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RELATIONSHIP BETWEEN CORTICOSTERONE AND BODY WEIGHT, ANDROSTENEDIONE AND INSULIN DURING THE PERIOD OF DELAYED OVULATION IN A VESPERTILIONID BAT, *SCOTOPHILUS HEATHI*

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The aim of the present study was to evaluate the relationship between corticosterone, body weight, insulin and androstenedione in order to understand the role of adrenal in contributing hyperandrogenism during delayed ovulation in *S. heathi.* The circulating corticosterone concentration in female *S. heathi* showed significant seasonal variation. The peak corticosterone concentration observed during August–September coincides with increased feeding activities in *S. heathi.* The present study noted a seasonal variation in relationship of corticosterone with insulin and androstenedione in *S. heathi.* An inverse relationship of corticosterone with insulin and androstenedione was found during August to December, but not during January to May. A seasonal variation in the effect of adrenocorticotropic hormone (ACTH) on adrenal corticosterone production *in vitro* was observed during reproductive cycle. Corticosterone production *in vitro* by adrenal declined significantly as compared to the control during quiescence in September. The finding suggests that adrenal attained the peak responsiveness to ACTH during September. ACTH significantly enhanced the androstenedione production by the adrenal *in vitro* during December, when the circulating androstenedione was also high in *S. heathi.* This suggests that the adrenal may also contribute to hyperandrogenism during the period of delayed ovulation in *S. heathi.* Further studies are required to reveal the unique pattern of seasonal relationship between corticosterone, insulin and androstenedione in *S. heathi.*

Keywords: Corticosterone – hyperandrogenism – ACTH – bat – insulin – body weight

INTRODUCTION

Scotophilus heathi (order: Chiroptera; family: Vespertilionidae) is seasonally monoestrous. In *S. heathi*, antral follicles are formed during the month of November but ovulation is delayed until early March [18]. Increased androstenedione produced by the ovary during the period from November to January was suggested to be responsible for suppression of follicular maturation and delayed ovulation in this bat [1]. Further studies on *S. heathi* demonstrated a close relationship between androstenedione concentration and increase in body weight due to the accumulation of adipose tissue (obesity) [2]. A similar association between hyperandrogenism (HA), obesity

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and anovulation is also well documented in women with polycystic ovarian syndrome (PCOS). Increase in serum androstenedione concentration during the period of weight gain and a decline in serum androstenedione concentration during the period of weight loss in *S. heathi* thus suggests that nutritional changes may be responsible for changes in gonadal steroidogenesis in this species.

The metabolic hormones, insulin and glucocorticoids have been known as the major physiological regulators of energy balance in mammals [6, 17, 24]. The anabolic actions of insulin on peripheral tissue are well established and plasma insulin also apparently serves as signal of body fat content to the central nervous system [24, 30]. Lipogenesis in adipose tissue is also amplified by insulin. Numerous studies suggested co-existence of hyperandrogenism and elevated insulin levels [12]. Recent studies in *S. heathi* also showed a close relationship between circulating androstenedione and insulin concentrations [11]. Hyperinsulinemia and increased insulin resistance have been associated with obesity [10]. The origin of insulin resistance is poorly understood. Its typical features, however, may also be observed during excess of glucocorticoids [4, 21]. Glucocorticoids exert multiple metabolic effects including the anabolic stimulation of lipogenic enzymes in the liver [5] and they have been linked to the development and maintenance of obesity in several laboratory rodents [17]. Cortisone may also act on the hypothalamus to promote feeding behaviour [14]. In laboratory rats and mice, the presence of corticosterone is necessary for increased food intake and gain in body mass.

