20 % and 53.3 % in diabetic rats at room temperature and diabetic rats under cold stress respectively than the control group.

The above results indicate that the highest mortality rate occurred in cold stress diabetic group and decreased in diabetic rats at room temperature by 33.33 % than the heat stressed diabetic rats.

Table 6: Mortality rateat week (1,2,3,4,5,6,7 and 9) in winter.

Groups	Winter									%
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	
G1				1		1			1	20 %
G2	3	1		1				1		40 %
G3	2		1						1	26.66 %
G4	3	2	2			1		1	2	73.3 %

G1 = Normal rat under Room temperature (control),G2 = Diabetic rat under Room temperature,G3 = Normal control rat under cold stress G4 = Diabetic rat under cold stress.

Blood Serum Parameters:

Serum T3 (Triiodothyronine) and Serum T4 (Thyroxine) Concentration:

Table (7) show that during summer after 9 weeks of starting the experiment T3 was significantly decreased in non-stressed diabetic rats, normal rats under heat stress and diabetic rats under heat stress compare to the control group. These results indicate that heat stress significantly decreased serum T3 in normal rats under heat stress and diabetic rats under heat stress, the results also indicate that serum T3 was significantly decreased in heat stress diabetic rats than diabetic rats under room temperature and normal rats under heat stress. Table (7) show that during winter after 9 weeks of starting the experiment T3 was significantly increased in normal rats under cold stress compare with control group. Meanwhile T3 was significantly decreased in non-stressed diabetic rats and diabetic rats under cold stress compared with control group and normal rats under cold stress. Table (7) show that during summer after 9 weeks of experiment start T4was significantly decreased in non-stressed diabetic rats, normal rats under heat stress and diabetic rats under heat stress compare to the control group. These results indicate that heat stress significantly decrease serum T4 in normal and diabetic rats. The results also indicate that diabetes induce more decrease in serum T4 than heat stress. The results in table (7) show that serum T4 was decreased in heat stress diabetic rats (2.55±0.241) than heat stress normal rats (4.495±0.878) but these decreases statistically not significant. Table (7) shows that during winter after 9 weeks to starting the experiment T4 significantly increased in normal rats under cold stress compare with control group. Meanwhile T4 was significantly decreased in non-stress diabetic rats and diabetic rats under cold stress compare with control group and normal rats under cold stress.

Table 7: Mean ±S.E for the effect of Diabetes on serum (T3 and T4) (nmol/L) in summer and winter..

Groups	T3				T4			
	Summer Mean±SE	DT.	Winter Mean±SE	DT.	Summer Mean±SE	DT.	Winter Mean±SE	DT.
G1	114.2±2.318	A	149.7±7.752	B	7.800±0.625	A	9.072±0.624	B
G2	67.59±1.591	C	78.07±1.784	C	3.157±0.567	B	3.272±0.483	C
G3	90.49±1.599	B	174.3±3.294	A	4.495±0.878	B	12.322±0.861	A
G4	58.21±1.140	D	63.43±1.454	D	2.552±0.241	B	3.087±0.086	C

G1 = Normal rat under Room temperature (control),G2 = Diabetic rat under Room temperature,G3 = Normal rat under heat stress (at summer) & under cold stress (at winter),G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter),S.E = stander error, Dt= Duncan's Multiple Range test between group.
Mean within each row with similar letters are not significant different at p ≥0.05.

