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THE IMPACT OF PREDATOR-INDUCED STRESS ON THE SNOWSHOE HARE CYCLE

RUDY BOONSTRA,[1] DAVID HIK,[1] GRANT R. SINGLETON,[2] AND ALEXANDER TINNIKOV[3]

[1]*Division of Life Sciences, Scarborough Campus, University of Toronto, 1265 Military Trail, Scarborough, Ontario, Canada, M1C 1A4*
[2]*Division of Wildlife and Ecology, Commonwealth Scientific and Industrial Research Organization, P.O. Box 84, Lyneham, Canberra, Australian Capital Territory 2602 Australia*
[3]*Institute of Cytology and Genetics, Novosibirsk 90, 630090 Russia*

Abstract. The sublethal effects of high predation risk on both prey behavior and physiology may have long-term consequences for prey population dynamics. We tested the hypothesis that snowshoe hares during the population decline are chronically stressed because of high predation risk whereas those during the population low are not, and that this has negative effects on both their physiology and demography. Snowshoe hares exhibit 10-yr population cycles; during declines, virtually every hare that dies is killed by a predator. We assessed the physiological responsiveness of the stress axis and of energy mobilization by subjecting hares during the population decline and low to a hormonal-challenge protocol. We monitored the population demography through live-trapping and assessed reproduction through a maternal-cage technique.

During the 1990s' decline in the Yukon, Canada, hares were chronically stressed—as indicated by higher levels of free cortisol, reduced maximum corticosteroid-binding capacity, reduced testosterone response, reduced index of body condition, reduced leucocyte counts, increased overwinter body-mass loss, and increased glucose mobilization, relative to hares during the population low. This evidence is consistent with the explanation that predation risk, not high hare density or poor nutritional condition, accounted for the chronic stress and for the marked deterioration of reproduction during the decline. Reproduction and indices of stress physiology did not improve until predation risk declined. These findings may also account for the lag in recovery of hare reproduction after predator densities have declined and thus may implicate the long-term consequences of predation risk on prey populations beyond the immediate effects of predators on prey behavior and physiology.

Key words: corticosteroid-binding globulin; cortisol; field endocrinology; cycles, population; gluconeogenesis and glucose metabolism; hypothalamic–pituitary–adrenal axis; immunosuppression; predation risk; snowshoe hare cycles; stress, chronic; sublethal effects; Yukon (Canada).

INTRODUCTION

Predators may have direct effects on prey populations by killing individuals and thus influencing prey dynamics (Boutin 1995, Sinclair and Pech 1996). Predators may also have indirect effects by affecting the behavior, reproduction, and foraging patterns of the remaining prey (Lima and Dill 1990, Korpimäki et al. 1994, Ylönen 1994). Snowshoe hare populations (*Lepus americanus*) show regular 9–11 yr cycles in population numbers throughout most of the boreal forest of North America (Elton and Nicholson 1942, Keith 1963, 1990, Krebs et al. 1986*b*) and predation plays a critical role in producing these cycles.

Two major hypotheses have been proposed to explain the role of predators in hare cycles, and both focus on the decline and the low that often follows the decline. The "Keith hypothesis" (1974, 1990) proposes that two sequential factors cause hare cycles: (1) the decline is initiated by winter food shortage at the peak, leading to poor nutrition, and this results in some starvation losses and a reduction in fecundity; and (2) the decline is maintained by predation acting in a delayed density-dependent manner, and this reduces hares to low numbers. Hare populations recover when predators become rare. The "predation hypothesis" (Krebs et al. 1986*b*, Trostel et al. 1987, Sinclair et al. 1988) proposes that predation alone is sufficient to account for the decline. It involves a delayed density-dependent interaction between predators and hares. Predators are proposed to drive the hares to low numbers, and recovery occurs when predators become rare. Evidence indicates: (1) that food shortage occurs in some but not all decline populations, and if it occurs, is restricted to a few months near the peak (Keith et al. 1984, Sinclair et al. 1988, Smith et al. 1988); (2) that predators are the major proximate cause of death during the decline (Keith et al. 1984, Boutin et al. 1986, Trostel et al. 1987, Hik 1995, Krebs et al. 1995); and (3) that addition of food does not prevent declines (Krebs et al. 1986*a*, *b*). Thus, the original Keith hypothesis appears incorrect. However, the pure predation hypothesis also

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appears incorrect for two reasons: (1) simultaneous removal of predators and addition of food resulted in much higher hare densities than either treatment by itself, suggesting a three-trophic-level interaction during the decline (Krebs et al. 1995); and (2) a pure predation hypothesis cannot account for the significant reduction in reproduction during the hare decline nor for lack of immediate recovery of the hare population after the predator populations have declined drastically at the end of the decline (Cary and Keith 1979) nor for the significant reduction in condition that occurs in hares during the decline (Keith 1990).

These two hypotheses have recently been reconciled by the "predation-sensitive foraging hypothesis" of Hik (1995). He argues that hares are able to assess predation risk in different habitats and, during the population decline, they attempt to minimize their probability of getting killed by restricting their activity to areas with dense cover. The correlate of choosing to live in areas of high protective cover is being forced to eat low-quality food, and with it, experiencing declining body condition that then limits reproductive fitness. Thus hares trade off reproduction (which requires high-quality forage prior to and during the breeding season) for survival during population declines.

If predation plays such a dominant role in hare ecology, it could also have major impacts on hare stress physiology, which then may directly affect reproductive fitness. Our study was designed to determine how hares in the decline are affected physiologically by living in a world where the probability of death from predation is extremely high and how this changes when most predators disappear. In vertebrates, the key to responding adaptively to environmental challenges is the *stress response* of the hypothalamic–pituitary–adrenocortical (HPA) feedback system (Fig. 1) (Selye 1946, Lee and McDonald 1985, Sapolsky 1992*a*, Pickering and Fryer 1994, Wingfield 1994). It is the set of "flight or fight" responses used to deal with stressors and then to return the body back to the homeostatic state. It can be called into play by environmental stressors (e.g., Wingfield et al. 1992, Dunlap and Wingfield 1995), by physical stressors such as attacks by a conspecific, or by psychological stressors such as the fear of an imminent attack (e.g., Mason 1968, Levine et al. 1989, Sapolsky 1992*b*). The stress response is designed to deal with acute, not chronic, environmental challenges (Munck et al. 1984). It is essentially catabolic in nature, mobilizing and shunting energy to muscles by stimulation of hepatic gluconeogenesis (the production of glucose by the breakdown of other body tissues—glycogen, fat, and protein) and the inhibition of a variety of body processes not required for short-term survival (Fig. 1). However, when activated chronically, especially by psychological rather than by physical stressors, the sustained stress response is severely deleterious and may affect long-term survival and fitness.

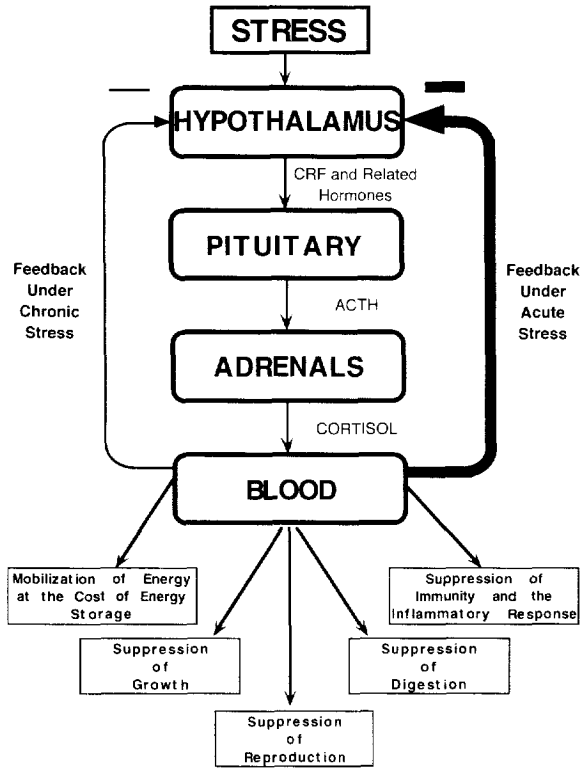

FIG. 1. Schematic representation of the hypothalamic–pituitary–adrenal feedback system response to a stressor, and the main effects on body processes (five boxes at bottom). A stressor causes the hypothalamus to release corticotropin-releasing factor (CRF) and other hormones, which causes the pituitary to release corticotropin (ACTH), and which in turn causes the adrenal cortex to release cortisol. Cortisol feeds back on the hypothalamus and pituitary to cause an inhibition of CRF release. Under conditions where the stressor is acute, the feedback mechanism operates efficiently, and the system rapidly returns to normal, resulting in effects on body processes that are only short term. Under conditions where the stressor is chronic, feedback signals are weak, and the system remains activated for longer periods, resulting in effects on body processes that can be long term and detrimental (Sapolsky 1992*a*).

Continuous exposure to high levels of glucocorticoids (principally cortisol in hares and humans, and corticosterone in voles and mice) can precipitate steroid diabetes, infertility, inhibition of growth, and impaired resistance to disease. This may be precisely the situation that hares find themselves in during the decline, continually trying to avoid being killed by predators, while at the same time having to find food and cover to deal with the severe environmental conditions of the boreal-forest winter.

We tested the hypothesis that hares during the decline are under chronic stress whereas those during the low are not. We used a protocol, a hormonal challenge, which allowed us to get an integrated picture of the animal's recent physiological past while at the same time permitting us to override the immediate stress response the hare was experiencing because of capture

and handling. This hormonal challenge involved two steps: first, the dexamethasone suppression test (Kalin et al. 1981, APA Task Force 1987), and second, the adrenocorticotropic hormone (ACTH) stimulation test. The *dexamethasone suppression test* is a method to assess whether the brain is registering glucocorticoid levels correctly (dexamethasone is an artificial glucocorticoid) and making the necessary negative-feedback adjustment by reducing ACTH and cortisol production. Stress has been found to either diminish (in baboons: Sapolsky 1983) or eliminate (in some Australian breeding male marsupials: McDonald et al. 1986, Bradley 1990) the suppressive effects of dexamethasone. The *ACTH stimulation test* has been used to probe the responsiveness of adrenals directly, and in humans (Miller and Tyrrell 1995), though not in most wild mammals studied (e.g., Sapolsky 1983, McDonald et al. 1986, cf. Harlow et al. 1992), chronic stressors cause high levels of ACTH and produce adrenals that are insensitive to ACTH; the result is that the production of glucocorticoids is diminished. This protocol or modifications of it has been successfully applied in comparative studies on stress in a wide variety of species in both the laboratory and the field (e.g., sparrows: Astheimer et al. 1994, deer: Smith and Bubenik 1990, lizards: Gist and Kaplan 1976, rats: Oitzl et al. 1995, humans: Schmider et al. 1995).

We made the following predictions in comparing decline- and low-phase hares, and predicted that these effects would not be seen in hares that are fed and protected from predators:

1) that chronic stress will cause hypertrophy of the adrenals (Miller and Tyrrell 1995) and thus a greater capacity to produce and to release cortisol when hares are challenged by stressors. Thus both the stress of capture and of the ACTH test should result in greater levels of cortisol in decline than in low hares.

2) that hares during the population decline will exhibit greater dexamethasone resistance (i.e., when dexamethasone is given, endogenous cortisol levels do not fall as rapidly in resistant animals as in normal ones) and an altered cortisol responsiveness to these hormonal challenge tests. In the blood, most cortisol is normally tightly bound to a carrier protein, corticosteroid-binding globulin (CBG). About 5–10% of the cortisol is unbound and free and it is only the free form that appears to be biologically active (Sitteri et al. 1982, Rosner 1990). The levels of corticosteroid-binding globulin are regulated by the production of glucocorticoids (cortisol in hares, corticosterone in voles and mice; Dallman et al. 1990), with chronically high levels of glucocorticoids causing a reduction in this binding protein. Thus, if hares are being chronically stressed in the decline, CBG levels should be lower and hence the amount of free cortisol should be higher.

