

Altered Energy Metabolism in an Irradiated Population of Lizards at the Nevada Test Site

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Field metabolic rates (via doubly labeled water), body compartmentalization of energy stores, and energy assimilation efficiencies were measured to assess all avenues of energy utilization in *Uta stansburiana* living in a low-level γ -irradiated plot in Rock Valley, Nevada. Comparison of energy budgets for radiation-sterilized females with those of nonirradiated control lizards revealed several substantial differences. Sterile females were heavier, mainly because they had extraordinarily large energy (fat) storage depots. Sterile females had much lower rates of energy expenditure via respiration and lower rates of energy intake by feeding. These differences are interpreted as indirect responses to radiation-induced sterility. Gastrointestinal tract function in sterile females was normal. There is little evidence of direct radiation effects on physiological functions other than reproduction. © 1985 Academic Press, Inc.

INTRODUCTION

In 1964, a 9 ha, circular, fenced plot was established in natural creosote-bush habitat in Rock Valley at the Nevada Test Site for the purpose of studying the effects of long-term, low-level γ radiation on native plants and animals. A 15-m-tall tower in the center of the plot contained ¹³⁷Cs and graded shielding that yielded free-air exposure rates ranging from 10 R/day near the tower to 2 R/day near the fence in January 1964. By 1973, free-air exposure rates had dropped to 8 R/day centrally and 1.5 R/day peripherally. Small animals living in this plot received tissue doses generally ranging between 10 and 60% of free-air exposure rates (1).

Uta stansburiana lizards living in the irradiated plot became sterile in their second or third year (1, 2). Other lizard species also became sterile. These included *Phrynosoma platyrhinos* (3), *Gambelia (Crotaphytus) wislizenii*, and *Cnemidophorus tigris* (4). Earlier studies indicated that only females became sterile, as evidenced by disappearance of their ovaries. Subsequent work has shown that older males were also sterile, although their testes did not disappear (5). The population density of *Uta* in the irradiated plot was sustained via reproduction by younger lizards which had not yet become sterile. This was also apparently true of *Cnemidophorus tigris*.

The apparent cause of sterility is directly through radiation damage to gametogenesis and the gonads, rather than indirectly through radiation effects on pituitary gland

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function (5). We wished to determine whether the radiation also affected the daily activity pattern (possibly through central nervous system damage) or gastrointestinal function. We also were curious about how sterile females handle their energy economy during the breeding season. Accordingly, we derived itemized energy budgets for sterile female *Uta stansburiana* for comparison with energy budgets of fertile females living in nearby, nonirradiated plots in Rock Valley (6). Field metabolic rates, which represent integrated daily costs of living and thus should reflect differences in daily activity patterns, were measured using doubly labeled water (7). Changes in body energy stores were assessed by measuring body mass and body composition. Sterile females were used in laboratory feeding experiments designed to measure assimilation efficiency, as an index to digestive tract function.

MATERIALS AND METHODS

Field work was done during the 1975 breeding season (April, May, and June) of 1975 in Rock Valley, Nevada Test Site, Nye County, Nevada. Simultaneous measurements were made on populations of *Uta stansburiana* living in the irradiated (I) plot and in unirradiated control (C) plots 1–2 km away. The *Uta* in these plots had been intensively studied, and their demography, habitat, and radiation doses have been described (8–10). Female *Uta* became sterile after accumulating doses of 500–1200 rad, which usually occurred sometime during their second year of life (2).

Seven cohorts were studied. In the control plot, the three cohorts were adult males, females in their first year (1 YR), and females that were in their second year or older (2 YR+). In the irradiated plot, the four cohorts were adult males, 1-YR females, 2-YR+ females that were still fertile, and 2-YR+ females that had become sterile. In the field, 2-YR+ females were judged as fertile or sterile by palpating the abdomen for ovaries, yolk follicles and eggs, or the presence of enlarged fat bodies. These diagnoses were confirmed later upon autopsy.

Field and laboratory procedures used for irradiated lizards were identical to those used for control lizards, and these are described in detail elsewhere (6). Briefly, respiration rates of free-ranging animals were measured using doubly labeled water (DLW). Lizards were captured, weighed, sexed, and marked with paint. They were injected with 10–50 μ l of water containing 30 at. % ^{18}O and 0.7 mCi/ml tritium and left for 1 hr to allow isotopes to equilibrate. A small blood sample was taken, and the lizards were then released where captured. They were recaptured at intervals of 8–55 days for weighing and blood or urine sampling. Isotope levels in water distilled from blood or urine samples were measured by proton activation analysis [for ^{18}O (11)] and by liquid scintillation counting (for tritium). The decline in tritium concentration in body water is a measure of water flux through the animal. Because ^{18}O is in isotopic equilibrium between water and CO_2 dissolved in body water (via carbonic anhydrase), ^{18}O concentration declines faster because it is lost as water and as CO_2 . Thus the difference between isotope washout rates is a measure of CO_2 production (7). Rates of CO_2 production were converted to units of energy metabolism using the factor 26 J/ml CO_2 , as derived from measurements of dietary chemical composition and assimilation (6).