These results are in agreement with Saha, (2012) who observed that the incidence of thyroid dysfunction was significantly higher under diabetic subjects (20%) than with non-diabetic. The incidences of hypothyroidism was significantly ($P<0.05$) increases in diabetic female group (6.66%) compared with non-diabetic female group. Nima (2011) observed that T3 and T4 levels were significantly higher in normal compared to diabetic ($p < 0.05$). At the same time, these levels remained significantly low in hypothyroidism subjects when compared with normal subject. The above results indicate that in normal and diabetic rat's thyroid gland responds to both heat and cold stress and this response is inversely related to ambient temperatures which whereas animals was exposed the results indicated that activity of thyroid gland decreased in diabetic rats than normal rats and this effect were more pronounced under heat or cold stresses than under normal condition. The decrease in activity of thyroid gland in diabetic rats may be due to the decrease in the energy availability to the thyroid gland under decreasing of Insulin hormone in diabetic rats (Khalil 2013) Insulin facilitates entry of glucose into muscle, adipose tissues and several of other tissues. The decrease of glucose lead to decrease of ATP and decrease the metabolism of the thyroid gland. Khalil (1990,2005,2012 and 2013)), Khalifa (1982), and Abd-El-Bary (1982), reported that most characteristic effect of the thyroid hormones is to increase energy production and oxygen consumption in most normal mammalian tissues. The conversion of chemical energy by digestion to potential kinetic form as function of the organism called calorigenesis. This processes normally regulated by number of hormones, including ACTH and GH, as well as thyroid hormone. The cost of living of organisms or basal metabolic rate (BMR) is determined by several factors, including age and sex however, thyroid hormones appear to be the chief regulatory of energy production and oxygen consumption. The calorigenic action of thyroid hormones probably occurs by the way of their effects on enzymes particularly the enzymes of mitochondrial respiratory chain. As a result, most tissue of the body with the exception of brain,

gonads, lymph nodes, adenohypophysis and spleen respond to the thyroid hormones by increasing metabolism activity that includes increase blood flow and respiratory rates. In the digestive tract, the thyroid hormones increase motility and absorption, as well as the metabolism of nutrients (carbohydrates, fats and proteins) and many vitamins that function as coenzymes. Thyroid hormones are necessary for the maintenance of normal protein synthesis rates. The early part of the biphasic response to thyroid hormone involves increase in formation of both structure and functional proteins such as enzymes, which increased initially following thyroid hormone stimulation, since they are responsible, ultimately, of any observed alterations in metabolic rates attributed to the hormones. Structural proteins, typically found in bone and muscle tissues, are increased during the 1st phase of hormone stimulation, but the continued T3 and T4 secretion leads to the second phase, characterized by increasing catabolism of structural body protein to be used as an energy source. Thyroid hormones are also permissive regulator for activities controlled principally by other hormones. These activities are bone formation, bone resorpation, water and electrolyte balance and hormonal control of the intermediary metabolism. At the same time, other hormones affect thyroid gland function either directly or through the respective release of trophic hormones.

Serum PTH (Parathyroid hormone) Concentration:

Table (8) shows that during summer after 9 weeks of starting experiment serum PTH was significantly increase in non-stressed diabetic rats and heat stressed diabetic rats compare with the control group. Meanwhile there were no significant differences in serum PTH between normal rats under heat stress and control rats.

Table (8) shows that during winter after 9 weeks of starting the experiment therewas no significant effect on serum PTH levels between rats under normal or cold stress, diabetic rats under cold stress and non-stress diabetic rats compare with control rats. The results also show that serum PTH were increased in diabetic rats under cold stress (17.65±1.26) than the other groups but this increase was statistically not significant.

The above results indicate that in normal rats heat or cold stress didn't show any significant effect on serum PTH. These results in turn indicate that heat or cold stress didn't affect on bone resorbtion in normal rats, which agree with those found by Abdel Hamid (2004) who reported that no significant differences in serum PTH levels of rats exposed to heat, indicating that exposure to heat in summer was of minor importance for osteoclastic reactions (i.e bone resorption).

The results in table (8) also show that in normal rats serum PTH levels tended to increase in winter than in summer and this result indicate that cold stress increased the osteoclasticreactions. Abdel Hamid (2004) reported that serum PTH levels tended to increase with the decrease in ambient temperature. in other words the osteoclastic reaction (i.e. bone resorptin) tended to increase with the daily repeated exposure to cold for a long period (2 month).

The above results indicate that during summer serum PTH level in diabetic rats groups was significantly increased than in normal rats groups. The significant increase in serum PTH in diabetic rats during summer may be due to the effect of diabeteson decreasing serum T3 and T4 or may be due to the effect of diabetes on calcium metabolism.

