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PHYSIOLOGICAL ECOLOGY OF THE ARCTIC GROUND SQUIRREL ENERGY METABOLISM, TORPOR, AND BEHAVIORAL ENDOCRINOLOGY

A

Thesis

Presented to the Faculty
of the University of Alaska Fairbanks
in Partial Fulfillment of the Requirements
for the degree of

DOCTOR OF PHILOSOPHY

By

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PHYSIOLOGICAL ECOLOGY OF THE ARCTIC GROUND SQUIRREL: ENERGY METABOLISM. TORPOR. AND BEHAVIORAL ENDOCRINOLOGY.

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ABSTRACT

I monitored seasonal changes in body condition and dates of immergence into and emergence from hibernation in a natural population of arctic ground squirrels (Spermophilus parryii kennicottii) living on the North Slope of Alaska. Age and sex differences in changes of body condition and hibernation chronology are attributable to sex differences in energetic costs associated with preparation for reproduction and differences in reproductive status within males. While winters are long and environmental conditions faced by arctic ground squirrels in northern Alaska are extreme, the timing of hibernation did not differ appreciably from patterns observed in Sciurid populations living in temperate latitudes. To determine the environmental conditions faced by S. parryii during hibernation and to investigate the effect of overwinter temperature on change in body condition of the individual, I instrumented 18 burrow sites with temperature-sensitive data loggers to record soil temperature at hibernacula depth. Burrow sites did not differ significantly in soil temperature over years, but significant thermal differences were observed among burrows. Burrows with more shrubby vegetation accumulated deeper snow and had higher overwinter temperatures than did windswept sites. Females hibernated at warmer sites than did males and adults hibernated at warmer sites than juveniles. Repeated measures of overwinter changes in body condition were not significantly correlated with winter soil temperatures for any age or sex class. To determine the energetic cost associated with hibernation under arctic conditions. I measured metabolic rate, respiratory quotient, and body temperature of arctic ground squirrels in steady-state torpor. At ambient temperatures below 0°C, body temperature remained constant and metabolic rate increased proportional to the gradient between body and ambient temperatures. With increased metabolic rate associated with decreased ambient temperature, animals switched from catabolism of exclusively lipid fuel to mixed fuels. To test the effects of male-male aggressive behavior on circulating testosterone and corticosterone concentrations during the breeding season, I staged encounters between free-living males and sampled them for blood following the interaction. Levels of testosterone and corticosterone significantly increased following the encounter compared to in control animals, and the magnitude of increase significantly correlated with the intensity of the interaction.

1. THE ANNUAL CYCLE OF FREE-LIVING ARCTIC GROUND SQUIRRELS: LIFE-HISTORY TACTICS OF A HIBERNATOR

Prepared for submission to the *Journal of Mammalogy* as "The annual cycle of free-living arctic ground squirrels: life-history tactics of a hibernator." By C. Loren Buck and Brian M. Barnes.

ABSTRACT

We monitored a natural population arctic ground squirrels (*Spermophilus parryii kennicottii*) on the North Slope of Alaska over 9 consecutive years for seasonal changes in body mass and 3 consecutive years for seasonal changes in body composition and for dates of immergence to and emergence from hibernation. Yearlings and adult females were at the lowest body mass of their active season at emergence in spring. Their mean body mass did not increase until later than 1 month after emergence and peaked in July (adult females) or August (yearlings). Adult males emerged from hibernation with body masses near the highest of the active season; their body mass decreased by 21% over the subsequent 10-day mating season but then reached a peak in August. Juveniles gained body mass during their active season, except for significant losses associated with dispersal from natal burrows. Females lost > 30% of their body mass during hibernation, but reproductive males emerged in spring without significant decreases in either fat or lean mass. Yearling and nonreproductive males were significantly lower in fat (38.5%) but not lean mass at emergence than at immergence, and females were significantly lower in fat (55.2%) and lean (20.5%) mass. Arctic ground squirrels entered hibernation over a > 1-month interval beginning in early August; females entered before males and adults of each sex before juveniles. The first reproductive males emerged 8 days before the first emergence of females. Fatter females emerged significantly earlier than leaner females.

Vaginal estrus was maximal 3 days post emergence. Nonreproductive males emerged last from hibernation. Mean ± *SE* days in hibernation was 240.1 ± 12.1 for adult females (69% of the year), 235.8 ± 10.3 for juvenile females, 230.3 ± 4.2 for nonreproductive males, 220.3 ± 12.5 for adult males, and 214.7 ± 6.5 for juvenile males. The timing of immergence to and emergence from hibernation for arctic ground squirrels did not differ significantly from that of sciurid populations in temperate latitudes.

INTRODUCTION

Most ground squirrels (*Spermophilus*) are obligate hibernators with endogenously timed annual cycles of body mass, reproduction, and a prolonged dormant season in winter when reserves of energy are conserved through profound reduction of body temperature and metabolic rate (Barnes and Ritter, 1993, Barnes and York, 1990; Kenagy, 1987; Wang, 1979). Timing of autumn immergence into hibernation depends on attainment of a suitable body mass (*S. beldingi*-- Morton *et al.*, 1974), and the amount of energy reserves an individual ground squirrel accumulates during the active season has been shown to be positively correlated with overwinter survival in several species (*S. richardsonii*--Michener, 1974, 1978; *S. columbianus*--Murie and Boag, 1984; *S. armatus*--Slade and Balph, 1974).

Sex and age classes of ground squirrels vary in timing of fattening during the active season and in the sequence of immergence into hibernation. Generally adult males immerge before adult females, followed by juvenile males and females (*S. tridecemlineatus*--McCarley, 1966; *S. beldingi*--Morton, 1975; *S. franklinii*--Murie, 1973; *S. richardsonii*--Michener, 1977; *S. saturatus*--Kenagy et al., 1989; *S. columbianus*--Murie and Harris, 1982; Young, 1990). In species in which males attain sexual maturity in their 1st year there are differences between the sexes in the timing of juvenile immergence. Juvenile males of *S. richardsonii* and *S. townsendii* tend to delay entering hibernation, perhaps to attain a larger body mass, enabling more successful competition for mating the subsequent spring (Michener, 1983; Rickart, 1982). Less commonly, adult females immerge before adult males in *S. armatus* (Knopf and Balph, 1977), *S. elegans* (Flagerstone, 1988), and in *S. parryii*, the arctic ground squirrel (McLean and Towns, 1981). McLean and Towns (1981) propose that male arctic ground squirrels remained active above ground late in the season to cache food and to maximize body mass at spring emergence by reducing the duration of hibernation. Michener (1984) suggested that late immergence also allows males to ascertain the locations of burrow sites of females, which would be advantageous in spring when locating estrous females.

In spring, adult males emerge from hibernation significantly earlier than females in most species of *Spermophilus*, although the sexes overlap to varying degrees in different species (Kenagy and Barnes, 1988; Knopf and Balph, 1977; Michener, 1983, 1984; Morton and Sherman, 1978; Murie and Harris, 1982). Exceptions to this sequence of vernal emergence have been reported for *S. variegatus* (Michener, 1984), some populations of *S. beecheyi* (Edge, 1931; Storer et al., 1944), and *S. tridecemlineatus* (McCarley, 1966). Age does not affect date of emergence in species in which yearlings become reproductively mature; however, if yearling males forgo reproductive development, they emerge last (Michener, 1984).

The arctic ground squirrel is the only species of its genus with a near Holarctic distribution and is the northern-most hibernator in North America, living in sporadically dense populations that reach the coast of the Arctic Ocean (Hall, 1981). At these high latitudes, the season of favorable environmental conditions for growth of juveniles and pre-hibernation fattening is greatly truncated. Mayer (1953) and Hock (1960) suggested that arctic ground squirrels at the northern-most part of their distribution experience the shortest active season and therefore the longest hibernation season and the most severe overwinter environment of any hibernator. Three studies have monitored chronologies of immergence and emergence and seasonal patterns of body mass change in this species in the field (Hock, 1960; Mayer and Roche, 1954; McLean and Towns, 1981). However, all but the study of Mayer and Roche (1954) were conducted on the sub-arctic subspecies, *S. p. plesius*. Galster and Morrison (1976) published data on body composition from captive arctic ground squirrels of the northern-most subspecies, *S. p. kennicottii*, at different times in their annual cycle. Information on changes in body fat and lean mass of free-living animals, however, is absent, and data are lacking on changes in body mass and composition of individual animals over the winter as well as changes in body mass in relation to the chronologies of immergence and emergence of different age and sex classes. In our study, we describe these features of an arctic population of *Spermophilus parryii kennicottii* and compare them to published values from temperate populations and species of hibernators.

MATERIALS AND METHODS

Study area.--Our study was conducted along the shores of Toolik Lake (68° 38'N, 149° 38'W) at an elevation of 809 m in the northern foothills of the Brooks Range, Alaska. The site is categorized as cotton grass (*Eriophorum*) tussock tundra. Greater than 98% of the vegetative biomass and productivity are attributable to only 10 species (Shaver *et al.*, 1986). The topography is gently rolling hills underlain by shallow permafrost. The growing season is brief, usually not more than 6 - 10 weeks (Chapin and Shaver, 1985). Winter is characterized by extreme sub-zero temperatures (< -20°C) for 7 - 8 months, frozen soils, snow, high winds, and darkness. Predators of ground squirrels on the study site include ravens (*Corvus corax*), ermine (*Mustela erminea*), long-tailed jaegers (*Stercorarius longicaudus*), snowy owls (*Nyctea scandiaca*), short-eared owls (*Asio flammeus*), golden eagles (*Aquila chrysaetas*), northern harriers (*Circus cyaneus*), gyrfalcon (*Falco rusticolus*), wolverines (*Gulo luscus*), red fox (*Vulpes fulva*), wolves (*Canis lupus*), and grizzly bears (*Ursus arctos*).

Capture and handling of animals.--Free-ranging arctic ground squirrels were live-trapped from 1993 (22 April -17 May, 24 June - 5 July, 13 August - 6 September), in 1994 (9 April - 10 May, 22 August - 22 September), and in 1995 (9 April - 12 May, 6 - 16 October) with tomahawk traps baited with carrot. Traps were set at known burrow sites dispersed along 4.0 km within a 0.81 km band on the north and east shores of Toolik

Lake. Burrow sites of arctic ground squirrels are easily identified on small rises on the tundra. In 1987, 53 burrow sites were identified and, over the next 8 years, 8 additional sites were discovered. Because much of the region is unsuitable for burrows, due to either shallow permafrost or rocky glacial moraine making digging difficult, the appearance of newly excavated burrows in the Toolik area was extremely rare. Between 40 and 80 traps were set in the early morning and examined every 1-3 h until traps were closed in late evening. Data on body mass changes of the population were collected during active seasons between 1987 and 1995 and are shown combined. Immergence and emergence dates of individuals and changes in body mass and body composition overwinter are from trapping

Trapped animals were transported to the Toolik Field Station of the University of Alaska Fairbanks, anesthetized by a 3-5 min exposure to methoxyflurane, and weighed with a Pesola spring scale to the nearest 1.0 g. Body mass and condition of individual ground squirrels at their last capture in autumn were used to indicate their pre-immergent status. Body mass and condition at emergence were measured at first capture in spring within 2 days of first appearance above ground. Animals were termed juveniles if trapped during the active season of their birth year and yearlings after their first season of hibernation. Animals that hibernated at least two seasons were termed adult. Males were scored as reproductive if testes were descended into a pigmented scrotal sac; reproductive males also had a strong musky odor. During late April and early May, vaginal lavages were performed on anesthetized females by pipetting 0.2 - 0.5 ml of saline into the vagina and removing it again with a short-stemmed Pasteur pipette. Lavage contents were placed on a slide and examined under a microscope under low power (10X). The percentage of cornified epithelial cells was estimated for a field of approximately 100 epithelial and leucocytic cells. Newly captured animals were ear-tagged and their pelage was dyed black in unique patterns for identification in the field. Beginning in autumn 1994, all newly captured animals also were implanted subcutaneously with a passive integrated transponder (Schooley *et al.*, 1993). Animals were released at the site of capture after recovery from anesthesia. Total time in the laboratory ranged from 1 - 3 h. All procedures used in this study were approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks.

Beginning in spring 1993, body composition of anesthetized animals was estimated by the total-body electrical conductance (TOBEC) method with an Emscan Model SA-2 (Springfield, IL). This device uses electrical conductivity of the total body to estimate fat-free mass of live animals (Walsberg, 1988). Fat mass was calculated as the difference between body mass and fat-free mass. Animals were anesthetized and weighed before being positioned in a restraining tube and inserted into the Emscan unit head first and ventrum up. Measures of conductance were repeated until three equal values were consecutively generated. Care was taken to ensure similar positioning between measures and among animals.

We generated a species-specific equation for predicting fat-free mass for *S. parryii kennicottii* with 17 animals. Animals used for calibration were directly euthanized and stored at -20° C. Frozen carcasses were shaved, cut into small pieces, and ground with a meat grinder until homogeneous. Approximately 30g portions were dried to constant mass in a convection oven at 60°C (12 - 16 h). Fat from three portions of each animal was extracted with a Soxlet apparatus with petroleum ether for 12 - 16 hours (Dobush *et al.*, 1985). Remains of lean tissue were dried to constant mass, averaged, and total fat mass was calculated by subtracting extracted mass from dry mass and extrapolating to the total animal. The regression equation formulated to predict lean mass was: lean mass = 185.81 + 0.267 * Emscan reading, yielding an $r^2 = 0.87$ for predicting lean mass and $r^2 = 0.82$ for predicting fat mass ($P < 0.001$).

Chronologies of immergence and emergence.--Dates of immergence were included only for animals that were resident at a burrow site in summer and autumn and captured again at that same site the following spring. The last day in autumn the animal was captured or seen was assigned as its immergence date. Some adult females had already immerged each year before trapping began in August, as indicated by a plugged burrow entrance from which they subsequently emerged the next spring. Dates of immergence for these individuals are listed as "< 13 August" in 1993 and "< 22 August" in 1994. Emergence dates for males were estimated by the first appearance of an exit hole in the snow at known burrow sites and subsequent trapping of the animal. In most instances detection of an exit hole and capture of the occupant occurred on the same day, and all males were trapped within 2 days of emergence. First emergence of females was estimated similarly. If the emergence hole of a female was not identified, the observation of dandruff on the animal when first captured indicated a recent (24 - 48 h) end to heterothermy (Barnes and Ritter, 1993; pers. obs.). The length of time in hibernation was calculated from immergence and emergence dates of individuals overwintering in 1993 - 1994 and 1994 - 1995.

Snow cover and depth.--We visually estimated snow cover daily in spring for the Toolik Lake area and recorded it as a percent of the area with snow within 1 km of the shore of Toolik Lake. Snow depth was measured on 12 April in 1994 and 1995 at the same 18 burrow sites and is reported as an average.

Statistical analyses.--Data are reported as mean ± *SE*. To produce a normal distribution, percentage data were arcsin-square root transformed before analysis. For comparisons between two groups, we used *t*-tests for independent measures and paired *t*-tests for repeated measures data. When assumptions of normality could not be met, we used Mann-Whitney rank sum tests (Zar, 1984). For multiple group comparisons with normally distributed data, we used one-way analyses of variance (ANOVA) to test for significant differences among groups and Tukey Tests for pair-wise comparisons. Multiple-group comparisons with non-normally distributed data were analyzed with Kruskal-Wallis tests to test for significant differences among groups and Dunn's method for pair-wise comparisons. We used a simple linear regression to examine associations between body mass and composition and emergence date. All differences were considered significant at $P < 0.05$.

RESULTS

Changes in body mass.--Juvenile animals first emerged from their natal burrows in the last week of June. Mean body masses on 24 June (males: 237 ± 8 g; females: 232 ± 32 g) almost tripled before immergence into hibernation in early to mid-September (males: 820 ± 71 g; females: 678 ± 104 g), yielding mean growth rates of 6 and 5 g/day, respectively (Fig. 1). By September, juveniles were similar to adult females in body mass, but still weighed significantly less than adult males ($F = 13.71$, *d.f.* = 64, $P < 0.001$). Although body masses of juveniles increased during their active season, both sexes decreased significantly in body mass from late July to early August (males $t = 2.94$, *d.f.* = 38, $P = 0.006$; females $t = 3.26$, *d.f.* = 53, $P = 0.002$), coinciding with the time juveniles began to be trapped away from their natal burrows.

Body mass of yearling males averaged 647 ± 18 g in April and 873 ± 13 g in

September, yielding a mean gain of 35.0% over the active season. Body mass of yearling females averaged 506 ± 11 g for all of April and 855.0 ± 45.8 g for the last week of August and the first week of September, a 68% gain in body mass during the active season.

Average body mass of adult males decreased by 21% from 941 ± 29 g after emergence in mid-April to 740 ± 23 g the first week of May; this decrease represents a 12 g/day mean loss per individual. Body mass gain was gradual through June and July but increased sharply in early August. Adult males peaked in body mass at 1026 ± 42 g in mid-August, and their body mass remained elevated until immergence into hibernation in September. Average body mass of adult females was lowest in spring (597 ± 9 g) and peaked at 1010 ± 47 g in late July. The decrease in mean body mass in August - September is likely due to early immergence of the largest females. Peak body masses of adult males and females did not differ (t = -0.433, *d.f.* = 15, P = 0.671), although females achieved maximum mass about 1 month prior to males.

Changes in body composition in the active season.--All sex and age classes emerged with substantial stores of fat (ca. 20%) which then decreased significantly in spring (Fig. 2). From mid-April to mid-May, estimated fat mass of reproductive males decreased from 194 ± 19 g to 48 ± 10 g (t = 6.71, *d.f.* = 29, P < 0.001), a 68.3 % loss, and percent body fat decreased from 22.0 % to 7.5% (t = 6.514, *d.f.* = 29, P < 0.001; Fig.

2). Estimated lean mass did not change significantly ($t = 0.392$, *d.f.* = 29, $P = 0.39$). Similarly, from late April to mid-May estimated fat mass of females decreased from 146 ± 11 g to 66 ± 9 g ($t = 5.73$, *d.f.* = 51, $P < 0.001$), a 54.8 % loss, and percent body fat decreased from 24.9 ± 1.6 to 10.4 ± 1.3 %. Estimated lean mass in females, however, increased 26.2 % (430 ± 11 to 542 ± 16 g) over the same time period. Estimated fat mass of nonreproductive males decreased significantly from late April to early May ($t = 3.18$, *d.f.* = 12, $P = 0.008$), but by mid-May percentage of fat had rebounded to levels not significantly different from measures in late April ($t = 205$, *d.f.* = 7, $P = 0.080$). Estimated lean mass of non-reproductive males did not significantly differ from late April to May ($t = 0.617$, *d.f.* = 7, $P = 0.56$).

All animals increased in fat mass and percent body fat in the weeks prior to immergence into hibernation (Fig. 2). Adult females averaged 41.5 ± 2.0%, juvenile females 32.1 ± 6.8 %, adult males 30.0 ± 1.7 % and juvenile males 23.2 ± 3.6 % body fat at last capture. Adult ground squirrels had a significantly higher percentage of body fat than juveniles ($t = 8.16$, *d.f.* =395, $P < 0.001$), and adult females were fatter than all other classes ($H = 75.38$, *d.f.* = 3, $P < 0.001$).

Dates of immergence and emergence.--In 1993 and 1994, females immerged before males and adults immerged before juveniles. Over 30% of adult females had immerged by the first date of trapping (13 August), and all had immerged by 11

September (Fig. 3a). Fewer than 5% of juvenile females had immerged prior to the first trapping day, and > 90% had immerged by the last day of trapping (22 September). Juvenile and adult males first began to immerge on 27 August, and 62.6% of juvenile males and 66.5% of adult males had immerged by 22 September. No animals were observed active above ground after 6 October.

Timing of emergence ranged from 12 April to 13 May. Reproductive males emerged before females and yearlings emerged after adults; nonreproductive males were the last to resume surface activity (Fig. 3b). Depth of snow on 12 April, the date of emergence of the first male in 1994 and 1995, was 24.4 ± 3.3 cm in 1994 and 36.5 ± 9.5 cm in 1995. Snow cover was still > 70% after all animals had emerged. In both years almost all reproductive males had begun surface activity prior to the emergence of the first female. The first nonreproductive male emerged after all other males and 70% of adult females and 35% of yearling females had appeared. Among females, date of first emergence was significantly correlated with body condition but not with body mass. Fatter females emerged earlier than leaner females of the same age class (adult females, $r^2 = 0.229$, $P = 0.001$; yearling females $r^2 = 0.163$, $P = 0.009$). Emergence dates of males were not significantly correlated with either body mass or composition.

Estimated length of hibernation.-- The duration of hibernation for females was significantly longer than that for reproductive males ($t = 4.21$, *d.f.* = 29, $P < 0.001$). The duration of hibernation was intermediate for nonreproductive males, and did not differ significantly from that for females or reproductive males ($F = 4.98$, *d.f.* $= 4$, $P = 0.004$). Adult and juvenile females did not differ significantly in duration of hibernation ($t = 0.91$, *d.f.* $= 22$, $P = 0.372$).

Body condition in successive autumns and springs.--Mean body masses of squirrels at time of immergence, based on combined data from autumn of 1993 and 1994, were 947 ± 37 g for 10 adult males, 733 ± 80 g for 5 juvenile males that were reproductive as yearlings, 765 ± 78 g for 6 juvenile males that were nonreproductive as yearlings, 851 ± 17 g for 15 adult females, and 607 ± 15 g for 16 juvenile females. Body masses of squirrels at emergence in spring averaged 922 ± 46.8 g for 10 adult males, 644 ± 44 g for yearling reproductive males, 520 ± 35 g for yearling nonreproductive males, 574 ± 17 g for adult females, and 423 ± 14 g for 16 yearling females (Fig. 4a).

Body masses of adult and juvenile males before and after hibernation were not significantly different (adult males $t = 0.53$, *d.f.* $= 9$, $P = 0.610$; juvenile males $t = 2.17$, *d.f.* $= 4$, $P = 0.095$), averaging 3 and 13.5%, respectively. Body masses of nonreproductive males (30%), adult females (33%), and juvenile females (30%) were significantly different before and after hibernation (nonreproductive males $t = 4.56$, *d.f.* $= 5$, $P = 0.006$; adult females $t = 15.57$, *d.f.* $= 14$; $P < 0.001$; juvenile females $t = 8.67$, *d.f.* $= 15$, $P < 0.001$).

Adult males were the only class that emerged from hibernation without a significant difference in estimated body condition as compared with pre-immergent measurements (lean mass change $t = 0.31$, $d.f. = 9$, $P = 0.765$, fat mass change $t = 1.23$, $d.f. = 9$, $P = 0.250$). Juvenile males, both reproductive and nonreproductive, emerged with significantly less lean mass (reproductive males $t = 2.94$, $d.f. = 4$, $P = 0.042$; nonreproductive males $t = 3.8$, $d.f. = 5$, $P = 0.013$), but without a significant reduction in fat mass (reproductive males $t = 0.13$, $d.f. = 4$, $P = 0.901$; nonreproductive males ($t = 1.80$, $d.f. = 0.133$, $P = 0.133$). Adult and juvenile females decreased significantly in both lean mass (juvenile females $t = 4.94$, $d.f. = 15$, $P < 0.001$; adult females $t = 5.44$, $d.f. = 14$, $P < 0.001$) and fat mass (juvenile females $\underline{t} = 4.12$, $d.f. = 15$, $P < 0.001$; adult females $t = 13.26$, $d.f. = 14$, $P < 0.001$) overwinter (Fig. 4b).

Vaginal estrus.-- At first capture in spring, females had only partial openings to their vaginae and unswollen labia; vaginal lavages yielded approximately 25% cornified epithelial cells and 75% leucocytes. In the next three days, the vaginae opened fully, the labia became swollen and crenulated, and the percentage of epithelial cells increased to > 50% indicative of vaginal estrus. The percentage of females in the population with > 50% epithelial cells peaked at 87.5% in late April and remained > 46% until after 1 May (Fig. 5). Thereafter, the number of females observed having lavages with > 50% epithelial cells decreased. Individual females with greater than 50% cornified epithelial cells occurred in the population between 22 April and 10 May. Sperm plugs were discovered in females between 28 April and 6 May.

Persistence of resident juveniles.--Of 32 juvenile males and 33 juvenile females caught and marked soon after they first emerged from their natal burrows in July 1993, 25% of males and 73% of females were trapped within the study area 2 months later in September. Of these, 62.5% of males and 58% of females successfully overwintered and were caught the next spring. Three of the five (60%) of these now yearling males still on the study site in spring were nonreproductive, with undescended testes and no scrotum.

