

INSULIN AND GLUCAGON RESPONSES IN THE HIBERNATING BLACK BEAR

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Abstract: To study the hormonal changes during hibernation in the black bear (*Ursus americanus*), 2 adult male bears were injected with insulin and with glucagon during their active and hibernating phases, and plasma urea, glucose, insulin, glucagon, and corticosteroids were measured before and after. The baseline urea values decreased during hibernation a pattern consistent with protein conservation. Baseline insulin values increased during the fall active phase, when bears are hyperphagic, returning to the normal range during early hibernation. Baseline glucagon levels increased during the fall hyperphagia phase and early hibernation and then tended to decrease at the end of hibernation. Baseline corticosteroid levels were lower during the summer active phase than during the other three periods. The insulin, glucagon, and corticosteroid responses to glucagon and insulin injection were variable, but in general were delayed during early hibernation. The plasma glucose response to insulin stimulation was also delayed during early and late hibernation but more so during early hibernation. The glucose response to glucagon stimulation was delayed to similar degrees during both early and late hibernation. These findings are consistent with decreased glucose utilization and increased lipolysis during hibernation. Furthermore, the apparent increase in glucose utilization at the end of hibernation when fat stores are nearly exhausted suggests a continuum of metabolic activity from early to late hibernation with a transition to the active phase by the end of hibernation.

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Metabolic changes are noted during hibernation in the black bear while the bear maintains nearly normal (within 2 C) body temperature (Nelson 1973, Nelson et al. 1975, Folk et al. 1976). Although the bear does not eat, drink, urinate, or defecate during the months of hibernation, it does not become uremic or dehydrated, and emerges from hibernation with a loss of 15 to 20% of prehibernation body weight. Body composition studies have shown that this weight loss is due almost exclusively to loss of body fat (Nelson et al. 1975, Lundberg et al. 1976). The blood glucose level decreases only slightly during the winter compared to glucose concentration in the summer, but in the active starving bear, blood glucose levels are markedly reduced (Nelson et al. 1975, Nelson 1978).

Current evidence suggests that in small animals, hibernation is controlled by the hypothalamic-pituitary axis. Adrenocorticotrophic (ACTH) blood concentrations decrease in winter hibernation in the European hedgehog (*Erinaceus europaeus*) (Hoo-Paris 1971), correlating with a decrease in sympathetic neurotransmitters (Sauerbier and Lemmer 1977). Ground squirrels show a marked reduction in thyroid function during hibernation coupled with increasing serotonin levels (Hudson and Deavers 1976, Hulbert and Hudson 1976). Experimentally, decreasing serotonin levels will disrupt hibernation (Spafford and Pengelley 1971).

In the black bear, the examination of hypothalamic-pituitary function is complicated by lack of specific antisera for bear pituitary hormones such as thyrotrophin (TSH), growth hormone (GH), and ACTH. Triiodothyronine (T_3) levels decrease during the winter, but the thyroid gland shows normal histology (Seal et al. 1970, Nelson et al. 1973). Previous studies involving measurements of TSH by bioassay and thyroxine (T_4) and T_3 indicated that the black bear manifests hypothalamic hypothyroidism during hibernation (Azizi et al. 1977). Whether hypothalamic hypothyroidism or a generalized hypothalamic hypofunction with secondary hypopituitarism accounts for the changes in the metabolism of carbohydrate, protein, and fat reported previously during hibernation is not known.

Insulin and glucagon stimulation studies were carried out during active and hibernating phases of the black bear's life cycle in order to further elucidate hypothalamic pituitary function during these periods. Plasma glucose, insulin, glucagon, and corticosteroids were measured during these tests. Measurement of GH and ACTH was also attempted, but lack of cross-reactivity with the specific human antisera used in the assay precluded assessment of these hormone levels.