Para thyroid hormone responds to the moment-by-moment fluctuation of Ca++ in the blood and other extracellular fluids at the following way ; when concentration of Ca++ in blood perfuse the parathyroid gland so that it drops below its set point (2.2 – 2.5 mmol/L) and the gland releases parathyroid hormone (PTH) into the circulation. PTH, in turn, affects the osteoclasts and the osteocytes by stimulating them to affect bone resorption, thus releasing Ca^{++} and phosphates from the bone into the circulation (Khalil 2013).

During summer heat stress significantly decreased serum T3 and T4. Thyroid hormones plays an important role in mineral metabolism Mosekilde (1990) and Allain (1993). Thus the significant decrease in T3 and T4 affect mineral metabolism. Sgal *et al*. (1989) showed that T3 produced arepid and concentration –related increase in Ca++ -ATPase activity. Vivek and Prasad (2003) demonstrated that Ca++ uptake into intestinal Brush Border Membrane Vesicles (BBMV) increased in response to thyroid hormone status and also showed that hypothyroid rats showed a significant decrease in initial uptake of Ca++. Thus heat stress decreased T3 and T4 as decreased in T3 and T4 lead to decrease in Ca++-ATPase activity. The decrease in Ca++-ATPase activity lead to decrease the absorption of calcium from the intestine. The decrease in calcium absorption lead to increase PTH secretion.

During summer in diabetic rats we showed more increase in serum PTH than the normal rats. These results may be the effect of diabetes on calcium metabolism. Sultan *et al*. (2008) showed that serum calcium levels of diabetic group were significantly lower compared with the control group, specially the uncontrolled group (9.96±1.9 mg /dl, $p< 0.05$). They also showed that diabetic group showed significant increase in levels of urinary calcium (270.66±41.7 and 300.56±55.67 mg) than the control group (244.23±51.5 mg), especially the uncontrolled one ($p< 0.05$ and $p< 0.001$) respectively.

The significant increase in the urinary calcium and significantly decreased in serum calcium lead to significant increase in serum PTH. The above results indicate that heat stress + diabetes have a double effect on increasing bone resorbtion.

During winter in normal rats cold stress didn't show any significant effect on serum PTH as compared with the control group. In diabetic rats serum PTH was increased in cold stress diabetic rats (17.65) than the normal rats and diabetic rats under room temperature but these results statistically not significant. The increase in PTH in cold stress diabetic rats may be due to the decreased in energy availability in the cell of the small intestine that transport calcium form the intestine to reach the blood circulation.

These results are in agreement with Caroline (2014) who observed increase of PTH levels with diabetic. Sultan (2008) observed that PTH level was higher in diabetic group compared with control group.(overweight, due to type 2 diabetes).

Serum Osteocalcin Concentration:

Table (8) shows that during summer after 9 weeks from start of the experiment there were no significant differences in serum Osteocalcin levels between normal rats under cold stress, diabetic rats under cold stress and non-stress diabetic rats compare with control rats.

Table (8) shows that during winter after 9 weeks from start of the experiment there was no significant differences in serum Osteocalc in levels between normal rats under cold stress, diabetic rats under cold stress and non-stressed diabetic rats compare with control rats.

The results also show that serum osteocalcin level in the control group 1.084 and 1.65 in summer and winter respectively, which means that bone formation was increased in winter than summer.

The results are in accordance with those obtained by Abdel Hamid (2004) who concluded that hyperthermia was effective on decreasing serum osteocalcin levels and in turn bone formation.

The above results also show that in summer serum osteocalcin was decreased in diabetic rat under room temperature (0.83) and diabetic rat under heat stress (0.89) than normal rat (1.08) and normal rat under heat stress but this effect were not significant. This results are in accordance with those obtained by Ranjbar (2012) who observed lower level of osteocalc in at diabetic groups compared with control group. Sultan (2008) observed lower level of osteocalc in in both diabetic groups (patients who were overweight, with type 2 diabetes) compared with control group.