DISCUSSION

As in other species of hibernating ground squirrels (Michener, 1984, Michener and Locklear, 1990), the pattern and timing of changes in body mass and condition in arctic ground squirrels at Toolik Lake, Alaska, depends on the sex and age of the individuals. When animals first emerged from hibernation in spring, yearlings and adult females had their lowest body mass of the season but adult males were near their maximum mass. Adult males then lost 21% of their body mass from mid-April to early May, while other classes maintained stable body masses. This difference between the sexes, together with differences in timing of body mass gain in summer and overwinter changes in body condition, reflect different breeding strategies, nutritional requirements, and possibly the use of food caches in the hibernacula by different sex and age classes.

Measures of body mass before and after hibernation revealed that, females and nonreproductive males decreased substantially in body mass while hibernating, but reproductive males first appeared in spring at body masses that did not differ significantly from their pre-immergent body mass. Male ground squirrels require a prolonged (1-4 week) interval of high, euthermic body temperatures after ending the heterothermic portion of hibernation for gonadal development (Barnes, 1996; Barnes *et al.*, 1986; Michener, 1992); the low body temperatures of hibernation appear to inhibit reproductive maturation. Their lack of overwinter loss of body mass suggests that male arctic ground squirrels spend this interval of pre-emergence euthermy sequestered within their hibernacula and feed from caches of food stored the previous autumn. During this time they seem to be able to fully compensate for body mass lost during the previous 7-8 months of hibernation.

Studies of male *S. parryii* in captivity show that males do lose body mass during the heterothermic season, decreasing in body mass on average by 48% (Galster and Morrison, 1976), an amount even greater than our measurements for females and nonreproductive males. Thus, in the field, reproductively mature adult males undergo an additional sub-cycle in body mass change, gaining an estimated 30% during several weeks of pre-emergence euthermy and then promptly losing 21% of that body mass during the subsequent mating season. Emergence at high body mass would provide males with endogenous body stores for use during the mating season, both reducing time needed for foraging when forage opportunities are limited, and conferring an advantage during competitive male - male interactions in which body size is positively correlated with dominance (McLean and Towns, 1981; Watton and Keenleyside, 1973). These conclusions are similar to those of McLean and Towns (1981) for a southern population of arctic ground squirrels in the Yukon Territory and those of Michener and Locklear (1990) for juvenile male Richardson's ground squirrels in Alberta, and offer a partial explanation for other studies that have demonstrated less overwinter body mass loss in males than females (Kenagy *et al.*, 1989; Mayer and Roche, 1954).

The loss of body mass by adult males during their first 3 weeks after emergence is consistent with depletion of cached food and with increased activity associated with mating. Vaginal lavages showed that this interval corresponds to estrus and mating in females. During this time, males were seldom seen feeding and appeared highly active, searching for females and interacting aggressively with other males. Females, on the other hand, remained near their burrow sites and were frequently observed digging around snow-free areas (pers. obs.). Our data are consistent with similar differences between the sexes in mating behavior in other populations of *S. parryii*, as well as other species of ground squirrels (Kenagy *et al*, 1989; McLean and Towns, 1981; Michener and Locklear, 1990).

All age and sex classes of squirrels lost fat during spring (Fig. 2). Snow cover remained near 100% until late April, and the loss of fat was consistent with poor foraging opportunities. Nonreproductive males were the first to replace their body fat, presumably because they did not incur the energetic costs of reproduction.

Juveniles gained body mass from their first appearance above ground in late June until the week of 1 August, when they lost 16 - 18% of their body mass. This was the time when juveniles first began to be trapped away from their natal burrows. Older classes continued to gain body mass during this period; thus, increased energetic costs, decreased foraging time associated with dispersal behavior, or both, rather than a change in availability of food, appears to have caused the loss in body mass. Juvenile males were three times more likely than females to disappear from the study area in late July and early August, suggesting male-biased dispersal (Holekamp and Sherman, 1989) or a greater risk of mortality in males.

The energetic expenses of lactation appear to delay body mass gain in yearling and adult female ground squirrels until after the end of June (Kenagy *et al.*, 1989). Male arctic ground squirrels began to increase in body mass in early June, coinciding with the beginning of green plant growth (Chapin and Shaver, 1985). Captive male and female arctic ground squirrels begin to gain body mass immediately after ending hibernation and peak in body mass within only 2 months (Boyer *et al.*, 1997). In the field, however, body mass gains associated with pre-hibernatory fattening began in July in adult and yearling females, but were delayed until mid-August and September in males.

Overwinter changes in body condition of individuals indicated that both lean and fat body mass decreased significantly during hibernation for juvenile males, adult females, juvenile females, and nonreproductive males (Fig 4b). Reproductive males, in contrast, showed no significant overwinter changes in lean or fat mass; presumably they metabolized fat and lean mass during hibernation as well, but restored these before emerging in spring. Both our estimates of loss of lean body mass during hibernation in the field, and those of Galster and Morrison (1976) for captive female and male arctic ground squirrels, suggest that muscle was significantly catabolized during hibernation in this species. Fat is thought to be the major metabolic fuel during hibernation (South and House, 1967), but protein can serve as a gluconeogenic source during prolonged fasts when triacylglyceride turnover and release of glycerol are low, as during torpor. Galster and Morrison (1976) concluded that replenishment of blood glucose from muscle catabolism occurs during hibernation but is confined to the euthermic phase of arousal intervals. Increased turnover of glucose may be associated with the continuous thermogenesis and elevation of metabolic rate in hibernation that accompanies overwintering in subfreezing soils (Barnes, 1989).

Because there is essentially no available forage when males first emerge in spring, males that enter hibernation without adequate food stores may be unable to mature reproductively and may hibernate longer. Nonreproductive males emerged last in spring and showed overwinter losses in body condition more similar to females than to reproductive males (Fig. 3b, 4a,b); these males may have had an inadequate supply of cached food. This proposed dependence of male reproductive maturation on the presence of adequate cached food is similar to the relationship between reproductive maturation and pre-hibernation body mass and fat stores in male golden-mantled and Belding's ground squirrels (Barnes, 1984; Forger *et al.*, 1986; Holmes, 1988). In those studies, smaller and leaner males, or males that had adipose tissue surgically removed prior to hibernation, failed or were delayed in their reproductive maturation during the subsequent spring. In our study, however, body mass and percent fat at immergence of yearling males that remained immature did not differ from those that became reproductive (Fig. 4a,b), suggesting that, under arctic conditions, both adequate body condition and food stores are required for male ground squirrels to attempt reproduction.

McLean and Towns (1981) trapped virtually only male arctic ground squirrels with cheek pouches full of seeds in August and September; males emptied these during quick trips to their burrow systems. Carl (1971) reported high levels of interaction and aggression among male *S. parryii* in autumn, which may be associated with behavioral defense of burrows with caches. Caching among several other species of hibernating ground squirrels has been observed to be an activity of males, but not of females (Kenagy *et al*, 1989--*S. saturatus*; Krogg, 1954--*S. parryii*; Michener, 1993--*S. richardsonii*; Shaw, 1926--*S. columbianus*). Caching behavior may therefore relate to the requirement for a prolonged interval of euthermy prior to emergence and thus be an additional cost of reproduction for male ground squirrels (Barnes, 1996; Barnes *et al.*, 1986; Michener and Locklear, 1990). Strict dependence on a cache for reproductive maturation, as hypothesized here for male arctic ground squirrels, is likely limited to environments with minimal foraging opportunities on emergence in spring.

The annual chronology of hibernation of this arctic population of *S. p. kennicottii* is similar to that reported for sub-arctic populations of *S. p. plesius* (Hock, 1960; McLean and Towns, 1981) and is remarkably similar to more temperate-dwelling species of *Spermophilus* including *S. columbianus* (51 °N; Murie and Harris, 1982; Young, 1990), *S. saturatus* (47° N; Kenagy *et al.*, 1989), and *S. lateralis* (40 °N; Bronson, 1979; Phillips, 1984). All of these, including our population, emerge from and immerge into hibernation during the same 2-3 weeks within each calendar year (Barnes, 1996). *S. richardsonii* in Alberta, Canada, which emerges and immerges as much as 1 month earlier than other species (Michener, 1992), is an exception to this general chronology. The environmental conditions faced by arctic ground squirrels upon emergence are more hostile than for other species, however, with snow cover and frozen soils lingering well into May. Early emergence from hibernation when environmental conditions are similarly unfavorable has been associated with high mortality of breeding adults in other species (Morton and Sherman, 1978).

In populations of *Spermophilus* for which multiple-year studies are available, timing of spring emergence can vary over 3 weeks from year to year (Michener, 1984). Delayed emergence has been correlated with proximate factors such as snow cover (Bronson, 1979; Phillips, 1984), snow depth (Morton and Sherman, 1978; Murie and Harris, 1982), air temperature (Knopf and Balph, 1977, Michener, 1977; Murie and Harris, 1982), and soil temperature (Iverson and Turner, 1972; Michener, 1978; Wade, 1950). The timing of spring first emergence of *S. p. kenocotti* was consistent in the years of this study, even though air temperature and snow cover varied substantially. The first male emerged from hibernation on 12 April in both 1994 and 1995 at Toolik Lake; mean air temperature on that date was -41.2°C in 1994 and but -23.6°C in 1995. Although snow cover was 100% on 12 April in both 1994 and 1995, mean snow depth was 24 ± 3 cm and 37 ± 10 cm, respectively. Hock (1960) reported similarly consistent timing of emergence for sub-arctic dwelling arctic ground squirrels (*S. p. plesius*). Relative inflexibility in timing of emergence and breeding may be adaptive in environments like the Arctic with short growing seasons, where animals emerging earlier would find intolerable conditions and delayed emergers would leave insufficient time for their offspring to grow and fatten in preparation for hibernation.

The mean duration of hibernation (215 to 240 days) we documented is among the longest described for *Spermophilus* (Barnes, 1996). Such prolonged dormant seasons combined with a requirement for continuous thermogenesis in very cold burrows throughout hibernation should result in high absolute costs of hibernation this species compared to other nonarctic species of hibernators. These costs may be somewhat offset in *S. parryii* by their relatively large body size and ability to store fat, which result in physiological efficiencies for thermoregulation and a relatively longer ability to withstand fasts (French, 1986; Morrison, 1960).

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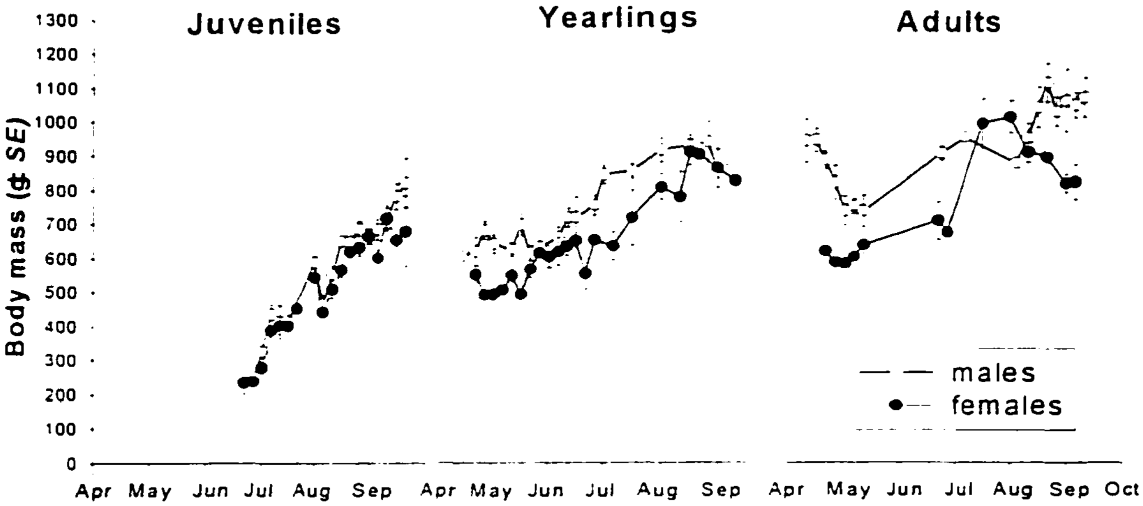

Figure 1.1.—Body mass change over the active season.

Body mass (5 day means ± *SE*) of arctic ground squirrels during their above ground active season at Toolik Lake, Alaska, 1987-1995. Data are based on 5 day pooled measurements from 1987 to 1995 and separate sex and age classes. Sample sizes for juvenile males = 6 - 40, median = 16; juvenile females = 2 - 50, median = 16; yearling males = 2 - 17, median = 6; yearling females = 1 - 52, median = 10; adult males = 2 - 24, median = 6; adult females = 3 - 60, median = 7

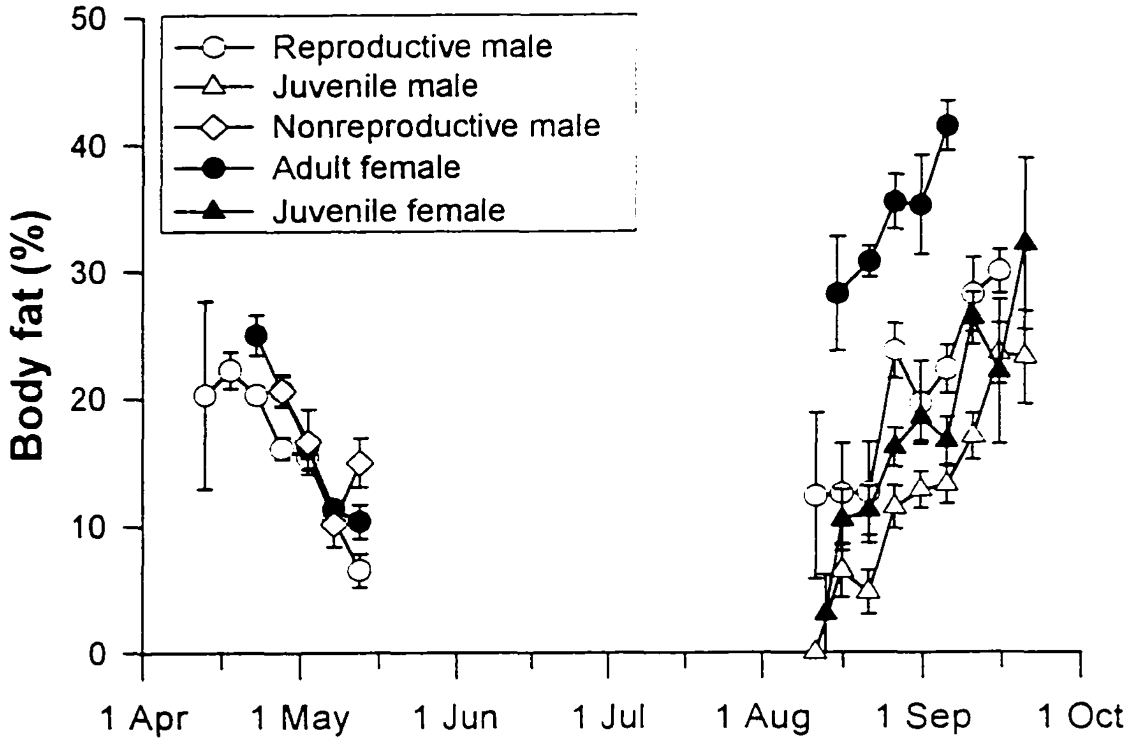

Figure 1.2.—Percent body fat change over the active season.

Body fat percentage (mean ± *SE*) of free-living arctic ground squirrels at Toolik Lake, Alaska, 1993-1995. Juveniles were born in May and nonreproductive males are yearlings that did not sexually mature. Reproductive male and female yearlings are combined with adults. Median N = 13 for adult males, 14 for juvenile males, 24 for adult females, 18 for juvenile females, and 8 for nonreproductive males.

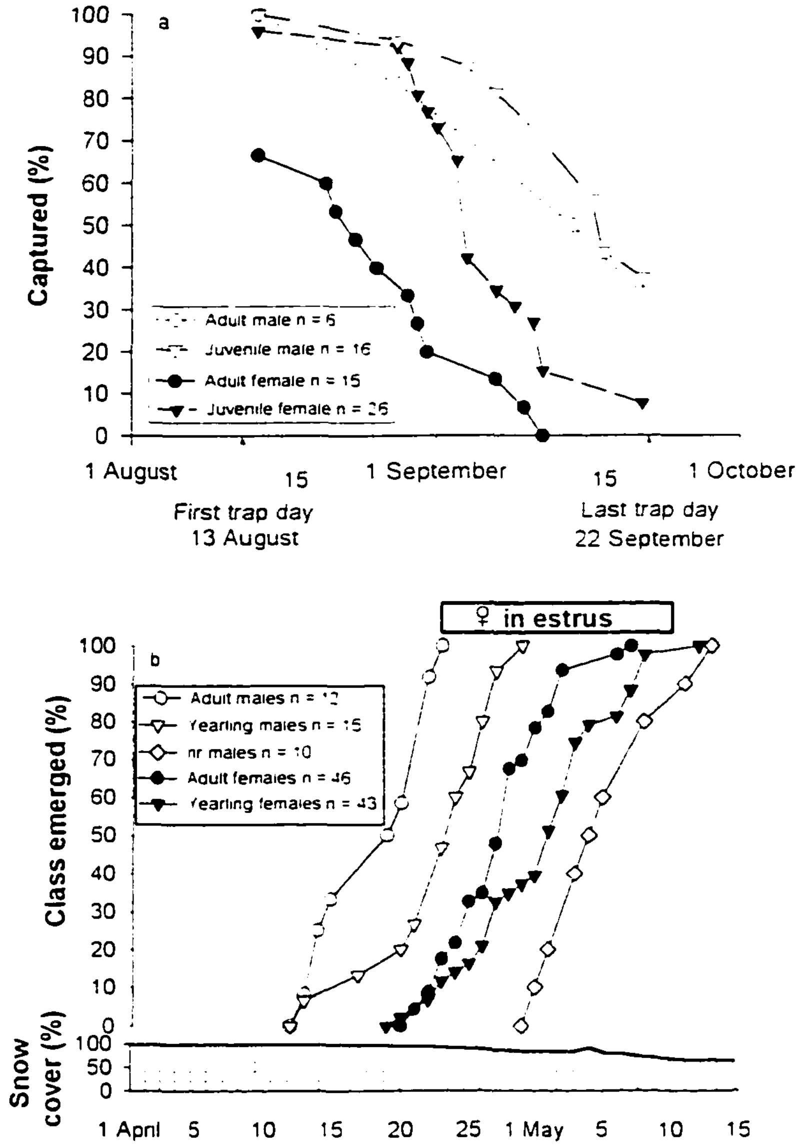

Figure 1.3.—Hibernation immergence and emergence schedules.

Hibernation immergence schedule (percentage ceasing above ground activity) in 1993 and 1994 a) and hibernation emergence schedule (percentage reappeared) in 1994 and 1995 b) of age and sex classes of arctic ground squirrels at Toolik Lake, Alaska. Also included in fig. 4b are percent snow cover from spring 1994 and 1995 and a bar labeled "females in estrus" marking when females with vaginal lavages showing >50% epithelial cells were trapped.

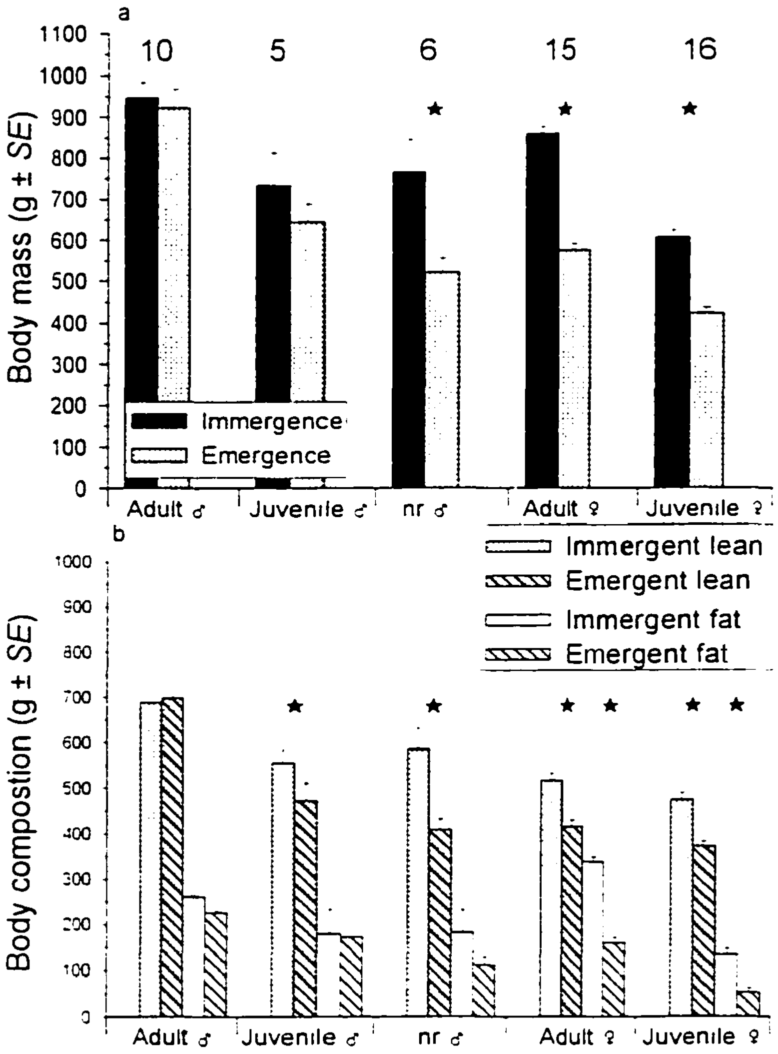

Figure 1.4.—Body mass and composition at immergence and emergence.

Mean ± *SE* body mass at immergence and emergence a) and body composition at immergence and emergence b) of age and sex cohorts of arctic ground squirrels caught in successive autumns and springs at Toolik Lake, Alaska, 1993-1995. Fig. 4a solid bars indicate body mass at immergence and shaded bars body mass at emergence. Fig. 4b lean and fat mass at immergence are indicated by solid bars and at emergence by hatched bars. Values are repeated measures estimated from the total body electrical conductance method. Immergent verses emergent mass measures that significantly differ within the cohort are designated by (*). Nonreproductive yearling males are listed as nr.

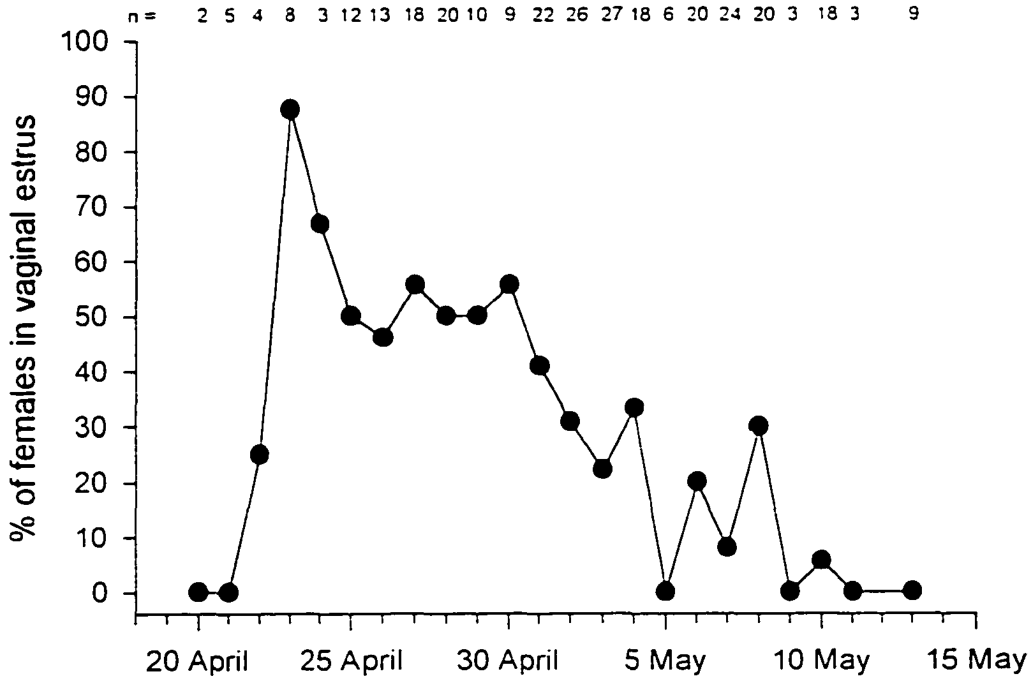

Figure 1.5.—Percentage of females displaying vaginal estrus.

Percentage of female arctic ground squirrels with vaginal lavages having > 50% epithelial cells (indicative of estrus) during the mating season at Toolik Lake, Alaska, 1993-1995. Sample sizes are listed above error bars.