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METHODS

Two adult male black bears were studied for 2 consecutive years (1977–1978) at the Institute Hills Farm and Research Center (Mayo Clinic) in Rochester, Minnesota. Bear 1, who weighed 190 kg at the beginning of the studies, had been in captivity at this institute for 8 years, and bear 4, who weighed 145 kg, had been confined for 3.5 years.

Summer bear quarters were double-mesh cages with a boxed-in corner for sleeping and shade. The bears were fed once daily and had free access to water.

Winter bear dens were located in a vegetable cold-storage cellar on the east side of a hill. Each bear was placed in a separate culvert with bars on either end and provided with straw bedding. The bears were not fed or given water while in the winter quarters.

Each bear, acting as its own control, was studied during the summer (June) and fall (October) active phases, and during early (January) and late (March) hibernation phases. In both active and hibernating experiments, blood samples were obtained in bears anesthetized intramuscularly with promazine and phencyclidine via a syringe and pole assembly.

Two baseline blood samples (30 ml/bear) were drawn at 30 minutes and 5 minutes prior to injection of insulin or glucagon and analyzed for plasma glucose, urea, insulin, glucagon, and corticosteroids.

The insulin stimulation experiment was performed by injection of 0.2 units (U) of insulin per kg of body weight into a femoral vein. A unit of insulin represents the biological activity of 1/22 mg of pure crystalline insulin. Additional blood samples were drawn from either the femoral vein or artery, starting on the side opposite the injection site. Samples were obtained at 5, 15, 30, 60, 90, 120, 150, and 180 minutes after administration of insulin and were analyzed for glucose, insulin, glucagon, and corticosteroids.

The glucagon stimulation experiment was done in the same manner; 2 mg of glucagon was inject-

ed intravenously. Serial blood samples were drawn as for the insulin stimulation experiment.

In the 1st year, the insulin stimulation experiment was performed 7 days prior to the glucagon stimulation experiment and in the 2nd year, 14 days after the glucagon stimulation experiment. This eliminated the possibility that the results obtained were related to the sequence in which the tests were done.

As a control experiment, insulin levels were also measured following intravenous injection of normal saline (2 ml) in 1 of the bears during the fall active period.

Glucose was determined using a Beckman glucose analyzer. Urea was measured by a standard enzymatic method utilizing urease (Marsh et al. 1957). Levels of insulin, glucagon, and corticosteroids (total of cortisol, corticosterone, and 11-deoxycortisol) were measured by previously described methods (Hales and Randle 1963, Murphy 1967, Faloon and Unger 1974).

Statistical analysis of the data utilized the standard *t*-test with significance determined at the 5% level.

RESULTS

The blood parameters measured were similar for the 2 bears during both active and hibernating experiments, so data are expressed as the mean of the values for both bears.

Baseline values

Several differences were seen in the prestimulation blood values during active and hibernation phases (Table 1). During the early hibernation phase, the mean glucose level decreased slightly but significantly ($P < 0.005$) from the active phase levels. During late hibernation, however, the glucose levels again rose to active phase levels. The decrease in plasma urea during hibernation was quite spectacular, and both early and late hibernation values were significantly lower than active values ($P < 0.001$). The highest mean blood insulin level was seen during the fall active phase, but there were no significant differences in insulin levels among any of the experimental phases. Glucagon levels varied widely, but they tended to be highest during the early hibernation phase; again, no statistically significant differences were noted among any of

Table 1. Blood values^a in 2 confined male black bears prior to insulin and glucagon stimulation experiments during active and hibernating phases.