Table 8: Mean ±S.E for the effect of Diabetes on serum PTH (pg/ml)and Osteocalcin(ng/ml) in summer and winter.

Groups	PTH				Osteocalcin			
	Summer Mean±SE	DT.	Winter Mean±SE	DT.	Summer Mean±SE	DT.	Winter Mean±SE	DT.
G1	9.77±1.667	B	12.50±2.087	A	1.085±0.042	A	1.650±0.184	A
G2	20.96±0.813	A	13.37±3.500	A	0.835±0.283	A	1.775±0.201	A
G3	9.55±1.132	B	13.91±1.426	A	1.103±0.106	A	1.237±0.289	A
G4	23.44±1.772	A	17.65±1.261	A	0.895±0.273	A	1.450±0.155	A

G1 = Normal rat under Room temperature (control),G2 = Diabetic rat under Room temperature, G3 = Normal rat under heat stress (at summer) & under cold stress (at winter), G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter),S.E = stander error, Dt= Duncan's Multiple Range test between group
Mean within each row with similar letters are not significant different at p ≥0.05

4.4. Serum LDH (Lactate dehydrogenase) Concentration:

Tables (9) show that during summer or winter seasons after 3, 6, & 9 weeks from start of the experiment serum LDH were significantly increased in non-stress diabetic and stress diabetic rats compare with the control rats and normal rats under stress. These results indicate that heat or cold stress had no significant effect on serum LDH, but diabetes significantly increase serum LDH. The increase in serum LDH in diabetic rats may be due to the decrease in the energy availability to the cells that lead to decrease the enzyme activity or cellular death which lead to increase serum LDH.

Lactate dehydrogenase is an intracellular enzyme that is widely distributed in the tissues of body, particularly in the kidney, heart, skeletal muscle, brine, liver, and lungs. Increases in the reported value usually indicate cellular death and leakage of the enzyme of cell (Frances *et al*., 2003).

These results are in agreement with Saddala (2012) who observed that the activity of LDH was significantly ($p < 0.01$) increased in the kidney in diabetic rats. El-Demerdash el at(2005)who found that inalloxan-diabetic rats the activities of plasma LDH, was significantly ($p < 0.05$) increased by 37%, relative to their normal levels.

Bernard *et al*. (1970) showed that there was an abrupt and marked increase in LDH levels in the fourth day coincident with the hepatic lipidosis and the other stressful conditions which accompanied the acute onset of severe diabetes. Again, the arteriosclerotic female breeders displayed the greatest increase in serum LDH levels, i.e., a 204 % increase, in the fourth day following the injection of Alloxan.

Table 9: Mean ±S.E for the effect of Diabetes on serum LDH (u/l) in summer and winter.

Groups	LDH											
	Summer Mean±SE						Winter Mean±SE					
	3 W.	dt	6W.	dt	9W.	dt	3 W.	dt	6W.	dt	9W.	dt
G1	163.95±11.70	B	169.25±22.45	B	160.78±19.98	C	153.60±5.20	B	176.50±7.837	B	196.68±10.31	B
G2	268.15±12.37	A	220.88±12.62	A	244.75±17.02	B	249.02±45.77	A	294.45±16.93	A	221.72±44.07	A
G3	161.80±8.91	B	182.62±7.14	B	150.22±9.37	C	148.95±6.18	B	179.78±27.82	B	209.05±5.41	B
G4	214.73±18.85	A	227.12±22.87	A	316.03±11.15	A	303.40±21.39	A	345.12±13.25	A	262.42±40.56	A

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature, G3 = Normal rat under heat stress (at summer) & under cold stress (at winter), G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter), Dt= Duncan's Multiple Range test between group, W. =weeks , S.E = stander error
Mean within each row with similar letters are not significant different at p ≥0.05

Serum Glucose Concentration:

Table (10) shows that during summer after 3, 6 and 9 weeks from start of the experiment serum glucose was significantly increased in non-stressdiabetic rats and diabetic rats groups compare with the control group. Meanwhile there was no significant differences between normal rat under heat stress and control group.

