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Effects of Cold Temperature on the Activities of Glutathione Peroxidase, Lactate Dehydrogenase and Free Radicals Production in *Uromastyx aegyptius*

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The purpose of this study was to evaluate the effect of long-term cold exposure on the activity of Glutathione Peroxidase (GPX), Lactate Dehydrogenase (LDH) and Free Radicals (FR) production in spiny tailed lizard, *Uromastyx aegyptius*. Fifteen animals were randomly assigned to two groups. The experimental group underwent whole body cold exposure (8-10°C) for seven weeks period. In comparison with the control group FR production and LDH activity were increased significantly ($p<0.05$) whereas GPX activity was decreased significantly ($p<0.05$) under cold exposure. The regression of LDH on FR was significant ($R^2 = 0.67$) indicting that FR affects LDH activity and there is a commonality exists between both variables. Based on the results of the present study it can be concluded that cold exposure induces acclimation in LDH activity. However the reductions of GPX reflect cold intolerance.

Key words: GPX, LDH, FR, cold acclimatization, cold intolerance, *U. aegyptius*

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INTRODUCTION

Cellular responses and adaptations to ambient temperature changes have attracted the attention of environmental physiologist through out the last two decades. Cellular processes are affected by oxidation when the productions of oxidants overwhelm antioxidants. Living Cell implies a balance of free radicals (FR) production, scavenging excess FR and repair of damage caused by FR. Adaptation to cold requires compensation such as glucose depletion and tissue hypoxia that aggravate the balance between FR production and removal and hence causing cellular toxicity.

Although long-term and short-term compensatory responses to cold tolerance of vertebrate have been described (Crockett and Sidell, 1990; Portner, 2002; Sommer and Portner, 2002; Zakhartsev *et al*., 2003) much less is known about cellular toxicity induced by Reactive Oxygen Species (ROS) and antioxidants enzymes in response to cold. Enzymes activities have been reliable tool in determining the cellular functions and adaptations (Kehrer and Lund, 1994; Baumeister *et al*., 2004).

Mitochondria swelling and hyperplasia is major source for Reactive Oxygen Species (ROS) that results in an increased reactive H_2O_2 and subsequent accumulation of FR (Humphries *et al*., 1988; Dawson *et al*., 1993; Cacciutteolo *et al*., 1999; Davidson and Schiestl, 2001; Haffor *et al*., 2002a, b; Lannig *et al*., 2003; Haffor, 2004). Glutathione peroxidase (GPX) is important antioxidant enzyme in the peroxidase system of cytchrom-C, Quinones and ascrobate that allow for the maintaining H_2O_2 at or nearby normal level (Paglia and Valentine, 1967). In addition, lactate dehydrogenase (LDH) prevent cytotoxicity (Ozernyuk *et al*., 1994; Danilenko *et al*., 1998; Fields *et al*., 2002; Haffor and Al-Johany, 2005). LDH regulates the byproducts of mitochondrial oxidative or reductive stress conditions via the maintenance of lactic acid at normal level by converting it to pyruvate.

It has been generally accepted that oxidative and reductive stress result in elevation of ROS. It is not clear nor reported whether adaptation to cold is mediated by changes in ROS and antioxidants.

Uromastyx egyptius are ectothermic that have increased activity at the upper limit of their preferred temperature zone in comparison with mammals mainly due to their physiological adaptation against dehydration (Al-Johany and Al-Sadoon, 1996; Al-Johany *et al*., 1996; Chiara *et al*., 2004). In addition *U. aegyptius* undergo hibernation for several months (Al-Johany *et al*., 1999) subsequently they undergoes seasonal metabolic fluctuation and hence cellular metabolic changes. We reasoned that adaptation of lizards to cold temperature involve imbalance between FR production and antioxidants scavenging processes. The purpose of this study was to examine the changes in FR production, GPX and LDH activities during cold acclimation in Lizards, *U. aegyptius*.

MATERIALS AND METHODS

Animals and experimental design: All animals were obtained from the central region in the Kingdom of Saudi Arabia and kept 3 days to adjust to laboratory condition. Fifteen adult lizards, *U. aegyptius*, matched by age and body weigh were divided randomly into two groups, six experimental and nine controls. The experimental group underwent whole body cold exposure (8-10°C) for seven weeks period. This temperature range was assumed to be within the cold tolerance range for *Uromastyx egyptius*. The control group was kept at the laboratory room temperature (24-25°C). All animals were fed mixed greens obtained from their native environment and all were kept under the same light conditions, 12 h light and 12 h dark.