3) that the mobilization of glucose in decline-phase hares will be increased and that of free fatty acids decreased. Excessive levels of glucocorticoids (such as cortisol) produced by chronic stress increase hepatic production and storage of glucose as glycogen by enhancing the liver's capacity for gluconeogenesis (Miller and Tyrrell 1995). This comes at the expense of peripheral tissues by decreasing their glucose uptake and utilization, by the release of gluconeogenic substrate (such as fats) from peripheral tissues, by increasing protein breakdown in several tissues such as muscle, adipose, and lymphoid, and by decreasing protein synthesis. Thus, if hares are more stressed in the decline than the low phase, the former should have a greater ability to mobilize glucose from larger liver stores and a reduced ability to mobilize free fatty acids.

4) that two indices of reproduction will be lower in decline-phase hares: (a) natality will be lower and (b) the testosterone response to stress in males (which is initially stimulated in dominant animals or those in good condition; Sapolsky 1985, Mann and Orr 1990) will not occur.

5) that indices of body condition and immune response will be lower in decline-phase hares.

Methods and Materials

The study area

The study was conducted in the boreal forest near the Arctic Institute Base at Kluane Lake in the southern Yukon, Canada (60°57′ N, 138°12′ W) (Boutin et al. 1995, Krebs et al. 1995). It is the site of a 10-yr study into the community dynamics of vertebrates in the boreal forest. The area has a heterogeneous vegetation structure with spruce covering 50% of the valley, shrub thickets 35%, and meadow 7%. The climate is cold continental, with snow cover occurring from October to early May and temperatures in February averaging −18.0°C (20-yr average, 1973–1992).

Breeding biology of hares

Since hormonal status affects reproductive condition, it is necessary to appreciate when these experiments took place relative to the breeding cycle of snowshoe hares. In the southern Yukon, males come into breeding condition in February and virtually all are scrotal by the end of February. In our study all males were in reproductive condition in all years. Females have their first estrus in mid-April and thus none were in breeding condition during our study.

Population changes in hares

Snowshoe hares were live-trapped on three Control grids and on two experimental grids, the Food grid and Predator Exclosure + Food grid. Food in the form of rabbit chow (16% protein) was added to both of the latter two areas at the rate of ~300 kg every 5–6 d from the summer of 1988 onwards. From the winter of 1990 onwards feed was distributed by hand under areas of high cover along four trapping rows. Thus the purpose of this aspect of the treatment was to prevent food

limitation during the decline. On the Predator Exclosure + Food grid, large mammalian predators (lynx and coyotes), but not raptors (Great Horned Owls and Goshawks), were excluded from a 1-km^2 area by a high-voltage electric fence (set up in 1988, Krebs et al. 1995). The purpose of this treatment was to provide both food and a relatively safe haven from a major source of mortality during the decline, predation by mammalian carnivores. Each live-trapping grid was 32.5 ha in size and trapped with 86 Tomahawk traps per grid with alfalfa cube bait for an average of five nights every March and November. All animals were ear-tagged on first capture (Number 3 monel tags, National Band and Tag Company, Newport, Kentucky, USA), weighted on Pesola scales (±10 g), sexed, sexual condition assessed, and right hind foot measured (in millimeters). Numbers were estimated using closed models from the program CAPTURE (Otis et al. 1978) and densities were calculated for a 60-ha area to take into account edge effect and are based on those reported in Boulanger and Krebs (1994).

Reproductive rates

The total number of young produced per female per year was determined using the maternity cage method reported in O'Donoghue and Krebs (1992). Krebs et al. (1995) summarized the results to 1992. In 1994, the same method was used on control females to examine their reproductive fitness during the low.

Experimental animals

Samples were obtained during the late winter of three years, two during the decline (22–28 February 1991 and 16–26 February 1992) and one during the low (24–27 March 1994). We were prepared to trap for 14 d in late February 1994, but, because of extremely cold conditions overnight (−25 to −45°C), trapping had to be delayed until March. Two groups of hares were obtained:

1) Control hares were a random sample of hares caught in the forest adjacent to the Alaska Highway over a 35-km transect. In 1991 and 1992 we were able to trap just off the highway, and in 1994 we trapped along an access road. All trapping sites were at least 1 km from the nearest experimental trapping grid.

2) Experimental hares were a random sample of hares captured on two trapping grids that were regularly supplemented with rabbit chow. In 1991 these hares came from the Food grid, and in 1992 and 1994 they came from the Predator Exclosure + Food grid. In fact, the hares on the Food grid in 1991 more closely resembled those from the Predator Exclosure + Food grid than they did "open" control populations because both of the former had similar high densities and overwintering survival at the times the samples were collected (see *Results: Population changes in snowshoe hares* and *Discussion: Alternative explanations: Predation,* below). Predators only discovered the high-density Food grid late in 1991, and thereafter we could not obtain sufficient samples from it.

To capture control and experimental hares, live-traps were prebaited with alfalfa cubes and rabbit chow for 2–3 d before being set. The general trapping methods used can be found in Krebs et al. (1986*b*). All traps were set between 1600 and 2400 and checked between 0600 and 0830. Trapping did not occur if overnight temperatures were forecast to fall below −20°C. To try to avoid the potentially complicating differences between the sexes, we initially attempted to use primarily males. However, in 1992 and 1994 we had great difficulty capturing sufficient numbers of hares and had to use all animals caught. This proved not to be a problem when we examined it statistically (see *Potential differences between males and females,* below). The animals were transferred to burlap bags and taken to a quiet, dimly lit laboratory heated to 5–10°C. In an attempt to try to standardize the conditions to which the hares were exposed prior to the experiment, hares were allowed to habituate to these laboratory conditions for ~3 h.

At the end of the experiment, experimental hares were released at their site of capture; two, four, and two hares were fitted with radiocollars in 1991, 1992, and 1994, respectively, to determine their post-treatment survival. In 1991 and 1992, control hares were killed by cervical dislocation and autopsied. In 1994, control hares were used for a breeding experiment in another study. All hares were weighed and the right hind foot was measured.

Injections

Each hare was bled five times from an ear artery. Blood from the first sample (called the "Base bleed," 0.5 mL) was used to obtain baseline estimates of blood parameters. This was immediately followed with an injection of 0.4 mg/kg of dexamethasone sodium phosphate (Sabex, Montreal, Canada) into an ear vein. The second bleed (called the "Dex bleed," 0.3 mL) occurred 2 h later, followed immediately by an intramuscular injection in the thigh of 40 μg of synthetic ACTH (Synacthen Depot, CIBA, Ontario, Canada). The remaining bleeds (all 0.3 mL) were conducted at 30, 60, and 120 min post-ACTH injection (called the P30, P60, and P120 bleeds, respectively) in 1991 and 1994 and at 30, 120, and 240 min post-ACTH injection in 1992. We changed the protocol in 1992 to see if blood parameters would change more significantly by 4 h post-ACTH injection than they had by 2 h. However, in 1994 we used the 1991 protocol because we were concerned that the extra 2 h might affect survival for control hares that were to be used in a subsequent breeding experiment.

Hematology

Blood was collected using 28-gauge needles (basal diameter: 0.35 mm, 1.27 cm long) into heparinized 0.5-

mL syringes (Lo-Dose U-100 Insulin syringes, Becton, Dickson and Company, Franklin Lakes, New Jersey, USA) and in 75 μL microhematocrit tubes. Measurement of glucose concentrations and preparation of blood smears were completed within 5 min and measurement of packed red-blood-cell volume (PCV) and staining of smears was generally completed within 0.5 h, and always within 1.5 h of blood collection. Hematology was conducted only on the first bleed of each animal, except for glucose concentrations, which were measured after every bleed. PCV was measured on two samples per hare after a 7-min centrifugation at 13 460*g* on an IEC Micro-Hematocrit Centrifuge, Model MB. The remaining blood was centrifuged at 8800*g* for 5 min in an Eppendorf Micro Centrifuge 5413. The separated plasma was then frozen at −20°C, transported to Toronto, and stored at −70°C until analysis.

Blood smears were stained using a modified Wright stain technique called Diff-Quik (Baxter Healthcare Corporation, Miami, Florida, USA). Blood smears were only made from experimental animals in 1991 and 1994; it was not done in 1992 because of problems with the stain freezing. All white-blood-cell counts were carried out by trained technicians at the Animal Care Centre, University of British Columbia (Vancouver, British Columbia, Canada). Differential white-blood-cell counts were based on counts of 100 leucocytes and the total number of leucocytes was estimated per milliliter.

Because control hares appeared anemic in the decline, we made a detailed examination for blood parasites in samples collected in 1991. Only those slides that had adequate and uniform staining were examined. Each slide was examined at 1000× under oil immersion using a compound light microscope (Zeiss). We scanned the complete tail of each smear. A minimum of 5 min was spent on each slide scan. Replicate slides were scanned in 70% of cases. In the remaining cases the replicate smear was stained or had overlapping layers of cells; in either case we could not screen them for identification of parasites.

Glucose concentration (in milligrams per deciliter) was determined with a glucose oxidase/peroxidase reaction (Accu-Chek III, Mannheim Boehringer, Mannheim, Germany). We compared the accuracy of the device against control solutions provided by the company and values were within 20% for the low control solution (51 mg/dL) and 10% for the high control solution (292 mg/dL); these are within the admissible range.

Hormone assays

We measured total plasma cortisol, maximum corticosteroid-binding capacity (MCBC—a measure of the corticosteroid-binding globulin [CBG] capacity), and plasma testosterone plus dihydrotestosterone by the radioimmunoassay as described in Boonstra and Singleton (1993). The intra- and inter-assay coefficients of variation for cortisol were 10% and 16%, respectively, and for testosterone, 5% and 6%, respectively.

To calculate the concentrations of free cortisol we used the calculation procedures outlined in Tait and Burstein (1964), and for these calculations we need to know three values: the albumin concentration in plasma (albumin also binds cortisol and has high capacity but low affinity), the ratio of albumin-bound to free cortisol, and the affinity constant of CBG for cortisol. Pure albumin was obtained through the trichloroacetic acid method described in Michael (1962). This albumin was then used as a standard to calculate the concentration of albumin in plasma by the chromographic method of Debro et al. (1957). We calculated that hares have 3.93 g albumin per 100 mL plasma. The ratio of albumin-bound to free cortisol in a 1% solution is 0.39. The CBG affinity constant for snowshoe hares was measured in a microdialysis system (Englund et al. 1969) modified to 12 chambers (A. J. Bradley, *personal communication*) using 60 μL samples of plasma diluted 1:5 with a phosphosaline buffer (0.05 mol/L, pH 7.4). In this system, equilibrium was established in 12 h at 37°C, following which the specific activity of dialysate and sac contents was measured in a scintillation counter. The concentration of CBG-bound and unbound cortisol was calculated by method described in Paterson and Hills (1967) and the CBG affinity constant calculated by the Scatchard analysis (Scatchard 1949). We calculated the CBG-binding constant of the snowshoe hare to be 5.05×10^7 L/mol. This is comparable to that obtained by Westphal (1967) for the laboratory rabbit 4.0×10^7 L/mol. This latter value was used for the 1991 animals in the paper of Boonstra and Singleton (1993) on snowshoe hare and thus the values given here are more accurate.

Free fatty acids

Free fatty acids (FFA) are mobilized under stress in rabbits in response to increasing ACTH levels, but not to increasing glucocorticoid levels (Rudman and DiGirolamo 1967, Desbals et al. 1970). A recent study has shown that FFA increase the binding of glucocorticoids to corticosteroid-binding globulin (Haourigui et al. 1993). Thus, increased FFA levels in stressed animals may reduce the level of free cortisol to which the cells are exposed. Because we became aware of the findings of Haourigui et al. after our other analyses had already been completed and thus little plasma remained, and because our method of measuring total FFA (measured using the technique of Laurell and Tibbling 1967) required 50 μL of plasma, we were able to quantify FFA levels in only a portion of our samples.