A close similarity between the ovaries of *S. heathi* during the period of delayed ovulation with polycystic ovarian syndrome (PCOS) in women has recently been described [2]. Polycystic condition has also been described in all types of congenital adrenal hyperplasia (CAH), and ovarian hyperandrogenism (HA) occasionally develop in the patients with CAH, thus suggesting a close relationship between adrenal and ovary during the condition of HA [3]. Whether adrenal gland either directly or indirectly contributes to hyperandrogenic condition in *S. heathi* during the period of delayed ovulation has not been studied. Mainly two types of abnormalities are described during HA or PCOS viz., adrenal enzyme dysfunction and adrenal androgen hyperresponsiveness to adrenocorticotrophic hormone (ACTH). Glucocorticoid excess secretion may result during adrenal enzyme dysfunction [3]. The aim of the present study was to evaluate the role of adrenal in contributing hyperandrogenism during delayed ovulation in *S. heathi.* Two different approaches were adopted. Firstly, we investigated the seasonal changes in the circulating corticosterone concentration and its relation to the body weight, and circulating insulin and androstenedione concentrations. Secondly, the effect of ACTH on adrenal corticosterone and androstenedione production *in vitro* was investigated to find out the adrenal responsiveness during different stages of ovarian cycle.

MATERIALS AND METHODS

All bats were trapped alive between August 1992 and April 1994 in areas adjacent to the Banaras Hindu University Campus (about 15 km from the laboratory in the Department of Zoology). Details of the study site and feeding activity of *S. heathi* were described earlier in detail [25]. These bats exhibit winter dormancy and torpidity from mid-December to late-January. During this period they exhibit hypothermia (showing rectal temperature less than in other periods of the year) and stop feeding (or foraging) [25]. Bats were generally trapped in each phase of the reproductive cycle between 0800 and 0900 hours directly from their roosting site. They were kept in cages made of wood and wire and transported to the laboratory. As soon as they reached the laboratory they were provided with water. The bats were generally sacrificed by decapitation between 1100 and 1200 hours within 30 min of their arrival in the laboratory. To minimize the stress body weight and other details of each bat were recorded after the bats were sacrificed. Stomach content of some of the bats was studied to determine recent feeding behaviour. During August, November and April bats showed recent feeding (post-parandial), whereas in December in February bats were in an unfed or fasting condition.

Studies in vitro

The *in vitro* study performed to determine the seasonal effects of ACTH on corticosterone and androstenedione production by the adrenal. Bats were sacrificed as soon as they were brought to the laboratory. The adrenal was dissected out immediately and cleaned in media-199 (Gibco). Adrenal (one per tube) were incubated with 1 μg ACTH (sigma) in 1 ml media-199 with 0.1% (w/v) BSA for 2 hours at 37 °C. Samples of media were frozen after the incubation at –20 °C until assayed for androstenedione and corticosterone.

Hormone assay

Insulin assay

The circulating concentration of insulin in the female bats was measured using a radioimmunoassay kit (RIAK-1) obtained from Bhabha Atomic Research Center, Mumbai, India. Verification of the insulin assay for the use in bat and detailed assay procedure was described previously [11]. Duplicate 0.1 ml of serum samples were used for the assay. All the samples were run in one assay. The intra-assay coefficient of variation was less than 7.8%.

Androstenedione assay

Circulating concentration of androstenedione was measured by radioimmunoassay according to the method of Abhilasha and Krishna [1]. Antibody for androstenedione was obtained from Dr. John Resko (Oregon Health Science University, Portland, USA). Radioactive androstenedione was purchased from Amersham. Steroids in the serum sample were extracted with 2 ml diethyl ether, the ether extract was decanted evaporated to dryness at 37 °C and resuspended in 0.01 M phosphate buffer saline gelatin (PBSG) for further analysis. Extraction efficiency was determined and found in the range of 75–95%.

Corticosterone

Corticosterone was measured as described by Wingfield et al. [29]. Serum samples (5 µl) were extracted with 2 ml of freshly opened diethyl ether. Ether extract was dried in a vortex evaporator and reconstituted in PBS. Extraction efficiency was determined and found in the range of 85–95%. Reconstructed extracts were incubated with primary antibody to corticosterone (ICN Biomedical Inc.) and (1,2,6,7-^{3}H) corticosterone (NEN Research Products, Boston, MA), and Dextron-coated charcoal was used to separate bound and free-labelled hormone. Intra- and inter-assay variations were less than 10%.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's test. Correlation coefficient and coefficient of determination (r^2) was used to compare the data. Data are expressed as mean ±SE.

RESULTS

Body weight and fat deposition

The variations in the body weight of *S. heathi* during different reproductive phases are described in detail by Abhilasha and Krishna [2].