Table (10) shows that during summer the mean glucose level was significantly higher in diabetic rat than normal rat group at all times. Table (10) shows that during winter after 3, 6 and 9 weeks from start of the experiment serum glucose was significantly increased in non-stress diabetic rats and diabetic rats group compare with control group. Meanwhile there was no significant differences between normal rats under heat stress and control rat group. Table (10) also showed that serum glucose was significantly increase in diabetic rat under cold stress compare with diabetic rats under room temperature

Table (10) shows that during winter the mean glucose level was significantly higher in diabetic rat than in normal rat group at all time. Moreover serum glucose was significantly increased in diabetic rat under cold stress than diabetic rat under room temperature.

These results are in agreement with Saha, (2012) who found that blood glucose was significantly increased in diabetic group compared with non-diabetic group. Sultan (2008) reported that blood sugar levels of both diabetic groups were significantly higher than in controls. Blood glucose levels are mainly regulated by insulin and glucagon hormones which are released from Langerhans islets of pancreas. Thus, insulin regulates blood glucose levels during the anabolic phase by transformation of blood glucose into glycogen in muscles and liver, glucagons regulates the blood glucose levels during the reverse catabolic phase.

The results also show that during winter serum glucose was significantly higher in cold stress diabetic rat than diabetic rat under room temperature these results may be due to the effect of adrenal cortex or adrenal medulla on increasing the secretion of cortisol and /or Epinephrine or nor Epinephrine.

Bethin *et al.*,(2000) observed that mechanisms by which neuroendocrine changes occur in response to stress are organized primarily in the brain, with hypothalamic secretion of hypophseal releasing hormones and control of the autonomic nervous system. Thus, stress increases sympathetic nervous system activity and the hypothalamic-pituitary-adrenal axis acts to enhance glucocorticoid secretion.

Kkalil (2013) reported that during stress Epenephrine and nor Epenephrine were secreted from adrenal medulla and reach to the pancreas Epinephrine and nor Epenephrine decreased the cyclic AMP in β-cells and thus decreased the insulin secretion of pancreas. At the same time Epinephrine and nor Epinephrine increased cyclic AMP in α-cells and thus increased the secretion of glucagon hormone of the pancreas. The effect of Epinephrine and nor Epinephrine on α-cells and β-cells of the pancreas may lead to the increase in serum glucose levels in cold stress diabetic rats.

The increase in serum glucose in cold stress diabetic rats may be due to the increase in serum corisol during cold stress. Kkalil (2013) reported that cortisol hormone increase the glucose level in blood by activating the gluconeogenesis process in the liver and decreased the glucose consumption by several body tissues (muscle tissue, adipose tissue .. etc.)

Table 10: Mean ±S.E for the effect of Diabetes on serum Glucose (mg/dl) in summer and winter.

Groups	Glucose											
	Summer Mean± SE						Winter Mean± SE					
	3 W.	dt	6W.	dt	9W.	dt	3 W.	dt	6W.	dt	9W.	dt
G1	89.25±4.49	C	91.00±4.70	B	104.75±3.03	B	113.00±2.38	C	111.75±4.26	C	106.25±2.42	C
G2	347.50±23.67	A	367.50±25.97	A	321.25±23.69	A	358.75±7.69	B	328.75±19.15	B	393.00±11.45	B
G3	76.00±7.42	C	86.25±5.86	B	86.75±5.00	B	120.50±4.11	C	132.00±8.05	C	115.00±2.12	C
G4	292.50±8.539	B	330.25±22.94	A	299.00±9.67	A	427.50±9.63	A	400.50±12.03	A	401.25±7.487	A

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature,G3 = Normal rat under heat stress (at summer) & under cold stress (at winter),G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter), Dt= Duncan's Multiple Range test between group, W. =weeks, S.E = stander error
Mean within each row with similar letters are not significant different at p ≥0.05.