Dry masses and amounts of chemical potential energy in abdominal fat bodies, reproductive tissues, extractable somatic lipid, and lipid-free somatic tissue were measured (via bomb calorimetry) in sterile females that were autopsied after field measurements were completed. We assumed percentage body composition remained constant during field measurements and estimated the proportions of chemical energy in marked lizards at the beginning of field measurements from their body masses at that time. Rates of energy flow to or from body compartments were estimated from the differences in body composition at the beginning and end of field measurements. The sum of energy utilization for respiration, storage in the body, and reproduction was taken to represent the rate of metabolizable energy gain from food. Ingestion rates were calculated as the ratio of metabolizable energy intake and the fraction of gross food energy that is metabolizable (not lost as feces and urine).

For the laboratory feeding experiment, five sterile female *Uta* were housed individually in plastic shoeboxes fitted with incandescent lights to provide a thermal gradient for 10 hr per day. Lizards were force-fed measured amounts of small *Tenebrio* larvae for 12 days, and all feces and urine were collected. Energy contents of food, urine, and feces were assessed with a bomb calorimeter. Assimilation efficiency

was calculated as (food energy consumed - feces energy voided)/food energy consumed, and metabolizable energy efficiency was calculated as (food energy consumed - feces and urine energy voided)/food energy consumed. The feeding experiment animals were given DLW to validate this method by comparing metabolic rates determined isotopically with those determined by the balance (input minus output) method.

Results are presented as means along with standard errors and number of measurements. Differences between means of body mass and field metabolic rate results were examined for statistical significance using the criterion of nonoverlap of 95% confidence intervals. Feeding experiment results and body composition results were tested for significant differences ($P < 0.05$) using the Z statistic, a form of the Student *t* test.

RESULTS

Body masses of irradiated males and fertile 2-YR+ females were similar to those of their counterparts in the control plot (Fig. 1). Sterile 2-YR+ females were significantly heavier than control (fertile, nonirradiated) 2-YR+ females. Surprisingly, irradiated 1-YR females had significantly greater masses than control 1-YR females. Sterile females lost body mass slowly and consistently during the study (mean mass 3.67 ± 0.09 g on 25 April, 3.54 ± 0.14 g on 21 May, and 3.26 ± 0.09 g on 11 June) in a manner similar to males in the irradiated and control plots (6). In contrast, 2-YR+ females in the control plots precipitously lost about 30% of their body mass on 22 April and again on 21 May, coincident with laying their first and second clutches of eggs.

Field metabolic rates (FMR) of irradiated males, fertile 1-YR females, and fertile 2-YR+ females did not differ significantly from those of their counterparts in the control plot, when FMRs were compared on a mass-specific basis (Fig. 1). However, sterile 2-YR+ females had significantly lower field energy expenditures than did

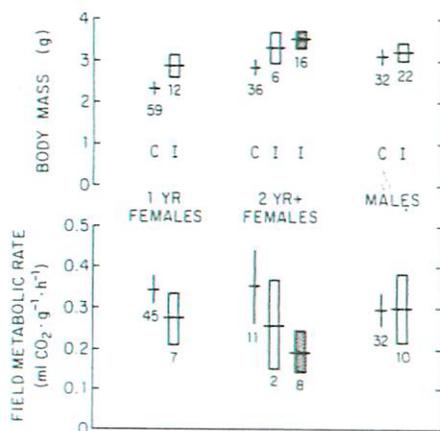


FIG. 1. Mean body mass and field metabolic rate (mass-specific) of *Uta stansburiana* lizards in the irradiated (I) plot and in the control (C) plot at Rock Valley during the interval 25 April to 11 June 1975. The shaded rectangles represent sterile 2-YR+ females. Open rectangles represent fertile cohorts living in the irradiated plot, and nonrectangular symbols represent cohorts in the control plot. Horizontal lines signify means and vertical lines or rectangles indicate 95% confidence intervals. Numbers below symbols show sample sizes.

control 2-YR+ females. The reduction in FMR associated with sterility in 2 YR+ females was about 45%.

The bodies of sterile females contained much more fat than did control females (Table I). Nearly 10% of live body mass of sterile lizards was fat, compared with only about 3% for fertile, nonirradiated females. This is reflected by a significantly higher dry matter content (lower water content) of sterile lizards.