2. INFLUENCE OF HIBERNACULUM TEMPERATURES ON OVERWINTER CHANGE IN BODY CONDITION OF ARCTIC GROUND SQUIRRELS

Prepared for submission to the *Journal of Mammalogy* as "Influence of hibernaculum temperatures on change in body condition of arctic ground squirrels overwinter" by C. Loren Buck and Brian M. Barnes.

ABSTRACT

Soil temperatures at hibernacula depth of free-living arctic ground squirrels *(Spermophilus parryii)* were recorded over 3 consecutive winters (1993 - 1996) at Toolik Lake, Alaska. Soil temperature means and minima (October - April) at 20 individual burrows averaged -8.9 and -18.8°C, respectively. Soil temperatures were lower than 0°C for on average > 6-months, which represents the duration of the hibernation season during which arctic ground squirrels must be continuously thermogenic. Soil temperatures near individual burrows did not differ from year to year, but significant differences in mean and minimum soil temperatures were observed among burrow sites. Burrow sites under more shrubby vegetation where deeper snow accumulated had significantly higher soil temperatures overwinter than windswept sites. Female ground squirrels hibernated at sites with significantly higher mean and minimum soil temperatures than did males, and adults hibernated at sites with significantly higher soil temperatures than did juveniles. Although ground squirrels occupying colder burrows were predicted to lose more body mass during hibernation than those in warmer burrows, neither overwinter change in body mass nor percentage of fat and lean were significantly correlated with overwinter measures of soil temperature for any sex and age class. The relationships between change in body condition of hibernating arctic ground squirrels and soil temperatures at hibernaculum depth may be confounded by the use of food caches, differing thermal conductance of

nests, or differences in hibernating efficiencies of individuals not related to the gradient between body and soil temperature.

INTRODUCTION

Arctic ground squirrels (*Spermophilus parryii*) are seasonal hibernators with a Holarctic distribution (Nadler *et al.*, 1982). Individual animals are active for 3-5 months, during which they reproduce, grow and fatten in preparation for hibernation (Mayer, 1953*a*, 1953*b*; Buck & Barnes, submitted for publication). Immergence into hibernation takes place from mid-August to mid-September in an overlapping sequence beginning with females (adults before juveniles) and then males (adults before juveniles). The next 7 - 9 months are spent sequestered in underground hibernacula. Emergence from hibernation begins in mid-April and lasts approximately 3 weeks with reproductive males emerging before females (Buck and Barnes, submitted for publication; McLean and Towns, 1981). Non-reproductive males (yearlings with undeveloped testes) are the last to resume surface activity (Buck and Barnes, submitted for publication)

Reproductively mature males first appear in spring with no significant loss in body mass compared to their autumn condition despite > 6 months of hibernation and a 1 - 3 week interval of euthermic body temperatures prior to emergence (Barnes, 1996; Barnes and Ritter, 1993). They are able recoup body mass lost over winter, presumably by eating during this interval from a food cache collected the previous summer. Females are not known to cache food before entering hibernation and emerge in spring with substantially reduced total body mass, fat mass and lean mass compared to pre-hibernation measures

(Buck and Barnes, submitted for publication; McLean and Towns, 1981). Change in body mass overwinter of females ranges from 11 to 47%, which represents a 0.71 – 1.47 g loss in body mass per day of hibernation (Buck and Barnes, submitted for publication). The sources of this variation in rates of overwinter body mass loss in females are not known.

The depths of burrows in most of the northern range of *S. parryii* are limited to about 1 m by permafrost (Carl, 1971). During subarctic and arctic winters hibernacula of arctic ground squirrels reach some of the lowest temperatures experienced by hibernating mammals. Mayer (1955) reported a minimum temperature of -26.7°C near nests of hibernating *S. parryii* in the Alaska Range, and Barnes (1989) recorded soil temperatures to -18.0°C at hibernaculum depth in the northern foothills of the Brooks Range, Alaska.

The thermal environment of the hibernaculum could be a major factor in determining energetic costs associated with hibernation for its occupant and, therefore, loss in body mass and change in body condition overwinter. Hibernating ground squirrels exposed to ambient temperatures that approach and decrease below 0°C increase their metabolic rate to keep from freezing, and rates of thermogenesis rise proportionally with increasing gradient between body and ambient temperature (Geiser and Kenagy, 1988; Heller and Colliver, 1974). Thus, substantial differences in the thermal environments of hibernacula sustained over a lengthy hibernation season should correspond to significant differences in energetic costs for the inhabitants.

The objectives of this study were to record soil temperatures at burrow sites of individual arctic ground squirrels to determine if temperatures varied among hibernacula or over years and to correlate these differences with changes overwinter in body mass and condition of the occupant. We hypothesized that ground squirrels hibernating in relatively colder sites would lose more body mass and emerge with poorer body condition compared to animals overwintering at relatively warmer sites. While age and sex classes differed significantly in hibernaculum choice with respect to both mean and minimum soil temperatures, overwinter soil temperatures did not significantly correlate with either change in body mass or composition of the burrow occupants. Confounding factors that may have contributed to this lack of correlation are discussed.

MATERIALS AND METHODS

Study area.--This study was conducted along the shores of Toolik Lake (68° 38'N, 149° 38'W, elevation 809 m) in the northern foothills of the Brooks Range, Alaska, 300 km north of the Arctic Circle. The topography is gently rolling hills underlain by shallow permafrost. The growing season for plants near Toolik Lake is brief, usually < 10 weeks (Shaver and Chapin, 1985) with freezing temperatures and snowfall possible each month of the year (pers. obs.). Snow in winter accumulates to depths of 10 to 75 cm and is composed of alternating layers of wind slab and depth hoar (Benson and Sturm, 1993; Sturm *et al.*, 1995). Winter is characterized by high winds, darkness, and extreme sub-zero temperatures for ca. 8 months at a time.

Animal handling.-- Free-ranging arctic ground squirrels were live-trapped with Tomahawk live traps baited with carrot in late summer (August and September) and in spring (April and May). We trapped at marked burrow sites along a 4.0 km transect within 1.8 km of the north and east shores of Toolik Lake. Between 40 and 80 traps were set in the morning and examined each 1 to 3 h until late evening. For this study we set traps daily in 1993 (13 August - 6 September), 1994 (9 April - 10 May, 22 August - 22 September), and 1995 (9 April - 12 May). Most animals for which data are reported in this study were captured twice weekly. Captured animals were transferred to the Toolik Field Station of the University of Alaska Fairbanks where they were weighed, assessed for reproductive status, and estimated for body composition by the total body electrical conductance method (TOBEC). Details of TOBEC and the calibration equation for *S. parryii* are provided in Buck and Barnes (submitted for publication). All procedures used in this study were approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks.

Locations of hibernacula.--Locations of individual hibernacula were determined by radio-telemetry of animals 1 to 3 weeks prior to immergence into hibernation. Ground squirrels were fitted with radiotransmitters (Model P2, AVM Instrument Company, Ltd., Livermore, CA) affixed to plastic wire-ties which served as neck collars. Transmitters operated at unique frequencies ranging from 150.000 to 159.999 MHz. The signal was transmitted from a 15.24 cm whip antenna and received with a portable receiver with a detached Yagi antenna (Model LA12-DS, AVM Instrument Co., Dublin, CA). In late summer animals were tracked to their sleeping nests after dark. A ground squirrel that was tracked to the same burrow site for at least 3 consecutive nights was considered the occupant of that site. The following spring, burrow sites were confirmed as hibernacula by locating the exit hole through the snow and subsequent capture of the same individual as in the previous autumn. Timing of immergence and emergence was inferred from sightings and success in trapping of marked individuals. Duration of hibernation for individuals was calculated as the difference in the number of days between dates of immergence and their subsequent emergence.

Body mass and condition change.--Body mass and percent fat and lean mass of individual ground squirrels on the date of their last capture in autumn were considered their immergent body condition. Similarly, measures at first capture in spring were considered their emergent condition. Emergent measures took place within 2 days of each animal's 1st appearance above ground as determined by disturbance of overlaying snow. We recorded burrow characteristics for 21 female and 11 male ground squirrel sites. Of those, we collected both immergent and emergent measures of body condition and dates of immergence and emergence for 14 females and 9 males.

Soil and air temperatures --In 1993 we instrumented 18 burrow sites with data loggers (Model Hobo-Temp II, Onset Computer Corp., Pocasset, MA; accurate ± 0.16°C at ice point) for semi-continuous recordings (1 reading per 4.8 hours) of soil temperature at the depth of the hibernaculum throughout the winter. Two additional sites were instrumented in 1995. In late August probes were inserted to the depth of maximum thaw of the active layer of the soil. Depth of the surface of the permafrost was determined by driving a stainless steel T-bar (1.3 cm diameter) with a sledgehammer through the soil active layer at five points surrounding each burrow site. The mean depth the metal rod could be driven was recorded as the depth of the permafrost table for that site.

Data loggers for recording soil temperature were encased in a 14.5 cm section of 5.1 cm diameter plastic pipe with a cap on one end to access the data logger. The lead of the thermister was threaded through a 1.5 m section of 1.3 cm diameter plastic pipe and its tip attached permanently with silicone to a cap at the end. This end was inserted through the soil to just above the surface of the permafrost table, leaving the data logger accessible above ground. The pipe containing the thermister probe was filled to the soil surface with granular salt to mimic soil thermal conductance and inhibit convection. Of the 18 hibernaculum sites outfitted with temperature loggers, data were recovered from all for the 1993-94 winter, 10 in 1994-95, and six in 1995-96. At 5 of the original 18 instrumented sites soil temperature data were collected for 3 consecutive years.

Snow-depth within 1 m of the data logger at each site was measured to the nearest 5 cm on 12 April 1994 and 1995. Measures of snow-depth were not made in spring 1996.

Mean temperature of the air was measured at the permanent meteorological station of the Arctic Tundra Long-Term Ecological Research site located at Toolik Lake Research Station. It was recorded hourly at 1 m above ground with a data logger (Campbell 21x Data Logger, Logan, UT) and averaged for the day. These data were accessed from the Arctic Tundra Long-Term Ecological Research web site: www.mbl.edu/html/ECOSYSTEMS/lterhtml/datbase1.html.

To estimate the effect of changing nest diameter on rates of heat loss from a hibernating ground squirrel, heat flow through a spherical nest was estimated from Kreith (1973) as:

$$Q = k(4\pi r_i)(4\pi r_o) * (T_2 - T_1) \cdot (r_o - r_i)$$

where Q is total heat flow in W/m2, k is the thermal conductivity (assumed constant), r_i is the radius of the squirrel (10 cm), r_o is the nest radius, T_1 is the soil temperature, and T_2 is the core body temperature of the hibernating squirrel (-2.9°C; Barnes, 1989).

Statistical analysis.--Temperatures, snow, and active layer depths are reported as mean ± standard error (*SE*). All percentage data were arcsin square root-transformed prior to analysis to meet the assumptions of normality for parametric tests. For multiple comparisons we used a one way analysis of variance (ANOVA) and the Tukey test for pair-wise comparisons (Zar, 1984). For temperature comparisons at a burrow site over years we used a Repeated Measures Analysis of Variance test. To determine any associations among changes in body, fat, and lean mass, and measures of soil temperature and to determine associations among variation in body, fat, and lean mass of individuals upon emergence and their emergence date and duration of hibernation, we used separate tests of simple linear regression. All differences were considered significant at $P < 0.05$.

RESULTS

Temperature at hibernaculum depth.--The depth of thaw of the active layer of the soil at 20 arctic ground squirrel burrow sites in the last 2 weeks of August averaged 97 ± 1.2 cm (range 80 - 104 cm; Table 1). Depth of the active layer among burrow sites was within a 15 cm range at all but 1 site. Soil temperature means and minima (mean T_S, minimum T_S) at all 20 burrow sites (October - April, 1993 - 1996) averaged -8.9 ± 0.4 (range, -4.7 to -13.5°C) and -15.8 ± 0.7°C (range -8.0 to -23.4°C), respectively. Soil temperature first decreased below 0°C ($T_S < 0$°C) on average on 26 October ± 3 days. Among these 20 sites and over 3 winters, mean and minimum T_S were positively correlated with spring snow depth (mean T_S, $r^2 = 0.49$, $P < 0.001$; minimum T_S, $r^2 = 0.53$, $P < 0.001$, Fig. 1) and with one another ($r^2 = 0.978$, $P < 0.001$), but not with date $T_S < 0$°C ($P = 0.08$). Depth of the soil active layer (permafrost table depth) was not significantly correlated either with mean or minimum T_S or with the date $T_S < 0$ °C (mean T_S, $P = 0.074$; T_S minimum, $P = 0.28$; $T_S <$ 0°C, $P = 0.46$).

Soil temperatures at hibernaculum depth were collected at 5 burrow sites and air temperatures at 1 site for 3 consecutive winters. Figure 2 shows temperature patterns over the winters of 1993 -1996 for the coldest and warmest of these burrow sites with annual changes in air temperature. Summary data are given in Table 2. Overwinter (October - April) mean and minimum T_S varied significantly among sites over all years ($P < 0.001$, $n = 5$), but did not vary significantly at any site among the 3 years (mean T_S, $P = 0.340$; minimum T_S, $P = 0.201$, Table 2). Air temperature (October - April) averaged -24.5°C and did not vary significantly among years ($P = 0.81$).

Loss of body mass and change in body condition overwinter --Repeated measurements of body mass and estimates of fat and lean mass loss overwinter were obtained for 14 female and 9 male arctic ground squirrels that hibernated at burrow sites instrumented to record soil temperature. Overwinter change in body condition was calculated as the difference between measures of body, fat, and lean mass of individuals at their last capture in autumn and when first captured after emergence from hibernation in spring. Males averaged the same body mass at first capture in spring as they did at last capture in autumn and showed no significant change in average body condition ($P = 0.82$, $n = 9$). Females weighed significantly less (on average 35% less, $P < 0.001$, $n = 14$) in spring than they had the preceding summer, accounted for by loss of 66.0% and 20.8% in fat and lean mass, respectively. For females, the duration of the hibernation season (difference between dates of immergence and emergence) varied among individuals (223 - 255 days), and the magnitude of body mass difference between immergence and emergence was correlated significantly with the number of days in hibernation ($r^2 = 0.34$, $P = 0.029$, $n = 14$). Females that hibernated longer lost more mass. Therefore, we calculated the rate of body mass and composition change over the hibernation season (Table 3). Individual females differed in rates of loss of body mass during hibernation by as much as 2.3-fold.

Soil temperature at hibernaculum depth and change in body condition.-- We performed separate linear regression analyses of mean and minimum T_S with differences between immergent and emergent body, fat, and lean mass to identify potential relationships between thermal conditions of hibernacula and overwinter changes in body condition of the occupant. There were no statistically significant correlations between the magnitude of body mass loss or change in composition overwinter with respect to mean or minimum T_S of the burrow site for any of the age and sex classes ($P > 0.05$). Because of the positive relationship in females between duration of hibernation and loss in body mass overwinter, we included hibernation duration with mean and minimum T_S in a multiple linear regression model and found no improvement in the predictability of body mass loss as indicated by r^2.

Site selection by age and sex classes.--Female arctic ground squirrels ($n = 21$) hibernated at sites with significantly higher mean ($P = 0.009$) and minimum T_S ($P = 0.009$) than did males ($n = 11$; Table 4). Adult females ($n = 14$) hibernated at burrow sites that were significantly warmer than sites of juvenile females ($n = 7$, mean T_S, $P = 0.046$, minimum T_S, $P = 0.024$). Adult males ($n = 6$) tended to hibernate at warmer burrow sites than did juvenile males ($n = 5$; $P = 0.057$), and juvenile females hibernated at significantly warmer burrow sites than did juvenile males ($P = 0.029$).

Over the 3 years of the study, 7 of 18 burrow sites were used exclusively by female ground squirrels and 7 exclusively by males (Table 1). All burrow sites used exclusively by females were located in areas with relatively tall, shrubby dwarf birch (*Betula nana*), and were consequentially sites with deeper snow. Only two of the seven burrow sites of males were in shrubby areas; four were in areas overlain with low tundra vegetation and one in a wind-scoured area with no vegetation. Two of the four burrow sites that were used by both male and female ground squirrels were in tundra vegetation and the other two in shrub areas. Most sites were in areas of fine soil or fine soil mixed with gravel, and 72% had a southerly exposure.

DISCUSSION

Burrow site and hibernacula conditions.--Hibernacula of arctic ground squirrels in our study were located in areas with an average active layer depth of 97 cm, whereas the depth of the active layer in the general Toolik Lake region averages 45 cm (pers. obs.). This observation is consistent with previous results (Mayer 1953*a*) showing that arctic ground squirrels select sites with a deeper than average permafrost table for digging their burrows. Although soil temperatures at burrow sites of arctic ground squirrels differed in mean and minimum T_S overwinter, all sites were very cold compared to conditions reported at burrow sites of lower-latitude hibernators, which only briefly or never reach freezing temperatures. Minimum soil temperatures collected at hibernaculum depth are -2.6 for *S. richardsonii* (Michener, 1992), ca. 0°C for *S. columbianus* (Young, 1990), 0.1°C for *Marmota monax* (Ferron, 1996), 0.5°C for *M. marmota* (Arnold *et al.*, 1991), and 2°C for *S. saturatus* (Kenagy *et al.*, 1989). Mean T_S were not reported in these studies. The highest mean T_S we recorded over three winters was -4.7°C, and the lowest was -13.5°C, with minimum temperatures of -23.4°C. Mayer (1955) reported soil temperatures at hibernacula of sub-arctic dwelling sub-arctic ground squirrels within the range that we recorded and minima of -26.7°C. We agree with Mayer (1953*a*, 1955), however, that hibernating in a burrow confers considerable thermal protection from the extremes in air temperature above (Fig. 2).

Burrow sites varied consistently and substantially from one another in temperature each year. Several factors could account for these differences. Variation among burrows in mean and minimum T_S were significantly correlated to differences in snow accumulation on the surface above the burrows (Fig. 1). For tundra snow, increases in accumulated snowcover are associated with increases in insulation due to increased depth and decreases in thermal conductivity (Sturm *et al.*, 1995). This was apparent in the relative warmth of burrows located in association with shrubby dwarf birch that act as wind breaks, preventing dispersal of fallen snow and collecting wind blown snow (Table 1).

The amount of water in the soil (percent saturation) also affects the pattern of change in soil temperature overwinter. Heat is released as water freezes, causing soil temperatures to remain at 0°C until all of the water in the soil is ice (Johnston, 1981). Once the soil is frozen, however, the facility with which it will undergo changes in temperature (thermal diffusivity) increases with percent saturation (Kerston, 1949). Burrows in wet soils, therefore, first decrease below 0°C later in the season compared to drier sites, but then cool faster. This first feature is apparent in the contrast between soil temperature patterns of the two burrow sites shown in Fig. 2, but not the second. This is likely due to the greater snow cover which insulated frozen soils from losing heat to the air above at the warmer burrow site (site 34) than at the colder site (site 31).

Finally, burrow sites could also vary in the thermal conductivity of the soil. Most of the burrow sites were overlain with a thin (7 - 12 cm) organic layer and located in fine soil and gravel (Table 1) and thus should have similar rates of thermal conductance through the soil. Only the coldest site (burrow site 31; Fig. 2), scoured by wind, lacked an organic layer and was in boulders and large cobble It is likely that conduction through and free convection around the boulders and cobble, combined with the little or no snow accumulation, resulted in the extreme cold recorded over 3 consecutive years at this site.

Burrow temperatures and overwinter body mass loss.--We presume that hibernating in cold burrows is more energetically expensive than hibernating in comparatively warmer burrows. Arctic ground squirrels thermoregulate to defend their core body from reaching no lower than -2.9°C and their head and neck no lower than 0°C during torpor (Barnes, 1989), and the lower critical temperature for euthermic arctic ground squirrels is reported to be 6 - 10°C (Chapell, 1981, Erickson, 1956; Withers, 1979). Therefore, for at least the duration of the hibernation season that T_S is below -2.9°C, arctic ground squirrels must be continuously thermogenic Mean T_S was lower than -2.9°C at male burrow sites for 71% (5.2 months) and at female sites for 66% (5.3 months) of their hibernation seasons. If thermal conductance does not differ significantly among individuals and their nests, and body temperatures during torpor are comparable among individuals, then rates of metabolism during torpor must increase similarly and proportionally with the increasing gradient between body temperature and T_S. In addition, the frequency of periodic arousal intervals is positively correlated with metabolic rate during torpor in ground squirrels (Geiser and Kenagy, 1988). Thus, animals in colder burrows with higher metabolic rates should arouse more often and spend more total time euthermic during the hibernation season than those in warmer burrows that have lower rates of metabolism. A higher metabolic rate during torpor, more frequent arousals to euthermy, and greater time spent euthermic predicts that arctic ground squirrels in colder burrows will expend more energy and hence lose more body mass overwinter compared to those in warmer burrows.

We tested this hypothesis by comparing means and minima of T_S of individual burrows to differences between immergent and emergent body, fat and lean mass, and rates of body, fat and lean mass loss (g/day) of the occupants of the burrows. There were no significant correlations between overwinter changes in body condition and soil temperature for either males or females. Such results were anticipated for males which are known to cache foods in their hibernacula (McLean and Towns, 1981) and undergo an extended (2 - 3 week) interval of euthermy prior to emergence (Barnes and Ritter, 1993; Barnes, 1996). During this time they eat and recoup body mass lost during the heterothermic season (Buck and Barnes, submitted for publication; McLean and Towns, 1981). Males in our study did not show overwinter losses in body, fat or lean mass upon emergence from hibernation in spring. Females, on the other hand, do not appear to cache prior to hibernation (Buck and Barnes, submitted for publication; McLean and Towns, 1981) and under captive conditions have only short (3 - 6 day) intervals of euthermy prior to emergence in spring (Barnes and Ritter, 1993). Neither female nor male arctic ground squirrels are known to eat during periodic arousals from torpor. Although there was significant and substantial (2.3 fold) variation in the percentage and rate of body mass loss among females overwinter (Tables 3 and 4), the parameters of body condition change we measured did not correlate with T_S overwinter.

One assumption of this study is that the soil temperatures recorded at maximum depth of thaw closely approximate thermal conditions experienced by the hibernating ground squirrel. Because arctic ground squirrels cannot dig into permafrost but otherwise place their nests as deep as they possibly can (Barnes, 1989; Carl, 1971, Mayer, 1953*b*), the maximum depth of the active layer approximates the lower boundary of the hibernaculum and thus the temperature of the soil surrounding the bottom of the nest. Soil temperature at this stratum should be relevant to estimating the thermal gradient between body and soil that arctic ground squirrels defend during hibernation. To avoid recording elevated temperatures due to thermogenic output of the hibernating occupant, we did not place the thermister probes directly into the nest chamber.

The lack of significant correlations between T_S and changes overwinter in female body condition could be due to several confounding factors including: 1) relevance of first and last capture measures of body mass and condition to conditions immediately before and after heterothermy, 2) differences in thermal conductance of nests, and 3) differences in hibernation efficiency related to dietary intake of polyunsaturated fatty acids during fattening before hibernation.

First, both immergent and emergent dates were inferred from capture success. Therefore, body mass and composition measures at last capture in fall and first capture in spring may not be strictly representative of values immediately pre- and post-heterothermy. The duration of time ground squirrels are euthermic underground in the fall after last capture or spring before first capture could have significant consequences on body mass and composition in either a positive or negative manner depending whether the animal was eating from a food cache. More precise information on body condition at the beginning and end of hibernation under field conditions will require better knowledge of when heterothermy begins and ends and by assessing animal body condition in close proximity to those dates.

Second, arctic ground squirrels may build nests with differing values of thermal conductance. Without knowledge of the thermal conductance properties of each of the nests of our subject animals, the influence of nest contents on overwinter body mass losses cannot be ruled out. However, we believe the following considerations make it unlikely that differences in nest thermal conductivity are responsible for obscuring relationships between hibernacula temperatures and overwinter body mass loss. Previous studies have shown that arctic ground squirrels construct nests of similar size and thermal conductivity. Captive animals that overwintered in self-dug burrows constructed nests close to 30 cm in diameter (Barnes, 1989), and Mayer (1953*b*) reports a range of diameters from 22.5 to 30 cm for nests dug in natural burrow sites near Point Barrow, Alaska, all consisting of dry grass and lichens. It is reasonable that arctic ground squirrels within our population used similar materials for nest construction to maximize insulation. Even if there was a variation in type and thermal conductivity of nest material selected, in a model of heat exchange for hibernating marmots (Webb and Schnabel, 1983), significant changes in conductivity (20% increase) were associated with < 1% change in metabolic rate. Building larger nests will decrease rates of heat loss, but calculations using a heat transfer equation adapted for a sphere (Kreith, 1973), shows a doubling of nest diameter (increasing from 30 to 60 cm) produces only a 25% decrease in the rate of heat loss and therefore a corresponding 25% difference in the energetic costs of hibernation and overwinter body mass loss. We consider it unlikely that arctic ground squirrels are building nests that differ in thermal conductance to the degree that would offset two-fold differences in burrow temperatures.