Serum corticosterone concentration and relationship with body weight and serum insulin and androstenedione concentrations

Serum corticosterone concentration in *S. heathi* showed a significant seasonal variation (Fig. 1) during reproductive cycle. Serum corticosterone concentration was high

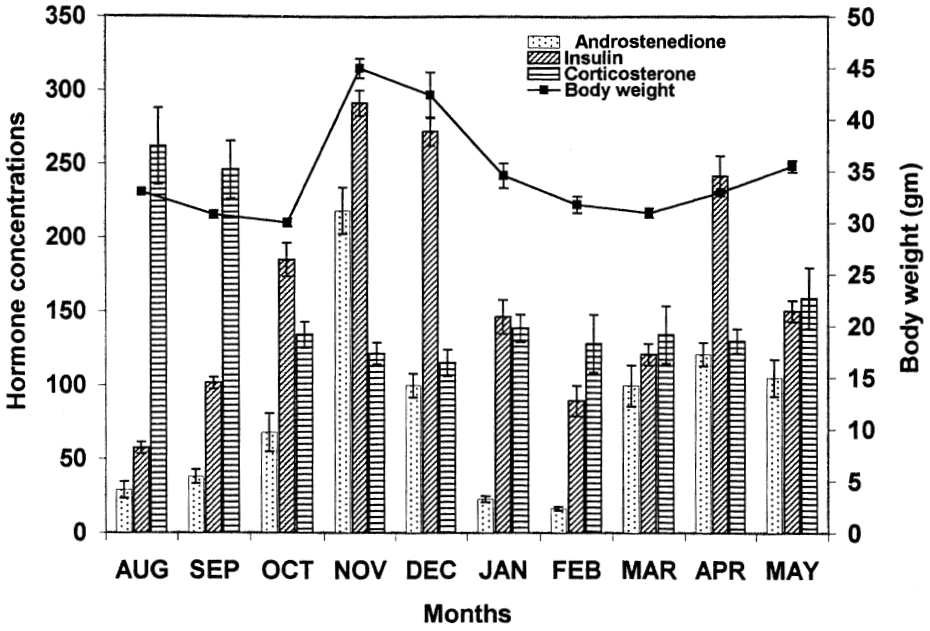

Fig. 1. Monthly changes in the body weight (gm) and circulating androstenedione (ng/ml), insulin (μU/ml) and corticosterone (ng/ml) concentrations of *S. heathi* during the period of delayed ovulation

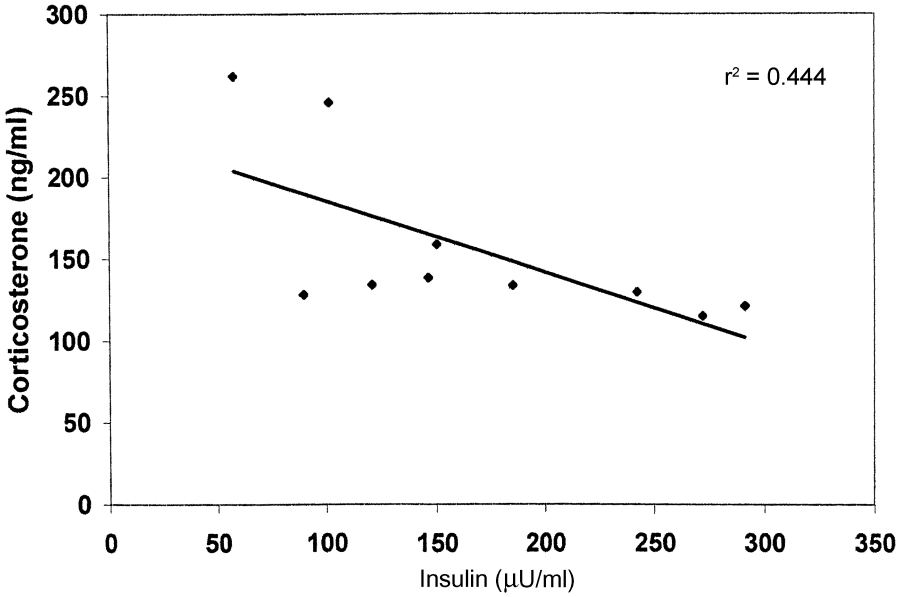

Fig. 2. Correlation between circulating corticosterone (ng/ml) and insulin (μU/ml) concentrations of *S. heathi*

