Interrelations of Fat Bodies and Liver to Reproduction in Female Acanthodactylus boskianus

KHAMIS ABDEL RAHEEM AND GHAZI DEHLAWI
Dept. of Biology, Fac. of Applied Sciences,
Umm Al-Qura University, Makkah, Saudi Arabia.

ABSTRACT. Studies were undertaken to examine the interrelationships of fat bodies to reproductive activity in the lizard Acanthodactylus boskianus. The data obtained indicate, that non-reproductive females had the highest percent of females with dissectable fat bodies. Reproductive females (classes II and III) showed various degrees of fat consumption. Winter animals exhibited a low percent of dissectable fat bodies while Summer animals showed a higher percent. This was attributed to the capacity for lipid replenishment, being greater in Summer. Excessive utilization of fat bodies becomes apparent when animals become ovigerous where the percent of animals with dissectable fat bodies becomes zero both in winter and Summer. The utilization of lipid stores during vitellogenesis is also indicated by the declines recorded in Lipidosomatic index as well as in hepatic lipid content. The total free amino acids in liver displayed a decline during vitellogenesis attributed to the synthesis of vitellogenin. Hepatic protein content declined during vitellogenesis which might be due to active export of proteins to the growing follicles. The importance of such changes in relevance to reproductive activity were discussed.

Introduction

Extensive investigations have been carried out on reproductive activity in reptiles. All such works stressed on the importance of fat bodies in vitellogenesis. This was reported in temperate lizards e.g. Anolis carolinensis[1], Dipsosaurus dorsalis[2,3], Sceloporous jarrovi[4,5] and Amphibolurus fordi[6]. Fat bodies were indicated[7] to decline in female Uta stansburiana, in late winter and early spring when deutoplasm was deposited in the ovarian follicles. Exirpation of the fat bodies in pre-breeding
females inhibited follicular development, while extirpation in females just becoming active, caused increased follicular atresia and retardation in yolk deposition.

Tropical species, displayed an inverse relationship between fat bodies and gonadal activity. This was demonstrated in *Agama agama*[^8], in *Ameiva festiva*, *Ameiva quadrilineata*[^9], *Sceloporus malachiticus*[^10] and *Anolis*[^11].

Utilization of lipids for vitellogenesis appears to be mediated by liver which was found to play an important role in this respect. This can be judged from the hypertrophy demonstrated in livers of female *Uta stansburiana* in which vitellogenesis had been induced by injecting estradiol-17 B. The same function ascribed to liver was also reported in *Takydromus tachydromoides*[^12] and *Sceloporus jarrovi*[^4]. It was also demonstrated[^13] that tropical lizard *Chamaeleo jacksoni*, exhibited a maximal liver weight at the onset of vitellogenesis, and reached a maximum during gestation.

Liver performs as well the important function of synthesizing a calcium binding phospho-protein, vitellogenin. Once synthesized, it is secreted to the circulation to be conveyed to the ovaries. It is generally found in blood of female lizards to an extent which depends on the nutritional state[^14-16] which promotes increased levels of vitellogenins in sera of females.

In Saudi Arabia, very limited works have been carried out to examine the reproductive activity in lizards. Moreover, works concerning functional relationship between fat stores and liver during vitellogenesis are lacking. Therefore, the present work was undertaken to examine the relationship of reproductive activity to liver in a semitropical lizard, *Acanthodactylus boskianus*.

### Material and Methods

#### Animal

The lizard used in this work is the semitropical lizard *Acanthodactylus boskianus* (Family: *Lacertidae*). Monthly trips were made to Wadi Fatima to the close surrounding of Makkah region, at the west of Saudi Arabia, where animals were caught by hand. Only adult females (SVL ≥ 6.8 cm) were used in this work.

#### Treatment

Animals were sacrificed the next day after collection. The Snout Vent Length (SVL) was measured to the nearest 1 mm. The body weights were recorded to the nearest 0.01 gm. Livers, fat bodies and ovaries were excised from sacrificed animals and were weighed to the nearest mg.