Statistical analysis

Data are expressed throughout as means ±1 SE. We used Cochran's test (Winer 1971) as recommended by Day and Quinn (1989) to test for homogeneity of variance. We found that the basic demographic and autopsy

data satisfied this criterion and hence used no transformations. The hormone data for the repeated-measures analysis did not, and we used the $\log(x + 1)$ transformation prior to any statistical analysis to make the variances homogeneous. In the repeated-measures analysis, we examined only those values from times that were constant across all 3 yr (i.e., Base, Dex, P30, and P120 bleeds). All ANOVAs were performed using SuperANOVA (Gagnon et al. 1990). A repeated-measures ANOVA was carried out on the hares challenged with the hormones. In a repeated-measures design, the values recorded from the same animal will be correlated with each other and thus are not independent as assumed by ANOVA. To compensate for this, we used the conservative Greenhouse-Geisser epsilon to adjust the degrees of freedom before calculating the probabilities as recommended by Keppel (1982). We used the Tukey-Kramer multiple comparison post-hoc test to examine the significance of main effects. A repeated-measures ANOVA could not be done for FFA levels (untransformed) as many samples had insufficient plasma. To assess whether FFA changed, sequential paired t tests (adjusted by sequential Bonferroni) comparing each of the times of bleeding were carried out. To test effect of treatment and year, a two-way ANOVA was carried out only on the P30 bleed. For the white blood-cell data we used a Mann-Whitney U test. We give P values between 0.10 and 0.05 and infer that these may be biologically, though not statistically, significant, possibly because of reduced-power sample sizes in some of our results (Yoccoz 1990).

Potential differences between males and females

Prior to carrying out the repeated-measured ANOVA to examine for year and treatment effects, we carried out an analysis on transformed values to determine if the sexes could be pooled. The analysis proceeded in two steps: first, we determined whether there was a sex effect in control animals by carrying out a two-way repeated-measures ANOVA (year × sex); and second, we compared only 1994 animals from both treatments by a two-way repeated-measures ANOVA (treatment × sex). At this level of the analysis we will only focus on sex effects (ignoring year and treatment effects, which will be dealt with below). We found no difference between males and females for free cortisol levels, for MCBC levels, for glucose levels, or for FFA levels, and thus we pool them for the subsequent analysis.

RESULTS

Population changes in snowshoe hares

Hares on control grids went through most of one cycle from 1987 to 1994, from increasing populations in 1987–1988 through to peak populations from 1989–1990, to declining populations from late 1990 to spring 1992, and finally low populations from autumn 1992 to the spring 1994 (Krebs et al. 1995; Fig. 2). During

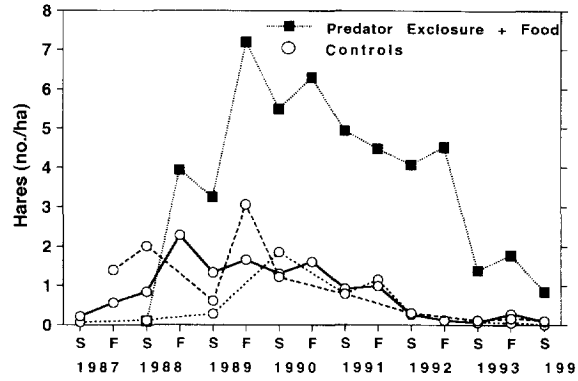

FIG. 2. Spring (S) and fall (F) densities of snowshoe hares in the southern Yukon on three control grids and on one Predator Exclosure + Food grid. The fence for the latter grid was built in 1988.

the peak of 1990, hare numbers in spring averaged 1.48 individuals/ha, which then fell 38% to 0.92 individuals/ha in spring 1991, a further 41% to 0.31 individuals/ha in spring 1992, a further 15% to 0.083 individuals/ha in 1993, and then showed little change to 0.077 individuals/ha in spring 1994. This drastic change in hare numbers was reflected in our ability to capture control hares for the experimental work. The percentage of traps capturing hares for stress tests dropped from 69% (31 hares caught in 45 trap nights) in 1991, to 10% (9/89) in 1992 and to 11% (16/141) in 1994. Thus our assessment of the ability of hares to respond to a challenge spanned the decline (1991 and 1992) and the low (1994).

In 1991 the experimental hares for the challenge experiments came only from the Food grid, and, though it was not protected from mammalian predators, the predators did not cue into these high hare densities that winter, and hence densities remained high. Densities on the Food grid in autumn 1990 were 6.60 individuals/ha and in spring 1991 6.57 individuals/ha. In 1992 and 1994 the experimental hares for the challenge experiments came from the Predator Exclosure + Food grid. Numbers on this grid in 1990–1991 were comparable to those on the Food grid and also showed a similar marginal decline over this period (Fig. 2). Thereafter, numbers also declined. They started at higher densities than control areas in spring 1990 (5.53 individuals/ha—3.7 times control densities), were much higher in spring 1992 (4.10 individuals/ha—13 times control densities) and were still much higher by spring 1994 (0.87 individuals/ha—11 times control densities).

Reproductive changes in snowshoe hares

The number of young produced per female declined markedly on control areas from a high of 16.4 animals in 1988 to a low of 3.3 animals in 1992 and then rebounded to 15.4 animals in 1994 (Fig. 3). The low values seen in control animals in 1991 and 1992 were not seen on the Predator Exclosure + Food grid, where

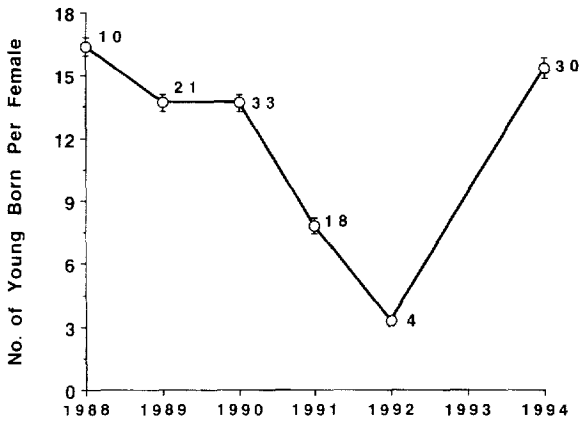

FIG. 3. Total reproduction of live young per female snowshoe hare per year (mean ± 1 SE) over the summer breeding season. Sample sizes are indicated beside each point.

the number of young per female remained comparable to 1988 control values (Krebs et al. 1995).

Summary of basic data

Ideally we would have liked to standardize our comparison among years and treatments by using only males. However, we had great difficulty obtaining hares in 1992 and 1994 because of the low densities and thus were forced to use both sexes in the challenge experiments. Fortunately, three control females had also been challenged in 1991, though we did not present their results in the study of Boonstra and Singleton (1993). Only males were challenged from the experimental grids in 1991 and 1992 and both sexes were used in 1994. Table 1 gives a summary of the basic demographic data of the experimental hares. To examine if there was a difference in mass between the sexes among years we performed a two-way ANOVA (year × sex) only on control hares and found no difference between the sexes ($F = 0.98$, df = 2, 31, $P = 0.33$). Thus data from both sexes were pooled and analyzed to examine for differences among treatments and years. There was both a treatment effect ($F = 6.47$, df = 1, 52, $P = 0.01$), with experimental hares weighing ~100 g more than control hares (1549 ± 27 g vs. 1456 ± 29 g, respectively [mean ±1 SE]) and a year effect ($F = 4.88$, df = 2, 52, $P = 0.01$) with hares from 1991 weighing significantly more than those from 1994 (by almost 150 g), but being similar to those from 1992. There was no interaction effect ($F = 0.34$, df = 2, 52). Mean pooled body masses for 1991, 1992, and 1994 were, respectively, for Control hares: 1525.5 ± 35.2 g, 1478.9 ± 69.1 g, and 1348.9 ± 46.1 g; and for Fed hares: 1591.4 ± 46.1 g; 1561.0 ± 36.1 g; and 1487.8 ± 56.0 g.

We carried out a similar analysis on right-hindfoot length, a size measure that should not be as condition dependent as mass is likely to be. In controls, though there was no year effect ($F = 2.16$, df = 2, 31, $P = 0.13$) or sex effect ($F = 0.47$, df = 1, 31, $P = 0.50$), there was a significant interaction effect ($F = 4.14$, df = 2, 31, $P = 0.02$). As indicated in Table 1, control males were similar in all years, but control females were similar to males in 1991, about 8 mm larger in 1992 and 5 mm smaller in 1994. We assumed that we were sampling the control-hare populations randomly and, if this is so, it would suggest that female hares were older than males in 1992 and younger in 1994. A two-way ANOVA (treatment × year, with all sexes pooled) indicated no treatment effect ($F = 1.36$, df = 1, 52, $P = 0.25$), no interaction effect ($F = 0.46$, df = 2, 52, $P = 0.63$), but a significant year effect ($F = 5.06$, df = 2, 52, $P < 0.01$), with 1994 hares being similar to those in 1991, but smaller than those in 1992. Thus the average animal was larger (and perhaps older) in 1992, than in 1991 or 1994, and in females on control areas, this was particularly marked.

Plasma cortisol levels

The repeated-measures ANOVA indicated that, averaged over the entire experiment (after the variance owing to within-subject effects had been removed), there was a significant difference in the levels of free cortisol and maximum corticosteroid-binding capacity (MCBC) between control and experimental populations and among years (Table 2) (interaction effects between

TABLE 1. Summary of the basic demographic data on hares used in the study (data are means ± 1 SE).

Treatment	1991		1992		1994	
	Males	Females	Males	Females	Males	Females
Control hares						
Sample size	8	3	3	6	6	3
Mass (g)	1546 ± 38	1470 ± 85	1337 ± 57	1550 ± 88	1352 ± 35	1343 ± 38
Right hind foot (mm)	132.4 ± 1.8	133.3 ± 3.7	131.3 ± 4.4	139.0 ± 1.53	134.3 ± 0.9	129.0 ± 2.1
Marrow fat (%)	63.9 ± 2.4	81.2 ± 5.7	45.4 ± 6.0	54.4 ± 3.7	57.5 ± 0.7†	54.7 ± 4.2
Experimental hares						
Sample size	10		10		5	4
Mass (g)	1591 ± 46		1561 ± 36		1442 ± 79	1545 ± 80
Right hind foot (mm)	135.4 ± 1.3		138.1 ± 1.1		131.2 ± 2.0	134.0 ± 2.7

† Percentage fat in 1994 hares was obtained from snared sample (3 males : 5 females).

TABLE 2. The average effects (in units of nmol/L) of the hormonal challenge on snowshoe hares (for details see *Materials and methods: Injections*). Data are means ± 1 SE; sample sizes are in parentheses (and are the same for both free cortisol and maximum corticosteroid-binding capacity [MCBC]).

Factor	Year†	Hormonal challenge (nmol/L)	
		Control hares	Experimental hares
Free cortisol‡	1991[a]	278.3 ± 40.4 (44)	274.5 ± 40.3 (40)
	1992[a]	301.8 ± 47.4 (36)	277.0 ± 40.3 (40)
	1994[b]	166.5 ± 30.6 (36)	108.0 ± 20.3 (36)
MCBC§	1991[a]	150.2 ± 7.6 (44)	178.3 ± 10.7 (40)
	1992[b]	191.9 ± 13.1 (36)	229.7 ± 15.0 (40)
	1994[b]	254.3 ± 20.1 (36)	261.2 ± 20.1 (36)
Testosterone	1991[a]	5.86 ± 0.81 (32)	9.88 ± 2.03 (40)
	1992[b]	2.76 ± 1.03 (12)	5.45 ± 0.94 (40)
	1994[a]	14.68 ± 3.80 (28)	10.09 ± 4.07 (20)

Notes: Only the Base, Dex, Post-30 min, and Post-120 min bleeds were included as these were constant across all years. Each hare was bled five times from an ear artery; for definitions, see Fig. 4 legend.

† Year effects for free cortisol and MCBC plasma levels, $P < 0.0001$; for testosterone plasma levels, $P < 0.003$. Years with identical lowercase superscript letters indicate means that are not significantly different.

‡ Treatment effect: $P = 0.06$.