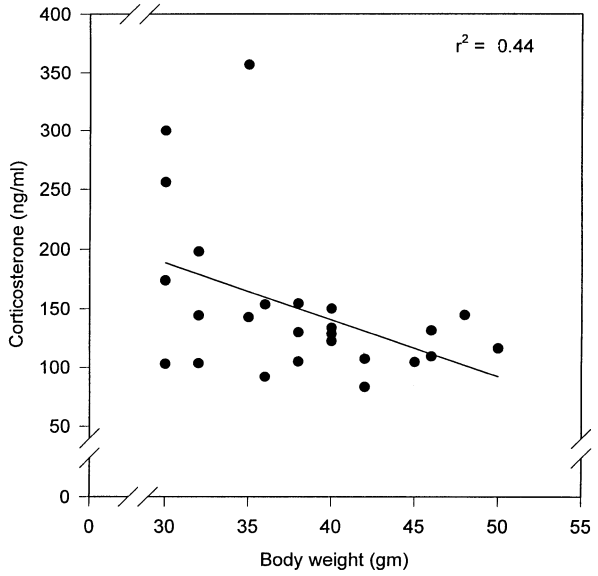

Fig. 3. Correlation between circulating corticosterone (ng/ml) concentration and body weight (gm) of *S. heathi*

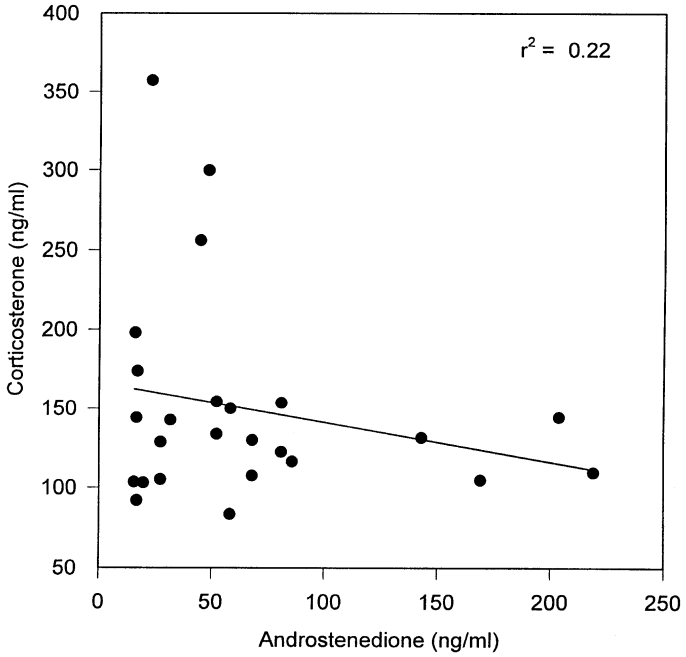

Fig. 4. Correlation between circulating corticosterone (ng/ml) and androstenedione (ng/ml) concentrations of *S. heathi*

during quiescence in August and September, before the accumulation of body fat and increase in body weight was noticed. Serum corticosterone declined significantly during October and attained a lowest concentration during December, when the heavy accumulation of adipose tissue was observed in *S. heathi***.** Serum corticosterone level remained low until April and increased significantly during May and June (Fig. 1). Variations in serum insulin and androstenedione concentration during the period of delayed ovulation are described earlier [11]. A negative correlation was observed between circulating corticosterone and insulin concentration (Fig. 2; $r^2 = 0.44$; $p < 0.05$) and with the body weight (Fig. 3; $r^2 = 0.44$; $p < 0.05$), but showed no significant correlation with androstenedione concentrations (Fig. 4; $r^2 = 0.22$;