#### Reproductive Status

Animals were assigned to one of four reproductive classes based on measurements and examinations of the ovarian follicles[^17], according to the following:

1. **Class I**

   Non-reproductive (No eggs in oviducts, ovaries contain small follicles ≦ 2.4 mm)
Class II
Reproductive (No eggs in oviducts, ovaries contain at least one yolk follicle, 2.5-7.0 mm).

Class III
Reproductive (No eggs in oviducts, ovaries contain at least one preovulating follicle in late vitellogenesis, 7.1-8.5 mm).

Class IV
Reproductive (animals contain corpora lutea in the ovaries and/or eggs in the oviducts).

Females in classes II, III and IV are considered reproductive, while females in class IV only were considered ovigerous.

Lipido, Gonado and Hepato Somatic Indices (LSI, GSI and HSI).
The weights of fat bodies, ovaries and livers (mg) were referred to the body weight (gm) x 100, to give LSI, GSI and HSI.

Methods
Liver samples were weighed then homogenized in distilled water (1:10) using Elvenjhem homogenizer, fitted with teflon pestle. Homogenation was undertaken at 4°C. Samples of liver homogenates were used for subsequent determinations.

1. Total lipids
Determinations were performed using the phosphovanillin method of Knight et al.[18].

2. Total proteins
Determinations were undertaken using the method of Lowry et al.[19].

3. Total free amino acids
Determinations were carried out using the method of Moore and Stein[20].

Statistical analysis
Values are given as averages ± standard error. Significance of the changes was tested using the student “t” test[21].

Results and Discussion
Studies undertaken on reproduction in reptiles, have pointed out the importance of fat stores for reproductive activity. Female reptiles were shown to put fat into their eggs at the expense of their fat bodies[2,7,9,12].

The study of the monthly variations in autopsied females with dissectable fat bodies revealed that a high percent occurred in the period March through August.
(100%) (Table 1). This occurred when a high percent of non-reproductive females was observed. This observation suggests that fat bodies are being developed and loaded with lipids when ample food was available.

### TABLE 1. Monthly variations in the percents of non-reproductive, reproductive, and ovigerous female A. boskianus and the percent of animals with dissectable fat bodies per each reproductive phase.

<table>
<thead>
<tr>
<th>Month</th>
<th>Autopsied Females</th>
<th>Non-reproductive class I</th>
<th>Reproductive class II + III + IV</th>
<th>Ovigerous class IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>% of animals with dissectable fat bodies</td>
<td>No.</td>
</tr>
<tr>
<td>Jan.</td>
<td>5</td>
<td>1</td>
<td>14.29</td>
<td>0</td>
</tr>
<tr>
<td>Feb.</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mar.</td>
<td>4</td>
<td>1</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Apr.</td>
<td>4</td>
<td>2</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>May</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>June</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Jul.</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Aug.</td>
<td>5</td>
<td>3</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Sept.</td>
<td>6</td>
<td>1</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Oct.</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nov.</td>
<td>6</td>
<td>3</td>
<td>50</td>
<td>33.33</td>
</tr>
<tr>
<td>Dec.</td>
<td>4</td>
<td>2</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

As animals became reproductive with follicle sizes ranging from 2.5 to 8.0 mm in classes II & III, consumption of fat bodies occurred. However the data recorded indicate a non-exhaustive consumption. The greatest decline in percent of females with dissectable fat bodies was recorded in winter animals (October-February) where replenishment of exhausted fat seems to be limited. Conversely, reproductive female A. boskianus, caught in the period March through September possessed a variable percent of animals with dissectable fat bodies, which tended to exhibit a value, higher than that recorded in winter animals. This strengthens our suggestion that, although reproducing females invariably consume lipid stores, however replenishment seems to take place during that period characterized by availability of food.

The heavy consumption of lipid stores becomes apparent when one considers the percent of ovigerous females (class IV) with dissectable fat bodies (Table 1). In these animals the percent was zero, except some irregular observations recorded in ovigerous females caught in July and August, which might be due to an early start of lipid replenishment after the reproductive cycle is over.