§ Treatment effect: $P = 0.04$.

treatments and among years were not significant) (Table 3). Experimental hares generally had lower free cortisol levels ($P = 0.06$) than control hares, with there being virtually no difference in 1991 and 1992, but a marked difference (64.5% of control values) in 1994. There was also a marked difference in free-cortisol levels among years, with 1991 and 1992 being similar and significantly higher (about 106%) than 1994 values (Table 2). Experimental hares had consistently higher MCBC levels (about 30–40 nmol/L in 1991 and 1992, but only 7 nmol/L in 1994) than control hares. There was also a significant difference in MCBC levels among each of the years, with values rising progressively from 1991 through to 1994 so that by 1994 they were ~80–100 nmol/L higher (58% higher than 1991 values). Thus, there are two major conclusions from the main-effects portion of the analysis: first, that experimental hares appeared to be better able to handle the hormonal challenge than control hares, as indicated by higher MCBC levels and slightly lower free cortisol levels; and second, that 1994 hares, independent of treatment, were in much better condition than 1991 and 1992 hares, and thus better able to respond adaptively to the hormonal challenge, resulting in higher MCBC levels and lower free-cortisol levels.

The within-subjects effects examining the responses to the injections showed similar changes for both free cortisol and MCBC (Table 3): first, there were highly significant changes over time in response to the injections; and second, there were highly significant differences among years, but for free cortisol, there was an interaction between time, treatment, and years. To simplify an examination of the response to the injections, we have plotted the data for the control and experimental hares separately on the same figure (Fig. 4).

With respect to changes in free cortisol levels, both control and experimental hares in all years responded rapidly both to the dexamethasone injection, with average levels falling to 3% of baseline levels (overall average baseline levels = 106.4 ± 12.9 nmol/L and Dex levels = 3.2 ± 0.5; means ±1 SE), and to the ACTH injection, with average levels increasing to 323% of baseline levels by post-30 min (343.6 ± 25.0) and to 466% of baseline levels by post-120 min (496.0 ± 27.3) (Fig. 4A). Fig. 4 also indicates that hares in 1994 in both treatments had much lower baseline levels initially and responded less dramatically to the ACTH injection than did the hares in the other two years, which were similar. Average baseline levels of free cortisol in 1994 were only 32.7% of 1991 levels and, in response to ACTH at P30, increased to only 39.8% of 1991 levels.

MCBC levels in both control and experimental hares in all years showed a similar lack of response to the dexamethasone injection, but this was followed by a dramatic, temporary increase after the ACTH injection (Fig. 4B). The overall average MCBC level increased 213% from 145.5 ± 5.4 nmol/L at the baseline bleed to 309.4 ± 13.3 nmol/L at the post-30-min bleed. By the post-120 bleed a marked decline had occurred to 237.8 ± 10.7 nmol/L, and the values from 1992 indicate that levels were approaching baseline levels by 4 h after the ACTH injection. These declining MCBC levels were not associated with declining free cortisol levels, which remained high to the end of the experiment. Thus the MCBC pulse appears to be a temporary response to the ACTH injection.

There were pronounced differences in MCBC levels among years, with baseline levels of hares from 1994 being marginally higher than those from 1991 and 1992 in all years (two-way ANOVA, [treatment × year] treatment effect: $F = 2.37$, df = 1, 52, $P = 0.13$; year effect: $F = 2.84$, df = 2, 52, $P = 0.067$; interaction effect: $F = 0.77$, df = 2, 52) and these differences becoming very marked after the ACTH injection (Fig. 4B). The difference in MCBC response between the

TABLE 3. Repeated-measures ANOVA testing differences in treatment (T; control vs. experimental) and year (Y; 1991, 1992, and 1994) over time in response to the hormonal challenge.

Source of variation	df	MS	F	P†
Free cortisol				
Treatment	1	0.32	2.90	0.06
Year	2	2.09	19.03	0.0001
T × Y	2	0.14	1.31	NS
Subject (Group)	52	0.11		
Time	3	53.63	1085.14	0.0001
Time × T	3	0.05	1.08	NS
Time × Y	6	0.32	6.50	0.0001
Time × T × Y	6	0.11	2.14	0.07
Error	156	0.05		
MCBC				
Treatment	1	0.16	4.53	0.04
Year	2	0.62	18.01	0.0001
T × Y	2	0.02	0.69	NS
Subject (Group)	52	0.03		
Time	3	1.52	340.57	0.0001
Time × T	3	0.01	1.26	NS
Time × Y	6	0.06	13.67	0.0003
Time × T × Y	6	0.004	0.83	NS
Error	156	0.004		
Testosterone				
Treatment	1	0.25	1.67	NS
Year	2	1.20	8.03	0.003
T × Y	1	0.27	1.79	NS
Subject (Group)	37	0.15		
Time	3	0.57	8.41	0.0001
Time × T	3	0.16	2.37	0.08
Time × Y	6	0.15	2.25	0.05
Time × T × Y	6	0.11	1.68	NS
Error	111	0.07		
Glucose				
Treatment	1	0.06	1.28	NS
Year	2	0.14	3.23	0.05
T × Y	2	0.17	3.65	0.03
Subject (Group)	51	0.04		
Time	3	0.28	46.20	0.0001
Time × T	3	0.01	1.82	NS
Time × Y	6	0.004	0.64	NS
Time × T × Y	6	0.01	2.09	0.08
Error	153	0.006		

Note: The probabilities of the within-subject (time) analysis were adjusted using the Greenhouse-Geisser estimate.

† NS = nonsignificant; however, the treatment values just above significance are given.

experimental and control hares to the ACTH injection became progressively less marked from 1991 through to 1994. At the post-30 bleed, experimental values in 1991 were 30.9% higher than control values in contrast to 16.3% and 0% in 1992 and in 1994, respectively. Thus it appears that the benefits of providing additional high-quality food to the experimental hares disappeared relative to control hares in 1994, in contrast to 1991 and 1992.

We examined if dexamethasone resistance occurred in our hares by comparing free cortisol levels at the baseline bleed with those at the dexamethasone (Dex) bleed. The difficulty with our experimental design is that the baseline estimates were obtained from hares that were already stressed from trapping and bringing them into the laboratory and thus were not equivalent to the resting levels, the reference point to which comparisons are usually made. A shot sample collected in February 1991 (Boonstra and Singleton 1993) had levels significantly below those obtained at our baseline (Base) bleeds. We assume that higher cortisol levels at the Base bleeds were correlated to higher resting levels and that those hares experiencing them were less able to cope with the stress of capture and handling. We correlated the free cortisol levels at the Base bleed with those at the Dex bleed and found a significant, positive relationship ($r = 0.50$, $F = 18.84$, df = 1, 56, $P = 0.0001$), which was primarily due to the strength of the relationship found in control hares ($r = 0.63$, $F = 18.16$, df = 1, 27, $P = 0.0002$) and less so to that found in experimental hares ($r = 0.35$, $F = 3.82$, df = 1, 27, $P = 0.06$). The subsequent ability to respond to the ACTH (free cortisol levels at the post-30-min bleed) was not correlated to cortisol levels at the Dex bleed ($r = 0.22$, $F = 2.82$, df = 1, 56, $P = 0.10$). Thus, animals having higher free cortisol levels at the Dex bleed were indicative of poorer quality animals. The three most resistant hares (having free-cortisol levels at the Dex bleed that all exceeded 10 nmol/L) were control 1991 animals. We conclude that dexamethasone resistance occurred in our hares, that the relationship was strongest in control hares, and that it was more pronounced in the first year of the decline, 1991, than later on.

Free fatty-acid levels

Free fatty-acid (FFA) levels were unaffected by Dex, but increased markedly with ACTH (Fig. 4C) (Base = Dex $\ll$ P30 = P60 $\gg$ P120 $\gg$ P240, compared by sequential paired t tests, $P < 0.05$). At P30 (two-way ANOVA, treatment × year), FFA levels were greater in experimental than control hares ($F = 4.74$, df = 1, 30, $P = 0.04$) and greater in 1994 than 1991 (1992 not different from either year; $F = 3.86$, df = 2, 30, $P = 0.03$). Over all years and treatments pooled, FFA levels at P30 were correlated to MCBC levels ($r = 0.56$, $N = 36$, $P = 0.001$). Thus FFA responded to ACTH, but not Dex, and levels were higher in 1994 than in 1991.

Plasma testosterone levels

The repeated-measures ANOVA indicated that, averaged over the entire experiment, there was no difference between control and experimental males, but there was a significant difference among years, with 1991 and 1994 being similar and different from 1992 (Table 2). It also indicated that there were significant changes over time, but interaction effects between time and treatment ($P = 0.08$) and time and year ($P = 0.05$) complicate the picture (Table 3). Fig. 5 indicates that in virtually all groups, the dexamethasone injection resulted in a decline in testosterone levels from average baseline levels of 11.06 ± 3.04 nmol/L to Dex levels of 4.43 ± 0.86 nmol/L (mean ±1 SE). The percentage decline progressively increased from 1991 through to

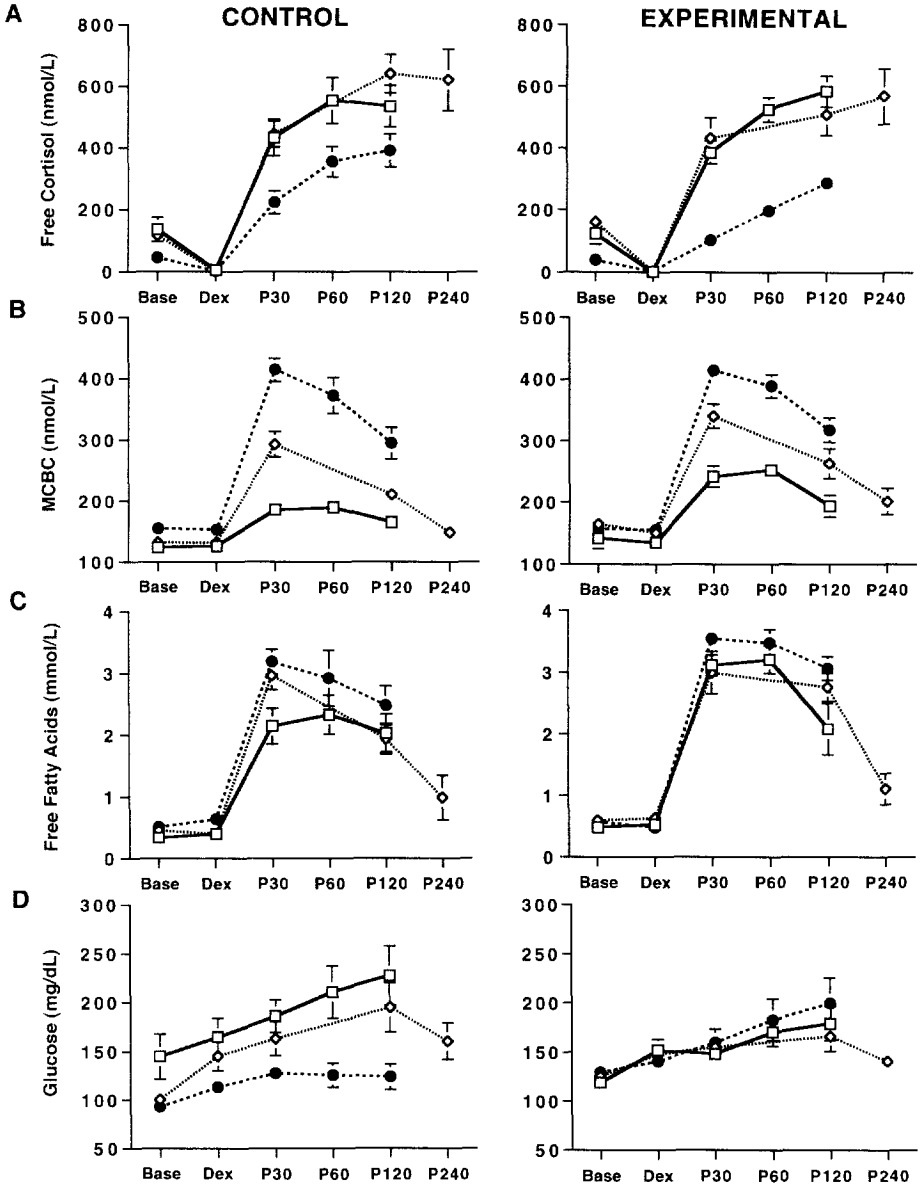

FIG. 4. Responses over time in plasma concentrations (means ± 1 SE) of (A) free cortisol, (B) maximum corticosteroid-binding capacity (MCBC), (C) free fatty acids, and (D) glucose in snowshoe hares from control and experimental areas during two years of population decline (□ = 1991, ◊ = 1992) and one year of a low (● = 1994). Base levels indicate initial values, Dex indicates values 2 h after the dexamethasone injection, and P30, P60, P120, and P240 indicate values 30, 60, 120, and 240 min after the adrenocorticotropic hormone (ACTH) injection.