Blood parameter	Active phase		Hibernating phase	
	Summer	Fall	Early	Late
Glucose (mg/dl)	74 ± 5 (63–82)	71 ± 2 (68–76)	60 ± 1 (59–62)	71 ± 6 (61–87)
Urea (mg/dl)	44 ± 5 (34–53)	44 ± 4 (36–55)	8 ± 1 (6–11)	12 ± 1 (9–14)
Insulin (μU/ml)	14 ± 6 (3.0–25)	53 ± 10 (24–66)	17 ± 3 (8.5–21.5)	32 ± 7 (20–44)
Glucagon (pg/ml)	78 ± 13 (57–100)	129 ± 27 (50–164)	175 ± 48 (93–303)	112 ± 31 (62–161)
Corticosteroids (μg/dl)	1.8 ± 0.2 (1.0–2.0)	7.4 ± 0.2 (1.3–13)	7.8 ± 2.5 (2.6–13)	7.6 ± 1.5 (5.4–12)

^a Mean ± standard error of mean of 4 values (range in parenthesis).

the phases. Corticosteroid levels were similar during fall active and early and late hibernation phases. However, the summer active corticosteroid level was significantly lower ($P < 0.02$) than the mean value for the other 3 phases.

Insulin Stimulation Experiment

The plasma insulin response to insulin injection, measured during the fall active and early hibernation phases, revealed a delayed disappearance of exogenous insulin during the hibernation phase. During both phases, maximum plasma insulin levels occurred at 5 minutes after insulin injection. The mean maximum values ($N = 2$) were 3600% of baseline during the fall active and 12,000% of baseline during the early hibernation phase. During the fall active phase, the insulin levels dropped to baseline values by 90 minutes, while during the early hibernation phase, the mean insulin level ($N = 2$) was still 800% of baseline at 180 minutes after insulin injection.

Insulin stimulation produced a prompt drop in plasma glucose levels (Fig. 1) with the lowest levels occurring within 30 minutes after insulin injection during the summer and fall active phases, and also during the late hibernation phase. In contrast, during the early hibernation phase, the glucose response was delayed and the maximum decrease was greater. The return to baseline glucose levels also took longer during the early hibernation phase than during the other 3 periods.

Plasma glucagon response to insulin stimulation (Fig. 2) during both early and late hibernation phases was delayed from that during the 2 active phases. This is demonstrated by both a later achievement of maximum glucagon values and by a failure to return to baseline levels by 180 minutes during the hibernation phase. The maximum glucagon level achieved during the summer active phase was much larger than the maximum value reached during any of the other 3 phases.

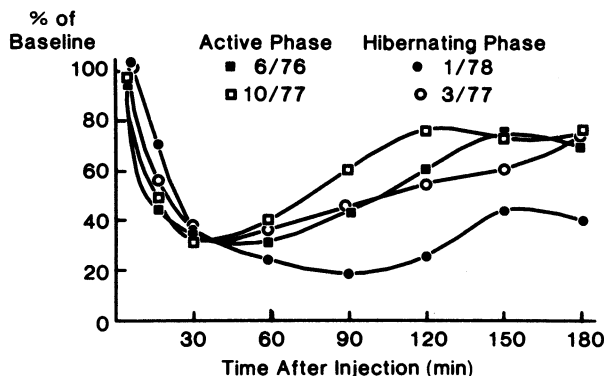


Fig. 1. Plasma glucose response (mean of 2 values) after insulin injection in 2 confined male black bears during active and hibernating phases.

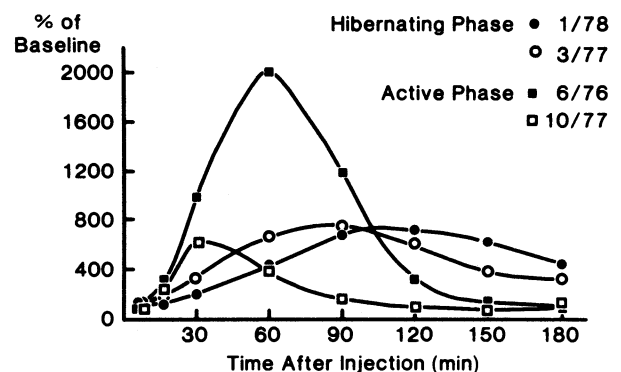


Fig. 2. Plasma glucagon response (mean of 2 values) after insulin injection in 2 male black bears during active and hibernating phases.