Third, diets rich in unsaturated fatty acids increase the efficiency of mammalian hibernation (Florant *et al.*, 1993; Frank, 1992; Geiser *et al.*, 1994). In laboratory experiments individuals fed diets containing high levels of the polyunsaturated fatty acid linoleic acid or the monounsaturated oleic acid during pre-hibernation fattening enter hibernation sooner, reach lower body temperatures and lower metabolic rates during torpor, and have longer torpor bouts than those with more saturated fat diets. Because arousals represent the major energetic expense during hibernation (Wang, 1979), animals with fewer arousal bouts and lower metabolic rates should expend less energy during the heterothermic season. Although diet-related differences in hibernation patterns have not yet been demonstrated in *S. parryii*, the effect has been shown in five species including two ground squirrels (Florant *et al.*, 1993, Frank 1992; Geiser and Kenagy, 1987, 1993; Geiser *et al.*, 1994; Thorp *et al.*, 1994). Polyunsaturated fatty acids are essential nutrients for mammals, and marmots and ground squirrels derive them from eating plants and seeds (Florant *et al.*, 1990; Frank, 1994). Natural diets and consequent depot fat concentrations of polyunsaturated fatty acids varied substantially in a field population of *S. saturatus* (Frank *et al.*, 1998), which raises the possibility that body mass losses overwinter in our population of arctic ground squirrels may have been affected by their diet.

Lastly, because ground squirrels of different age and sex classes were found at burrow sites that differed significantly in soil temperature may indicate an ability to discriminate and a preference of burrows based on their thermal characteristics. Female ground squirrels hibernated at warmer sites than males, and adults occupied warmer sites than juveniles. Carl (1971) describes an intense period in late summer of overt territoriality and aggression among arctic ground squirrels that is associated with competition for and guarding of hibernacula sites. Relative qualities of sites to ground squirrels may differ between the sexes and access by juveniles to preferred sits may be limited by competition with adults. Thermal conditions may be only one of several preferred features that include drainage, availability of food, and cover for protection from predators, particularly for newly emerging young of the year

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Table 2-1—Burrow site characteristics.

Site characteristics of burrows and age (adult = A; juvenile = J) and sex of the arctic ground squirrel occupant at Toolik Lake, Alaska. Vegetation acronyms are: SH = shrub; T = tundra; WS = wind scour. Soil acronyms are: FS = fine soil; FSG = fine soil and gravel; C = cobble; GC = gravel and cobble; CB = cobble and boulder. Blank spaces represent years for which data were not collected for that particular site

					$\bar{X}$ snow depth (cm)		Soil temperature $\bar{X}$ and minimum (°C)						Occupant class	
Site	Active layer (cm)	Aspect	vegetation class	Soil class	1994	1995	1993 - 1994		1994 - 1995		1995 - 1996		Age	Sex
1	100	S	SH	FSG	35	50	-8.9	-15.7	-7.0	-12.3	-7.2	-12.3	A,J	♀
4	92	S	SH	FSG	30	20	-9.4	-17.5			-8.7	-15.6	A,J	♀
5	98	SW	T	FSG	10	30	-12.0	-20.9	-11.2	-18.8			J	♂
6	97	W	SH	C	35	60	-8.2	-15.1	-6.7	-11.8	-11.0	-18.8	A	♂, ♀

11a	101	W	SH	FS	30	15	-8.6	-16.3					A,J	♀
11b	98	SW	SH	FS	20	15	-9.4	-17.7					A	♂
12	93	SW	SH	GC	15	15	-10.6	-18.8	-10.6	-16.1			A,J	♀
13	102	NE	SH	FSG	45	100	-6.6	-11.5	-4.7	-8.0			A	♀
16	80	NW	T	FS	10	10	-10.5	-18.8					A	♂
18	98	S	T	FSG	10	10	-10.6	-19.1	-9.7	-16.6			J	♂, ♀
21	94	SE	T	FS							-5.7	-12.8		
22	90	SE	T	FSG							-11.0	-21.0		
23	100	SW	SH	FSG	45	30	-11.7	-19.5			-5.6	-9.7	J	♂, ♀
31	89	NW	SH	CB	10	0	-13.5	-23.4	-13.0	-21.9	-12.2	-20.8	J	♂
32	103	SE	T	FS	10	10	-12.4	-21.6					A	♂, ♀
33	100	SW	T	FSG	35	10	-10.6	-19.1	-9.7	-16.1	-8.9	-15.9	A,J	♂, ♀
34	96	SE	SH	FSG	40	45	-5.6	-11.2	-5.5	-9.7	-5.0	-8.7	A	♀
39	100	W	SH	FSG	40	45	-5.6	-11.2	-5.8	-11.2			A	♀

46	102	W	SH	FSG	10	10	-7.2	-13.4	A	♂
53	104	SW	T	FSG	10	10	-8.8	-15.6	A	♂

Table 2-2—Mean and minimum soil temperatures

Mean and minimum soil temperatures (October - April) of five arctic ground squirrel burrow sites and annual air temperatures collected from 1993 to 1996 near Toolik Lake, Alaska.

	YEAR							
	1993-1994		1994-1995		1995-1996		3 year	
Site	X	Minimum	X	Minimum	X	Minimum	X	Minimum
1	-8.9	-15.7	-7.0	-12.3	-7.2	-12.3	-7.7	-13.4
31	-13.5	-23.4	-13.0	-21.9	-12.2	-20.8	-12.9	-22.0
6	-8.2	-15.1	-6.7	-11.8	-11.0	-18.8	-8.6	-15.2
34	-5.6	-11.2	-5.5	-9.7	-5.0	-8.7	-5.4	-9.9
33	-10.6	-19.1	-9.7	-16.1	-8.9	-15.9	-9.7	-17.0
Overall mean	-9.4	-16.9	-8.4	-14.4	-8.9	-15.3	-8.9	-15.5
Air	-24.9	-42.3	-25.7	-46.5	-24.7	-43.4	-25.1	-44.1

Table 2-3—Body fat and lean mass loss of female arctic ground squirrels per day of hibernation.

Daily body, fat, and lean mass loss ($\bar{X}$ ± SE; range) of female arctic ground squirrels (n = 14) during hibernation near Toolik Lake, Alaska. Data are combined for 1993 - 1995.

	Mass lost overwinter (g/day)	
	$\bar{X}$ ± *SE*	Range
Body mass	1.1 ± 0.07	0.7 - 1.6
Fat mass	0.6 ± 0.07	0.3 - 1.2
Lean mass	0.5 ± 0.08	0.09 - 1.1

Table 2-4—Average means and minima of Ts of burrow sites of age and sex classes of arctic ground squirrels.

Average means and minima of Ts of burrow sites of age and sex classes of arctic ground squirrels at Toolik Lake, Alaska (1993 - 1995). Different superscripts indicate a significant difference (Tukey Test, P 0.05) in either mean Ts or minimum Ts between classes.

		Soil temperature	
Class	n	$\bar{X} \pm SE$ (°C)	Minimum ± SE (°C)
Adult ♀	14	-7.7 ± 0.06^{a}	-13.7 ± 1.0^{a}
Juvenile ♀	7	$-9.8 \pm 0.60^{b,c}$	$-17.6 \pm 0.98^{b,c}$
Adult ♂	6	-9.4 ± 0.61^{c}	-17.0 ± 1.2^{c}
Juvenile ♂	6	-11.9 ± 0.55^{d}	-20.2 ± 0.98^{d}

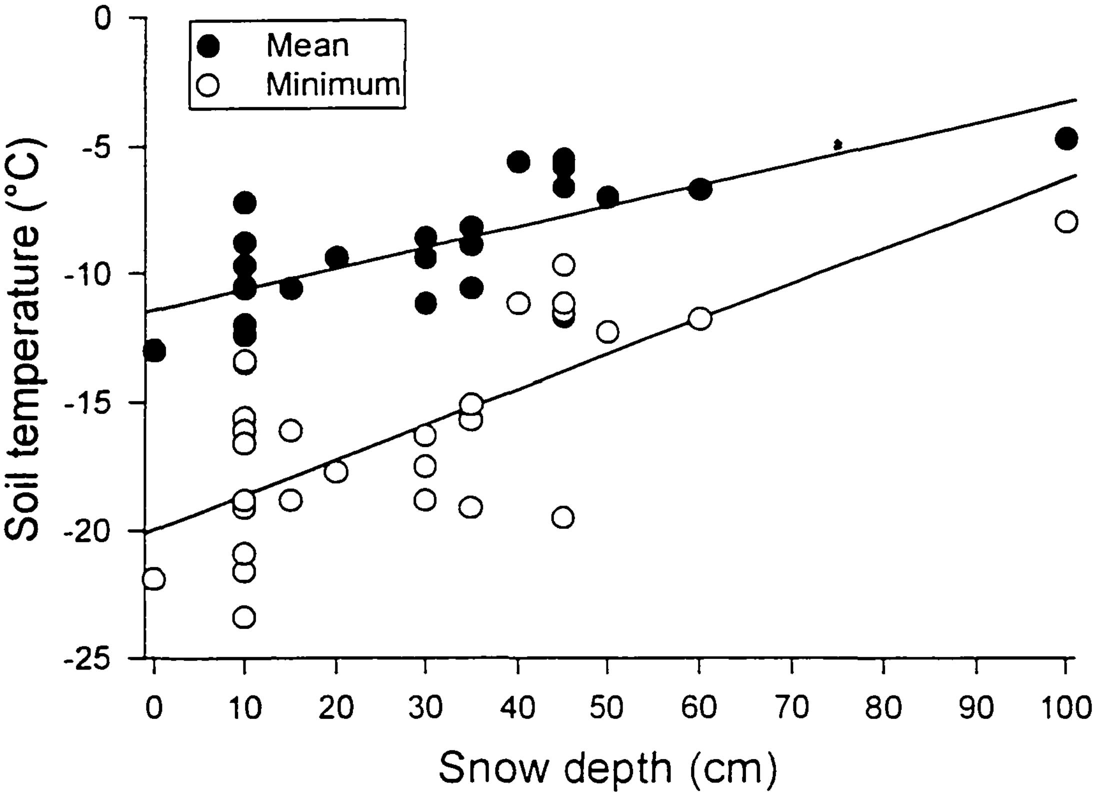

Figure 2.1 —Soil temperature at burrow sites versus snow depth.

Relationship between soil temperature at arctic ground squirrel hibernacula and snow depth on 12 April at Toolik Lake, Alaska (1993 - 1995). Both mean and minimum soil temperatures were significantly correlated to snow depth (mean, $r^2 = 0.49$, $P < 0.001$; minimum, $r^2 = 0.53$, $P < 0.001$).

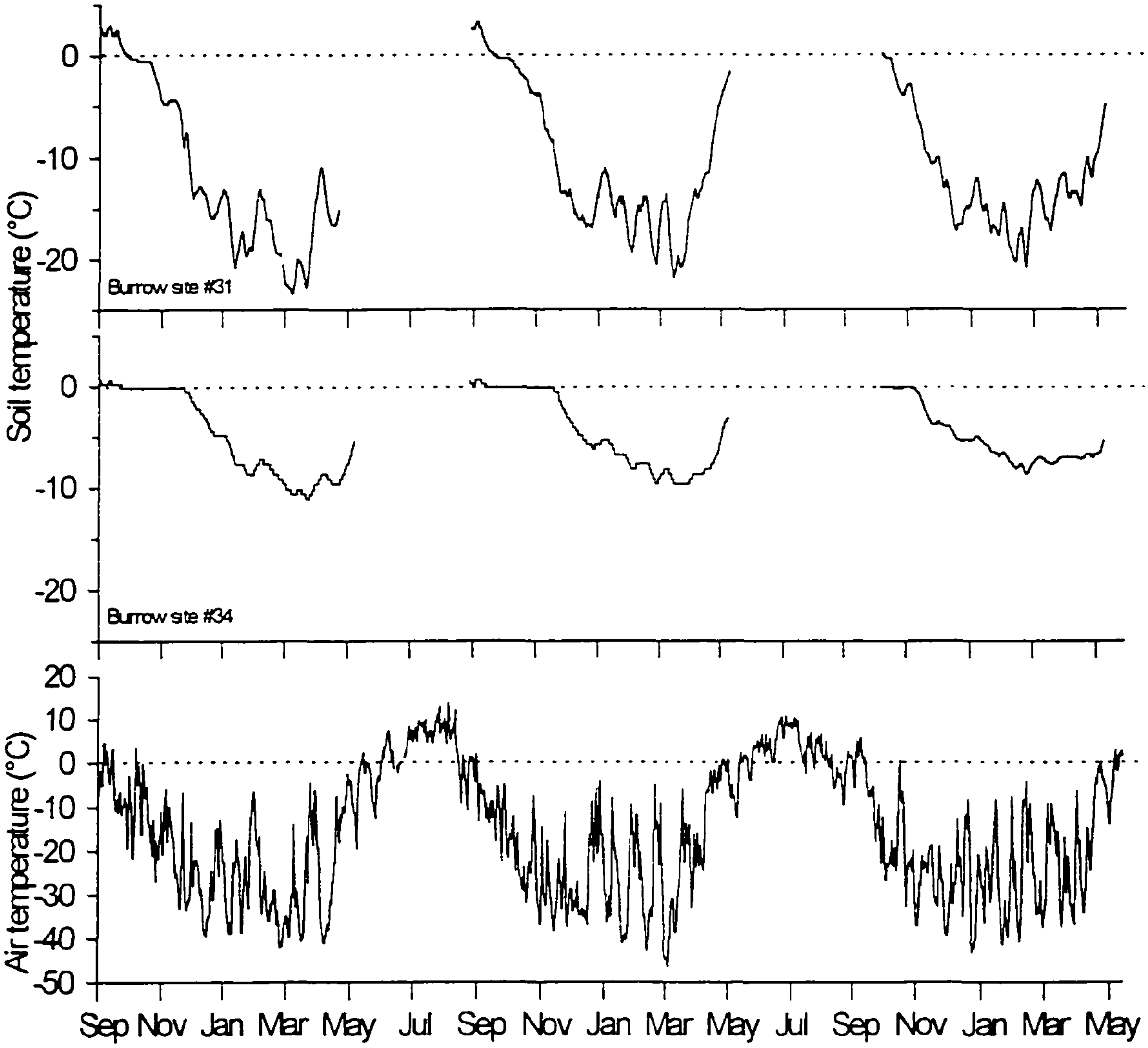

Figure 2.2 —Soil and air temperature patterns over three consecutive years.

Air temperature (bottom) and soil temperature patterns at hibernaculum depth of the coldest (top) and warmest (middle) burrows of arctic ground squirrels monitored 1993-1996 at Toolik Lake, Alaska.

3. AMBIENT TEMPERATURE EFFECTS ON METABOLIC RATE, FUEL USE, AND TORPOR IN AN ARCTIC HIBERNATOR

Prepared for submission for the *American Journal of Physiology* as "Ambient temperature effects on metabolic rate, fuel use, and torpor in an arctic hibernator" by C. Loren Buck and Brian M. Barnes.

ABSTRACT

Arctic ground squirrels (*Spermophilus parryii*), the northern-most hibernating mammal, live and overwinter under extreme ecological conditions that include substantially subfreezing hibernaculum temperatures. During steady-state torpor they display ambient temperature (T_a) dependent patterns of body temperature (T_b), torpid metabolic rate (TMR), and metabolic fuel use as determined by respiratory quotient (RQ). At T_a's 0 to -16°C, T_b remains relatively constant and TMR is negatively correlated to T_a, increasing proportional to the gradient between T_b and T_a > 15-fold from a minimum of 0.0115 ± 0.0012 ml O2 $\cdot g^{-1} \cdot h^{-1}$. At $T_a > 0$°C, T_b increases directly with T_a, however, TMR does not significantly change from T_a values of 4 to 12°C, suggesting there is temperature-independent inhibition of metabolism over this temperature range. The overall change in TMR from T_a of 4 to 20° equates to a Q_{10} of 2.4, but within this T_a range Q_{10} changes from 1 to 14.1. Arctic ground squirrels apparently use different metabolic substrates during torpor depending upon T_a. At T_a 4 and 8°C, RQ averaged 0.70 ± 0.013, indicative of exclusive lipid catabolism, but RQ increased significantly to > 0.85 at T_a -16 and 20°C consistent with recruitment of non-lipid fuels. RQ is negatively correlated with maximum torpor bout length. For $T_a < 0$°C this relationship supports the hypothesis that availability of metabolic fuels limits torpor duration in hibernating mammals.

INTRODUCTION

Hibernating mammals reduce their energy requirements during a prolonged dormant season by becoming hypometabolic and hypothermic. Torpor during hibernation has been described in detail for a number of mammalian species and is characterized by a profound reduction of body temperature and metabolism (Barnes and Ritter, 1993; Wang, 1979). Proposed physiological mechanisms for reducing the rate of metabolism during torpor (TMR) include Q_{10} effects on enzyme kinetics (Geiser, 1988; Snapp and Heller, 1981; Tucker, 1965), suppression by acidosis (Malan, 1986), combined temperature-dependent and independent inhibition (Geiser, 1988; Song et al., 1995, 1997), temperature differential (ΔT) effects (Heldmaier and Ruf, 1992), and low mass-specific apparent thermal conductance (C) during torpor (Snyder and Nestler, 1990).

Torpor (hypothermia and hypometabolism) during hibernation is not continuous; rather, it is punctuated by periodic arousals to euthermia. The functional significance of these arousal episodes is not known but hypotheses generally relate to either body temperature or metabolic rate-dependent processes. The metabolic hypothesis states that arousal episodes are required to rectify a metabolic imbalance that arises during hibernation. Periods of euthermia either clear metabolic waste products or make available metabolic fuels, processes that may be inhibited during torpor (Willis, 1982; Dark and Ruby, 1993). Therefore, TMR dictates torpor bout length (TBL). Alternatively, it has been proposed that low body temperatures during hibernation may result in a slowly functioning circadian clock and that arousals are simply a function of the circadian cycle. Accordingly, TBL is independent of TMR.

Although ample comparative data have been published on MR and metabolic fuel use of mammalian hibernation during torpor at T_a > 0°C, only a few studies have been done at T_a just slightly below 0°C (Arnold et al., 1991; Geiser and Kenagy, 1988; Heller and Colliver, 1974) and none in the conditions that prevail in hibernacula in the Arctic (-5 to -25°C; Buck and Barnes, submitted for publication). To determine the energetic cost associated with hibernation under arctic conditions, we measured TMR, respiratory quotient, and body temperature of arctic ground squirrels (*Spermophilus parryii*) in steady-state torpor at sub-zero T_a's to -16°C. To investigate relations between TMR and T_a further and to consider the potential effects of temperature and temperature independent inhibition, the range of measurements were extended to T_a's above zero to 20°C. In addition we measured TBL of undisturbed arctic ground squirrels at Ta values ranging from -16 to 20°C.

METHODS

Animals—Arctic ground squirrels (*S. p. kennicottii*) were live-trapped in July in the northern foothills of the Brooks Range, Alaska, near the Atigun River (68° 38' N latitude) and transported to the animal holding facility at the University of Alaska Fairbanks. Animals were individually maintained in metal cages (45.7 X 30.5 X 20.3 cm) at a photoperiod of 12L:12D and ambient temperature of 5 ± 2°C prior to the beginning of the experiment. Food (Mazuri Rodent Chow, carrots and sunflower seeds) and water were provided *ad libitum*.

Body Temperature—To record core body temperature (T_b) temperature-sensitive radiotransmitters (Model VMH-BB, Minimitter Co., Sunriver, OR) were surgically implanted into each animal's peritoneal cavity at least 1 month prior to the start of metabolic measurements. Transmitters were calibrated to the nearest 0.1°C against a precision mercury thermometer in a water bath at 0 and 20°C. Animals were held at T_a 20°C following surgery for seven days and then transferred to 15L plastic respirometry chambers housed in a thermally-controlled environmental chamber. Cotton batting was provided for nesting material and cages were filled to 5 cm with absorbent wood chips. The signal from the transmitter was received with a model RA1010 radio receiver placed below each cage that was interfaced to a computerized system of data acquisition (Dataquest III, Minimitter Co., Sunriver, OR) and recorded once per 15 minute interval. All procedures were approved by the University of Alaska Fairbanks' Institutional Animal Care and Use Committee.

Respirometry— At T_a values of 4, 0, -4, -8, and -16°C (each ± 0.5°C) repeated measures of rates of oxygen consumption and carbon dioxide production were recorded during steady state torpor of the same eight animals (4 male, 4 female). At T_a values of 8, 12, 16, and 20, we were not able to record from all eight original animals, because arctic ground squirrels are less likely to display torpor, and are more easily aroused at T_a's >

4°C. After at least 1 month following the beginning of hibernation, measurements of TMR were begun 4 days after T_b of the subject animal had decreased to below 30°C during entry into a torpor bout. At T_a values of -16, -8, -4, 0, 4, 8, 12, 16, and 20°C, TMR of animals that were within their nests was measured. TMR of animals removed from their nests and placed on woodchips was additionally measured at T_a values of -8, -4, 0 and 4 °C. A flow rate of 125 - 300 ml min^{-1} (depending on mass and TMR of the animal) of dried air through the respirometry chamber was controlled with a flow rotometer and measured with a mass flow meter (Model 229H, Teldyne Hastings-Raydist, Hampton, VA). Oxygen content of dried air leaving the respirometry chamber was determined with a single channel oxygen analyzer (Ametek Applied Electrochemistry S-3A, Pittsburgh, PA). Carbon dioxide content of air leaving the respirometry chamber was measured with a carbon dioxide analyzer (Beckman Model 864 Infrared CO2 Analyzer, Fullerton, CA). Rates of oxygen consumption and carbon dioxide production were recorded each 1 min over 5 h of torpor for each animal at each T_a. Rates of oxygen consumption and carbon dioxide production were defined for each T_a as the mean rate measured over the last 1 h of (60 measures) steady-state torpor, that is, constant T_b and rate of oxygen consumption for 2 or more consecutive hours. CO_2 and O_2 analyzers were calibrated to outside air and span gas (20% O_2 and 0.5% CO_2) at 1 h intervals during each trial. Equation 3b of Withers (1977) was used to calculate VO_2. The mass specific apparent conductance and Q_{10} of metabolic rate were calculated as $C = TMR/(T_b - T_a)$

and $Q_{10} = (\mathrm{TMR}_1/\mathrm{TMR}_2)^{(10/T_b{}^1 - T_b{}^2)}$, respectively

With a separate group of wild-caught arctic ground squirrels, the maximum torpor bout length (TBL) of animals left undisturbed within their nests was determined for T_a -16, -8, -4, 0, 4, 10, 16 and 20°C. TBL was calculated as the number of hours $T_b < 30°C$ as measured by radio telemetry. The longest bout of torpor for each individual for each T_a was recorded as its TBL and averaged with values of TBL of other animals. TBL was based on torpor bouts from the middle of the hibernation season by excluding initial, penultimate, and ultimate torpor bouts in TBL calculations.

Data are presented as mean ± standard error (*SE*). For statistical evaluations of multiple group comparisons we used a one way analysis of variance (ANOVA), and pair-wise comparisons with a Tukey test. A Student's *t*-test was used for between group comparisons, and non-parametric comparisons were analyzed with a Mann-Whitney rank sum test. To test for significant relationships between variables, we used a simple linear regression model. TMR results for T_a's > 0°C were log-transformed to meet the assumptions of equal variance for regression analysis. We compared the coefficient of determination (r^2) to select between linear and curvilinear models. RQ data were arcsin square root transformed prior to analysis to meet the assumptions of normality for parametric tests. In reporting sample sizes, "*N*" represents the number of animals and "*n*" the total number of measurements. Because the regression analyses violate the assumptions of independence and may underestimate alpha, differences were considered significant at $P < 0.01$. Differences were considered significant at $P < 0.05$ for the non-regression analyses.

RESULTS

Rates of metabolism and conductance—We measured metabolic rate and T_b of arctic ground squirrels in steady-state torpor at T_a values of -16 to 20°C and observed different thermoregulatory responses depending on whether T_a was greater or less than 0°C. At T_a's < 0°C all animals increased rates of metabolism and thermogenesis to maintain a constant T_b. At T_a's > 0°C animals did not thermoregulate (Fig. 1). At T_a = 0°C, three of eight animals had ΔT of 1.20 to 1.26°C, indicative of thermoregulation, whereas in the remaining animals ΔT ranged from 0.010 to 0.70°C. We considered torpid animals to be thermoregulating if T_b - T_a (ΔT) was > 1°C.