1994, when levels fell an average of 39.8%, 46.6% and 74.1%, respectively.

The response to ACTH varied markedly between treatments and years, and hence the interaction effects (Table 3). It is obvious from Fig. 5 that control males in 1994 were markedly different from those in 1991 and 1992. To try to tease out what was going on, we examined only controls hares through a repeated-measures one-way ANOVA, and again there is a significant main effect of years ($F = 4.70$, df = 2, 45, $P = 0.03$), but now 1991 and 1992 are similar and significantly different from 1994. Testosterone levels continued to fall immediately after the ACTH injection at the post-30-min bleed in 1991 (from 5.19 ± 1.69 to 4.55 ± 0.68 nmol/L; mean ±1 SE), showed a modest increase in 1992 (from 0.73 ± 0.16 to 2.16 ± 0.83 nmol/L), and a dramatic transitory pulse in 1994 (from 6.28 ± 3.77 to 20.15 ± 8.54 nmol/L). The 1994 levels then fell progressively to reach slightly higher levels by the post-120-min bleed (8.25 ± 1.73 nmol/L) than those of the Dex bleed. Experimental males in all years showed an increase in response to the ACTH injection,

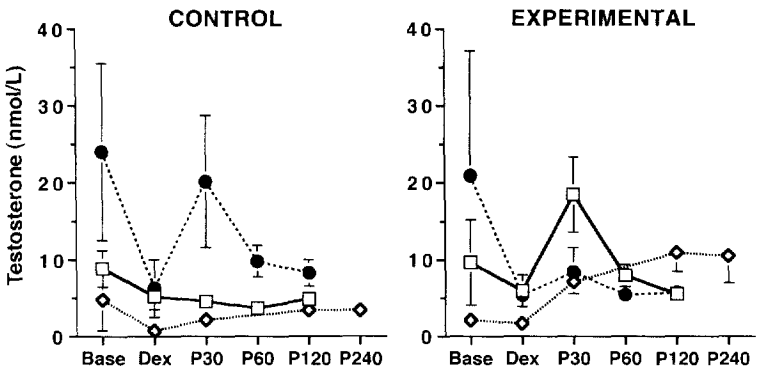

FIG. 5. Responses over time in plasma concentrations of testosterone (means ± 1 SE) in male snowshoe hares from control and experimental areas during two years of population decline (□ = 1991, ◇ = 1992) and one year of a low (● = 1994). See Fig. 4 legend for additional details.

with the most dramatic occurring in 1991. In both 1991 and 1994 it was also transitory, but in 1992 levels showed no decline but remained high 4 h after the ACTH injection. In summary, testosterone levels in all animals declined in response to dexamethasone, and in control 1994 males and in experimental 1991 and 1994 males testosterone levels showed a transitory, 30-min increase before falling again.

Plasma glucose levels

Two lines of evidence indicate that control hares during the decline had the highest glucose levels and the greatest ability to mobilize and sustain a glucose response (and thus must have been much more stressed) than those from the low. First, we determined whether there was a difference in baseline glucose levels by a two-way ANOVA (treatment × year). Control hares had significantly lower average baseline glucose levels (115.00 ± 10.10 mg/dL) than experimental hares (126.87 ± 5.04 mg/dL; mean ±1 SE) (F = 5.37, df = 1, 52, P = 0.02), but the picture was complicated by marginal differences among years (P = 0.08) and by interaction effects between years and treatments (P = 0.07). Fig. 4D indicates that baseline levels were different in control hares among years (F = 4.02, df = 2, 26, P = 0.04), with 1994 hares having levels significantly lower than those from 1991 and 1992, which did not differ from each other. Baseline levels in experimental hares were similar in all years (one-way ANOVA, F = 0.04, df = 2, 26, P = 0.96).

Second, we examined for differences in ability of hares to mobilize glucose in response to the injections (both Dex and ACTH mobilize glucose from liver glycogen reserves [Dallman et al. 1989]) with a repeated-measures ANOVA. The analysis (Table 3) and graphs (Fig. 4D) indicate that plasma glucose was mobilized over the time course of the experiment. However, the significant interaction effects (at both the main-effects level and the within-subjects level over time, Table 3) complicate the interpretation, and Fig. 4D clearly indicates that differences between treatments occurred. To tease out the differences in response by control and experimental hares to the injections, we performed a one-way repeated-measures ANOVA on each treatment separately. In control hares, there was a significant year effect (F = 5.07, df = 2, 78, P = 0.01), with animals from 1991 (overall mean = 180.7 ± 12.0 mg/dL) having levels significantly higher than those from 1994 (114.3 ± 4.5 mg/dL); those from 1992 (150.8 ± 10.2 mg/dL) had levels intermediate and not different from those in 1991 or 1994. In experimental hares, animals in all years were similar (overall mean = 150.8 ± 6.2 mg/dL) (F = 0.06, df = 2, 78, P = 0.94). In contrast, control hares had a greater ability to mobilize glucose than experimental hares in 1991, a similar ability in 1992, and a much lower ability in 1994. A decreased ability to mobilize glucose in the 1994 control hares must indicate that they had much lower liver glycogen reserves than control hares from 1991 and 1992, and thus that they were much less stressed. Thus the decline-phase hares (1991 and 1992) had a much greater capacity to respond to both the stress of capture and handling and to the ACTH test than did the low-phase hares (1994).

Hematology

The changes in the major white-blood-cell types are presented in Table 4. Within years, we found no difference between control and experimental hares. Between years, we found significantly lower counts of neutrophils, lymphocytes, and monocytes from hares in 1991 than from those in 1994 and significantly higher counts of eosinophils in 1991 than in 1994.

Packed red-blood-cell volume (PCV) is an integrative index of body condition in which higher values have been linked to better condition (Franzmann and LeResche 1978, Seal and Hoskinson 1978, Lochmiller et al. 1986, Hellgren et al. 1993). To assess how PCV values changed over the study, we first determined whether there were differences between the sexes. We carried out a two-way ANOVA (treatment × sex on control shot [shot in 1991 to assess the effect of the

TABLE 4. Number and major cell types of leucocytes per milliliter estimated from blood smears from snowshoe hares collected during the first year of population decline (1991) and during the population low (1994).

Leucocytes	Year	Control hares	Experimental hares	All hares
Neutrophils	1991	3022 ± 200 (12)	2928 ± 385 (9)	2982 ± 195 (21)****
	1994	5164 ± 380 (16)	4269 ± 480 (9)	4842 ± 305 (25)
Lymphocytes	1991	1561 ± 104	1623 ± 135	1587 ± 81***
	1994	2252 ± 248	2372 ± 226	2295 ± 176
Monocytes	1991	38.2 ± 11.4	5.8 ± 5.8	24.4 ± 7.7***
	1994	123.8 ± 33.0	124.4 ± 28.2	124.0 ± 23.1
Eosinophils	1991	36.7 ± 12.6	82.5 ± 36.1	56.3 ± 17.3**
	1994	3.4 ± 3.4	6.1 ± 6.1	4.4 ± 3.0

Notes: Control and experimental samples were obtained from the first sample of the experimental protocol. Data are means ± 1 SE. Sample sizes are in parentheses, are given for neutrophils only, and are the same for all other cell types. Mann-Whitney U test was used for comparisons. Treatment effects within year: none.
Year effects: ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (comparison of overall means only).

stress of capture and handling, see Boonstra and Singleton 1993]), control stressed, and experimental stressed hares. We found no significant effect of sex ($F = 0.20$, df = 1, 68) and no interaction effect ($F = 0.005$, df = 1, 68), though we did find a significant treatment effect. Thus we pooled males and females for this analysis. To assess whether there were treatment and year effects, we carried out a two-way ANOVA on hormonally challenged hares only. There were significant differences between treatments (experimental ≫ control: $F = 34.9$, df = 1, 59, $P < 0.0001$), among years (1992 = 1994 ≫ 1991: $F = 15.3$, df = 2, 59, $P < 0.0001$), and an interaction effect ($F = 3.4$, df = 2, 59, $P < 0.04$) (Fig. 6). PCV levels in control hares were consistently lower than those in experimental hares, but showed a continuous improvement from 1991 through to 1994, when their levels converged on those of hares from the experimental area. Those on the experimental area also improved from 1991 to 1992, but not thereafter. Thus, control hares during the first and second years of the decline were anemic relative to experimental hares, but this difference disappeared during the year of the low.

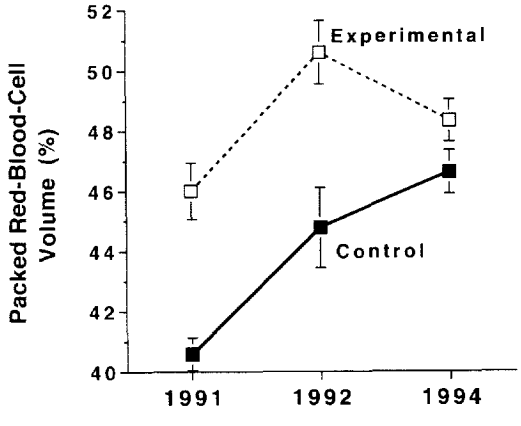

FIG. 6. Changes in packed cell volume of red blood cells in snowshoe hares from control and experimental populations during two years of population decline (1991 and 1992) and one year of a low (1994). Packed cell volumes were measured at the Base bleed.

One possible explanation for the anemic condition of the 1991 hares was a blood parasite such as *Babesia* spp., *Eperythrozoon* spp., or *Hemobartonella* spp. We made a detailed examination of the blood smears of all control animals collected in February 1991 ($N = 12$ individuals) and in May 1991 ($N = 12$ individuals) (Boonstra and Singleton 1993) and of those shot for baseline levels at both times. We found no evidence of blood parasites in any of these samples, and thus rule out blood parasites as an explanation for the relative anemia in 1991.

Survival of radio-collared and tagged experimental hares

The experimental hormonal challenge might have been severe enough to affect long-term survival. In 1991, of the two hares from the experimental population radio-collared and released, one survived for more than 287 d and the other for at least 34 d, when radio contact was lost. Two of the hares that were only ear-tagged lived a minimum of 28 d after release (as determined from capture in traps). Thus at least 4 of 10 hares in 1991 lived a minimum of another 4 wk subsequent to the experimental treatment. In 1992, the four radio-collared hares all lived at least 5 wk, with the range being 35–301 d. Three other hares that were only ear-tagged lived a minimum of another 4 wk. Thus at least 7 of 10 hares in 1992 were alive at least one month later. In 1994, one radio-collared hare survived for >275 d; the other died the week after the experiment of unknown causes (however, a trapping session occurred that week, it was captured again, and so it may have succumbed from the combined effects of the experiment and of trapping, i.e., not having sufficient time to recover from the effects of the experiment). Two of the hares that were only ear-tagged lived a minimum of another 183 d. Therefore, 3 of 9 (33%) hares survived on the grid at least until the autumn trapping session, and this compares with 9 of 25 (36%) hares that were not part of our experiment that survived from the spring trapping session to the fall trapping session. Thus the experimental treatment was not se-

TABLE 5. Overview of predictions and results of the hormonal-challenge experiments on the hypothalamic–pituitary–adrenal (HPA) axis. In the snowshoe hare cycle, decline years were 1991 and 1992, and the low year was 1994.