Table 2. Plasma corticosteroid levels (mean of 2 values, in $\mu\text{g}/\text{dl}$) following intravenous injection of insulin (0.2 units/kg of body weight) or glucagon (2 mg/kg) in 2 confined male black bears during active and hibernating phases.

Phase	Minutes after insulin injection				Minutes after glucagon injection			
	0	60	90	120	0	60	90	120
Active:								
Summer	1.5	24	24		2	6.2	3.5	
Fall	7.2	9.6	4.6	3.3	7.6	6.8	14	4.7
Hibernation:								
Early	3.6	13	12	14	12	12	11	7.2
Late	5.8	18	23		9.2	12	22	

Plasma corticosteroid response to insulin stimulation (Table 2) paralleled the glucagon responses, with an increase in corticosteroid levels at 60 minutes for all study phases. Again, the maximum value achieved was greatest during the summer active phase.

Glucagon Stimulation Study

Plasma glucagon levels after glucagon injection were measured during the fall active and early hibernation phases. The mean maximum glucagon level ($N = 2$), which occurred 5 minutes after injection, was 1900% of baseline during the fall active phase and 2500% of baseline during the early hibernation phase. Return to baseline glucagon levels during the hibernation phase was delayed, with a mean level of 700% of baseline ($N = 2$) at 180 minutes after injection compared to a mean level of 240% of baseline ($N = 2$) at 180 minutes during the active experiment.

The degree of corticosteroid response to glucagon injection (Table 2) was much lower than the response seen after insulin injection for all 4

of the experimental phases. Indeed, it appeared that during the early hibernation phase, there was no corticosteroid response at all.

Plasma glucose response to glucagon administration (Fig. 3) was similar in the summer and fall active phases. The glucose responses seen during the early and late hibernation phases were also similar to each other; however, the maximum glucose levels achieved were higher, and both the rise in glucose levels and the return to baseline values were delayed when compared to the active phase experiments.

The insulin levels following glucagon injection (Fig. 4) exhibited a more variable response than any of the other parameters measured. Although the maximum insulin levels occurred at different times for all of the study periods, they were similar in degree for all of the phases except the late hibernation phase. The insulin response during the late hibernation phase appeared to be suppressed. During the early hibernation phase, the return to baseline values was delayed when compared to the pattern for the other 3 phases.

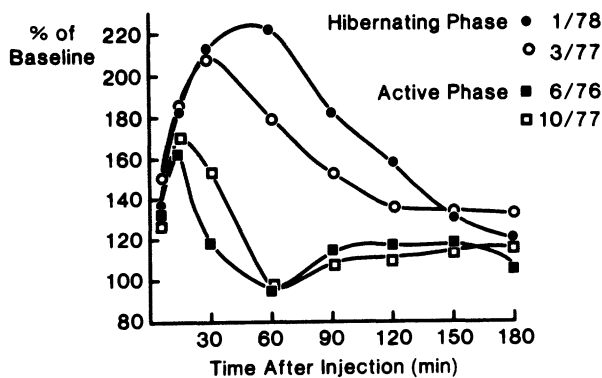


Fig. 3. Plasma glucose response (mean of 2 values) after glucagon injection in 2 male black bears during active and hibernating phases.

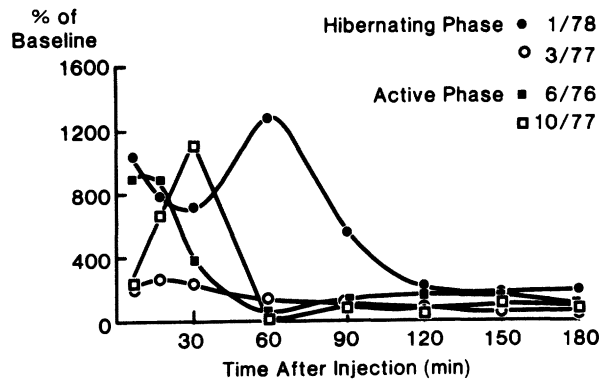


Fig. 4. Plasma insulin response (mean of 2 values) after glucagon injection in 2 male black bears during active and hibernating phases.

