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ORIGINAL ARTICLE

Fuelling of TCA cycle in hepatic cells *Marwari* goat during ambient temperature associated stress

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The present study was launched to assess the effect of extreme ambient temperature associated stress on fuelling of TCA cycle in hepatic cells of *Marwari* goat. Based on the fact that whenever a hepatocyte needs fuel for TCA cycle, the activity of enzyme glutamate dehydrogenase (GD) increases making alpha-ketoglutarate available for TCA cycle, 600 apparently healthy *Marwari* goats of either sex, between 6 months to 3 years of age were screened and blood samples were collected during moderate, cold and hot ambient temperature periods to determine the serum glutamate dehydrogenase enzyme and glucose concentration. The mean value of serum GD was significantly ($p \leq 0.05$) higher during cold and hot ambient temperature periods in comparison to overall moderate mean value. However, the rise was greater in cold (2.20 times) than hot ambient temperature (1.19 times). The serum GD activity was higher in male and younger animals. Serum glucose concentration showed a reverse trend as compared to serum GD activity. The results indicated that in cold condition associated stress the fuelling to TCA cycle was more than moderate and hot ambient temperature periods. Serum GD activity was also found related with glucose homeostasis. Further the study has shown that variations in the enzyme levels are not always pathological and while interpreting clinical data, a clinician must consider these variations.

key words: Ambient temperature /cold/ glucose/glutamate Dehydrogenase/ hot/Marwari goat

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To determine the energy balance of the animals at cellular levels, various tests are performed. One simple way is to determine the levels of enzyme regulators involved in these reactions like glutamate dehydrogenase enzyme. Glutamate dehydrogenase enzyme is located in the mitochondria and is an

important branch-point enzyme between carbon and nitrogen metabolism (Stillman et al., 1993). It catalyses the reversible NAD $(P)^+$-linked oxidative deamination of L-glutamate into alpha ketoglutarate and ammonia, making alpha-ketoglutarate available to tricarboxylic acid cycle (TCA cycle).

The TCA cycle is the central metabolic hub of the cell. It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or dicarboxylic acid.

It is involved in the chemical conversion of carbohydrates, fats and proteins into carbon dioxide and water to generate a form of usable energy. One source of oxidative energy is through catabolism of amino acids which are derived from the normal breakdown of cellular proteins, ingested proteins or break down of body proteins *in lieu* of other fuel sources. Whenever a hepatocyte needs fuel for TCA cycle, the activity of enzyme glutamate dehydrogenase (GD) increases making alpha-ketoglutarate available for TCA cycle (Lehninger *et al*., 1993). The mechanism involves conservation of energy by Krebs reactions which can be illustrated by looking at the fate of glutamic acid, or glutamate. Glutamate enters the intermembrane space through the porins. A transport mechanism in the inner membrane called the glutamate-aspartate exchange carrier takes the glutamate molecule into the matrix. The enzyme complex known as glutamate dehydrogenase binds the glutamate molecule, a molecule of oxidized nicotine adenine dinucleotide (NAD), and a water molecule. Off comes the amino group, and glutamate is partially oxidized to alpha-ketoglutarate.

Glutamate dehydrogenase enzyme is also important in normal glucose homeostasis. It enhances oxidation of glutamate which is transformed to alpha ketogluterate, a substrate of TCA cycle, which thereby stimulates insulin secretion (Tanizawa *et al.,* 2002**).** In this way higher serum GD activity helps in the peripheral utilization of glucose under the influence of insulin.

Marwari breed of goat has a significant status in the economy of arid tracts in India. Vast variations in the ambient temperatures ranging from very low to high produce stress to these animals. Therefore physiological mechanisms are modulated to sustain the life. Despite of immense quality characteristics of *Marwari* goat very little scientific has been given to explore these mechanisms. Looking towards the significance of glutamate dehydrogenase enzyme in energy metabolism and paucity of work on this aspect in *Marwari* goat, the present study was launched to assess the effect of extreme ambient temperature stress on fuelling of TCA cycle.