	Predictions†	Variable‡	Results§	
			Year	Treatment‖
Main effects on HPA axis				
Dexamethasone resistance	Decline > Low	Free cortisol	1991 = 1992 > 1994	Cont. > Exp.
ACTH¶ stimulation	Decline > Low	Free cortisol	1991 = 1992 > 1994	Cont. > Exp.
	Decline < Low	MCBC	1991 = 1992 < 1994	Cont. < Exp.
Physiological consequences if hares were stressed in decline				
Energy mobilization	Decline > Low	Glucose	1991 = 1992 > 1994	Cont. < Exp.
	Decline < Low	FFA	1991 = 1992 = 1994 (1991 < 1994)	Cont. < Exp.
Reproduction				
Females	Decline < Low	Natality	1992 < 1991 < 1994	Cont. < Exp.
Males	Decline < Low	Testosterone	1992 = 1991 < 1994	Cont. = Exp.
Immune response	Decline < Low	White blood cells#	1991 < 1994	Cont. = Exp.
Condition index	Decline < Low	PCV	1991 < 1992 = 1994	Cont. < Exp.
	?	Mass	1991 = 1992 > 1994	Cont. < Exp.
	?	RHF	1992 > 1991 = 1994	Cont. = Exp.

† A question mark (?) indicates that either no prediction was made with respect to the parameter or the direction could not be predicted.
‡ MCBC = maximum corticosteroid-binding capacity; FFA = free fatty acids; PCV = packed red-blood-cell volume; RFH = right hind foot.
§ Results for both year and treatment effects are approximate, especially if there were interaction effects.
‖ Cont. = control; Exp. = experimental.
¶ ACTH = adrenocorticotropic hormone.
# Counts only available for 1991 and 1994 hares.

vere enough to compromise survival of experimental hares over the short term.

DISCUSSION

The hares in the boreal forest of the southern Yukon went through one cycle in numbers from 1987 to 1994 (Krebs et al. 1995). Hares increased from the mid-1980s to a peak in spring 1990, declined ~40% over the winters of both 1990–1991 and 1991–1992 and an additional 15% over 1992–1993, and reached a low in spring 1993 that then lasted until 1994 (Fig. 2). Mortality patterns during the decline echoed these changes, with annual mortality being >95% from 1990 to 1992 and diminishing to 64% in 1993–1994 (Krebs et al. 1995). Thus, animals for our challenge experiments came from two decline winters and one low winter. The low winter was followed by a summer of good reproduction (Fig. 3) and population growth (C. J. Krebs, D. Hik, C. Doyle, and S. Boutin, *unpublished data*).

Our major conclusion is that snowshoe hares during the population decline (1991 and 1992), but not during the low (1994), were chronically stressed and this directly affected their ability to maintain homeostasis and ultimately their fitness. Because our findings are complex, we have summarized them and the predictions in Table 5 and we discuss each in turn. However, it must be recognized that in all years and in both treatments, hares always demonstrated a hypothalamic–pituitary–adrenal (HPA) feedback system that continued to be responsive to both the dexamethasone (Dex) and the ACTH injections, and thus adrenal exhaustion as proposed by Selye (1946) never occurred.

Caveats

There are three caveats that must first be addressed before we discuss the results. First, could the fact that the 1991 and 1992 hares were tested in late February and the 1994 hares were tested in late March have affected our results? We do not think so for three reasons. (1) No major changes in reproductive status occurred in hares from February to March—all males were reproductive (testes scrotal) at both times and no females were (they would have bred in mid- to late April). In addition, snowshoe hares are not territorial and thus do not show the type of pre-breeding establishment and defense of areas that may affect the hormonal profile of territorial species (Boutin 1984, Graf 1985, Boutin et al. 1986). (2) Weather conditions may vary from year to year and this may have influenced the response of the animals to the challenge tests. Though weather is generally milder in late March than in late February, conditions in February 1994 were much colder than in February 1991 and 1992, and thus any amelioration of conditions in March 1994 may have counterbalanced the milder conditions of February 1991 and 1992. (3) Perhaps there is some factor not related to weather or to obvious changes in reproductive biology that could explain the improvement in physiological condition in hares from February to March that was not related to the phase of the hare

cycle (decline vs. low). If we assume that there is indeed some sort of progression in physiological condition from February through to May, then we can test whether animals collected in between those months are somewhere in between them in physiological condition. To examine this idea, we compared control hares collected in February (1991), in March (1994), and in May (1991) (the 1991 May results were published in Boonstra and Singleton 1993) (note that we assume here the null hypothesis of no differences between years). In each of these analyses, in the overall comparisons in the responses of the hares in the different months, March 1994 was significantly different from February 1991 and May 1991, which were similar to each other (free cortisol: lower in March 1994, $P = 0.0089$; MCBC: higher in March 1994, $P = 0.0003$; glucose: lower in March 1994, $P = 0.0068$). Thus, the physiological response of the hares sampled in March was not part way in between the response of hares sampled in either February or in May. Rather, they were in significantly better physiological condition than hares collected at either of the other two times. We conclude that the difference of ~30 d between the collection times in late February (1991 and 1992) and in late March (1994) cannot account for the results we obtained, and thus that the differences we observed were related to the phase of the cycle from which the hares came.

Second, sample sizes were small and this may have affected the results. Clearly, it would have been better to increase the number of hares sampled each year. However, by the low of the cycle the boreal forest was virtually empty of hares, with densities falling from 150 animals/km^2 at the peak to 8 animals/km^2 at the low, and only with considerable effort were we able to get the sample sizes we did. Theoretically, low sample size would have increased the standard errors of our estimates and decreased our probability of finding significant effects if such effects were really there. However, in spite of our sample sizes, the analyses indicate that for much of the data standard errors were extremely tight, resulting in probabilities that were often very or highly significant (Tables 3 and 4; Figs. 4 and 5).

Third, was the experimental protocol we followed the optimal one (trapping, bringing hares to a field laboratory, and then sequentially injecting them with Dex and ACTH), as it may have stressed the animals too much and compromised our results? It is clear from the comparison of the physiological profile of shot hares from 1991 with that of initial bleeds of stressed hares from 1991, that trapping and handling hares stressed them (Boonstra and Singleton 1993). However, from our radiotelemetry data, our procedure, though stressful, did not appear to compromise their long-term survival. Other protocols utilized in field endocrinology must be considerably less stressful than our method. All basically involve capturing resting animals and immediately beginning a series of sequential bleeds. For example, Sapolsky (1983) darted baboons from behind and used their response to the anesthetic as a standardized stressor to discriminate between dominants and subordinates. Astheimer et al. (1994) used a capture (mist netting) and handling protocol as a standardized stressor in sparrows and effectively discriminated among classes of birds. Dunlap and Wingfield (1995) used a noosing protocol as a standardized stressor in lizards with equally good effect. However, it is clear from recent brain research that stressors in rats start affecting the biochemistry associated with brain glucocorticoid receptors within 60 min (Herman and Watson 1995). Thus, if other vertebrates respond in a similar manner, all protocols, including ours, must rapidly affect brain organization. However, since virtually all field endocrinology studies are *comparative* in nature (i.e., we are usually less interested in the absolute effect of our protocol on the animals then in the differences among classes of animals), what is critical in all protocols is that the procedure be standardized and that there be no systematic bias across time. Similar comparative assessment has been carried out in human medicine with good effect (e.g., Schmider et al. 1995). In addition, in virtually all studies of all small mammals and many of the larger ones (ungulates and carnivores), animals must be trapped, and since one cannot be there instantaneously to begin the bleeding procedure after capture, a protocol is needed that gets around the problem of the delay between capture and bleeding. As well, severe conditions, such as those occurring during the winter in the Yukon, preclude bleeding animals in the field. Thus, in situations where one cannot eliminate the stress response of animals prior to bleeding, what is required is a protocol that standardizes the present and essentially overrides it to get an integrated picture of the past. The sequential challenge with Dex and ACTH is a particularly good probe of the function of the hypothalamic–pituitary–adrenal (HPA) axis, has been used to good effect in many other studies (see *Introduction* for citations), and, as the results from our study attest, was also extremely effective in snowshoe hares in permitting us to discriminate among animals from different years and treatments (Tables 2 and 3; Figs. 4 and 5).

Changes in HPA response

Free cortisol levels were higher both at the baseline bleed and following the ACTH injection during the decline-phase years (1991 and 1992) than during the low year (1994) (Fig. 4A, Table 3). These results are consistent with the former hares being chronically stressed whereas the latter were not. Chronic stress causes adrenal hypertrophy in most, but not all, species of mammals (Christian 1980, Harvey et al. 1984, Miller and Tyrrell 1995), and though we did not measure adrenal gland size, our results imply that hypertrophy had occurred as there was clearly a much greater capacity

to respond both to the stress of capture and handling and to the ACTH test.

Free cortisol levels showed dexamethasone resistance during the decline, but not the low, and then reached significantly higher levels in response to the ACTH injection during the decline than the low and on the experimental grids than the controls (Fig. 4A, Table 5). The dexamethasone response was predicted for the decline and is typically seen in animals that have been chronically stressed. For example, in male dasyurid marsupials that have just finished the intense breeding season and shortly before they all die, the HPA axis is resistant to Dex injections (McDonald et al. 1986, Bradley 1990). The cortisol response to the ACTH injection was dramatic. The hares clearly were responding to the ACTH at all times and thus the adrenals were not less sensitive to ACTH, as they become in humans (Miller and Tyrrell 1995). Similar results to ours have been found by Sapolsky (1983) working on baboons (subordinates, who did show Dex resistance, responded similarly to dominants when given ACTH) and by McDonald et al. (1986) working on the dasyurid marsupials (males during the breeding season continued to respond to ACTH in the same manner as males before the breeding season). In contrast, Harlow et al. (1992), working on cougars that had been repeatedly chased by dogs, found these cougars to show a diminished response to ACTH in comparison to control animals. In the cases of the hares, baboons, and dasyurids, all had been exposed to stressors that they would regularly encounter in nature and thus should be adapted to, while in the case of cougars it is unlikely that they are adapted to repeated hunts with trained dogs.

The shift in the free cortisol response to ACTH occurring over our study (decreasing from the decline to the low) was directly tied to the shift in the opposite direction of the maximum corticosteroid binding capacity (MCBC). During our study, MCBC levels changed from low values in 1991 to high values in 1994. Since it is the free, non-CBG-bound cortisol that is biologically active, higher levels of free cortisol would mean greater impact on the biological areas listed in Fig. 1. Corticosteroid-binding globulin (CBG) is the primary transport protein in plasma (Rosner 1990) and levels typically show little variation over the short term, though a variety of stressors resulting in an increase in glucocorticoid levels can cause CBG levels to fall (Kattesh et al. 1980, Levin et al. 1987, Schlechte and Hamilton 1987, Frairia et al. 1988). Our results indicate that though CBG levels may remain constant following an ACTH injection (Boonstra and Tinnikov 1998), the binding capacity of the plasma for cortisol (our MCBC values, Fig. 4B) does not and rapidly increases over the short term (30–240 min). This is directly related to the rapid mobilization of free fatty acids (FFA) (Fig. 4C), which has been shown to increase CBG binding capacity in rats (Haourigui et al. 1993). As plasma FFA levels fall, so do the MCBC levels. Free cortisol levels remained high or increased, primarily because the ACTH we injected is a long-lasting form, whereas the FFAs were being utilized by the liver.

Thus the depressed MCBC levels seen in hares during the decline relative to the low indicate that the latter were better buffered from the potentially deleterious effects of free cortisol than the former. In addition, CBG is thought to act as a reservoir of glucocorticoids, so that there can be a rapid release of glucocorticoids in response to an environmental stressor (Rosner 1990). Free cortisol is normally rapidly excreted by the kidneys. Thus, the higher MCBC levels in the hares from the low could also be adaptive in that it served to maintain the cortisol signal by retaining it in the plasma compartment at levels significantly higher than basal levels for a longer period of time, thus keeping the animals in a state of readiness for a short time subsequent to the initial stressor. In nature continued vigilance after an initial challenge, either because of a predation attack or a social interaction with conspecifics, would make sense and keep the stress axis "primed" for a subsequent challenge.