Serum Alkaline Phosphates (ALP) activities:

Table (11) shows that during summer or winter seasons after 3, 6 and 9 weeks from start of the experiment, serum ALP activities were significantly increased in non-stressed diabetic rats and stressed diabetic rats group compare with the control. Meanwhile there were no significant differences between normal rats under heat stress and control group.

Table (11) shows that serum alkaline phosphates (ALP) activities were high at diabetic groups than any other groups during summer or winter seasons at all times.

These results are in agreement with Ranjbar *et al*., (2012) who observed that bone serum alkaline phosphatase activities significantly higher with diabetic groups(diabetic patients with chronic disease such as chronic renal failure and diabetic Patients) compared with control group. Sultan *et al*., (2008) showed that PTH level in all diabetic patients was found to correlate positively with ALP level (r= 0.54, P<0.01) and negatively with serum calcium (r = -0.65, P<0.01). Radhia *et al*., (2012) observed a significant increase in the level of Alkaline phosphatase (ALP) for diabetic groups compared with control group. Sultan,(2008) reported that the detected elevation in ALP level could be explained by the prolonged exposure to PTH which eventually increase ofosteoblastic activity.

Table 11: Mean ±S.E for the effect of Diabetes on serum ALP (U/L) in summer and winter.

Groups	ALP											
	Summer Mean±SE						Winter Mean±SE					
	3 W.	dt	6W.	dt	9W.	dt	3 W.	dt	6W.	dt	9W.	dt
G1	205.70±11.60	B	213.56±14.78	B	217.78±9.38	B	125.59 ±11.92	B	117.34±10.15	B	120.18±9.80	B
G2	304.55±12.05	A	321.03±15.72	A	321.03 ±6.45	A	270.64 ±9.41	A	271.90±14.65	A	241.40±10.32	A
G3	216.55±26.54	B	236.96±44.22	B	225.26 ±37.10	B	115.46 ±11.05	B	132.64±17.70	B	114.00±14.56	B
G4	329.70±21.52	A	326.04±17.91	A	268.74 ±78.13	A	237.71 ±14.82	A	259.86±13.77	A	257.13±9.40	A

G1 = Normal rat under Room temperature (control),G2 = Diabetic rat under Room temperature,G3 = Normal rat under heat stress (at summer) & under cold stress (at winter),G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter),Dt= Duncan's Multiple Range test between group,W. =weeks ,S.E = stander error.
Mean within each row with similar letters are not significant different at p ≥0.05.

Serum ALT (Alanine transaminase) activities:

Table (12) shows that during summer or winter seasons after 3, 6, & 9 weeks from the start of the experiment serum ALT was significantly increased in non-stressed diabetic rat and stressed diabetic rats compare with the control rats and normal rats under heat stress. These results indicate that heat or cold stresses had no significant effect on serum ALT, but diabetes significantly increase serum ALT activities.

The above results indicate that elevated liver enzymes in rats with diabetes due to that diabetes is a chronic disease, and always lead the disease to complications, affects all the body cells, and show symptoms of infection of cells in important organs such as liver. Accompanying diabetes many of the imbalances in the liver fat accumulation: the accumulation of fat inside liver cells occurs (what is known as fatty liver) which sometimes accompanied by inflammation in the liver and increase liver enzymes. Lack discipline of blood sugar may occur which accumulate glycogen in the cells of liver probably due to the large disparity in level of blood sugar.In this case increases the size of the liver and liver enzymes rise

These results are in agreement with Radhia (2012) who found that Alanine amino transferees (ALT), significantly increase in diabetic groups compared with control group. El-Demerdash (2005) observed that the activities of plasma AST&ALT, were significantly ($p < 0.05$) increased by 49& 60, %, respectively in Alloxan-diabetic rats as compared to the control group. They also indicated that diabetes might induce hepatic dysfunction. Celik *et al*. (2002) observed that ALT was slightly higher in the diabetic group than in the control group.