Saline Control Study

There was a slight increase in the plasma insulin level 5 minutes after injection of saline in 1 bear during the fall active phase (from 23 μ U/ml at time 0 to 40 μ U/ml at 5 minutes). Insulin levels returned to the baseline value by 14 minutes and remained there until the end of the 60-minute experiment. Such an increase would account for little, if any, of the changes seen during the stimulation tests.

DISCUSSION

Hibernation in the black bear produced changes in basal and stimulated levels of plasma glucose, insulin, glucagon, and corticosteroids. These alterations may be related to hypothalamic hypofunction triggered by external environmental factors. Whether the changes described were influenced by biochemical or hormonal factors, released in response to external stimuli but not measured, could not be ascertained from these studies. Previous measurements of serum thyroxine and thyrotrophin during active and hibernating periods indicated hypothalamic hypothyroidism (Azizi et al. 1977).

In the present studies, basal urea levels during hibernation were significantly decreased, a finding consistent with previous reports of protein conservation during hibernation (Nelson et al. 1975, Lundberg et al. 1976). Basal levels of glucagon and corticosteroids tended to be higher during hibernation, which is consistent with the adaptation of the bear to starvation. This adaptation apparently permits the bear to utilize fat stores rather than protein for energy needs, with a consequent decrease in blood urea.

The delay in insulin disappearance during hibernation was consistent with the prolongation of the decrease in plasma glucose observed during hibernation after the intravenous administration of the insulin. These findings suggest that insulin degradation is impaired during hibernation by mechanism(s) as yet unknown. One possible explanation is that the decrease in the glomerular filtration rate during hibernation (Brown et al. 1971) results in a decrease in insulin clearance by the kidney. It would be interesting to speculate that the delay in insulin disappearance may help

to promote protein conservation and modulate the release of free fatty acids to be utilized for energy during hibernation.

The glucose response to insulin stimulation suggests that in early hibernation, the levels of the antagonistic hormones are reduced or the responses of these hormones to stimulation are suppressed, suggesting hypothalamic hypofunction. The rise in plasma glucagon after insulin administration was prolonged during hibernation, perhaps in response to the prolonged decrease in plasma glucose levels during hibernation. However, the tendency for the plasma glucagon levels to remain elevated with insulin stimulation is also consistent with fat mobilization during this period. The delay in glucagon disappearance after glucagon stimulation during hibernation was consistent with the findings during insulin stimulation. The rise in plasma glucose with glucagon administration during hibernation was delayed and relatively higher than in the active phase of the bear's life cycle. This difference in plasma glucose response between hibernating and active phases may be related to the delay in the disappearance of injected glucagon during hibernation. The findings suggest a decrease in glucose utilization during hibernation as might be expected when an exogenous fuel or energy source is not available or utilized. This decrease in glucose utilization is accompanied by protein conservation and fat utilization as the sole source of energy, a most remarkable phenomenon. Ketosis does not develop as a consequence of fat combustion in hibernating black bears, presumably as a result of increased triglyceride turnover (Nelson 1980).

The rise in plasma insulin after glucagon injection was most marked in the fall active, hyperphagic phase, prior to hibernation. During early hibernation this rise in plasma insulin decreased until in later hibernation and summer active phases the patterns of plasma insulin response were quite similar. These findings describe a shifting continuum of metabolic activity from early to late hibernation to the active phase.

The corticosteroid levels after stimulation with insulin and glucagon suggest hyporesponsiveness of the pituitary-adrenal axis as the bears go into hibernation. This hyporesponsiveness may in turn be a consequence of hypothalamic hypofunction.

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