Mobilization of energy

Glucose mobilization differed dramatically in control hares, being much greater in 1991 than 1994, when little response occurred (Fig. 4D). This evidence supports the prediction that hares during the decline were stressed and had chronically high levels of cortisol, while those in the low were not. Glucocorticoids, such as cortisol, promote gluconeogenesis (the production of new glucose through the breakdown of other body tissues), and this glucose will be stored in the liver as glycogen (Miller and Tyrrell 1995). For example, in a recent study on dogs, chronic cortisol administration increased liver glycogen two-fold (Goldstein et al. 1993). Since both Dex and ACTH mobilize glucose from liver glycogen stores, a greater mobilization must mean greater stores. Gluconeogenesis also comes at the expense of peripheral tissues, by inhibiting non-hepatic glucose utilization, by promoting substrate delivery to the liver, and by conversion of protein to glucose. The latter may help to explain why overwintering loss in mass occurs in hares during the decline (Keith and Windberg 1978). This loss cannot be accounted for by non-protein sources, as the winter body reserves of hares are minimal (Whittaker and Thomas 1983). Experimental hares, which were food supplemented, showed a similar glucose response in all years (Fig. 4D) that was less than that of 1991 control hares but more than that of 1994 control hares. Thus, access to high-quality food may have compensated to some extent for exposure to higher cortisol levels.

Free fatty acids (FFA) are one of the energy substrates delivered to the liver during the stress response for gluconeogenesis (Miller and Tyrrell 1995), and in hares only ACTH causes this mobilization (Fig. 4C).

Though our data are less complete than for other parameters (for reasons indicated in *Materials and methods*, above), they are sufficient to indicate that FFA values were significantly lower in controls in 1991 than in the 1994 (1992 was intermediate). Reduced availability of FFA in the decline is consistent with the argument of chronic activation of the HPA axis. The Fed animals generally had higher FFA values than controls, though both groups converged by 1994. Changes in plasma FFA levels have been linked to changes in conformational structure of CBG in rats, causing increased cortisol binding (Haourigui et al. 1993), which should cause an increase in MCBC. In addition, preliminary data (Boonstra and Tinnikov 1998) indicate that binding by the most abundant plasma protein, albumin, is also stimulated by increased FFAs, which should also increase MCBC. However, Fig. 4B clearly indicates that the MCBC responses were still less dramatic than the FFA responses in 1991 and 1992. Part of the answer may lie in the type of FFA (saturates vs. unsaturates) being released by the ACTH. Haourigui et al. (1993) indicate that only unsaturated FFAs are able to increase CBG binding in FFA, but we had insufficient remaining plasma in the samples to identify the individual acids.

Suppression of reproduction

Reproductive output per female per year mirrored the changes in survival during the decline and low, with values being 25–50% lower during the decline than the increase and then recovering during the low (Fig. 3). Cary and Keith (1979) found similar changes in hares in Alberta. Reproduction is known to be inhibited by a variety of physical and psychological stressors (Munck et al. 1984, Greenberg and Wingfield 1987, Chatterton 1990). Over the 3 yr of our study, maximum natality occurred during the low when females had the smallest mass (Table 1) and appeared to be the youngest (based on our index of age—the right hind foot). Cary and Keith (1979) found the same relationship. This is contrary to the conventional pattern that large body size and greater age generally results in greater natality (Stearns 1992). Though other factors such as poor nutrition may also be linked to declining reproduction in mammals (Bronson 1989), our results are consistent with the hypothesis that stress played a role in the pattern we observed.

Male reproduction can also be inhibited by a variety of either psychogenic or physical stressors that cause an increase in glucocorticoid levels (Greenberg and Wingfield 1987, Levine et al. 1989). In all years, the dexamethasone injection suppressed testosterone levels (Fig. 5) and this was expected because Dex is a glucocorticoid. Dexamethasone suppression of testosterone has also been observed in other wild mammals (marsupials: Vinson and Renfree 1975, McDonald et al. 1986, Bradley 1990; baboons: Sapolsky 1985) and in laboratory animals (Bambino and Hsueh 1981). The dramatic difference among years followed the ACTH injection: control males in 1994 showed the transitory increase in testosterone levels (as indicated by high levels at the post-30-min bleed), males in 1991 and 1992 did not. In the experimental males, testosterone levels increased temporarily in all years following the ACTH injection, though it was most dramatic in 1991. A similar transitory response has been observed in other studies. Sapolsky (1985) stressed dominant and subordinate olive baboons (*Papio anubis*) with immobilization and found that during the first hour testosterone levels rose in dominants but fell in subordinates. Mann and Orr (1990) stressed good-condition laboratory rats by restraint stress and also found a transitory increase in testosterone levels lasting 1 h. We interpret our results to mean that 1994 control hares and experimental hares at all times were less stressed than 1991 and 1992 control hares.

Suppression of immune responses and effects on condition

Higher levels of free cortisol should act as an immunosuppressant (Keller et al. 1984, Munck et al. 1984, Kelley 1985), and we see this reflected in lower levels of white blood cells involved in the immune response (neutrophils, lymphocytes, and monocytes, Table 4) in 1991 than in 1994, with both control and experimental hares being similar. Eosinophil counts, however, go in the opposite direction, being higher in 1991 than 1994. Dieterich and Feist (1980) have done the only other hematology study in snowshoe hares, but their data span only the peak (1972) and first year of the decline (1973). In the latter year, they found cell counts that were roughly similar to ours, though their neutrophil levels were much lower. Eosinophilia is known to be associated with parasitic worm infections (Bullock and Rosendahl 1984) and the latter were found to be common in declining hare populations in Alberta (Keith et al. 1986). Cary and Keith (1979) found that a variety of endoparasites were highest during the decline. D. Hik and A. R. E. Sinclair (*personal communication*) corroborates this evidence, also finding high levels of infection by coccidia in the decline hares and low levels in those from the low.

The second aspect of hematology that improved markedly from the decline to the low was the red-cell packed cell volume (PCV) (Fig. 6), a condition indicator (Lochmiller et al. 1986, Hellgren et al. 1993). Though mass and right hind foot also change (Table 1), they may be confounded by age effects, and hence we discuss changes in them below (see *Long-term consequences and maternal effects*). PCV increased from 40.6% in 1991 to 46.6% in 1994 in controls when levels converged on those found in experimental hares (48.3%). These levels were obtained from the Base bleed and represent levels of hares already stressed by handling. A sample of hares, shot in February 1991, had significantly lower PCV levels (36.4 ± 1.4%, N =

24 animals) than control hares (40.6 ± 0.5%, $N = 12$ animals, $P = 0.04$) (R. Boonstra and G. R. Singleton, *unpublished data.*). The most likely explanation for this difference is that the spleen, a storage site for erythrocytes, contracted in response to an adrenalin surge caused by the stress of handling and capture and released erythrocytes into the blood (Guthrie et al. 1967, Cross et al. 1988), causing the temporary increase in PCV. The adaptive argument for this sudden release is that increased numbers of erythrocytes increase the oxygen capacity of the blood immediately at a time that the animal needs it for fleeing or fighting. Clearly, control hares in 1991 had a much reduced ability to mobilize erythrocytes than those in 1994. Dieterich and Feist (1980), from a shot sample of hares in Alaska (peak and decline hares), reported values similar to ours (ranging from 31 to 43% over their eight different sampling times, mean 39%). The changes in PCV from 1991 to 1994 may in part be related to changes in nutrition (e.g., as found in collared peccaries [Lochmiller et al. 1986] and in black bears, [Hellgren et al. 1993]). However, in the laboratory glucocorticoids inhibit erythrocytes (Zalman et al. 1979, Leung and Gidari 1981), and thus stressors may be also affecting red-blood-cell levels directly.

Alternative explanations

The above evidence is consistent with the hypothesis that the decline hares were exposed to chronically higher levels of some environmental stressor(s) that stimulated the HPA axis to produce higher levels of cortisol and that had detrimental effects on other physiological processes (Fig. 1). There are three potential causal mechanisms that could explain our results: first, the negative effects of high density; second, the negative effects of insufficient and/or low-quality food; and third, the detrimental effects of high predation risk. We will discuss each in turn and argue that only high predation risk during the decline adequately accounts for the evidence.

Density.—High density, acting through intense social interactions and competition, may detrimentally affect the HPA system through chronic stimulation. Christian's "stress" hypothesis (1980) recognized this explicitly, and he argued that the effects of high density acting through spacing behavior should cause poor reproduction and survival, and eventually a population decline. There is little field evidence that this occurs (Krebs and Myers 1974, Lee and McDonald 1985, but see Boonstra and Boag 1992). If the results from control populations were simply the reflection of changes in density, then the experimental populations, which had densities between 4 and 13 times those of the control populations, should have been even more stressed and less able to handle the challenge tests. They were not. By virtually every measure (MCBC and free cortisol levels, Figs. 4A and B, and testosterone levels, Fig. 5; glucose response in 1992 and 1994, though not in 1991, Fig. 4D), the hares from higher density experimental populations performed better than the lower density control populations. Keith (1990) also found no relationship between adrenal gland size (heavier ones being an indication of more stressed animals) and density in the hare populations. Finally, hares in winter show no significant spacing behavior that is likely to be affected by density (they are not territorial and home ranges overlap broadly) (Boutin 1984). We conclude that density changes cannot explain the physiological changes we observed.

Food.—Lack of food or lack of the right quality of food during the decline may explain our results. Reduced food intake in mammals has been found to cause an increase in corticosteroid levels in some (Harris et al. 1994), though not all, studies (Miller and Tyrrell 1995). For example, Sapolsky (1986) found that during a drought, when food was at a premium, wild male olive baboons spent considerably more time foraging and walking to find food but that cortisol levels were unaffected. In snowshoe hares, there are five arguments against the hypothesis that changes in food can explain our results. First, all our estimates of forage availability indicate that overall food quantity was not limiting at any time, though foraging behavior may have limited access to it (Smith et al. 1988, Hik 1995). There is no evidence to indicate that there were significant changes in forage quality (Sinclair et al. 1988). Second, if hares are prevented from experiencing either an absolute or relative food shortage, as on the experimental grids, they should be in good condition throughout the decline and low, and should show a similar stress response in all years. In fact, food addition clearly did have distinct benefits in terms of energy levels, as experimental hares had a similar glucose response in all years (Fig. 4D). However, their hormonal stress response, though generally better than control hares, was not similar in all years, but rather showed a progressive improvement from 1991 to 1994, with increasing MCBC levels (Fig. 4B) and declining free cortisol levels (Fig. 4A). The improvement in the stress response of the experimental hares paralleled that of the control hares, and both converged by 1994. Third, chronic stressors are known to depress the immune response (Keller et al. 1984, Kelley 1985, Miller and Tyrrell 1995), and if lack of food was acting as a chronic stressor then our index of the immune response, leucocyte counts, should be higher in experimental than control hares. They were not, being similar on both treatments in both years, but being significantly lower in 1991 than 1994 for all cell types except for eosinophils (Table 3). The latter are known to increase in response to increased nematode infections, which were higher in 1991 than 1994 (D. Hik and A. R. E. Sinclair, *unpublished data*). Fourth, body mass is also expected to decline under high stress levels because of gluconeogenesis (Miller and Tyrrell 1995). Body mass decreased overwinter during the decline on both control areas and on experimental areas that were

fed, but not protected from mammalian predation; no decrease occurred on fenced areas from which mammalian predators were excluded whether or not they were fed (Hik 1995). Thus food by itself was insufficient to prevent body-mass loss. No overwinter decrease in body mass was observed in the low (C. J. Krebs, D. Hik, C. Doyle, and S. Boutin, *unpublished data*). Fifth, overwintering body-mass loss has been observed during the decline in both our populations (Hik 1995) and in Alberta (Keith and Windberg 1978, Cary and Keith 1979), but it is an enigma why mass loss should have any affect on summer reproduction (which it appears to), as females in summer must be relying primarily on food taken in during that time, as their body reserves are extremely small. Whittaker and Thomas (1983) have shown that hares in winter have only sufficient internal reserves to survive 2–4 d without feeding. We conclude that lack of food cannot explain our results.