Statistical analysis: Mean group differences for FR, GPX and LDH were evaluated using unpaired t-test with homogenous variance. A multiple regression model for FR versus LDH relationship was generated using least square linear regression model. Residual sum of squares criterion was used to evaluate the validity of the regression model.

Blood sample collection: Three to 5 mL of blood samples were collected into heparinized syringe by heart puncture technique. Blood samples from the control and the experimental group were used for FR D-ROM method according to Fenton reaction as described by Haffor (2004). The remaining blood samples from the two groups were used for the determination of GPX and LDH activities using Randox protocol (Randox, England).

RESULTS

Results showed that Free radicals production was higher significantly ($p<0.05$) by 45.3% in experimental group, as compared with the control group (Table 1). It is important to emphasize that these values of FR are considered within normal range for mammals. The activity of LDH was increased significantly ($p<0.05$) in the experimental group, indicating that cold stress causes cellular damages (Table 2). On the contrary the GPX was lower ($p<0.05$) in the control group which indicates that ROS causes cellular damages that resulted in higher FR production (Table 3) which overwhelmed the antioxidants system. These findings were supported by a significant

Table 1: Glutathione peroxidase in Blood (μU L^{-1}) of the two groups

Animal	Control group	Cold temperature treated group
1	5623.7	2934.11
2	6601.72	2689.6
3	8313.3	3154.16
4	7579.78	Died
5	7090.76	4621.22
6	6846.23	2298.38
7	6601.74	---
8	6357.23	---
9	5623.71	---
Average	6737.574	3139.494
SD	865.4296	887.1002

Table 2: Lactate dehydrogenase activity (U L^{-1}) in blood of the two groups

Animal	Control group	Cold exposure group
1	113.33	369.36
2	76.32	460.25
3	83.26	469.23
4	74.01	Died
5	138.77	451.35
6	106.39	483.26
7	104.08	---
8	69.39	---
9	99.45	---
Average	96.11111	446.69
SD	22.51637	44.80774

Table 3: Free radical (UCARR)* production in blood in the two groups

Animal	Control group	Cold treated group
1	101	112
2	99	151
3	98	165
4	102	Died
5	182	142
6	125	281
7	142	---
8	102	---
9	103	---
Average	117.1111	170.2
SD	28.50633	64.91302

* One unit CARR is = 0.08 g Vol % of H_2O_2

Table 4: Regression models of FR versus LDH in the two groups

		Groups	
		Control	Cold
Source of regression		FR-LDH	FR-LDH
Model relationship			
	R	0.813	0.816
	R^2	0.660	0.665
Model analysis			
	Model	4762.071	11212.517
	MSR	306.254	1880.761
Fisher's ratio			
	F	15.549	5.962
	Pr > F	0.004*	0.092
Parameter			
	Alpha	24.472	-366.202
	Beta	0.969	1.231

linear regression (R^2 = 0.62) of FR on LDH (Table 4). Although the relationship deviated from linearity, the activity of GPX decreased by 50% in the experimental group (Fig. 1 and 2). This regression model was evaluated using residual sum of squares which departed symmetrically from zero at all levels of LDH (Fig. 1 and 2).

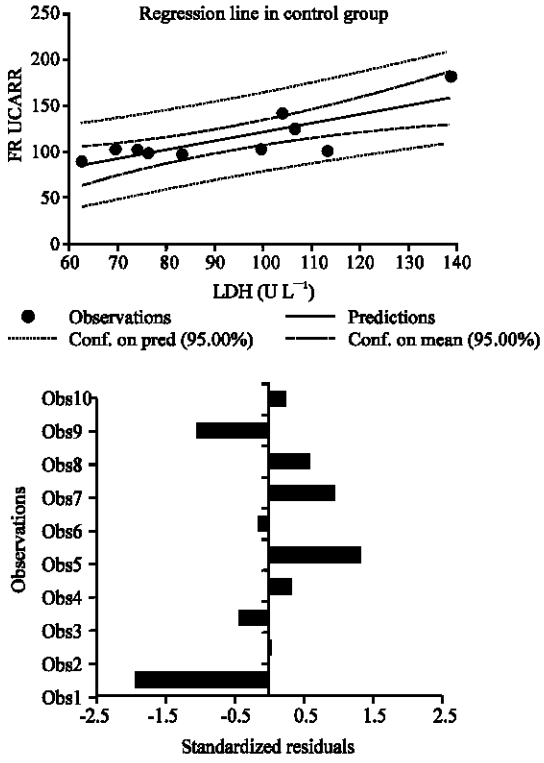

Fig. 1: Least square regression line (top) and standardized residual histogram (bottom) for the FR-LDH relationship in the control group