In the laboratory feeding experiments, assimilation efficiencies in sterile females were slightly, but not significantly, higher (0.919 ± 0.007 , $N = 5$) than in control lizards (0.909 ± 0.012 , $N = 10$). Similarly, metabolizable energy efficiencies in sterile females were also slightly (not significantly) higher (0.830 ± 0.009) than in control animals (0.812 ± 0.011). Metabolic rates of sterile females, as determined with DLW (0.225 ± 0.020 ml $\text{CO}_2/\text{g hr}$), were similar to those estimated by the balance method (0.231 ± 0.013). The mean error in the DLW values was -0.006 ± 0.010 ml $\text{CO}_2/\text{g hr}$, or $-3.0 \pm 5.0\%$ of the balance method values. This error is not significantly different from zero error. Thus DLW is an accurate method for measuring metabolic rate in sterile female *Uta*.

Rates of energy flow through individual, 2-YR+ females were much higher in fertile, nonirradiated lizards than in sterile lizards during the breeding season (Table II). Fertile females consumed food energy more than twice as fast as sterile females, and fertile females had higher per animal metabolic rates (by about 40%). Fertile females ended the breeding season with about the same body composition as they had 2 months earlier and were able to convert about 18% of their metabolizable energy intake into offspring. Sterile females, on the other hand, obviously did not

TABLE I
Body Composition of Radiation-Sterilized and Nonirradiated 2-YR+ Female *Uta stansburiana* Lizards in Mid-June 1975

	% Live mass		
	Mean	SE	P
Dry matter			
Sterile	33.3	0.7	
Control ^a	26.5	0.7	<0.01
Dry abdominal fat bodies			
Sterile	3.57	0.55	
Control	0.48	0.38	<0.01
Extractable somatic lipid			
Sterile	6.20	0.26	
Control	2.76	0.62	<0.01
Dry ovaries and eggs			
Sterile	0	0	
Control	0.34	0.31	>0.05

Note. Sample size is five for both groups.

^a Values for nonirradiated animals taken from (6).

TABLE II
Energy Flow during the Breeding Season in Sterile and Fertile 2-YR+ Female *Uta stansburiana*
Living in Rock Valley, Nevada Test Site

	Energy flow (J/animal/day)	
	Sterile (3.52 g)	Fertile (2.83 g)
Respiration	422	602
Somatic tissue	-33	-3
Somatic lipid	-22	9
Fat bodies	-11	4
Ovaries and eggs	0	138
Metabolizable	356	750
Ingestion	429	953

reproduce, but they lost energy stores from their bodies at a slow rate. This is similar to the energy budgets of male *Uta*, which also did not consume quite enough food energy to maintain body condition constant during the breeding season.

DISCUSSION

Field metabolic rate measurements represent the sum of all energetic expenses a lizard must pay during a 24-hr period. For small, diurnal, insectivorous lizards such as *Uta stansburiana*, 80–90% of total 24-hr energy expenditure occurs during the daytime activity period (12). Thus any deleterious effects of γ radiation that cause changes in the amount of time each day that a lizard is abroad and warm or changes in intensity of activity while abroad should be readily detectable by measuring field energy expenditure (13).

The substantially lower FMR of sterile females (Fig. 1) could suggest a direct effect of radiation on daily behavior patterns. We feel this interpretation is not correct. The measured FMR of sterile females (422 J/animal/day) is very close to the FMR predicted for a nonirradiated 2-YR+ female that is not reproducing or growing (409 J/animal/day) (6). Thus the higher FMR of fertile *Uta* probably reflects their added costs associated with reproduction, such as obtaining and processing additional food, converting food chemicals into yolk in follicles, finding nest sites, and laying eggs. Sterile females were not paying these additional costs for reproduction. This analysis suggests that any radiation effects on behavior or FMR of sterile lizards are indirect, resulting from ovarian dysfunction rather than from direct effects on the central nervous system. This interpretation is consistent with evidence indicating that pituitary function in sterile *Uta* from the irradiated plot was also not impaired (5).

Gastrointestinal tract function was not detectably altered in sterile females. Assimilation and metabolizable energy efficiencies were not statistically distinguishable from those of control lizards.

The unusually high fat content of sterile females appears to be a reasonable consequence of sterility, given that they retained large appetites after becoming

sterile. These lizards clearly consumed more energy than they needed for respiratory costs and stored the excess as fat. High feeding rates also occurred in fertile females in the irradiated plot, with the excess energy going primarily to reproduction. It is possible that sterile females were obese because they had abnormal appetites due to radiation effects. We cannot evaluate this possibility at present, because very little is known about normal physiological mechanisms that regulate appetite in reptiles.

The results of this study provoke other questions which are unanswerable at present. For example, why did sterile females lose body mass slowly (as did males), and why were 1-YR females in the irradiated plot larger than 1-YR females in the control plot? We suspect that answers to these questions may involve the relationship between food supply and food demand, and the extent of intraspecific competition among *Uta* lizards in the control and irradiated plots. These are difficult parameters to measure in the field, but they are central to understanding energy and material flow through consumers in ecosystems. Unfortunately, the irradiation experiment in Rock Valley has been dismantled, so it is no longer possible to study the issues raised above.

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