Observations made on autopsied females revealed that no yolky ova would be recorded in the presence of corpora lutea. When only corpora lutea degenerate, yolky follicles make their appearance, implying that a time lapse should separate each two consecutive clutches, while in the same time reproductive activity remains invariably continuous throughout the year.
It appears that the clutching profile noted in *A. boskianus* allows sufficient time for fat bodies to develop and suitable replenishment of lipids consumed with reproduction. Other reptiles where clutches are produced without time lapse between each two consecutive clutches, fat bodies do not seem to be important for reproductive activity[7]. These animals were indicated to exhibit little if any replenishment of fat between clutches. It appears that in such animals, lipids are transferred to ovaries, which will furnish the requirements necessary for each clutch. The several clutches produced in this case, apparently take place with yolky ova in the presence of corpora lutea.

Measurements of the indices of lipidosomatic, gonadosomatic and hepatosomatic in *A. boskianus* carried out in the present work have revealed that LSI declines as non-reproductive females pass to the reproductive phase (classes II, III and IV), assuming its minimal value in the ovigerous females. Apparently, lipids are mobilized both to liver and ovaries indicated by the increases recorded in HSI and GSI. However as animals become ovigerous, a sharp decline would occur in GSI while a moderate decline takes place in HSI (Fig. 1). These observations support the above-mentioned view that fat bodies are important for reproduction in *A. boskianus*.

![Graph showing variations in GSI, HSI, and LSI](image-url)

Fig. 1. Variations in the indices GSI (---), HSI (-----) and LSI (△) in *A. Boskianus* during the four reproductive classes. Numbers are the sizes of observations.
The biochemical analyses of vitellogenic macromolecules and ultrastructural information as a function of season or estrogen stimulation are however meagre\cite{23}. Since liver has a primary role in the synthesis and the supply of important vitellogenic substance\cite{12,14,16}, examinations of the changes in lipids, proteins and total free amino acids in liver of *A. boskianus* were determined both seasonally as well as a function of the reproductive status.

The monthly records of hepatic lipid content (Fig. 2) revealed that liver exhibited three waves of high lipid content; January through April, July through October and November through January. When these changes are considered in the light of monthly variations in the percent of reproductive animals with dissectable fat bodies, one would observe that the high hepatic lipid content coincides with the relatively high percent of females with dissectable fat bodies. This relationship might indicate that as stated above, lipid stores are mobilized to liver beside ovaries. This strengthens the view of the role of liver in vitellogenesis and gonadal activity. Support of this view can be withdrawn when one considers the changes in hepatic lipid

![Graph](image-url)
content versus reproductive classes of *A. boskianus*. The data obtained (Fig. 3) demonstrate that a relatively high lipid content occurs in non-reproductive females (follicle size $\leq 2.4$ mm). However as vitellogenesis proceeds a moderate decline occurs in hepatic lipid content, which exhibited a very low level as females become ovigerous.

**FIG. 3.** Levels of hepatic proteins (▲ _ _ _ ▲), total free amino acids (●——●——●) and total lipids (●——●) versus different reproductive classes in *A. boskianus*. Vertical bars are S.E.M. Numbers are the sizes of observations.
It appears that fat mobilization to ovaries occurs either directly from fat stores or indirectly via liver, since good supply of lipids to vitellogenic ovaries is essential to meet the demands of the ovaries. It was demonstrated that lipid bodies, consisting of phospholipids develop a very complex structure and increase in amount with the growth of follicles. These phospholipids apparently are utilized for the construction of cellular lipoprotein membranes of cell organelles and various other metabolic activities concerned with maturation of follicular epithelium and oocyte[24-26].