Predation.—Finally, higher predation risk may result in hares being more stressed psychologically during the decline than the low because of failed attacks on themselves, successful attacks on nearby hares (hares may emit a high-pitched scream when attacked), or frequent encounters with predator sign. Three lines of evidence indicate that predation risk was indeed higher during the decline and lower after it. First, the majority of hares (83%) died because they were killed by predators (Krebs et al. 1995) and thus the proximate cause of the decline was predation. Second, the numbers of the main predators of hares on our study area were high during the hare decline and low after it: from 1990 to 1992 there were 46 Great Horned Owls and Goshawks per 100 km^2 in the valley and these declined to 22 birds/100 km^2 in 1994; respective values for lynx and coyotes were 19.9 in 1990, 25.6 in 1991, 12.7 in 1992 and 3.5 in 1994 (Boutin et al. 1995). Third, overwinter survival on control areas (1 October to 1 April) (obtained from Kaplan-Meier survival estimates using staggered entry design of radiocollared hares [Krebs et al. 1995]) was extremely low during the decline, but improved markedly in the low, being 0.12, 0.005, and 0.50 in winters of 1990–1991, 1991–1992, and 1993–1994, respectively. A parallel change in survival occurred on the Food grid (1990–1991: 0.43) and Predator Exclosure + Food grid (1991–1992: 0.46, 1993–1994: 0.68), with survival being consistently better on these grids than on the control grids (Krebs et al. 1995). In 1991, hares on the Food grid were exposed to both avian and mammalian predators, yet, as indicated above, neither group appeared to have focused their efforts on this grid, and hare survival was 3.5 times that on the controls. In 1992 and 1994, hares on the Predator Exclosure + Food grid were exposed only to avian predators (which cause ~40% of all deaths on control areas in the decline [Krebs et al. 1995]), and hare survival was much higher in 1992 than on controls and slightly higher in 1994. The 20% survival improvement seen on the Predator Exclosure + Food grid from the winter of 1991–1992 to that of 1993–1994 also indicates that this grid, too, experienced greater predation loss in the former winter, though much less than on the controls. Thus, predation pressure was particularly intense during the decline (being much more severe on the controls than on the experimental grids) and ameliorated during the low. This change in predation pressure paralleled the physiological changes and was inversely related to natality (Fig. 3).

There are two lines of evidence to show that the hares in our study area were responding to increased predator risk during the decline. First, there was a marked shift in foraging locations over the decline (Hik 1995). In 1991, while males continued to forage in open shrub and spruce habitat, females started to shift their habitat use to closed spruce habitat. By 1992, both sexes were using closed spruce habitat heavily and avoiding open sites with high-quality food. By 1994, both sexes were again using open habitats (O'Donoghue et al. 1998*a*). Second, the diet on control areas changed markedly from 1991 to 1992, from the high-quality shrubs found in more open habitat (willow and birch) to the low-quality spruce found in high-cover areas. In the winter of 1990–1991, willow was consumed at 42% of the feeding sites and spruce was consumed at 58% (Hik 1995). In contrast, in the winter of 1991–1992, willow was consumed at 8% of the feeding sites and spruce at 91%. Thus spruce consumption increased markedly in the second year of the decline and this increase was also found by Smith et al. (1988).

The extensive data collected by Keith and his students in Alberta (summarized in Keith 1990) reinforces our conclusion that predation risk has major effects on hare physiology and reproduction, but it also suggests that the effects of this risk may start operating even earlier than the decline. They found that the maximum natality in hares occurred during the first year or two of the increase phase, and that this was coincident with little or no overwintering body-mass loss. However, natality started to decrease and overwintering body-mass loss started to increase shortly thereafter and a full 2–3 yr before the first decline year. It is unlikely that reduced food is the explanation at this early phase of population growth. These changes in natality and body mass are, however, coincident with increases in predator numbers, and this suggests that hares are extremely sensitive to changes in predator abundance.

Why should the perception of increased predation risk be stressful to hares? First, it is clear that the HPA system is just as responsive to psychological stressors as it is to physical ones (Mason 1968). Second, the crucial factor with respect to the intensity of response to psychological stressors is related to the lack of control and predictability that the animals experience. Weiss (1984) showed that lack of predictability increases the adrenocortical response in rats. Rats in two groups were shocked with electricity, with one group

receiving a signal indicating the impending shock, the other receiving no signal. The former had much lower levels of adrenocortical response. The second group may be analogous to the situation in which hares find themselves, particularly during the decline when predators are abundant. Hares cannot predict when the next attack will come, and as a result they are forced into a state of continuous high vigilance. We propose that it is this constant need for high vigilance together with the occasional reinforcing predator attack that causes hares in the decline to be more stressed than those during the low.

Can the addition of high-quality food mitigate the effects of high predation risk? Our results indicate that the hares on the Food grid and on the Predator Exclosure + Food grid were more stressed in 1991 and 1992 than in 1994 (Fig. 4A and B, Table 4), but two body-condition indicators, their glucose (Fig. 4D) and PCV response (Fig. 6), were largely unchanged. In addition, natality on the Predator Exclosure + Food grid remained high (Krebs et al. 1995). Clearly the risk was less on this grid as only avian predators had access to the hares here. Ideally, it would have been beneficial to have detailed information on natality on grids where just food was added or where just predators were removed, but because of manpower limitations and insufficient numbers of hares on these latter areas, these data could not be obtained. The presence of overwintering body-mass loss (a good correlate of the decline in natality [Royama 1992]) in populations that were fed, but not protected from mammalian predation, and the absence of body-mass loss in populations that were protected from mammalian predation, but not fed (discussed above and in Hik [1995]) is consistent with the argument that it was reduced predation risk on the Predator Exclosure + Food grid that permitted the higher natality and the lower loss of body mass. Nevertheless, the data are suggestive that access to high-quality food may reduce the effects of predator-induced stress.

Long-term consequences and maternal effects

Are there any long-term consequences to hares from being chronically stressed by high predator risk and does this play any role in generating population cycles? Two areas may be affected: first, there may be an increase in hare vulnerability to predation, accelerating the rate of decline, and second, there may be a decrease in hare fecundity, delaying the rate of recovery after the predator populations collapse. Hares may become more vulnerable for three reasons: a loss of muscle mass, an increase in developmental asymmetry, and a decline in cognitive function. First, the loss of protein caused by the shunting of energy to the liver (gluconeogenesis) and away from maintenance and repair of muscle will cause myopathy, resulting in muscle weakness and fatigue (Sapolsky 1992*a*). In hares, muscle mass is lost over the winter prior to and during the population decline (Keith and Windberg 1978, Cary and Keith 1979, Hik 1995) and this may reduce the hare's ability to maneuver when pursed by a predator. Second, developmental asymmetry may increase during the decline because of chronic stress and this may also reduce the ability of hares to escape from predators. Witter and Lee (1995) found evidence suggesting that Starlings, stressed by the perception of higher predation risk caused by reduced cover, had increased asymmetry of feather growth. Zakharov et al. (1991) found that developmental stability (or symmetry) in the common shrew was highly correlated to cyclic changes in population density in Siberia and positively correlated to breeding success. There is no evidence for such changes in hares. Third, severe or prolonged stressors, particularly acting through high glucocorticoid levels, have deleterious effects upon broad aspects of cognition, and these may be directly related to changes in the morphology of the hippocampus, a region of the brain central to learning, memory, and spatial ability (McEwen and Sapolsky 1995). Some of these changes may be reversible, though in cases of severe, long-term stress, hippocampal neurons are lost (de Kloet and Joëls 1996). In humans, hippocampal volume appears inversely related to the length of time of the chronic stress (e.g., combat stress, long-term depression, etc.) and some of these changes appear irreversible (Sapolsky 1996). There is no evidence in any species from the wild for the role of chronic stress on brain hippocampal function. Thus, we do not know whether any or all of the above factors act to affect hare vulnerability during the decline. However, indirect evidence suggests that vulnerability of hares increases during the decline. From a detailed analysis of the functional response of lynx at Kluane Lake, O'Donoghue et al. (1998*b*) found that the kill rate per lynx at a given hare density was greater during the decline than during the increase. This suggests that the hares are indeed more vulnerable in the decline and that the above mechanisms, all caused by chronic stress, may act to explain this increased vulnerability.

Hare fecundity changes dramatically over the cycle (Keith 1990, Krebs et al. 1995, this study), being particularly low during the decline, and this may be directly related to the high predation risk hares experience. However, the low phase following the decline, which can last 1–3 yr (Keith 1990), remains an enigma (Boonstra et al. 1998). Why don't hare populations rebound with high rates of reproduction as soon as predator populations have collapsed and food is abundant? We propose that reproductive fitness of females is affected by the degree of predator-induced stress they have experienced in the past, possibly aggravated by a shift in age structure toward older individuals, and that this has direct effects on brain organization, particularly at the level of the hippocampus (Sapolsky 1992*a*, de Kloet and Joëls 1996), which then affects breeding success and progeny fitness. In rats, chronic stress causes loss of hippocampal neurons in old, but

not in young animals (Seckl and Olsson 1995). In hares, Cary and Keith (1979) found that as the decline progressed, the population became composed of older and older animals. Maximal reproductive rates did not occur again until the population was composed of young, low-body-mass hares. Our limited evidence in Table 1 also suggests that the 1992 control females were old whereas the 1994 control females were largely young, low-body-mass animals; subsequent information on reproduction from this latter cohort indicates that they did extremely well (Fig. 3). We suggest that population recovery does not occur until young are born to mothers that have not been stressed by high predation risk. Thus, long-lasting maternal effects may be a consequence of the experience of intense, long-term exposure to high predation risk.

Recent evidence shows that maternal effects are pervasive and are capable of altering the mean and variance of a population's phenotype, thus affecting population quality. Maternal effects, the influence of environmental conditions experienced by mothers on the growth, survival, and fitness of offspring, are well known from both the laboratory and the field (Bernardo 1996, Rossiter 1996), and have been postulated to play a role in the population demography of insects (e.g., Rossiter 1994), reptiles (e.g., Massot and Clobert 1995), and mammals (e.g., Boonstra and Hochachka 1997). In laboratory rodents (mice and rats), stressors experienced by the mother, particularly during pregnancy, can have life-long consequences for progeny fitness and behavior (e.g., Crump and Chevins 1989, vom Saal et al. 1990). In laboratory studies on wild mammals, perception of increased predation risk in red-backed voles (*Clethrionomys rutilus*) by exposure to mustelid odor has been found to depress embryo growth (Ylönen et al. 1992) and juvenile growth and maturation (Heikkilä et al. 1993). In field studies on ungulates, stressors acting through poor maternal condition while pregnant have negative consequences for offspring fitness (e.g., Albon et al. 1987, Mech et al. 1991, Post et al. 1997). In fluctuating meadow voles, Mihok and Boonstra (1992) found that the decline-phase animals brought into the laboratory had much lower rates of reproduction than increase-phase animals. Thus, maternal experiences in mammals can directly affect progeny fitness and may have long-term demographic consequences.

If hares are periodically chronically stressed by high predation risk, what should be done next? We propose four lines of research. First, to establish that hares in the decline and low have intrinsically lower reproductive fitness than those from the increase and peak and that this affects their progeny, hares from these periods should be bred in the standardized conditions of the laboratory and the survival, growth, and reproductive fitness of their progeny determined. Second, foraging behavior changes during the decline and we suggest this is directly related to predator avoidance. An examination of vigilance behavior, how it is learned, and how it changes over the cycle would elucidate this link between ecology and physiology. Third, if developmental stability is affected by stress, the chronic stress hares experience during the population decline would be an ideal situation in which to examine its impact on developmental stability. Fourth, if the stress experienced during the decline causes organizational changes in brain structure (particularly in the hippocampal area, a critical area instrumental in regulating the HPA axis) that then affects cognition, the ability to maintain homeostasis, and reproductive fitness, major changes in brain structure should be obvious (changes in corticosteroid and sex-steroid receptor site levels and in neuronal densities). The brains of hares from the various phases of the cycle should be compared using the techniques developed for laboratory animals to examine whether changes occur in neural density and structure, receptor levels, and receptor mRNA levels (e.g., van Eekelen et al. 1991, Matthews and Challis 1995, MacLusky 1996).

Populations experiencing chronically high levels of predator mortality should also experience a number of sublethal effects induced by high predation risk (Abrams 1992). These sublethal effects should be reflected in a cascade of behavioral and physiological effects in the prey that are a consequence of high predation risk and that may ultimately trade off long-term reproduction for short-term survival. We present evidence for this for the first time in a field population of a wild mammal. These effects should be pervasive in any population experiencing high predator-induced mortality and should be investigated.

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