MATERIALS AND METHODS

To carry out the study, 600 apparently healthy *Marwari* goats of either sex, between 6 months to 3 years of age were screened during various ambient temperature periods.

Blood samples were collected during slaughtering (jugular vein) from private slaughter houses (Bikaner, Rajasthan). Sampling was carried out in morning hours during moderate (mean maximum ambient temperature was 29.08±0.12^{o}C), hot (mean maximum ambient temperature was 44.50±0.05^{o}C), and cold (mean minimum ambient temperature was 3.01 ± 0.05^{o}C),ambient temperature periods. Blood was collected directly into the clean, dry test tubes without any anticoagulant and serum was harvested. Supernatant clear serum was collected and stored at 4^{o}C in the refrigerator until analysis (King, 1965). In each ambient temperature period

200 animals were taken and grouped into male (100) and female (100). Further each group was

divided according to age as 6-9 months (100) and 1-3 years (100).Serum glutamate dehydrogenase activity was determined by spectrophotometric method (King,1965) and serum glucose was determined by Folin-Wu method as described by Oser (1976).

The mean value obtained during moderate ambient temperature period was considered as control value and in each category the mean values were compared with the respective moderate mean value as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Mean ±SEM values of serum GD and glucose during different ambient temperature periods, sex and age groups are presented in table 1.

Table 1. Mean ± SEM values of serum glutamate dehydrogenase (GD) and glucose in *Marwari goats*

S.No.	Effects	GD, UL^{-1}	Glucose, mmol UL^{-1}
1.	Overall (600)	28.90±0.71	2.95±0.01
2.	Ambient temperature periods		
(A)	Moderate (200)	19.71±0.70	3.35±0.01^{b}
I	Sex		
(i)	Male (100)	23.11±0.70	3.14±0.01^{c}
(ii)	Female (100)	15.99±0.80^{c}	3.56±0.01
II	Age		
(i)	6-9 months (100)	24.88±0.71	3.12±0.01^{c}
(ii)	1-3 Years (100)	14.53±0.40^{d}	3.58±0.01
(B)	Hot (200)	23.47±0.80^{b}	3.0±0.02
I	Sex		
(i)	Male (100)	27.5±0.90	2.9±0.01^{c}
(ii)	Female (100)	19.6±0.72^{c}	3.1±0.02
II	Age		
(i)	6-9 months (100)	26.31±0.70	2.8±0.01^{c}
(ii)	1-3 Years (100)	20.62±0.72^{d}	3.2±0.02
(C)	Cold (200)	43.51±0.72^{b}	2.50±0.01^{b}
I	Sex		
(i)	Male (100)	48.5±0.73	2.40±0.01^{b}
(ii)	Female (100)	38.5±0.70^{c}	2.60±0.01^{b}
II	Age		
(i)	6-9 months (100)	49.5±0.55	2.30±0.01^{b}
(ii)	1-3 Years (100)	37.5±0.53^{d}	2.70±0.02^{b}

(i) Figures in the parenthesis indicate number of animals.

(ii) Superscript ‘b’ indicates a significant difference ($p \leq 0.05$) between moderate and hot and moderate and cold mean values.

(iv) Superscript ‘c’ indicates a significant ($p \leq 0.05$) difference between male and female mean values within an ambient temperature period.

(vi) Superscript ‘d’ indicates a significant ($p \leq 0.05$) difference between 6-9 months and 1-3 years old animals within an ambient temperature period.