During vitellogenesis, the liver was found to exhibit a decline in its protein content as vitellogenesis proceeds (Fig. 3). Then, it increased when animals became ovigerous. On the other hand monthly fluctuations were recorded which gave peak values at March, July and November. On the other hand, while the total free amino acid content of liver exhibited monthly fluctuations (Fig. 2), it showed a significant decline during vitellogenesis (classes II, III). It then increased when animals were in the ovigerous stage (class IV). These results clearly indicate that liver is actively utilizing free amino acids for the synthesis of proteins important for vitellogenesis. However, it appears that liver once synthesizes such proteins would deliver it to the circulation, which explains why the hepatic protein content exhibited a decline during vitellogenesis. These changes are clearly indicative of adaptation of liver for providing various substances such as lipids, proteins and even amino acids to the growing follicle via the circulation. The follicular epithelium by specific uptake mechanisms (as well as through biosynthetic capacity) can maintain the nutritional requirements of the growing previtellogenic oocyte in the reptilian ovary.

In this connection we might refer to the pronounced increase which would occur in α-globulin electrophoretic component after estrogen administration to Sceloporus cyanogenys[27] which might be interpreted as demonstrating vitellogenin induction. A plasma component was also noted in the serum of Dipsosaurus dorsalis[28]. It might be assumed in this respect that the liver in A. boskianus under the action of estrogen would synthesize vitellogenin and immediately convey it to the circulation. Vitellogenin was also demonstrated in different species of reptiles, e.g. Thamnophis sauritus and T. elegans[29], Pseudemys scripta and Uta stansburiana[30,31].

It appears that vitellogenin reaches the growing follicles by means of the capillary network embedded within the thecal layer of the follicle[32]. It would then leave the capillaries and translocates to the oocyte surface[33]. It was reported that in amphibia, vitellogenin appears to be incorporated by an extensive micropinocytotic process[16,34,35]. The information in this connection in reptiles is however lacking and need an extensive work to clarify the process.

References

Interrelations of Fat Bodies and Liver.


العلاقات التبادلية للأجسام الدهنية والكبد بالتكاثر في أنش السحلية

خميس عبد الرحيم و غازي يحيى دهولي
قسم الأحياء ، كلية العلوم التطبيقية ، جامعة أم القرى ، مكة المكرمة ، المملكة العربية السعودية

أجريت الدراسات لقياس العلاقات بين النشاط التكاثري في السحلية أكاثوداكتيلاس بوسكياناس. وقد أدت النتائج على أن أعلى نسبة إناث تحتوي على أكساد دهنية كبيرة وجديدة في الإناث غير المتكاثرة. أما الإناث المتكاثرة (المرآة التكاثرية 202)، فقد أُنشئت بها درجات استهلاك الأجسام الدهنية بشكل مختلف حيث كانت نسبة الإناث ذوات الأجسام الدهنية الكبيرة صغيرة في الشعراوية كبيرة في الصيف. وقد أعزى ذلك إلى القدرة على تعويض الأجسام الدهنية ومتناولة ومتخلفة أثناء الصيف. وينبغي استهلاك الأجسام الدهنية عند دراسة الحيوانات التي تمر بمرحلة الإباضة حيث كانت نسبة الحيوانات ذوات الأجسام الدهنية منفية (صفر).

وبدراست التغيرات في محتوى الكبد لكل من الدعوين الكلية وال악ام الأمينية الكلية وكذلك البروتينات الكلية سواء شهرياً على مدار العام أو بالنسبة للمراحل التكاثرية المختلفة التي تمر بها الأنثى، فقد وجد أن الدعوين تستهلك أثناء عمليات تكوين المخ (المرآة التكاثرية 202) حيث يتخفض محتوى الكبد من الدعوين. كما أن الخضاضات محتويات الكبد لكل من الاقسام الإمينية والبروتينات أثناء تكوين المخ مما يعزز الاعتدال باستهلاك الأحماض الأمينية سواء لإمداد المبيض بحاجاته أو تكوين البروتينات فينظم، وهو لازم لعملية تكوين المخ. وبدلاً من ذلك نخفض درجات التكاثر على زيادة تصور البروتينات أثناء تكوين المخ والتي يتوفض معدناً في الحيوانات التي تمر بمرحلة الإباضة حيث يتوفض محتوى الكبد من البروتينات والأحماض الأمينية، تحولت لدورة جديدة.