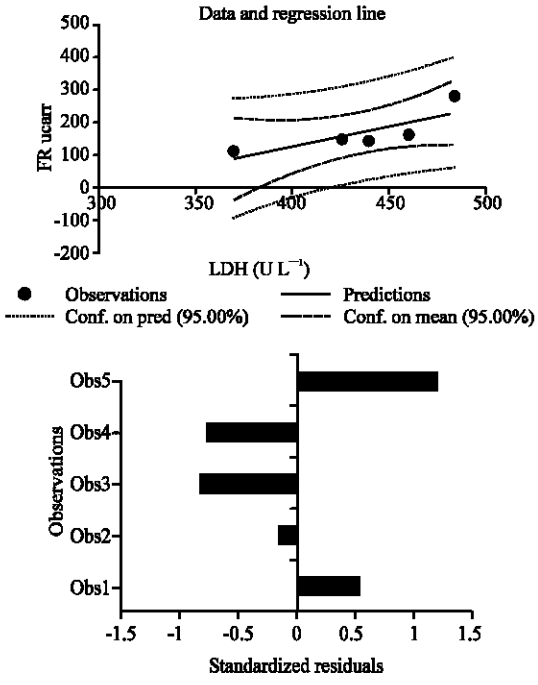

Fig. 2: Least square regression line (top) and standardized residual histogram (bottom) for the FR-LDH relationship in the cold treated group

DISCUSSION

Results of the present study indicated that cold exposure for seven weeks resulted in an increased activity of LDH associated with elevation in FR in *U. aegyptius*. Elevated LDH signifies imbalance between lactate and pyruvate mainly due to in inadequate oxygen supply. Thus shortage in oxygen delivery can be a potential mechanism of adaptation to cold. However in ectothermic animals such as *U. aegyptius* long-term temperature shifts can possibly lead to compensatory changes in the activity of glycolytic and mitochondrial enzymes. It was shown that the change is glycolytic and mitochondrial enzymes affects LDH activity (Clarke, 1991). Moreover, Battersby and Moyes reported that both Citrate Synthase (CS) and sytocrom c oxidase (COX) activities increased in parallel during cold acclimation in trout (Battersby and Moyes, 1998). The authors concluded that the observed increase was due to changes in mitochondrial volume and concentrated cristae. As the origin of oxidative stress and reductive stress is the mitochondrial respiratory electron transport chains (Epe, 1996; Al-Johany and Haffor, 2005). It can be suggested that the cellular damages induced by cold temperature loop is mediated by mitochondrial stimulus. Therefore, the observed changes in LDH activity should represent a progressive acclimatization to cold secondary to changes in glycolytic and mitochondrial enzymes. Previous work in which mitochondria pathological changes such swelling, hypertrophy, hyperplasia and concentrated cristae caused increased in FR production (Bolli *et al*., 1989; Sun *et al*., 1996; Zhou *et al*., 1996). Furthermore, the increased LDH activity induced by cold is supported by studies conducted on other species that showed during cold acclimation, of *Z. viviparus* from the North Sea showed a significant increase of CS activity, implying oxidative stress (Lucassen *et al*., 2003). Clearly, change in CS activity demonstrates mitochondrial responses to cold. The findings of increased FR production in the present study establish new physiological concept that can explain the association of Reactive Oxygen Species (ROS) and cold acclimatization. On the other extreme, it was shown that there was an increase in ROS production during 42°C exposure (Al-Johany and Al-Sadonn, 1996) which indicate the influence of unidentified parameters other than temperature, during cold exposure. As time course of the present study reflects an adaptation process it suspected that other factors could have affected LDH. During adaptation there is a sufficient time for enzymetic changes induced by both translational and posttranslational processes (Battersby and Moyes, 1998). The finding of lower GPX activity of the present study suggests that cold stress did not act as cellular stimulus to activate antioxidant enzymes. Thus depression of the antioxidants defense in terms of a reduction of GPX activity reflects cold intolerance of *U. aegyptius*, rather than adaptation process.

CONCLUSIONS

Based on the results of the present study it can be concluded that long term cold exposure does not stimulate antioxidant defense system, in terms of GPX activity, yet it does aggravate the formation of ROS which is considered as cytoplasm cellular stimulus for LDH in *U. aegyptius*.

RECOMMENDATIONS

Further research is needed regarding the cold stress limits and the period of adaptation within which *U. aegyptius* showed cold intolerance at which GPX deflection point is well defined.

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