At T_a < 0°C, T_b averaged -0.42 ± 0.12°C and did not change significantly with changing T_a ($P = 0.071$, $N = 8$, $n = 24$). The lowest T_b recorded was -1.97°C at T_a of -8°C. From a minimum value of 0.0115 ± 0.0012 ml O2 • g^{-1} • h^{-1} at T_a 4°C, TMR increased by a factor of 15.8 to 0.1820 ± 0.0244 ml O2 • g^{-1} • h^{-1} at -16°C. TMR was positively correlated with ΔT ($r^2 = 0.95$, $P < 0.001$, $N = 8$, $n = 24$, Fig. 2) but not T_b ($r^2 = 0.008$, $P = 0.685$, $N = 8$, $n = 24$).

At T_a's > 0°C, T_b increased linearly with T_a ($r^2 = 0.99$, $P < 0.001$, $N = 9$, $n = 35$;

Fig. 1). TMR was not significantly different between T_a values of 4 to 12°C ($P > 0.05$, $N = 9$, $n = 24$), even though T_b had increased on average 7.9°C. In a stepwise linear regression including T_b, T_a and ΔT, the overall increase in TMR over the T_a range 4 to 20°C was significantly correlated only to T_b and best described with an exponential regression ($r^2 = 0.56$, $P < 0.001$, $N = 9$, $n = 35$, Fig. 3). TMR remained unchanged at T_a values of 4 and 8°C averaging 0.0115 ± 0.0005. At T_a 16 and 20°C TMR was significantly increased ($P < 0.05$) compared to T_a 4°C. TMR averaged 0.0179 ± 0.0051 ml $O2 \cdot g^{-1} \cdot h^{-1}$ at $T_a = 16$ and 0.0466 ± 0.0073 ml $O2 \cdot g^{-1} \cdot h^{-1}$ at T_a 20°C. The overall increase in steady-state TMR from T_a values of 4 to 20°C reflects a Q_{10} of 2.4; however, Q_{10} differed within this range of T_a from 1 to 14.1 (Table 1). At $Ta > 0$ ΔT averaged 0.61 ± 0.12°C and was not significantly correlated to TMR ($P = 0.184$, $N = 8$, $n = 35$).

Conductance (C) of torpid arctic ground squirrels in their nests at $T_a \leq 0$°C averaged 0.0121 ± 0.0005 ml $O2 \cdot g^{-1} \cdot h^{-1} \cdot {}^{\circ}C^{-1}$ and was not significantly correlated to either T_a ($r^2 = 0.013$, $P = 0.597$, $N = 9$, $n = 24$) or T_b ($r^2 = 0.114$, $P = 0.106$, $N = 9$, $n = 24$). At $T_a > 0$°C, C was significantly greater than at $T_a < 0$ (Mann Whitney Rank Sum Test, P = 0.026) and averaged 0.043 ± 0.009 ml $O2 \cdot g^{-1} \cdot h^{-1} \cdot {}^{\circ}C^{-1}$. Conductance at $T_a > 0$ was not significantly correlated to either T_b ($r^2 = 0.270$, $P = 0.026$, $N = 11$, $n =$

43) or T_a ($r^2 = 0.173$, $P = 0.012$, $N = 11$, $n = 43$)

Influence of a nest—We compared T_b and TMR of eight torpid arctic ground squirrels hibernating without nest material at T_a values of 4 to -8°C. As defined by ΔT, three of eight animals were thermoregulating at T_a 0°C (ΔT = 1.17 to 1.27°C), all were thermoregulating at T_a -4 and -8°C (ΔT = 3.38 to 7.98°C), and none was thermoregulating at 4°C (ΔT = 0.16 to 0.80°C). There was no difference in TMR of animals hibernating with a nest versus without a nest at T_a 4°C ($P = 0.559$, $N = 8$, $n = 16$) and 0°C ($P = 0.286$, $N = 8$, $n = 16$). At $T_a < 0$°C, TMR of animals hibernating without a nest was significantly higher (32.3% at -4°C and 35.8% at -8°C) than TMR of torpid animals with a nest at these same T_a values ($P < 0.05$, $N = 8$, $n = 32$). This difference can be attributed to significantly higher C for animals without a nest at both T_a -4°C (mean C in a nest = 0.01073 ± 0.0014, without a nest = 0.0170 ± 0.0011, $P < 0.001$, $N = 8$, $n = 16$) and at T_a -8°C (mean C in a nest = 0.01239 ± 0.0003, without a nest = 0.0194 ± 0.0005, $P < 0.001$, $N = 8$, $n = 16$).

Fuels of metabolism—To identify the metabolic fuels used during torpor, we calculated the respiratory exchange quotient (RQ) for torpid ground squirrels at different T_a's. RQ's were 0.70 ± 0.01 at T_a 4 and 8°C and increased at both lower and higher T_a's, reaching significantly higher values of 0.86 ± 0.02 at -16°C ($P < 0.05$) and 0.88 ± 0.09 at 20°C ($P < 0.05$). At T_a's < 0°C, RQ positively correlated with TMR ($r^2 = 0.409$,

$P < 0.001$, $N = 8$, $n = 24$; Fig. 1, 5a) but not T_b ($r^2 = 0.017$, $P = 0.540$, $N = 8$, $n = 24$). At T_a's > 0°C, RQ was positively correlated with T_b ($r^2 = 0.523$, $P < 0.001$, $N = 8$, $n = 35$; Fig. 4b), and in a step-wise regression model addition of TMR or T_a did not significantly improve the fit.

Torpor bout length—Maximum TBL of undisturbed animals was longest at T_a = 0°C, averaging 16.1 days, and did not significantly differ from TBL at T_a -4 to 4°C (P > 0.05; Fig. 5). TBL was significantly shorter at T_a's that were ≤ -8°C and ≥ 10°C compared to T_a 0°C.

To investigate the interrelationships of TBL to TMR, T_b, and RQ at differing T_a's, linear step-wise regression analyses were performed. Averages of maximum TBL at T_a values from -16 to 20°C from one group of arctic ground squirrels were regressed with averages of MR, T_b, and RQ of another group during torpor at the corresponding T_a. TBL was negatively correlated to RQ ($r^2 = 0.61$, $P = 0.039$, $n = 6$, Fig. 6), and addition of MR or T_b did not significantly improve the fit of the model.

DISCUSSION

Arctic ground squirrels hibernating in ambient temperatures ranging from substantially subfreezing conditions to room temperature showed changing interrelationships among T_a, T_b, TMR, and RQ that revealed differing physiological responses to heterothermy as T_b and requirements for thermoregulation changed. At T_a's below 0°C metabolic rate increased linearly with progressively decreased T_a while T_b remained constant. Above 0°C, T_b increased directly with T_a while metabolism remained minimal and constant until T_a and T_b increased above 12°C when TMR began to increase with Tb. Apparent use of metabolic fuels also changed over this range of T_a's, as indicated by a changing steady-state RQ.

This uncoupling of metabolic rate from T_b and change in sources of energy over a wide range of T_a provides an opportunity to investigate alternative hypotheses of what limits torpor duration in heterothermic endotherms and thus the function of periodic arousal episodes. In arctic ground squirrels a decrease in the duration of torpor under laboratory conditions was significantly correlated with the use of non-lipid fuels (Dark and Ruby, 1993). This suggests limitations in carbohydrate or protein stores may constrain TBL in mammals (Galster and Morrison, 1970).

Thermoregulation during torpor --When exposed to T_a's below 0°C, arctic ground squirrels in steady-state torpor maintained a constant T_b by increasing metabolic heat production proportionally with decreases in T_a. The T_a at which heterothermic arctic ground squirrels began to defend T_b (T_{tc}) was very close to 0°C; 5 of 8 animals held at this temperature maintained minimal TMR and ΔT. The remaining 3 and all animals at T_a -4°C had elevated TMR and ΔT indicative of thermoregulation. Although arctic ground squirrels can supercool their abdomen to -2.9°C, head and neck temperatures remain ≥ 0°C (Barnes, 1989), which would require increased levels of heat production at $T_a < 0°C$, the magnitude of increase is dependent on T_a and the insulation provided by the nest. At T_a of -16°C TMR was almost 16-fold higher than minimum values at $T_a > 0°C$.

T_a's of -16°C and lower are relevant to hibernating arctic ground squirrels. Minimum hibernaculum temperatures in burrows on the North Slope of Alaska during winter range from -18 to -25°C (Mayer, 1955, Barnes, 1989) and from semi-continuous recordings from 18 burrow sites averaged -8.9°C overwinter (Buck and Barnes, submitted for publication). Because the lower critical temperature of euthermic arctic ground squirrels is 6-10°C (Withers, 1979; Chappell, 1981), animals overwintering in these < 0°C burrows must be continuously thermogenic whether they are torpid or euthermic. From our study, an animal in an average temperature burrow without a nest would have a TMR 10-fold higher than if hibernating at $T_a > 0°C$ (0.1 vs. 0.01 ml O2 g^{-1} h^{-1}), a nest would lower this to a 6.5-fold increase in TMR and decrease lower critical temperature to near 0°C (Chappell, 1981). These considerations illustrate the substantially elevated energetic costs of hibernating under arctic conditions encountered by arctic ground squirrels compared to hibernators at lower latitudes where burrow temperatures only briefly or never decrease below freezing (Arnold, 1991; Ferron, 1996; Kenagy et al., 1989; Michener, 1992; Young, 1990). Arctic ground squirrels meet these challenges in part through a larger body size compared to other hibernating congeners (maximum body size

1.5 kg vs. < 0.6 kg in other species; Michener, 1984), which offers a proportionally greater ability to store fat combined with a lower metabolic rate compared to smaller species (Morrison, 1961). The minimum TMR we measured in this species is among the lowest measured in hibernating endotherms (Geiser and Ruf, 1995).

At $T_a \leq 0$°C, conductance was minimal and did not change with decreases in T_a. Achieving and maintaining minimal C at $T_a \leq 0$°C minimizes the metabolic heat production needed to maintain a constant T_b. That arctic ground squirrels do not display increased C with increased TMR associated with decreased T_a differs from marsupial hibernators which reportedly lack brown adipose tissue and therefore rely on shivering thermogenesis during torpor at $T_a < T_{tc}$ (Song *et al.*, 1995, 1997). Song *et al.* (1997) suggest that in marsupials increased C with progressively increased requirement for thermogenesis at $T_a < T_{tc}$ may be due to muscular shivering and increased peripheral circulation. Rodents during hibernation rely extensively on non-shivering thermogenesis and apparently do not shiver during steady state torpor (Lyman, 1958); therefore, different patterns of C with respect to T_a may be related to different mechanisms of thermogenesis between rodent and marsupial hibernators.

Torpor at T_a 0°C.—TMR of arctic ground squirrels was minimal at T_a 0 to 12°C and did not significantly increase until T_a and T_b were ≥ 16°C (Fig. 1, 3). T_b increased passively with T_a, averaging 0.67°C greater than T_a at Ta > 0°C. T_a's from near 0 to

12°C represent a thermal neutral zone for torpid arctic ground squirrels

Temperature effects on tissue and whole organism rates of metabolism have Q_{10} values of 2 - 3 (Roberts and Smith, 1967, Geiser and McMurchie, 1984, Aloia and Raison, 1989). Several hibernating species show Q_{10} values of metabolic rate well above three during the entry phase of torpor, suggestive of additive effects of temperature dependent and independent metabolic inhibition of metabolism (Geiser, 1988, Malan, 1986, Song et al., 1995, 1997, Storey, 1990). Although the overall increase in TMR from T_a 4 to 20°C is represented by a Q_{10} of 2.4, Q_{10} across these T_a's range from 1 (T_a 4 to 8°C) to 14.1 (T_a 16 to 20°C, Table 1). The lack of a significant change in TMR from T_a of 4 to 12°C suggests a temperature-independent mechanism of metabolic inhibition over that range of T_a. Suppressing TMR over this range of T_b maintains maximum energy savings of torpor over a range of T_a's, although T_a's > 0°C occur over only a brief part of the hibernation season at the northern limit of the distribution of this species (Buck and Barnes, in review). At higher T_a values (16 to 20°C) temperature-independent mechanisms are apparently overcome, thus resulting in exceptionally high Q_{10} values of TMR from T_a 16 to 20°C.

At T_a values > 0°C, thermoregulatory heat production was absent, as indicated by T_b and TMR, and heat loss was facilitated by significantly increased average C. Values for C that we calculated for torpid arctic ground squirrels without a nest at T_a 0 and 4°C were lower by 64 and 55%, respectively, than predicted values (Snyder and Nestler, 1990). However, Snyder and Nestler (1990) based their prediction on measures of TMR obtained from animals held at T_a of 6°C and that averaged ~200g less than the animals in our study. That animals in this study were at a slightly lower T_a (0 and 4°C) and were significantly larger could account for the lower than predicted observed C.

Metabolic fuels of hibernation— Hibernators reportedly use fat as the exclusive metabolic substrate during torpor (Bibikov, 1989; Davis, 1976; Dark and Ruby, 1993, , Dark and Miller, 1998; Lyman et al., 1982; Snapp and Heller, 1981; South and House, 1967). However, RQ results in this study suggest that the metabolic fuel used by arctic ground squirrels during steady-state torpor depends on T_a. An RQ value of 0.70 indicates exclusive fat catabolism and 1.0 of exclusive carbohydrate catabolism; RQ values between 0.70 and 1.0 can reflect either catabolism of protein or mixed fuel use (Ferrannini, 1988; Kleiber, 1975). The RQ of arctic ground squirrels during torpor significantly correlated to TMR at T_a's < 0°C and to T_b at T_a values > 0°C. Observed shifts in RQ with T_a are not thought to be representative of transitory changes in respiratory exchange because respirometry was done on animals in steady-state conditions (constant T_b and TMR) on the fourth day of a torpor bout and values represent means obtained over a 1 h interval. Changes in RQ may be reflective of retention or release of CO_2 rather than shifts in substrate use. Resulting changes in relative acidosis of blood have been linked to metabolic inhibition during hibernation, but these changes are transitory (Krilowitz, 1985; Nestler, 1989; Malan, 1986) Changes in CO_2 flux occur over short time intervals (minutes) during entry into or arousal from torpor and are therefore unlikely to have contributed to the differences in RQ reported here. At T_a's of 4 and 8°C RQ was ~0.70 and TMR was minimal. At T_a's either $>$ 8°C or $<$ 4°C, RQ was shifted upward to maxima of 0.88 at T_a of 20°C and 0.86 at T_a of -16°C.

Why do metabolic substrates shift from primarily lipids at low and high T_a's? At subzero T_a's an increase in glucose use in arctic ground squirrels may be related to increases in TMR supporting thermogenesis (Zimmerman, 1982). Elevated activity of heart muscle, erythrocytes, and brain (all glucose-utilizing tissues) may be associated with higher rates of metabolism at lower T_a's, and elevated non-shivering thermogenesis in brown adipose (and other) tissues may require increased catabolism of carbohydrates (Gibbons *et al.*, 1985; Cooney *et al.*, 1986). Increased glucose uptake in thermogenic brown adipose tissue (BAT) is required to supply oxaloacetate (via conversion to pyruvate) during citric acid cycle oxidation (Cannon et al., 1978), and glucose can act as a substrate for lipogenesis in BAT and the liver (Himms-Hagen, 1990; Saggerson *et al.*, 1988). Use of glucose for lipogenesis in fasting ground squirrels would spare white adipose tissue stores at the expense of catabolising lean tissue for gluconeogenesis. A requirement for protein-derived carbohydrate use during hibernation is indicated by 30-40% losses in lean body mass observed over the hibernation season in arctic ground squirrels investigated in the laboratory (Galster and Morrison, 1975) and the field (Buck and Barnes, in review), and progressive decreases in plasma and liver carbohydrate stores (to 50%) during torpor bouts which are replenished during arousal episodes (Galster and Morrison, 1970, 1975). Glycerol release from the metabolism of triacylglycerols is estimated to be insufficient to provide for glucose requirements over a torpor bout in arctic ground squirrels, when they are at low metabolic rates (Zimmerman, 1982). Galster and Morrison (1975) estimate that one-third of the glucose used during torpor is replaced via gluconeogenesis from amino acids, and Zimmerman (1982) estimates that TMR of arctic ground squirrels would have to reach 0.2 ml O2/g/h with only fat oxidation in order to prevent depletion of glycogen via gluconeogenesis from glycerol. Our results indicate that TMR this high would be reached at T_a of approximately -20°C (Fig 1). Ambient temperatures this low do occur in the soils where hibernacula of arctic ground squirrels are located, but they are not sustained for long intervals (Buck and Barnes, submitted for publication), suggesting an additional need for gluconeogenesis. Nonetheless, higher rates of glycerol release and its combustion as carbohydrate may contribute to the upward shift of RQ as TMR increases at lower T_a's.

These arguments for an increase in RQ at T_a's below 0°C cannot altogether explain its increase at $T_a > 8$°C. At T_a 12°C RQ is significantly higher compared to measurements at T_a 4 to 8°C, without a significant increase in TMR and likely no thermogenesis. Overall increases in RQ and TMR at $T_a > 0$°C are not proportionate to their changes at $T_a < 0$°C. At $T_a > 0$°C RQ is most directly related to changes in T_b, suggesting a changing relationship of use of metabolic fuels as tissue temperatures rise.

Torpor bout length--. Our results suggest that impetus for the periodic arousal to euthermy in the arctic ground squirrel may vary as a function of T_a. Of the parameters we measured (TMR, T_b, T_a, RQ), RQ was the only one significantly correlated to TBL from T_a -16 to 20°C, suggesting that shifts in metabolic fuel use have physiological consequences for the hibernating arctic ground squirrel. Animals held at T_a values associated with more rapid glucose utilization, as indicated by higher RQ, needed to arouse most frequently (Fig. 6). These results support Galster and Morrison's (1975) hypothesis that arctic ground squirrels become glucose-limited during torpor and must arouse in order to replenish carbohydrate stores via gluconeogenesis. Although shifts in RQ and TBL are of equal magnitude at T_a's both above and below 0°C, absolute rates of catabolism of carbohydrate differ substantially between the two temperature ranges because TMR is significantly lower at T_a's > 0°C. For example, at T_a -16 and 20°C TBL did not differ statistically, yet the rate of catabolism of carbohydrate was 3.6-fold higher at T_a -16°C (Kleiber, 1975). That arctic ground squirrels having RQ's of 0.70 still aroused each 16 days and that arctic ground squirrels held in semi-natural conditions display longer TBL that is not significantly affected by decreased T_a < 0°C (Barnes and Ritter, 1993) suggest TBL may be influenced by multiple factors.

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Table 3-1—T_a, T_b, TMR, and Q_{10} of hibernation.

Ambient temperature (T_a), average core body temperature (T_b), and average maximum torpid metabolic rate (TMR) of arctic ground squirrels during steady-state torpor and corresponding Q_{10} values of TMR between different T_b measurements.

Group	1	2	3	4	5
T_a =	4	8	12	16	20
T_b =	4.66	8.22	12.56	17.11	20.73
TMR = (ml O2/g/h)	0.0115	0.0115	0.0137	0.0179	0.0466

Q_{10} values

Q_{10} (1 - 2) = 1.0	Q_{10} (2 - 3) = 1.5	Q_{10}(3 - 5) = 4.45
Q_{10} (1 - 3) = 1.25	Q_{10} (2 - 4) = 1.64	Q_{10} (4 - 5) = 14.1
Q_{10} (1 - 4) = 1.43	Q_{10} (2 - 5) = 3.06	
Q_{10} (1 - 5) = 2.4	Q_{10} (3 - 4) = 1.8	

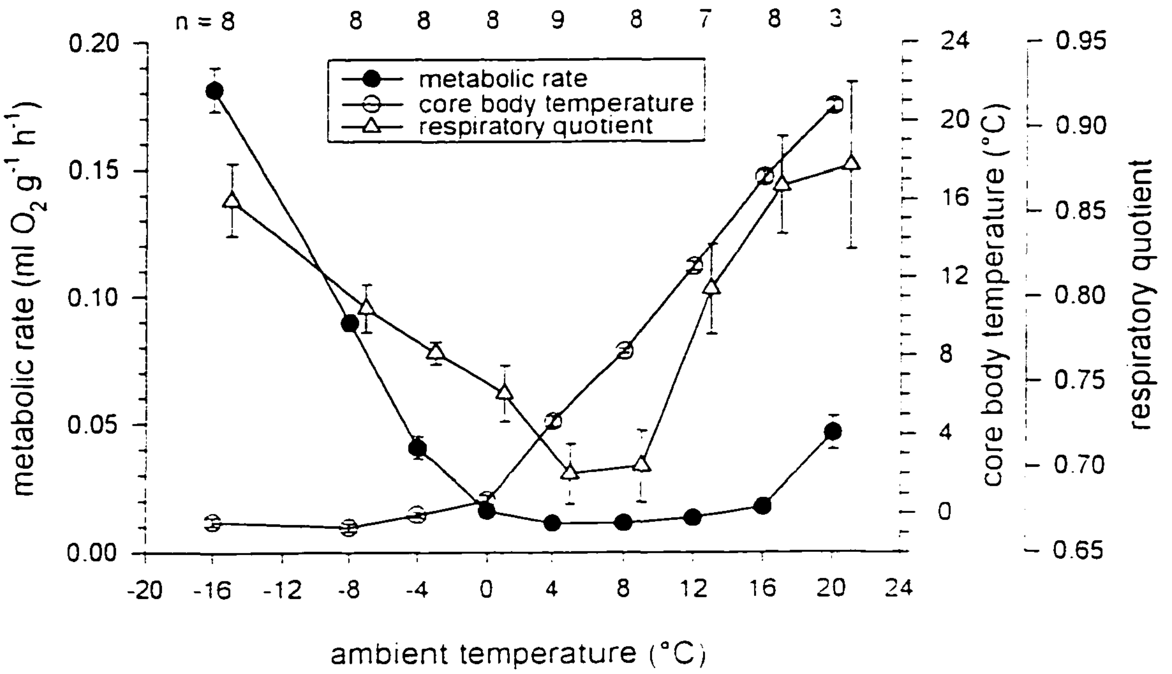

Figure 3.1—Ambient temperature, metabolic rate, core body temperature, and respiratory quotient during torpor.

Ambient temperature (T_a), torpid metabolic rate (TMR), core body temperature (T_b), and respiratory quotient (RQ) of arctic ground squirrels during steady-state torpor. Data are presented as means ± *SE*. Sample sizes are included in figure.

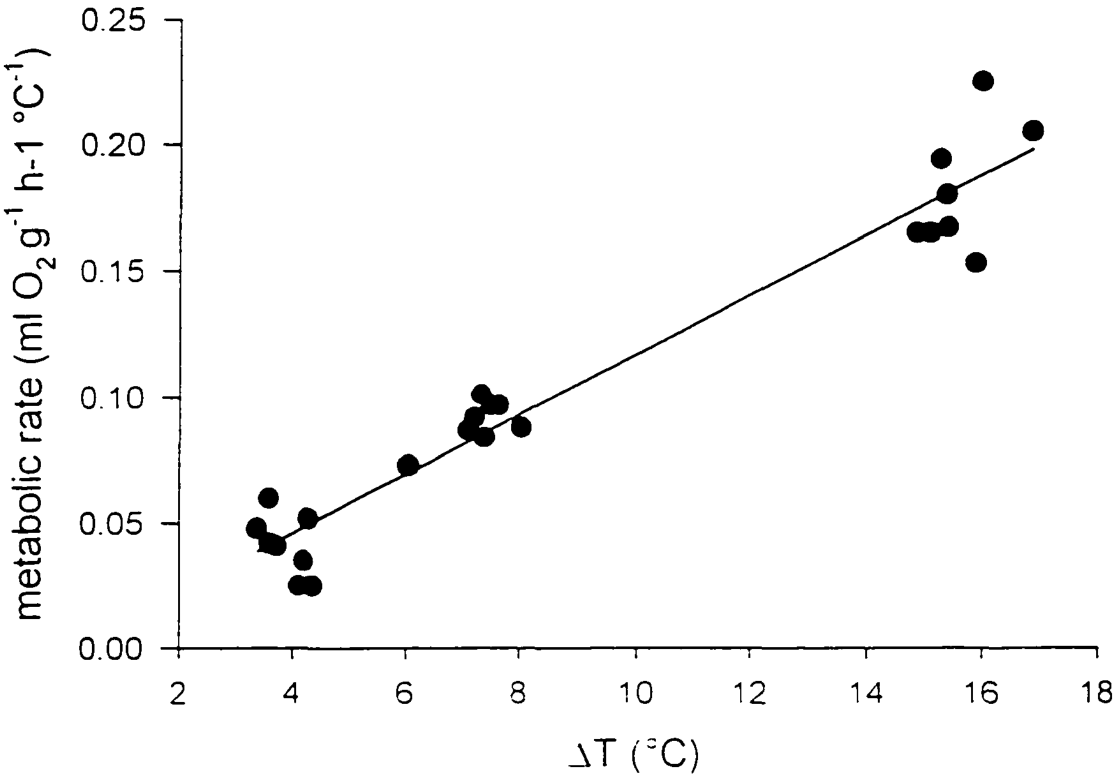

Figure 3.2 —ΔT versus TMR at T_a's < 0°C.