The mean value of GD was significantly ($p \leq 0.05$) higher during cold and hot ambient temperature periods in comparison to overall moderate mean value. However, the rise was

greater in cold (2.2 times) than hot ambient temperature (1.19 times).This clearly showed that GD activity during cold increased in otherwise healthy animals to meet out energy requirements of the body and for thermoregulation. Its activity increased to provide more fuel for TCA cycle by making alpha-ketoglutarate available (Lehninger *et al*., 1993). Therefore blood levels of this enzyme can be used to assess fuelling of TCA cycle. Simultaneously the changes in glucose concentrations also showed a link with serum GD activity.

Variation in the serum GD levels becomes more important from energy stand point of view as GD is allosterically regulated by the cell's energy state. During the formation of alpha ketoglutarate, GDP and ADP positively regulate GD in mammals, and GTP, ATP, leucine, and coenzyme inhibit the enzyme. Therefore, when the level of ATP is high, conversion of glutamate to alpha ketoglutarate is limited; however when the cellular energy charge is low, glutamate is converted to ammonia and alpha ketoglutarate (Banerjee *et al*., 2003). This happens in cold conditions when energy requirement is high to meet the demand for thermoregulation and metabolism. GD plays a central role in amino group metabolism. The effect of varying ambient temperatures on metabolism is well documented (Locher *et al*., 2008). Increased GD activity was perhaps to meet the fuelling requirement of TCA cycle in stress periods as extreme ambient temperature periods are considered as stressors for the animals. Increased glucocorticoids due to ambient temperature related stress (Kataria *et al*., 2000) could be the possible cause of higher GD activity.

In each ambient temperature period, sex and age wise variations was also observed in GD activity and glucose concentration. The mean value of serum GD was higher in male animals as compared to female animals. Probably the higher cortisol levels in males could be the proper explanation for higher GD levels, modulating the energy processes (Lehninger *et al*., 1993). In each ambient temperature period, the mean value of serum GD decreased significantly ($p \leq 0.05$) with the advancement of age. Higher GD activity probably helped the young animals in maintaining high BMR (Locher *et al*., 2008).The variations in serum glucose showed a reverse trend as compared to serum GD activity.

In present study the value of the serum GD was higher (range was from 15 to 43 UL^{-1}) than the reported values in other animals (Boyd,1962). It is because of the fact that GD is highly concentrated in liver of goat (Tennant, 1999) and is associated with microsomal function of hepatocytes (El Samani *et al*.,1985).Due to its higher concentration in the goat, the estimation of GD as a liver function test is being emphasised in goat (Tennant, 1999). Sener and Malaisse (1980) suggested that oxidative deamination reaction of GD feeds the tricarboxylic acid (TCA) cycle by converting L-glutamate to-ketoglutarate, whereas the reductive amination reaction supplies nitrogen for several biosynthetic pathways.

An increased serum GD activity was related with a decrease in serum glucose concentration. This showed the association of serum GD with glucose homeostasis. Many earlier researchers (Maechler and Wollheim, 1999) have correlated the serum GD activity with insulin stimulation. In pancreatic-cells, GD is involved in the regulation of insulin secretion, especially amino acid–stimulated insulin secretion and in this way it is also important in glucose homeostasis. Glutamate was suggested to be a mediator of glucose-stimulated insulin secretion, acting directly on insulin secretory

granules (Maechler and Wollheim, 1999).In present study, during cold and hot ambient temperature periods, when serum GD activity increased, the serum glucose concentration decreased. The pattern of change in glucose concentration substantiated the fact that GD functions as an enzyme to regulate glucose concentration also through hormone insulin. Therefore lower serum glucose concentration in hot and cold ambient temperatures indicated towards its greater peripheral utilisation to meet out the energy requirement of the body.

These results indicated that in cold condition fuelling to TCA cycle was higher than moderate and hot ambient temperature periods. Serum GD values showed variations due to changes in ambient temperatures, sex and age, each having the relevant physiological significance. Low glucose concentration during hot and cold ambient temperature periods indicated a high rate of peripheral utilization. Further the study has shown that variations in the enzyme levels are not always pathological and while interpreting clinical data, a clinician must consider these variations.

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