Table 12: Mean ±S.E for the effect of Diabetes on serum ALT (U/L) in summer and winter.

Groups	ALT											
	Summer Mean±SE						Winter Mean±SE					
	3 W.	dt	6W.	dt	9W.	dt	3 W.	dt	6W.	dt	9W.	dt
G1	69.75±6.15	B	84.40±5.55	B	78.07±6.63	B	27.60±1.17	B	18.32±1.072	B	22.14±0.53	B
G2	74.85±1.28	A	90.60±9.94	A	90.68±2.74	A	43.62±0.56	A	50.76±7.96	A	50.93±6.42	A
G3	79.59±6.72	B	83.25±5.35	B	72.94±6.32	B	27.53±1.77	B	28.67±1.86	B	27.17±0.76	B
G4	89.04±3.89	A	84.40±5.067	A	90.04±5.01	A	48.75±5.60	A	40.70±3.63	A	67.50±12.81	A

G1 = Normal rat under Room temperature(control),G2 = Diabetic rat under Room temperature, G3 = Normal rat under heat stress (at summer) & under cold stress (at winter), G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter), Dt= Duncan's Multiple Range test between group, W. =weeks, S.E = stander error
Mean within each row with similar letters are not significant different at p ≥0.05.

4Serum AST (Aspartate transaminase) activities:

Tables (13) showed that during summer season after 3, 6, & 9 weeks of starting of the experiment serum AST was significantly increased in non-stressed diabetic rats and stressed diabetic rats compare with the control rats and normal rats under heat stress. The result also indicate that serum AST was significantly increased in diabetic rats under heat stress compare with all groups. Tables (13) show that during winter season after 3,6,& 9 weeks of starting the experiment serum AST were significantly increased in diabetic rat under cold stress and diabetic rat compare with the control rats and normal rats under cold stress. The result also indicates that serum AST had no significant difference between control groups and normal rats under cold stress**.**

The above results indicate that elevated liver enzymes in rats with diabetes due to that it is a chronic disease, that always lead to complications, affects all the body's cells, and show symptoms of infection of cells in important organs such as liver. Diabetes associate with many imbalances in liver that accumulate fat inside liver cells (what is known as fatty liver) and sometimes accompanied with inflammation in liver. The increase of liver enzymes might cause lack discipline of blood sugar which may accumulate glycogen in the liver cells probably due to the large disparity in the level of blood sugar. In this case size of the liver increases and liver enzymes rise.

These results are in agreement with El-Demerdash (2005) who observed that the activities of plasma AST&ALT were significantly ($p < 0.05$) increased by 49& 60,%, respectively in Alloxan-diabetic rats as compared with the control group. They also indicated that diabetes may induce hepatic dysfunction. Celik *et al*. (2002) observed that AST&ALT, were slightly higher in the diabetic group than in the control group. Abdel Hamid (2004) observed that in summer and winter there is no significant effect on serum AST & ALT levels between the normal group and stressed cold and heat groups.

Table 13: Mean ±S.E for the effect of Diabetes on serum AST (U/L) in summer and winter.

Groups	AST											
	Summer Mean±SE						Winter Mean±SE					
	3 W.	dt	6W.	dt	9W.	dt	3 W.	dt	6W.	dt	9W.	dt
G1	22.73±1.3	C	36.21±11.81	C	24.51±2.51	C	41.57±2.34	B	38.77±1.56	B	38.62±0.99	B
G2	38.24±3.94	B	45.40±2.22	B	41.80±3.60	B	64.88±1.57	A	51.17±1.69	A	52.80±2.52	A
G3	27.75±0.89	C	27.68±0.42	C	30.56±1.53	C	54.74±1.88	B	39.70±1.20	B	41.63±5.53	B
G4	57.57±1.96	A	52.40±3.16	A	58.65±0.99	A	70.65±8.64	A	52.97±1.75	A	56.86±2.81	A

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature,G3 = Normal rat under heat stress (at summer) & under cold stress (at winter),G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter), Dt= Duncan's Multiple Range test between group, W. =weeks , S.E = stander error .
Mean within each row with similar letters are not significant different at p ≥0.05.