Relationship of gradient between core body temperature and ambient temperature (ΔT) and torpid metabolic rate (TMR) of arctic ground squirrels during steady-state torpor T_a's < 0°C.

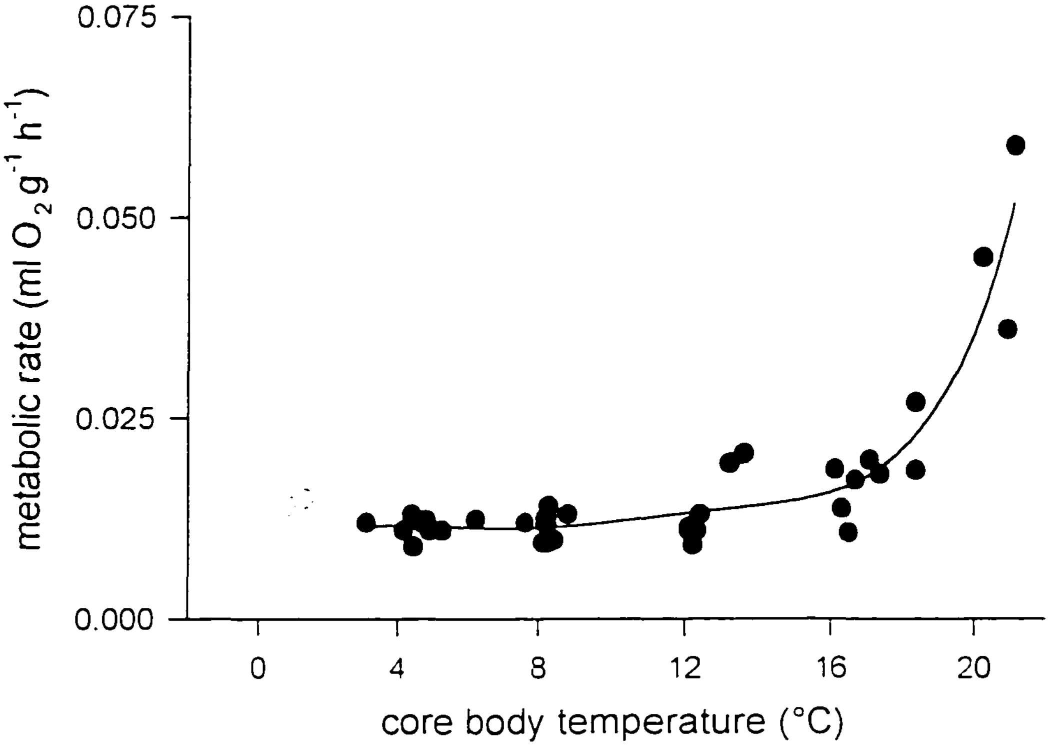

Figure 3.3—Tb versus TMR at $T_a < 0°C$

Core body temperature (T_b) and torpid metabolic rate (TMR) of arctic ground squirrels during steady-state torpor at ambient temperatures (T_a) $\leq$ 0°C. The exponential fit of the regression was significant ($r^2 = 0.56$, $P < 0.001$). Open circles represent animals held at 0°C; 3 of 8 of those animals were thermoregulating as indicated by a gradient between core body temperature and ambient temperature (ΔT) greater than 1°C.

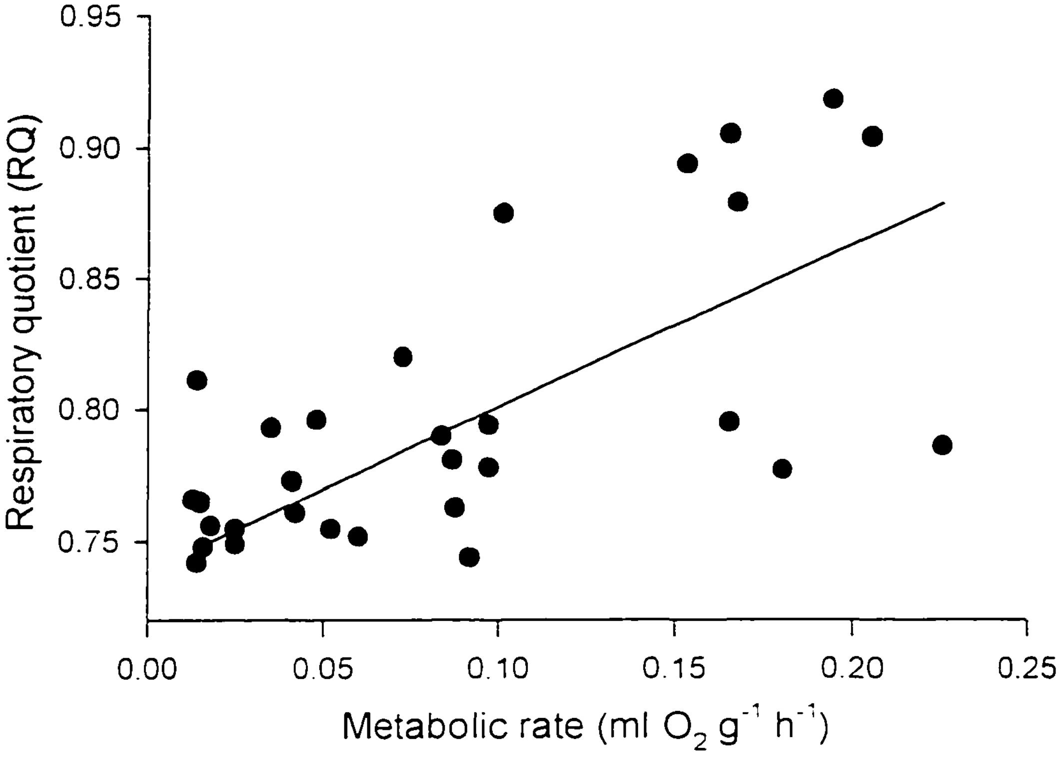

Figure 3.4—RQ versus TMR at T_a < 0°C.

Relationship between torpid metabolic rate (TMR) and respiratory quotient (RQ) of arctic ground squirrels during steady-state torpor at ambient temperatures (T_a) less than 0°C.

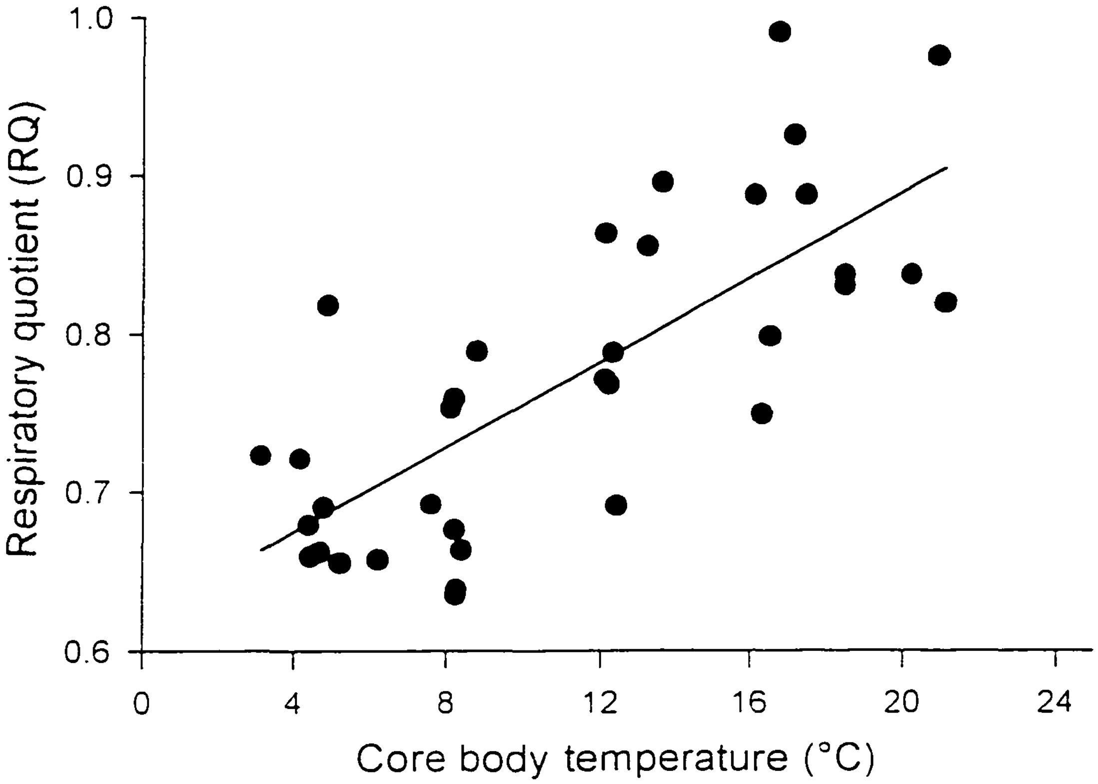

Figure 3.5 —RQ versus T_b at T_a > 0°C.

Relationship between core body temperature (T_b) and respiratory quotient RQ at ambient temperatures above 0°C.

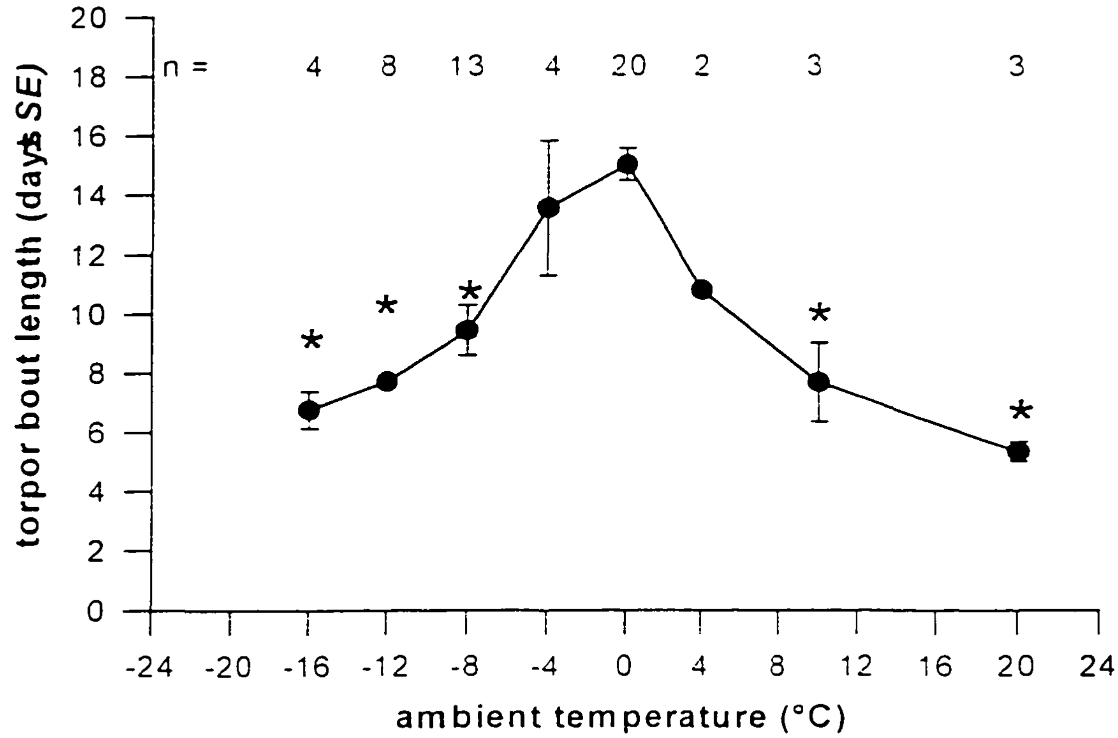

Figure 3.6 —TBL versus T_a.

Mean ± *SE* maximum torpor bout length (TBL) of arctic ground squirrels hibernating at different T_a's. Points that significantly differ from TBL at ambient temperature (T_a) of 0°C are indicated by an asterisk. Sample sizes are included on the figure.

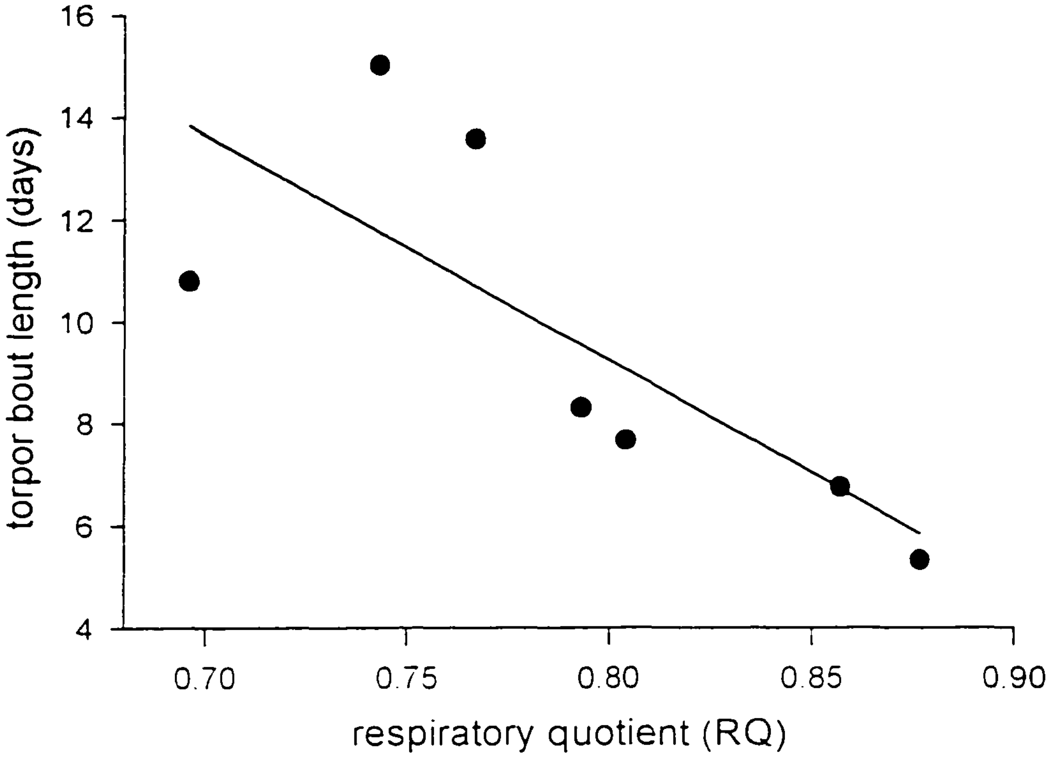

Figure 3.7 —TBL versus RQ.

Relationship between mean torpor bout length (TBL) in days and respiratory quotient (RQ) of the arctic ground squirrel

4. PLASMA ANDROGEN AND CORTICOSTERONE IN FREE-LIVING ARCTIC GROUND SQUIRRELS: INFLUENCE OF STAGED MALE-MALE ENCOUNTERS

Prepared for submission to *Hormones and Behavior* as "Plasma androgen and corticosterone in free-living arctic ground squirrels: influence of staged male-male encounters" by C. Loren Buck and Brian M. Barnes.

ABSTRACT

In a field study on the North Slope of Alaska we determined plasma levels of androgen and corticosterone for male arctic ground squirrels (*Spermophilus parryii*) after emergence from hibernation in spring, throughout the mating season, and prior to their immergence into hibernation in late summer. In mid-April reproductively active males emerged with testes descended into the scrotum and with intermediate levels of plasma androgen. Androgen levels remained constant at 2.86 ± 0.29 ng/ml for about 1 week before significantly increasing ($P < 0.05$) to the active season maximum of 4.60 ± 0.42 ng/ml. A lesser post breeding peak in late summer of 2.65 ± 0.17 ng/ml was observed before levels decreased to the annual minimum of 1.74 ± 0.57 ng/ml prior to entrance to hibernation. Corticosterone levels did not vary significantly across season and averaged 4.31 ± 0.10 ng/ml. We tested the responsiveness of plasma androgen and corticosterone to challenges by conspecific males by staging dyadic aggressive encounters between males and comparing plasma levels of androgen and corticosterone of these animals to unmanipulated animals. The effect of staged encounters on androgen level depended on time of year. Androgen levels in spring increased after a staged encounter ($P = 0.046$) and were positively correlated to the relative intensity of aggression during the encounter ($r^2 = 0.24$, $P = 0.002$), whereas staged encounters in late summer had no significant effect on androgen level ($P > 0.05$). Corticosterone levels in both spring and late summer were significantly increased by staged encounters ($P = 0.001$) and were positively correlated to

the relative intensity of the encounter ($r^2 = 0.12$, $P = 0.02$). The manipulations with conspecific males during peak mating support the challenge hypothesis (Wingfield, Hegner, Dufty, and Ball, 1990) for this polygynous mammal, but response of androgen secretion to male encounters varied over the rest of the season.

INTRODUCTION

Ground squirrels (*Spermophilus*) display endogenous circannual rhythms of body condition, hibernation, and reproductive function, including plasma levels of reproductive steroid hormones and gonadotropins (Barnes, Kretzmann, Zucker, and Licht, 1986, Licht, Zucker, Hubbard, and Boshes, 1982; Kenagy, 1980, 1986; Heller and Poulson, 1970). Kenagy (1986) described two major effects of environmental information on the expression of circannual rhythms in ground squirrels The first is to entrain the period length to one year and ensure appropriate phase relationships between physiological and behavioral cycles and events in the annual environmental schedule (e.g., to time hibernation to occur in winter, reproduction to occur in spring). Second, environmental information, including the animal's social environment, can act on a shorter time scale to modulate the phasing and magnitude of the physiological change, thereby retaining a responsiveness to short-term variation in conditions within the context of the long-term endogenous rhythm. Such fine-tuning by environmental conditions is thought to precisely define when seasonal events such as arrival at breeding grounds, mating, shifts from territorial behavior to parental care, or fattening for migration or hibernation occur within the annual cycles of animals (Barnes and York, 1989; Wingfield, 1980)

Among environmental cues that fine-tune reproductive hormone levels are stimuli that modulate plasma androgen above the concentrations necessary to support gonadal maturation. In some bird species these include aggressive interactions among conspecific males and response to sexual soliciting behaviors of conspecific females (Moore, 1982, 1983; Wingfield, Ball, Dufty, Hegner, and Ramenofsky, 1987, Wingfield, 1988). Once androgen levels are increased, the intensity and frequency of the behavior are promoted, thereby establishing a positive feedback loop enabling the increase of androgen concentrations to maximum physiological levels which are maintained until the stimulation is removed (Wingfield, et al., 1990). Wingfield et al., (1990) hypothesized that this responsiveness of circulating androgen levels in males to territorial or aggressive challenges by conspecific males depends on the mating system of the species Monogamous males that provide parental care are predicted to be more responsive in change of androgen levels than polygynous males that maintain maximum physiological levels of androgen throughout the mating season

Agonistic interactions between males can also increase circulating glucocorticoid hormone levels as part of the generalized stress response (Sapolsky, 1992), which in turn can suppress reproductive function by either reducing secretion of androgen or the endocrine sensitivity at target tissues (Sapolsky, 1985, Knapp and Moore, 1997, Wingfield, Newman, Hunt, and Farner, 1982, Wingfield, O'Reilly, and Astheimer, 1995). Such a reciprocal relationship between corticosterone and androgen may mediate stress-induced changes in reproductive behaviors (Moore, Thompson, and Marler, 1991), although these mechanisms also may be modulated in species according to breeding strategy and latitude (Astheimer, Buttemer, Wingfield, 1995, Wingfield et al., 1995).

Much of the theory and empirical data relating to the fine-tuning of hormone levels and timing of reproduction in seasonally breeding animals comes from investigations in non-mammalian vertebrates. The generality of hypotheses regarding the effects of mating and social systems, breeding strategies, and latitudes on patterns of circulating hormone levels, however, requires much additional information, including field data from mammalian species with easily identifiable behaviors and the experimental ability to manipulate social interactions while frequently sampling blood. Diurnal sciurid rodents offer an attractive system for these types of studies, especially those living in environments where they can be easily viewed, trapped and retrapped and with sharply defined breeding seasons and behaviors.

The arctic ground squirrel (*Spermophilus parryii*) has a single, brief mating season each spring confined to the two-weeks over which females emerge from hibernation (Buck and Barnes, in review). Arctic ground squirrels are polygynous, territorial, and show high levels of aggression among males (Carl, 1971, Hock, 1966). There are two seasons of overt male-male aggression in arctic ground squirrels. The first occurs shortly after emergence from hibernation in spring and is associated with acquisition and guarding of mates, when agonistic interactions between males are severe, leading to frequent wounding and, in one documented circumstance, death of an antagonist (Carl, 1971; Barnes, Benassi, and Buck, unpublished obs). A second period of intense male-male aggression occurs in late summer and extends until immergence into hibernation. This aggression occurs in the absence of females and appears to be associated with defense of burrow sites (hibernacula) in which males cache food for use the subsequent spring (McLean and Towns, 1981; Buck and Barnes, in review). The intensity of fighting and the appearance of wounds in males during this pre-immergence period can exceed those seen during the spring mating season (Carl, 1971). In a field study of arctic ground squirrels in the northern foothills of the Brooks Range, Barnes (1996) showed two peaks in circulating androgen levels in males, one in spring and one in late summer, that coincide with these periods of heightened male-male aggression. During both periods individual variation in plasma androgen levels was high, and high levels of circulating androgen continued to occur in individual males trapped throughout the active season until just before immergence into hibernation

In the present study seasonal changes in circulating levels of androgen and corticosterone were measured frequently in male free-living arctic ground squirrels during the two periods of male-male aggression: early spring and late summer To test the effect of challenges by conspecific males on circulating concentrations of both hormones, we staged aggressive encounters between males, scored the encounters for relative aggression, and examined the effect of the interactions on levels of both hormones.

METHODS

This study was conducted along the shores of Toolik Lake (68° 38' N, 149° 38' W, elevation 809 m) in the northern foothills of the Brooks Range, Alaska. For details on study area see Buck and Barnes (a,b in review). All procedures used in this study were approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks.

Animal handling.—Free ranging arctic ground squirrels were live-trapped with Tomahawk traps (19 X 19 X 51 cm) baited with carrot. Between 40 and 80 traps were set at marked burrow sites along a 4.0 km transect within 1.8 km of the North and East shores of Toolik Lake each morning and examined each 1 to 3 h until they were closed in late evening. For this study we trapped from 9 April to 10 May and 22 August to 22 September in 1994, and 9 April to 12 May in 1995. Most animals were trapped twice weekly once they had emerged from hibernation. Captured animals were transferred to the Toolik Field Station of the University of Alaska Fairbanks where they were exposed to 3 - 5 minutes of methoxyflurane, sampled for 0.5 - 2.0 ml of blood via cardiac puncture with a heparinized 25 gauge needle on a 3 cc syringe, and assessed for reproductive condition. Additionally, animals were weighed with a Pesola spring scale to the nearest 5 g and analyzed for body condition with the total body electrical conductance method for other studies (Buck and Barnes, in review). Whole blood samples were centrifuged at 2000 rpm for 10 minutes within 8 h of being drawn. Plasma was separated from cells using Pasteur pipette and stored at -70°C until assayed within the year. Details on animal handling techniques appear in Buck and Barnes (a,b, in review).

Time in trap.—Because of the nature of capturing animals in the field, the duration that animals were in a trap before being sampled for blood varied from 1 to 4 hours. To investigate the influence on circulating hormone concentrations of how long an animal remained in its trap before blood was sampled, animals were observed entering traps and varying lengths of time allowed to lapse (range 15 minutes to 4.5 hours) before the animal was collected, anesthetized and sampled for blood. This was done in spring (5-11 May) and summer (7-9 August). There were no significant correlations between time in trap and plasma androgen or corticosterone levels either in spring or summer or overall (linear regression, $P > 0.05$; androgen $n = 15$; corticosterone $n = 36$).