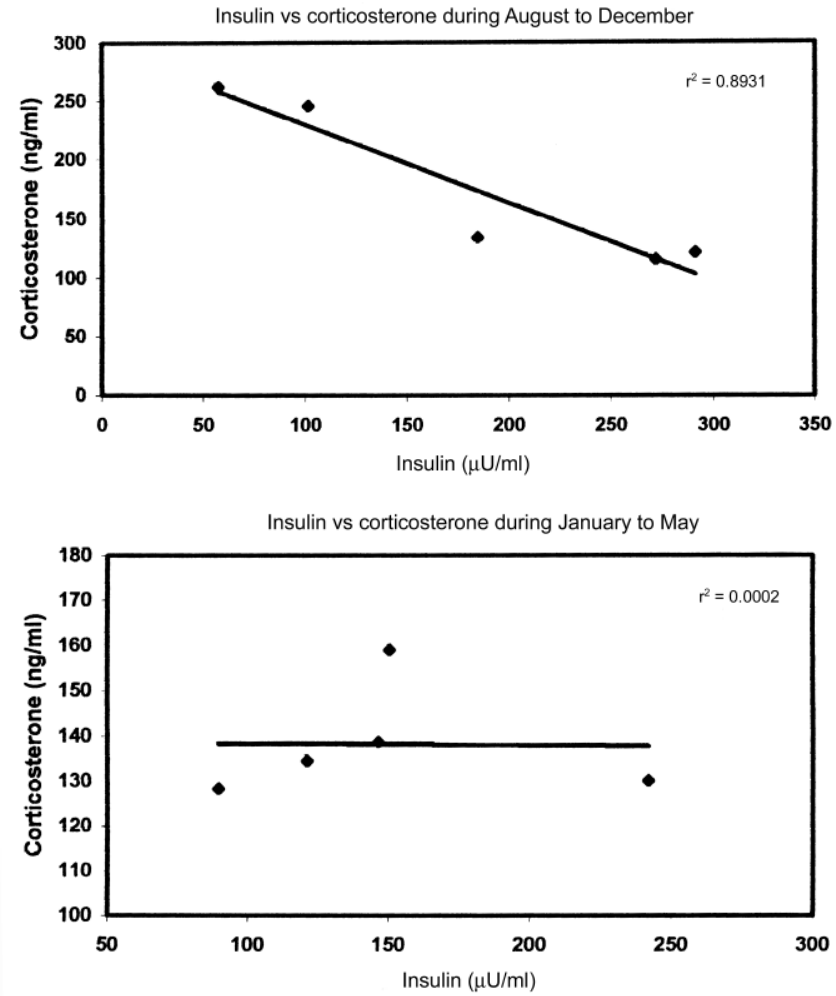

Fig. 5. Seasonal variation in relationship between circulating corticosterone (ng/ml) and insulin (μU/ml) concentrations of *S. heathi*

$p > 0.05$). The relationship between corticosterone and insulin showed highly significant inverse correlation between August and December (Fig. 5; $r^2 = 0.89$; $p < 0.01$), but showed no significant correlation between January and May (Fig. 5; $r^2 = 0.0002$; $p > 0.05$). An inverse relationship was also found between corticosterone and androstenedione during August to December ($r^2 = 0.50$; $p < 0.05$), but not during January to May ($r^2 = 0.08$; $p > 0.05$).

Seasonal effects of ACTH on corticosterone and androstenedione production by adrenal in vitro

The present study showed a clear seasonal variation in the ability of the adrenal to produce corticosterone and androstenedione *in vitro* in response to ACTH stimulation (Table 1). During most of the reproductive stages, corticosterone and androstenedione production *in vitro* by the adrenal increased in response to ACTH as compared to the respective control. However, corticosterone production by the adrenal declined in response to ACTH as compared to control during September prior to accumulation of adipose tissue, whereas androstenedione production by the adrenal declined in response to ACTH as compare to control during February prior to ovulation.

Table 1
Seasonal variations in the ACTH stimulated adrenal androstenedione and corticosterone production *in vitro* in the female *S. heathi*

Months	Androstenedione (ng/ml)		*t*-test	Corticosterone (ng/ml)		*t*-test
	control	ACTH		control	ACTH	
September	4.52±1.08[cd]	6.36±0.2[d]	S*	7.14±4.30[d]	1.60±1.29[b]	S*
November	2.62±0.98	6.18±2.40[d]	S*	3.04±1.58	7.78±2.58[ac]	S*
December	1.22±0.99[a]	5.82±2.15[d]	S*	1.05±0.76[a]	1.86±0.69	NS
February	1.25±0.34[a]	1.03±0.09	NS	0.46±0.09[a]	4.79±1.52[b]	S**
ANOVA (p value)	<0.025	<0.10		<0.01	<0.05	

Values are mean ± SE of five samples. Values are significantly different by Duncan's as compared with [a] September, [b] November, [c] December, [d] February. NS = non-significant; S = significant. Values (p value) are less than *0.01 or **0.001.