Serum Creatinine Concentration:

Tables (14) show that during summer or winter seasons after 3,6,&9 weeks of the start of experiment, serum creatinine was significantly increased in non-stressed diabetic and stress diabetic rats compare with the control rats and normal rats under heat or cold stresses. These results indicate that heat or cold stress had no significant effect on serum creatinine, but diabetes significantly increases serum creatinine.

May the high creatinine level in diabetic animals is due to rise of sugar level in the blood which leads to increase work of the kidneys, thereby increasing the overall stress. In addition, lack of energy resulted of a lack of thyroid hormones led to increase the content of creatinine and the weakness of kidneys.

These results are in agreement with El-Demerdash (2005) who observed that levels of creatinine was significantly ($P< 0.05$) increased in plasma of Alxoan –diabetic rats compared to control group. Also indicted that diabetes could lead to renal dysfunction.

Ranjbar, (2012), observed that creatinine level significantly high in diabetic groups compared with control group. Radhia,(2012), found that increase level of serum creatinine significantly differ in diabetic groups compared with control group.

Table 14: Mean ±S.E for the effect of Diabetes on serum Criatininin(mg/dl) summer and winter

Groups	Criatinin											
	Summer Mean±SE						Winter Mean±SE					
	3 W.	dt	6W.	dt	9W.	dt	3 W.	dt	6W.	dt	9W.	dt
G1	0.57±0.06	B	0.55±0.009	B	0.52±0.026	B	0.53±0.53	B	0.51±0.02	B	0.53±0.01	B
G2	0.72±0.23	A	0.66±0.007	A	0.88±0.043	A	0.74±0.64	A	0.75±0.088	A	0.79±0.06	A
G3	0.52±0.026	B	0.51±0.027	B	0.65±0.058	B	0.50±0.50	B	0.52±0.026	B	0.55±0.10	B
G4	0.91±0.022	A	0.68±0.090	A	0.77±0.069	A	0.73±0.63	A	0.73±0.09	A	0.79±0.06	A

G1 = Normal rat under Room temperature (control), G2 = Diabetic rat under Room temperature, G3 = Normal rat under heat stress (at summer) & under cold stress (at winter),G4 = Diabetic rat under heat stress (at summer) & under cold stress (at winter), Dt= Duncan's Multiple Range test between group, W. =weeks, S.E = stander error
Mean within each row with similar letters are not significant different at p ≥0.05

Conclusion

In normal rats heat stress significantly decrease serum T3 and T4. Meanwhile cold stress significantly increase serum T3 and T4. These results indicate that thyroid gland responds to both heat and cold stresses and this response is inversely related to ambient temperatures in which the animals is exposed. Thyroid hormones are also permissive regulators for activities controlled principally by other hormones. These activates bone formation and bone resorption. In diabetic rats heat stress caused more significant decrease in serum T3 and T4. Also in diabetic rats cold stress significantly decrease T3 and T4hormones. The significant decrease in T3 and T4lead to increase bone resorption.

In normal rat heat or cold stresses didn't show any significant effect on serum PTH. Meanwhile in diabetic rats serum PTH significantly increase compared with the control group. Serum PTH was increased in cold stress diabetic but not significantly.

In normal and diabetic rats heat or cold stresses didn't show any significant effect on serum osteocalcin. These results indicate that normal or diabetic rats under heat or cold stresses didn't show any significant effect on bone formation.

In normal rats heat or cold stress didn't show any significant effect on serum glucose. Diabetes significantly increased serum glucose moreover cold stress which significantly increase serum glucose in diabetic rats than diabetic rats under room temperature.

In our experiment we suggest that in diabetic animals heat or cold increased bone resorption and this effect leads to bone diseases.

The present results suggest that it is worth to carry out further studies to detect how to protect animals from the side effects of heat or cold stresses in diabetic animals.

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