Timing of emergence.—Timing of emergence from hibernation was estimated for individual animals in spring 1994. Emergence of resident males was estimated by the first appearance of an exit hole in the snow and subsequent capture of the individual. In most instances observance of the exit hole and capture of the occupant occurred on the same day, and all males were trapped within 2 days of emergence. Female emergence was estimated similarly unless the emergence hole could not be identified. In these situations, the observation of dandruff on the animal when captured indicated recent (24 - 48 h) end to heterothermy (Barnes and Ritter, 1993).

Classes of male ground squirrels.—In this study we recognize three classes of male arctic ground squirrels in spring of 1994: Resident, Non-resident, and Non-reproductive. Resident males emerged within the area that we trapped and were reproductively mature (testes descended into the scrotal sac). Non-resident males were reproductively mature males that emerged from someplace outside of the trapping area and immigrated to the study area and were subsequently captured. Presumably the dates of emergence of these males were similar to those of resident males. Non-reproductive males emerged within the study area but failed to attain reproductive maturity as indicated by a non-evident scrotum. No distinction among groups of males was made for late summer because all animals are non-reproductive at that time of year and delineation between resident and non-resident status can be made since almost all males are dispersing at that time of year (pers. obs.).

Radioimmunoassay.—Plasma samples were measured for concentrations of corticosterone and total androgen by radioimmunoassay (RIA). Plasma samples (100 µl for androgen and 75 µl for corticosterone) stored at -70°C were thawed and vortexed. To estimate hormone recovery after extraction, approximately 4000 cpm of ^{3}HT-labeled androgen or corticosterone (NEN, Boston, MA) were added to each sample and left at room temperature for two hours. Five ml of newly distilled dichloromethane were added to each sample, and extraction tubes were vortexed for 15 s and allowed to sit for 2 h for separation of the organic and aqueous phases. The organic phase was drawn off, placed in a warm water bath (38°C), and dried with stream-filtered N_2 gas. Dried samples were resuspended in warm, phosphate buffered saline with gelatin by vortexing for 15 s and shaking for 2 h at room temperature before being transferred to and held covered for 16 h at 4°C. Following resuspension, an aliquot of each sample was collected for analysis of recovery efficiency. Duplicates of samples were made by dividing suspension volumes in half. An androgen antibody solution (diluted 1 : 20,000; provided courtesy of Dr. Niswender, Ft. Collins) or corticosterone antibody solution (diluted X : X, from where) was added to each sample tube and tubes for standard curves except for total counts and background tubes, and approximately 18,000 cpm of either labeled androgen or corticosterone was added to all tubes. Tubes were vortexed and incubated for 16 h at 4°C prior to the addition of dextran-coated charcoal to all but the total counts tubes. Tubes were shaken, allowed to sit for 10 minutes, and centrifuged at 3000 rpm for 10 minutes, after which the supernatant was decanted into liquid scintillation vials and counted for radioactivity. The androgen antibody has a cross reactivity of 69% with dihydrotestosterone. Intraassay and interassay variation, determined from repeated measures of high and low plasma pool concentrations of hormones, was 6.3% and 13.1%, respectively for androgen, and 5.1% and 14.5%, respectively, for corticosterone.

Staged encounters.—Ground squirrels in this portion of the study were either in a cage or free within their habitat. Caged animals were captured away from the range of the target male less than 3 hours prior to the staged encounter and held within a Tomahawk trap (19 X 19 X 51cm) until they were brought and within 10 m of the free male. The Tomahawk trap is large enough to not restrict the movement of the caged male and does not obstruct visibility for both caged and free males.

The response of the free male to the caged male was categorized from 0 to 3 depending on the relative intensity of the encounter as determined by the types of male-typical behaviors displayed. A score of 0 indicates that the free male neither approached the cage and postured nor left the site when presented with the caged male. A score of 1 indicates that the free male approached cage and displayed an aggressive posture when presented with the caged male but did not physically contact the cage at any point. A score of 2 indicates the free male aggressively contacted the cage but displayed a limited number of aggressive postures. And finally, a score of 3 indicates the free male displayed the full suite of male-typical aggressive behaviors. Aggressive encounters were allowed to continue for 30 minutes and were terminated by capture of the free male with an insect net. Blood was drawn by cardiac puncture from both the caged and free males within 10 minutes of the staged encounter. In none of the staged encounters were either the caged or free animals injured.

Statistical analyses. Data are expressed as means ± standard errors (*SE*). To test for significant differences in hormone concentrations between spring 1994 and 1995, we used a 2-way analysis of variance (ANOVA). Peaks in hormone concentration were analyzed with repeated measures ANOVA's and differences were compared with Tukey post hoc tests. Non-normally distributed data were analyzed with a Kruskal-Wallis ANOVA on ranks and post hoc analyses performed with Dunn's method. For between group comparisons we used a Student's *t*-test if the data were normally distributed and a Mann-Whitney rank sum test for non-normally distributed data. To determine associations between hormone concentration and the time interval that animals were held in traps, between hormone level and the relative intensities of the encounters, and between androgen and corticosterone levels, we used a simple linear regression. Differences were considered significant at $P < 0.05$.

RESULTS

Seasonal changes in hormone levels in the field.—Plasma levels of androgen but not corticosterone varied significantly over the active season in male arctic ground squirrels (for androgen, Kruskal-Wallis ANOVA on ranks, $P < 0.001$, Fig. 1a), for corticosterone, (ANOVA, $P = 0.078$; Fig. 1b). At first capture after emergence from hibernation, testes of reproductively active males were enlarged and descended into the scrotum, and plasma androgen concentrations were at intermediate levels. After remaining constant for one week, androgen levels increased to the seasonal maximum during the last week of April (Dunn's method, $P < 0.05$) and declined during the first week of May. Androgen levels showed a diminished secondary peak in August before declining to the active season minimum (Dunn's method, $P < 0.05$) in mid-September, as the last animals immerged into hibernation. Plasma levels of corticosterone showed no significant seasonal changes during the sampling periods, with concentrations in individuals ranging from 1.11 to 9.31 ng/ml.

In non-reproductive males, those that emerged from hibernation with undeveloped testes which remained small and undescended during spring, androgen levels during 1 - 12 May averaged 0.921 ± .015 ng/ml ($N = 7$) and were significantly less than mean levels in reproductive males over the same time interval (3.42 ± 0.017 ng/ml; n = 76, t-test, $P < 0.001$).

To evaluate changes in circulating androgen in reproductive males during spring in the context of their changing social environment, values from April and May 1994 are shown in Fig. 2 overlaying that year's chronologies of emergence from hibernation of resident males and females (animals marked from the previous year and with hibernacula on the field site) and the first appearance of non-resident males (previously untrapped and unmarked). Mean androgen levels remained constant and at intermediate levels in males as they emerged prior to the first emergence of females and appearance of non-resident males. Levels then peaked two weeks later on 1 May coincident with when approximately 75% of females and non-resident males had appeared and been trapped on the study site. The statistical significance of the 1 May peak in androgen levels was established by comparing serial measurements made on 5 individual resident males sampled in three periods: 18-20 April, 21 April - 1 May and 2-9 May (repeated measures ANOVA, period effect: $P = 0.02$). Although plasma androgen levels charged significantly with date, there was no significant correlation between plasma androgen concentration and the number of days individual males had been active above ground since emerging (linear regression, $P > 0.05$; data not shown)

Staged encounters: behavioral responses. –In mid April, prior to the emergence of females, males were usually found in the close vicinity of the opening to their hibernaculum. When these males were approached with a caged male in an attempt to stage an encounter, in all instances (6/6) the free male either left the burrow site or entered the burrow and did not reappear within the test interval (30 - 40 minutes). After the appearance of the first female, males began to be found in association with females, typically at the burrow site of the female. When an encounter was attempted with these males, they responded by approaching the caged male in 29 of 33 instances. If, however, there were no females evident in the immediate vicinity, the free male made no attempt to encounter the caged male (18/18). In late summer and autumn males were not in association with females but they responded to staged encounters at their burrow site with approaches 20 of 21 instances.

In both spring and late summer the responses of the free male to an encounter were predictable in their occurrence, involving a suite of male-typical behaviors. Based on the response of the free male to the caged male, staged encounters were categorized in four levels (0 to 3) by the authors in order of increasing intensity. Because occurrence of specific behaviors were sequential, an encounter that received a score of 3 reflects all of the behaviors of the lower scores in addition to progressively more agonistic behaviors.

A staged encounter was scored 0 if the free male did not depart but neither approached nor postured (see below) when the caged male was placed nearby (< 10 m). The free male generally remained in one place in an alert, upright posture apparently attentive to the caged male. The caged male in these circumstances remained stationary and low to the ground.

We assigned a score of 1 to the encounter when the free male approached and displayed male typical postures. Typically, within 1 minute of placing the caged male on the tundra, the free male approached to investigate the cage. Before reaching the cage, he stopped, sat back on his haunches and began grooming about the head and neck. Grooming began with fore paws pulling from the ears forward across his cheeks and then changed to using fore paws in a spreading motion down the back and to the tail. This grooming pattern appears to be related to spreading scent from facial cheek glands. After intense grooming, the head was glossy with secretions. Special attention to grooming was also given to the tail, maximizing pilo erection. The caged male also groomed similarly after the approach of the free male. Once grooming was complete, the free male adopted a stiff-legged walking gate with tail straight and pilo erect while approaching closer to the cage. This behavior was accompanied by high-pitched vocalizations by both the caged and free animals that were only heard during a male-male encounter.

Typically the free male made several circuits around the cage in this posture prior to rubbing his head (cheek glands) along the cage. The encounter was scored as a 2 if the free male contacted the cage. After marking the cage, the free male would place his lateral surface against the cage and push. The caged male would reciprocate by placing and pushing his body against the inside of the cage against the free male. Loud growls from both the caged and free male accompanied the side-to-side behavior. Commonly the cage was moved several meters during the side-to side-pushing.

If after several minutes of pushing and growling the free male turned and faced the caged male and either charged and contacted the cage or jumped on top of the cage, the encounter was scored a 3. The charge resulted in moving or turning the cage off its base. When on the cage the free male generally walked its length several times before jumping off. The caged male reacted by quickly turning over on his back, with paws out-stretched, growling and teeth-grinding. Following either the charge or climbing on top of the cage, the animals resumed side-to-side-pushing alternating by charges and jumping on top of the cage. If unfrozen soil was available, in some instances the free male would push dirt with his fore paws at the caged male The relative intensities of the staged encounters in spring (N = 18) and late summer (N = 36) did not differ significantly (Mann Whitney rank sum test, P = 0.792)

Staged encounters in both spring and autumn were terminated after 30 min. when the free male was captured with an insect net. Both animals were then anesthetized in the field with Metophane and sampled for blood (1 ml) within 10 minutes of the end of the encounter. Plasma samples were analyzed for androgen and corticosterone

Staged encounters: endocrine responses. To investigate the effect of staged encounters between conspecific males on their circulating hormone concentrations, we compared androgen and corticosterone levels in plasma sampled from males after they had engaged in encounters staged during three time intervals spanning the reproductive season (18 - 20 April, N = 9; 21 - 30 April, n = 14; 1 - 9 May, N = 13) with levels in plasma from 8 other male arctic ground squirrels trapped and sampled serially on the same dates but in the absence of staged encounters In a 2-way ANOVA with date and encounter as factors, both date (P = 0.005) and encounter (P = 0.046) had a significant effect on androgen levels. Although plasma androgen levels in all males increased to a significant peak in late April, plasma concentrations were significantly higher in males involved in an experimental encounter compared to those that were not (Tukey Test, $P < 0.05$; Fig. 3a) This effect was due to a responsiveness of circulating androgen levels in males challenged either early (18-20 April) or late (1-9 May) in the reproductive season and not during the middle of mating (21-30 April), when androgen levels were maximally elevated in both experimental and control males. Within animals involved in staged encounters, the free male had significantly higher plasma androgen levels after the encounter compared to the caged male (overall means for free males 5.41 ± 0.55 ng/ml, n = 19 vs. overall means for caged males 4.00 ± 0.31 ng/ml n = 18, t-test $P = 0.033$). Plasma androgen levels following the staged encounter positively correlated to increasing relative intensities of the interactions ($r^2 = 0.24$, P = 0.002; Fig. 4).

In late summer, staged encounters had no significant effect on circulating androgen levels (2 way ANOVA with date and encounter as factors, $P > 0.05$; Fig. 3b), nor did androgen levels differ between caged and free animals (t-test, $P = 0.228$) or correlate significantly with relative intensity levels of aggression ($P = 0.622$).

Since plasma corticosterone level showed no significant changes over the season in males, its responsiveness to challenges between conspecific males was assessed by comparing levels in the same 8 males sampled within 5 days before, 10 minutes following, and within 5 days after staged encounters performed in both spring and late summer. Plasma corticosterone was significantly higher immediately following an encounter compared to the days before and after (repeated measures ANOVA, $P = 0.001$, Fig. 5). Caged and free males did not significantly differ in plasma levels of corticosterone immediately after the encounter (t-test, $P = 0.128$). Plasma corticosterone levels were positively correlated to increasing relative intensities of encounters ($r^2 = 0.12$, $P = 0.020$; Fig. 6).

DISCUSSION

Our results indicate that free-living male arctic ground squirrels modulate circulating androgen and corticosterone concentrations according to season and social stimuli, including agonistic interactions between conspecific males. The intensity of aggressive interactions and its relationship to hormone levels, however, varies with season.

Seasonal changes in hormone levels: Androgen.--Plasma levels of androgen in male arctic ground squirrels significantly changed over the active season and followed patterns generally similar to those found in previous field studies of ground squirrels, with the major peak associated with reproductive activities in spring and a secondary peak in late summer (Holekamp and Talamantes, 1991, 1992, Barnes, 1996). Plasma androgen was at intermediate levels when arctic ground squirrel males were first sampled in mid-April within 1-2 days of emerging from hibernation. Mean levels then rose to a peak on 1 May, coinciding with the midpoint of when newly emerged females were displaying morphological estrus and when matings were occurring (Barnes, 1996, Buck and Barnes, in review). This is similar to the pattern in golden-mantled ground squirrels (*S. saturatus*) in the Cascade Mountains (Barnes, 1996) but differs from that in California ground squirrels (*S. beecheyi*) for which Holekamp and Talamantes (1991, 1992) describe plasma androgen levels rising to a peak and then declining before the onset of matings. Holekamp and Talamantes (1992) attribute the pattern they observe to a hypothesized importance for direct stimulation by androgen of behaviors related to space use and territoriality in males before females appear and to a temporal lag between plasma levels of androgen and effects on agonistic and sexual behaviors In contrast, agonistic interactions, breeding behaviors, and peak levels of circulating androgen happen simultaneously in male arctic ground squirrels.

In late summer average plasma androgen levels were elevated to levels that were 57% of the peak levels in spring, before decreasing to the active season minimum just before immergence of males into hibernation in mid September. These results are similar to previous results with the same population; although peak levels in late summer reported then (Barnes, 1996) represented the active season maximum and were nearly 2-fold greater than the levels reported here Elevated plasma androgen in late summer also has been reported for male *S. saturatus* (Barnes et al. 1986) and *S. beecheyi* (Holekamp and Talmantes, 1991). This increase in circulating androgen occurs when testes are aspermatogenic (HoleKamp and Talmantes, 1991; Barnes, 1996) and largely in the absence of females (who immerge earlier into hibernation), but when in arctic ground squirrels conspecific male aggression and fighting can reach seasonal highs (Carl, 1971; Buck and Barnes, submitted for publication). Elevated androgen levels during late summer and fall may facilitate behaviors in males such as dispersal, burrow guarding, caching, and defense of caches and foods (Barnes, 1996; Buck and Barnes, in review; Holekamp and Talmantes, 1992). In ground squirrels androgen stimulates growth and secretory activity of male scent glands (Kivett, 1976). Scent marking, which we observed frequently in males during late summer, is associated with each of these behaviors. Elevated androgen could be supporting a combination of behavioral and physiological functions. Holekamp and Talmantes (1991) associated elevated androgen in *S. beecheyi* males in fall with initiation of penile and testicular development.

Corticosterone.—There were no significant differences in plasma corticosterone across the sampling period. In free-living *S. saturatus* corticosterone levels are lowest in spring, peak in mid-summer, and decrease to low levels again prior immergence into hibernation in late summer (Boswell, Woods, and Kenagy, 1994). Because we did not sample during June and July, we do not know if arctic ground squirrels show a similar peak in plasma corticosterone in mid summer. However, levels that we report for the spring and late summer are lower than for *S. saturatus* over the same time intervals (Boswell et al., 1994).

Fine temporal controls over hormone levels.-- The seasonal pattern of plasma androgen in free-living arctic ground squirrels is similar to the circannual pattern observed in laboratory-held ground squirrels (Licht et al., 1982, Barnes et al., 1986), yet field-caught males vary substantially from one another in plasma levels of androgen within a season. Presumably, the modulation of hormone levels either above or below the endogenously controlled levels are under social environmental influence.

Social environment.—Following their emergence from hibernation in mid-April, resident males had intermediate androgen levels that were associated with early termination of heterothermy, reproductive maturation, and emergence from hibernation

(Barnes Kretzmann, Zucker, and Licht, 1988, Barnes, 1996; Hall, Bartke, and Goldman, 1982) and were sedentary and solitary (Hock, 1966). Although most of the resident males had emerged from hibernation by 21 April, males seldom ventured more than a few meters from the entrance of their hibernaculum and no woundings from fighting were noted until after 21 April, coincident with the emergence of the first females. By 1 May greater than 70% of females and non-resident males had appeared and androgen level of resident males had increased by 69%. All males during this time were in close association with females, and naturally occurring male-male aggression was high. By 3 May all resident males we examined displayed wounding from fights by

Challenge experiments: Spring.—The social stimulation male arctic ground squirrels perceive, specifically male-male aggression, can influence circulating levels of hormones and is supported by the spring-time experiments that simulated a challenge by a conspecific male to territorial and mate-guarding males. Encounters in mid-April and early May resulted in significant rises in circulating levels of androgen in both caged and free males compared to levels in unmanipulated males. A responsiveness of circulating androgen to male-male encounters may explain why in late April males not in staged encounters also had elevated androgen, if they were spontaneously engaging in encounters with other males before being trapped and sampled for blood. Most matings in this population occur in late April (Buck and Barnes, submitted for publication) and all reproductive males we handled then had multiple fresh wounds indicative of recent fights.

Alternatively, all reproductive males may show maximally elevated circulating androgen levels in late April regardless of recent social experiences

Wingfield et al. (1990) hypothesized that, among seasonally breeding vertebrates, the duration that circulating levels of androgen remain maximal in males and the responsiveness of plasma androgen to male-male interactions depends on the breeding system of the species. Polygynous males that provide little or no parental care may maintain high plasma androgen levels longer than do monogamous males that only increase androgen levels in response to acute challenges by conspecific males for territories or for mates. The present data represent a partial test of this hypothesis in small mammals. The result that males in late-April have high levels of plasma androgen and do not respond to staged encounters by increasing androgen still further supports the "challenge hypothesis" (Wingfield et al., 1990) for this polygynous, aggressive and territorial species. However, both before and after the peak breeding period males have only intermediate levels of androgen These levels do respond to challenges suggesting a complex control over circulating androgen even in polygynous species Sustained high androgen levels and/or the energetically expensive and dangerous behaviors that they stimulate may be prohibitively costly for males (Wingfield et al., 1990). The mating season has been shown to be the most energetically expensive time of the year for free-living *S. richardsonii* (Michener and Locklear, 1990) and *S. saturatus* (Kenagy, 1989). Like *S. richardsonii* and *S. saturatus*, male arctic ground squirrels in spring show increased activities associated with mating but little foraging behavior (McLean and

Towns, 1981, Buck and Barnes, submitted for publication) and lose considerable body mass (21%) during the three-week mating season (Buck and Barnes, submitted for publication). That reproductive males continue to lose body and fat mass through early May while non-reproductive males gain mass suggests the activities associated with high plasma androgen have adverse energetic consequences.

Late summer.—Aggressive interactions between males in late summer occurred naturally as evidenced by wounding and were elicited in staged encounters. Unlike in spring, plasma androgen level of animals sampled directly after a staged encounter did not differ from levels of unmanipulated animals. Males in late summer were just as likely to engage in agonistic behaviors as in spring, and agonistic interactions did not differ in intensity from behaviors measured in spring encounters. Although encounters in late summer varied in relative intensities, intensity was not correlated with plasma androgen level. There were no females present at any of the staged encounters in late summer because most of the females had immerged into hibernation prior to 1 September, whereas most of the males were still active on the surface through the middle of September (Buck and Barnes, submitted for publication). Therefore, aggressive interactions between males in late summer were not associated with guarding females from other males, rather they were in defense of burrow sites. Similarly, investigations with several species of birds have shown that androgen is not directly involved in stimulating aggressive interactions in non-breeding males (Wingfield and Marler, 1988), but is involved in aggression associated with reproduction (Dettami and Reyer, 1984; Wingfield and Ramonofsky, 1985).

Corticosterone. -Staged encounters between males had a modulating effect on plasma corticosterone levels in both spring and late summer. Average plasma levels of corticosterone immediately after a staged encounter did not vary between spring and late summer. Levels were significantly higher in encounter animals than in unmanipulated animals. Although we do not know the time course of increase and decrease nor the magnitude of maximum levels, serial plasma samples taken from the same group of animals within 10 minutes after a 30 minute encounter were significantly higher than levels either five days before or five days after the encounter. Moreover, levels five days before and after the encounter did not significantly differ. Plasma corticosterone level of animals after an encounter significantly correlated to the relative intensity of the encounter.

Staged encounters had an effect in spring but not late summer either because androgen levels were responding more to the presence of estrous females than to challenges by conspecific males or that increases in plasma androgen is inhibited in late summer. We are unable to dissociate the effects of females on male androgen concentrations since encounters could only be elicited in the presence of a female. Androgen levels were significantly lower in unmanipulated males on the fringes of the breeding season even though these males were with females suggesting that male-male interactions do indeed influence plasma androgen. Because high plasma androgen is associated with increased activity, body mass loss, and mortality, inhibition of androgen secretion in late summer may be associated with pre-hibernation fattening, food caching, and therefore increased survivorship. Further investigations, including manipulation of hormone levels are necessary to further understand the relationships between androgen, corticosterone and seasonal aggression in male arctic ground squirrels.

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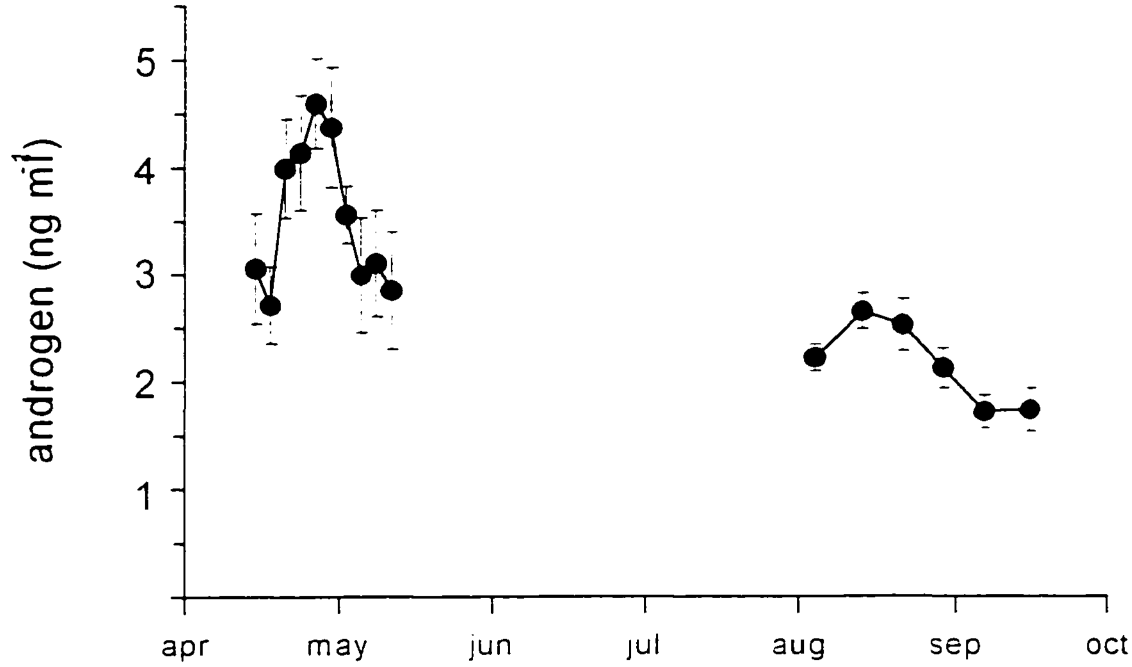


Figure 4-1 —Active season changes in plasma androgen concentration.