DISCUSSION

The present study showed a significant seasonal variation in the circulating corticosterone concentration in female *S. heathi.* Serum corticosterone concentrations have been reported for several temperate zone bat species [15, 27, 28]. However, this study presents the only report of serum glucocorticoid concentrations in any female tropi-

cal vespertilionid bat species. The significant seasonal variation in serum corticosterone concentration in male *S. heathi* was recently published [19]. The corticosterone concentrations presented here are higher than those reported for other microchiropterans [28]. These higher values could be due to stress during capture and handling, as demonstrated by Widmaier et al. [28], although precautions were taken to minimize stress prior to killing the bats. However if corticosterone levels were increased by stress, stress may not have affected the pattern of seasonal variations in corticosterone concentration shown in the present study, because nearly identical capture procedure, handling and timing of sacrificing the bats were adopted during each phase. Besides corticosterone, extremely high concentrations of cortisol were also reported, particularly in megachiropterans [28]. Circulating cortisol concentrations could not be measured in the present study because of a lack of sufficient serum sample.

The significance of the seasonal pattern of corticosterone in female *S. heathi* is not quite clear. The peak corticosterone concentration observed during August–September may be related with the increased feeding activities reported during this period in *S. heathi* [25]. The presence of high corticosterone was shown to be necessary for increased food intake and gain of body mass in laboratory mice [6]. Adrenalectomy prevents the complete expression of hyperphagia and gain of body mass in several laboratory rodents, and this can be reversed by peripheral or central administration of corticosterone [17]. Corticosterone is also necessary for increased food intake and body mass in lean rats rendered underweight by food restrictions [14]. Corticosterone has been shown to promote feeding behaviour by acting on the hypothalamus [14]. Lowest concentration of serum corticosterone in female *S. heathi* was found during early phase of winter dormancy in December, which coincided with the period of maximum fat deposition and low food intake in this species [25]. The present study thus does not correspond with the finding in other bat species, *Myotis lucifugis*, where mobilization of fatty acid from deposited fat coincided with the elevated glucocorticoid level. This difference could be due to the difference in the type of glucocorticoid measured in the two studies. *M. lucifugus* exhibited lower levels of cortisol during months of activity and higher levels during hibernation.

An important finding of the present study is the seasonal variations in relationship between serum corticosterone and insulin concentration in *S. heathi*. Corticosterone showed an inverse relationship with insulin ($r^2 = 0.89$; $P < 0.01$) between August and December, but not during January to May ($r^2 = 0.0002$; $p > 0.05$). This finding thus supports the earlier reports of insulin-agonistic effect of cortisol and other corticosteroids in rats and humans [7, 8, 26]. Glucocorticoid is an important glucose counter-regulatory hormone and an increased activity of this or the hypothalamic-hypophyseal-adrenal axis was suggested to be responsible for the development of obesity and insulin resistance in human [9, 16]. The inverse relationship between corticosterone and insulin during August to December coincided with the period of intense feeding activity and accumulation of adipose tissue in *S. heathi*. Our recent studies on *S. heathi* demonstrated the occurrence of hyperinsulinemia and insulin resistance in this species during recrudescence in November [11]. It thus appears that such a relation-

ship may be required for intensive feeding activity, fat deposition and insulin resistance in *S. heathi* prior to winter dormancy. A short peak of insulin noticed during April in *S. heathi* may be due to increased feeding activity. Lack of any corresponding changes in the corticosterone concentration during April further support the seasonal changes in the relationship between corticosterone and insulin in *S. heathi*. The seasonal variations observed in the relationship between corticosterone and insulin in *S. heathi* is unique and requires further investigation.

The factors responsible for insulin resistance are poorly understood. Its typical features, however, may also be observed during glucocorticoid excess. The association between glucocorticoid and insulin resistance has also been demonstrated in patients with Cushing's syndrome [22]. Based on these findings, it may be hypothesized that high circulating concentration of corticosterone observed in *S. heathi* during September, immediately preceding the fat deposition, may be one of the factors responsible for developing insulin resistance in this species. Insulin resistance and consequently hyperinsulinemia may be responsible for increased androstenedione synthesis from adrenal together with the ovary of *S. heathi* as also reported in women with Cushing's syndrome [23]. A very low circulating level of dehydroepiandrosterone observed in *S. heathi* suggests androstenedione as a major andogen produced by adrenal in this species (unpublished data). The high androstenedione produced was shown to be responsible for PCO-like features in the ovary and delayed ovulation in *S. heathi* [1].

The present study showed an increase in circulating concentration of androstenedione during October, which coincides with a sharp decline in corticosterone level. However, lack of significant correlation between circulating corticosterone and androstenedione concentrations rules out any direct involvement of glucocorticoids in influencing androstenedione synthesis in *S. heathi*. Further analysis showed an inverse correlation between corticosterone and androstenedione concentration during August to December ($r^2 = 0.50$; $p < 0.05$), but not during January to May ($r^2 = 0.08$; $p > 0.05$). Since during August to December corticosterone showed much higher level of correlation with insulin as compared with androstenedione and it is also well known that insulin stimulates androstenedione synthesis in *S. heathi* [11], relationship between corticosterone and androstenedione might be indirectly through insulin. A few studies in the past though have suggested the stimulation of androgen production as a consequence of lowered glucocorticoid concentration [13]. It is interesting to mention here that that the effect of insulin on androstenedione production in *S. heathi* was also found to be seasonal [11].

The present study showed a seasonal variation in the effect of ACTH on adrenal corticosterone and androstenedione production *in vitro*. ACTH enhanced corticosterone production *in vitro* by adrenal as compared with the control during most phases of the cycle. During quiescence in September, however, corticosterone production *in vitro* by the adrenal in response to ACTH declined significantly as compared with the control. Since circulating corticosterone concentration was very high during this period, the *in vitro* decline in corticosterone synthesis in response to ACTH stimulation suggest homologous downregulation of ACTH receptor. These findings thus

suggest that adrenal gland during the quiescence phase may be at the peak level of responsiveness for ACTH. Such findings further suggest that high circulating corticosterone concentration in *S. heathi* during September may be because of increased adrenal responsiveness for ACTH. The rapid increase in the production of androstenedione by the adrenal *in vitro* in response to ACTH suggests that the adrenal may also be a source of androstenedione in *S. heathi*. ACTH significantly enhanced the androstenedione production by the adrenal *in vitro* during December, when the circulating androstenedione was also shown to be high in *S. heathi* [1]. This suggests that the adrenal may also contribute to hypreandrogenism during the period of delayed ovulation in *S. heathi*. Mild adrenal responsiveness to ACTH induced androstenedione synthesis was observed during September (quiescence phase), this coincided with the low circulating androstenedione concentration during this period in *S. heathi*. Interestingly the androstenedione production by adrenal in response to ACTH stimulation *in vitro* declined during preovulatory period in February. This finding thus suggests that the adrenal also contributed in lowering the circulating androstenedione concentration during preovulatory period. The factor causing seasonal variation in the adrenal responsiveness to ACTH is not clearly known. Increased adrenal responsiveness to ACTH induced androstenedione production during December may be due to hyperinsulinemia shown in *S. heathi* during this period [11]. Increasing adrenal responsiveness to ACTH in response to hyperinsulinemia has earlier been shown in human [20]. The present study further suggests that the ACTH, which has a well-established role in regulating glucocorticoid production, is also involved in the regulation of adrenal androgen production in *S. heathi*.

In brief, the present study showed a seasonal variation in the pattern of serum corticosterone concentration and showed inverse correlation with insulin during August to December but not later. The inverse relationship between corticosterone and insulin prior to winter dormancy may be important for increased food intake, fat deposition and development of insulin resistance. The resulting hyperinsulinemia causes increase production of androstenedione. The *in vitro* study suggests increased adrenal responsiveness for ACTH during September and this may be responsible for increased corticosterone production during this period. The *in vitro* study further suggests that adrenal may also be an important source of androstenedione during the period of delayed ovulation in *S. heathi*. Further studies on the seasonal shift in the relationship between corticosterone, insulin and androstenedione noticed in *S. heathi* may provide some important information on phenomena such as insulin resistance, fat deposition etc.

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