Active season changes in plasma concentrations of androgen for male arctic ground squirrels sampled near Toolik Lake, Alaska. Values are means ± *SE*; animal *N*'s for testosterone means range from 5-22, median = 14. Because there were no significant differences between years (2-way ANOVA, year and date interaction *P* = 0.34), data are shown pooled for 1994 and 1995.

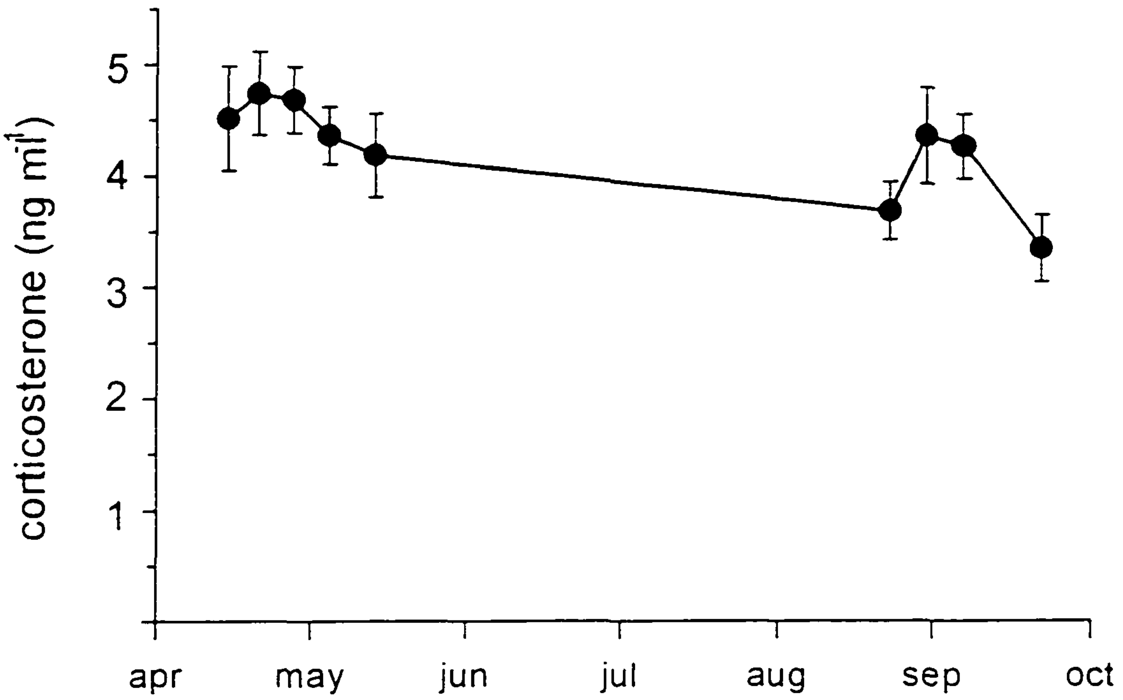


Figure 4-2 —Active season changes in plasma corticosterone concentration.

Active season changes in plasma concentrations of corticosterone for male arctic ground squirrels sampled near Toolik Lake, Alaska. Values are means ± *SE*, animal *N*'s for corticosterone means range from 4-26, median = 15. Since there were no significant differences between years (2-way ANOVA, year and date interaction *P* = 0.34), data are shown pooled for 1994 and 1995.

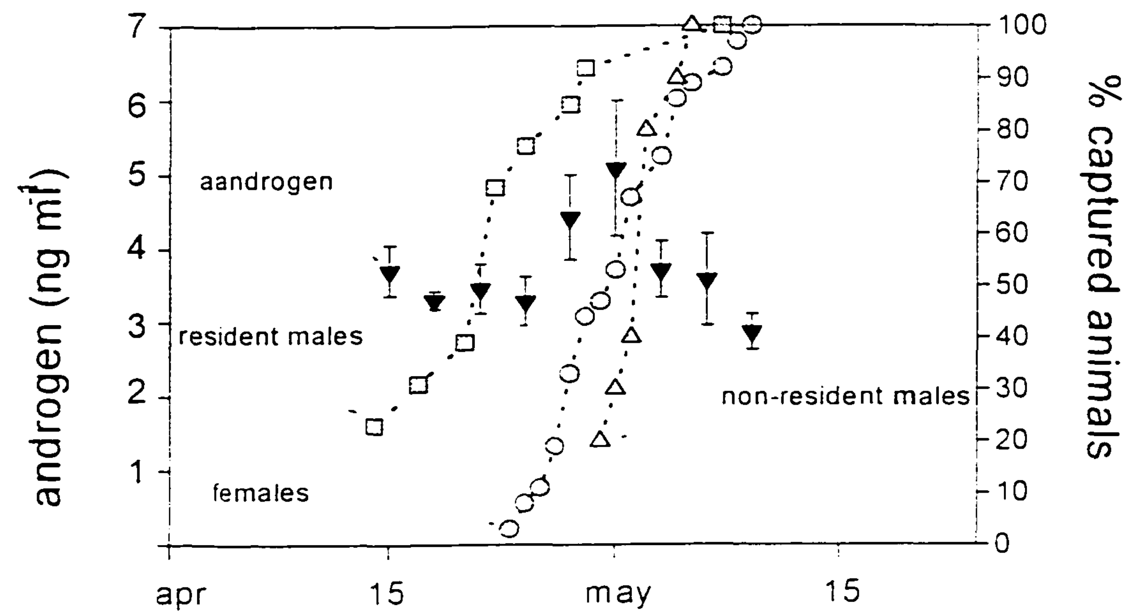


Figure 4-3 —Emergence and appearance chronologies and plasma androgen concentration of resident males.

Chronologies of emergence from hibernation for resident males and females and the first appearance of non-resident males (see text) at the study site in 1994 and change in average plasma androgen levels in resident males. Chronology data are shown as cumulative percent of total animals captured; $N = 10$ for resident males, $N = 26$ for resident females, and $N = 7$ for non-resident males. Number of animals sampled for means of testosterone levels ranged from 3-9 and median = 6.

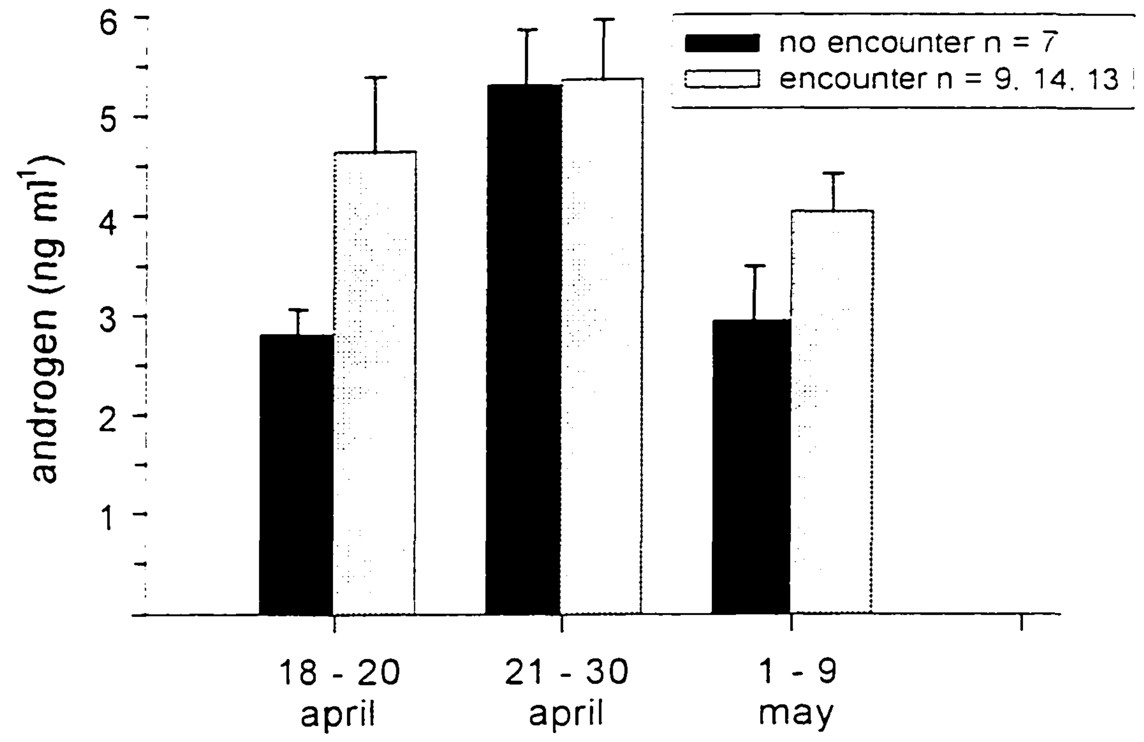


Figure 4-4 —Plasma androgen concentration of encounter and no encounter animals in spring.

Androgen levels in plasma from male arctic ground squirrels sampled during three date intervals spanning the breeding season either immediately following a staged encounter with a conspecific male or in unmanipulated males. Means for males engaged in an encounter combine both the caged and the free male (N = 9 , 14, and 13 for the 3 consecutive intervals); means for unmanipulated males represented repeated measures in 8 individual males. 2-way ANOVA was significant for both encounter (P = 0.005) and date (P = 0.046).

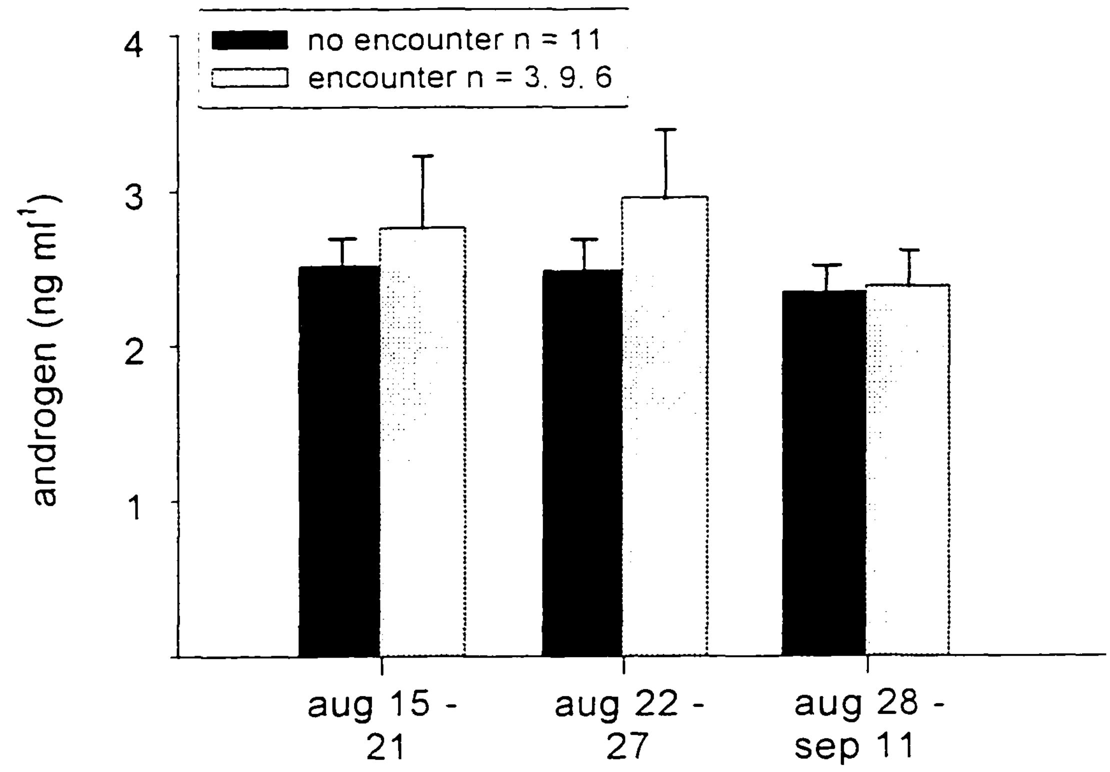


Figure 4-5 —Plasma androgen concentration of encounter and no encounter animals in late summer.

Effect of staged encounters on androgen levels during late summer compared to unmanipulated males sampled over the same date intervals. Means for male engaged in an encounter include both caged and free animals ($N = 3, 9, 6$); means for unmanipulated males represent repeated measures for 11 animals. 2-way ANOVA insignificant with date and encounter as factors.

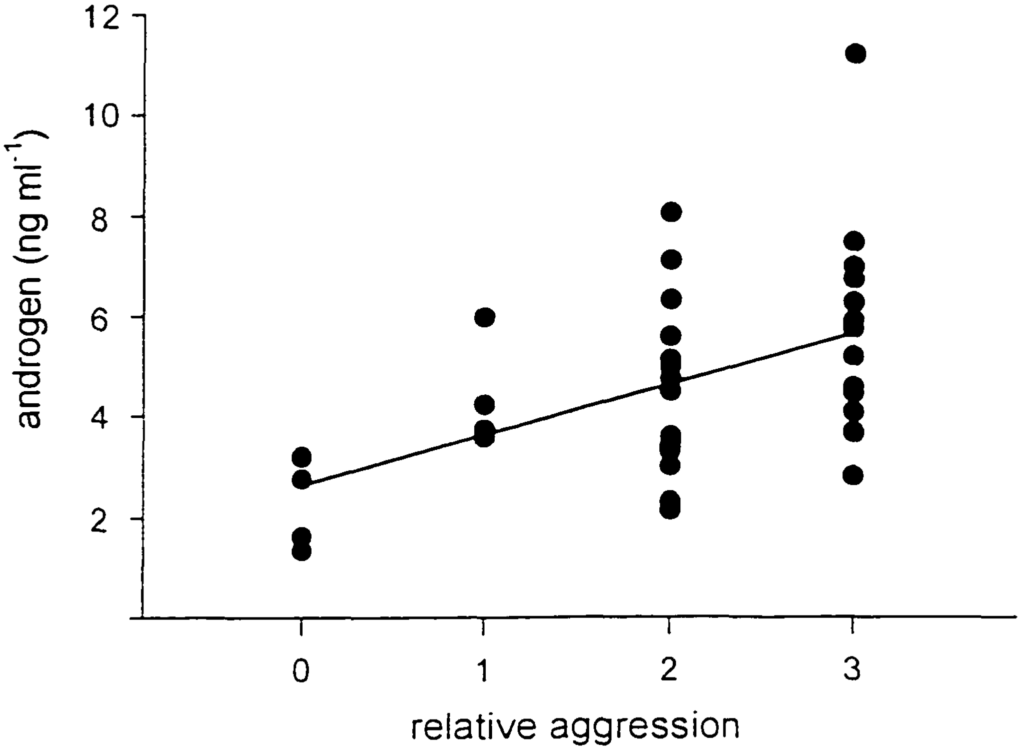


Figure 4-6 —Plasma testosterone versus relative intensity of encounter.

The relationship of relative intensity of the encounter and circulating androgen concentration following a staged encounter between male arctic ground squirrels at Toolik Lake, Alaska in spring 1994 and 1995 ($r^2 = 0.24$, $P = 0.002$). Androgen concentrations are from both caged and free animals sampled within 10 minutes of the encounter

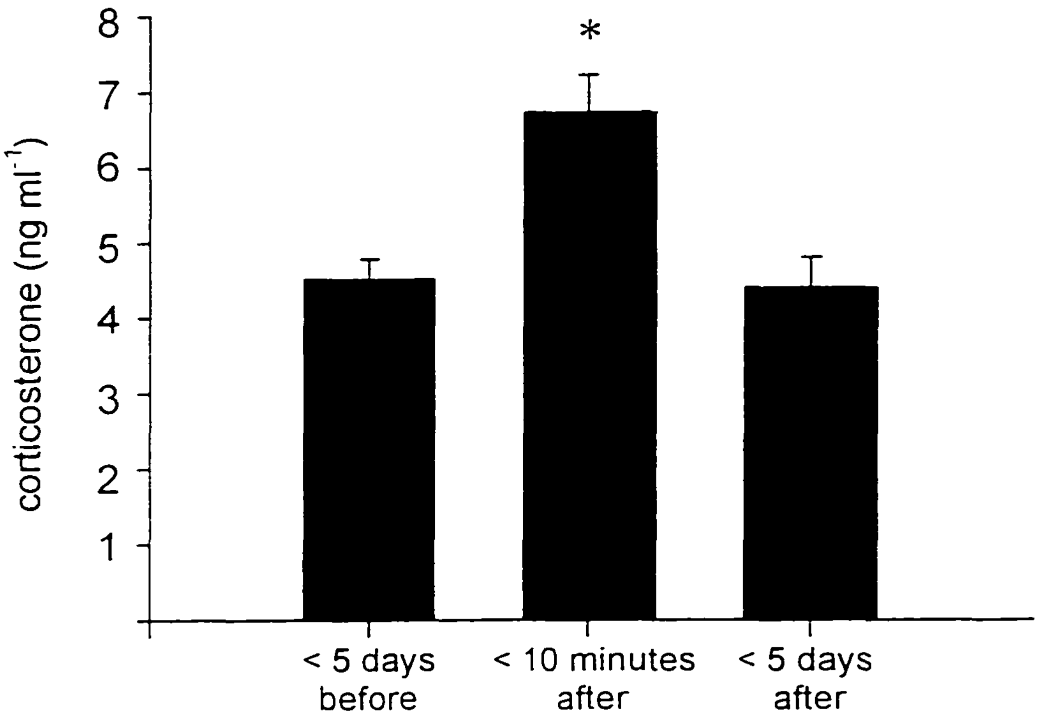


Figure 4-7 —Plasma corticosterone concentration before and after staged encounters.

Plasma concentrations of corticosterone sampled serially from the same 8 male arctic ground squirrels less than 5 days before, within 10 minutes after, and within 5 days after a staged encounter at Toolik Lake, Alaska in late summer 1994. Corticosterone levels of animals 10 minutes after a staged encounter were significantly higher than 5 days before and 5 days after ($P < 0.05$).

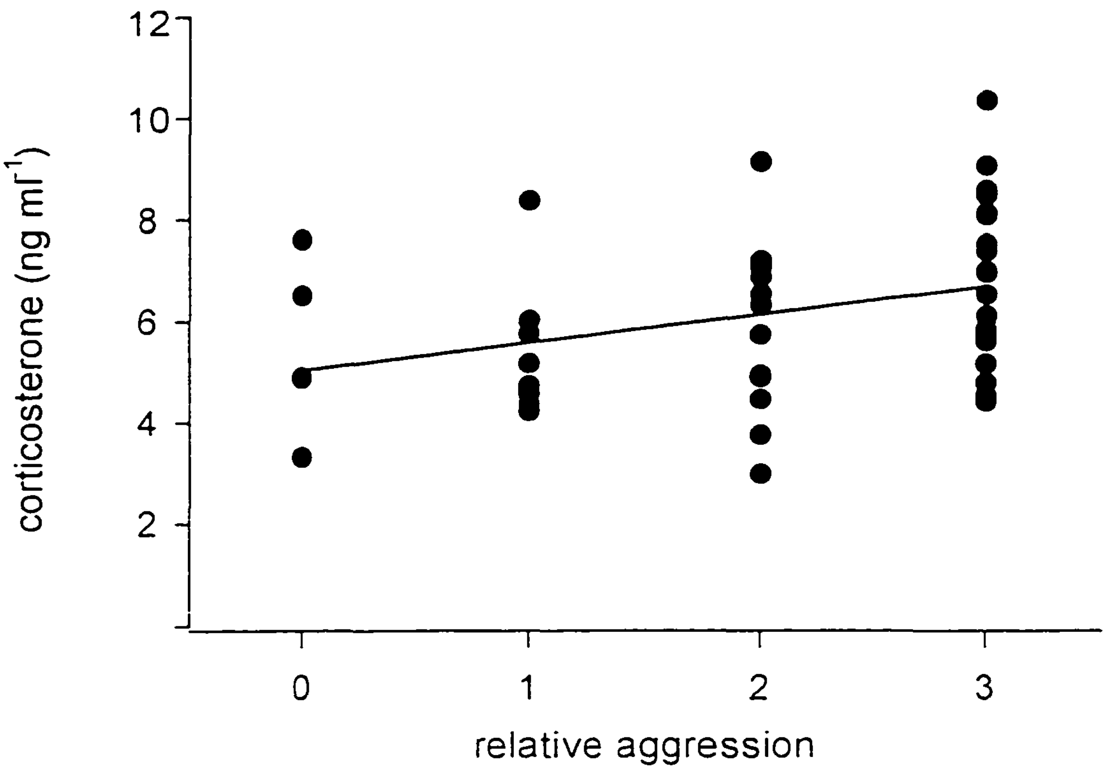
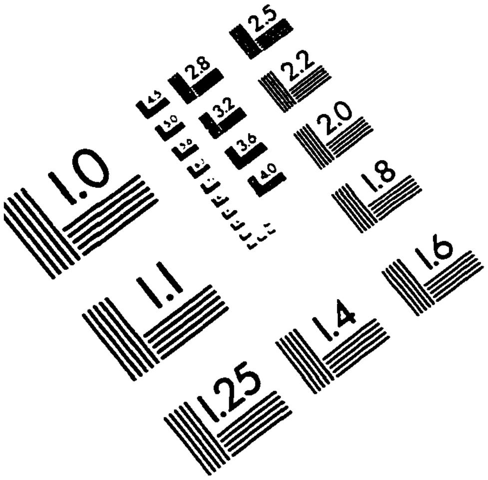
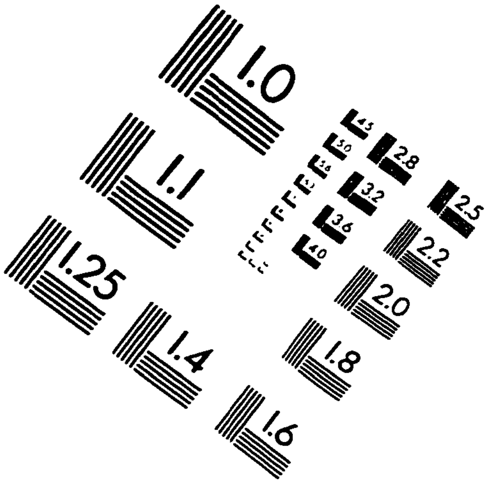


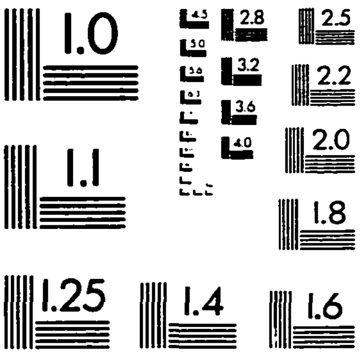
Figure 4-8 —Plasma corticosterone concentration versus relative intensity of staged encounter.

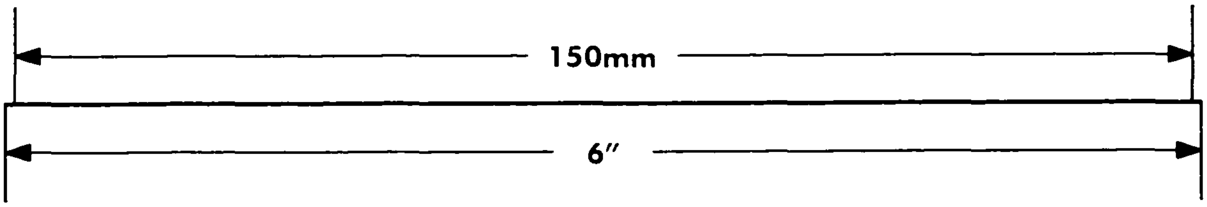
The relationship of relative intensity of the encounter and circulating corticosterone concentration following a staged encounter between male arctic ground squirrels at Toolik Lake, Alaska in spring 1994 and 1995 ($r^2 = 0.12$, $P = 0.020$). Corticosterone concentrations are from both caged and free animals sampled within 10 minutes of the encounter.

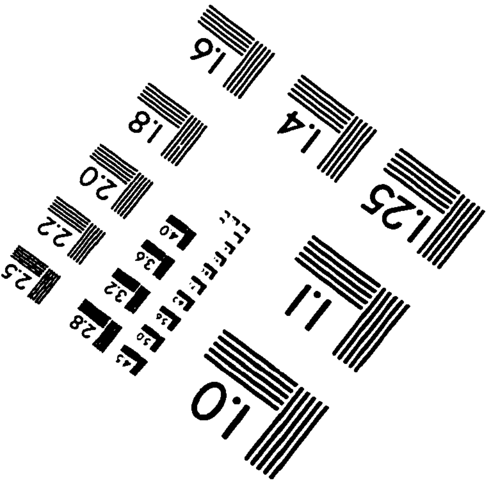
IMAGE EVALUATION TEST TARGET (QA-3)











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