Protein and fat metabolism in hibernating bears[1]

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ABSTRACT

Hibernation in the bear (*Ursus americanus*) is unique in that it is continuous for 3 to 7 months and occurs at near normal body temperature, yet the bear does not eat, drink, urinate, or defecate. During hibernation there is no loss of lean body mass because amino acids enter protein synthetic pathways at increased rates producing reciprocal decreases in entry into the urea cycle. The urea that is formed is hydrolyzed and the nitrogen released is combined with glycerol to form amino acids, which reenter protein synthetic pathways. Body fat supplies the substrate for metabolism (4000 kilocalories/day). Ketosis does not occur. Metabolic water is sufficient to maintain normal hydration. About 100 ml of urine is filtered daily by the kidneys but the bladder wall transports water and solute back into blood at a rate about equal to their entry into the bladder. The bear cannot duplicate its winter adaptation in summer when housed in the cold and dark. During hibernation the bear shows hypothalamic hypothyroidism and increased testosterone production. These changes appear necessary for developing the selective states of anabolism and catabolism found in the hibernating bear. — **Nelson, R. A.** Protein and fat metabolism in hibernating bears. *Federation Proc.* 39: 2955–2958; 1980.

In an evolutionary sense, hibernation in the bear represents perhaps the most refined response to starvation of any hibernating or nonhibernating mammal. The bear is considered a hibernator because its reactions are similar to those of small hibernating animals that show a decreased heart rate, metabolic rate, and body temperature (6). Nevertheless there are several reasons why its hibernation is unique. The bear hibernates at a near normal body temperature, 31 to 35 C. Its dormancy is continuous from 3 to 7 months. Although expending about 4000 kilocalories per day (calculations based on body fat utilization rates), the bear neither eats, drinks, urinates, nor defecates. It is easily aroused into a mobile, reactive state, aware of its surroundings, able to defend itself. Female bears give birth to cubs and nurse them under these stressful conditions (13).

When considering these features of hibernation in contrast with those of small mammals, the adaptation of the bear in winter can be best described with one word, extraordinary. For instance, the 13-lined ground squirrel hibernates only 4 to 10 days at a time. It undergoes periodic arousals and must have food present because while aroused it eats, urinates, and defecates. While hibernating, its body temperature is near 0 C; it cannot protect itself from predators (6).

Although the bear starves throughout hibernation there is no net formation of the common metabolic end-products of protein metabolism in the blood. The concentrations of amino acids, total protein, urea, and uric acid remain unchanged throughout winter as do blood volume, hydration, and hematocrit (4, 17). However, blood creatinine concentration increases from about 1 mg/dl to 3 mg/dl early in hibernation but then shows no further change. The amount of creatinine required to produce this increase represents only about 1 or 2 days of usual production (17).

There is no evidence of intestinal storage of nitrogen in feces. Four fecal samples obtained from four bears on the first day after hibernation showed practically no nitrogen in the samples, values ranging between 0.13 and 3.9 g (17).

Since nitrogeneous end-products do not accumulate in blood and are not excreted in urine or stored in feces, lean body mass must remain constant. This conclusion was supported by body composition studies using the D_2O dilution technique (15); lean body mass remained essentially unchanged throughout hibernation (9, 16).

Therefore, combustion of fat must supply the energy for hibernation metabolism. Data collected in hibernation support this conclusion. The respiratory quotient (RQ) decreases from 0.78 to between 0.62 and 0.69 (1, 17). Blood concentrations of cholesterol, triglycerides, fatty acids, and phospholipids increase and remain elevated until the bear emerges from hibernation and resumes activity in the spring (17, 18). The repeatable finding of a decrease in RQ below that of 0.71 (representing pure fat combustion) suggests that fixation of CO_2 is occurring, thus reducing respiratory losses of carbon.

Also, since fat appears to be the only substrate utilized for energy requirements, metabolic water produced from it must be the only source of water for replenishment of respiratory losses. Since total body water, red cell and plasma water, hematocrit (17), and blood volume (4) remain within expected normal limits, metabolic water is produced in sufficient quantities to meet these needs. De-

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spite no urination during hibernation, about 100 ml of urine is filtered daily by the kidneys (4). However, the bladder transports the water and solute back into the bloodstream at a rate about equal to their entry into the bladder; thus no urination occurs (16).

PROTEIN METABOLISM

The ability to preserve lean body mass and to maintain normal levels of blood alanine despite months of no food or water, with body temperature near normal, appears limited to the bear. To date, no other animal, hibernating or nonhibernating, has been shown to possess these abilities.

The importance of inhibiting the net production of urea, the principal end product of protein catabolism, was demonstrated by injection of urea into the bloodstream of hibernating bears. It induced diuresis, caused intracellular dehydration, and clearly disrupted hibernation (17).

Despite conservation of lean body mass and absence of net production of urea during hibernation, there is metabolic activity: anabolism and catabolism of proteins occur (1, 9), urea is formed and degraded (16), and amino acids enter into protein synthesis (1, 9) and are transaminated and catabolyzed (1). However, there are profound changes in the rates of these processes, which appear essential for maintenance of the state of hibernation.

The rate of protein turnover increased three- to fivefold in hibernation when calculated using labeled albumin as an indicator (9). This finding was confirmed by studies of incorporation of ^{14}C-labeled leucine (9) and alanine (1) into plasma proteins. Per unit time, considerably more leucine and alanine were incorporated into plasma proteins during hibernation than during active states, although circumstances were similar in respect to the amount of activity injected, blood concentrations of leucine and alanine, plasma concentration of protein, hematocrit values, and total body water (1, 9).

On the other hand, the rate of formation of urea, calculated from the disappearance of [^{14}C]urea from blood, was greatly slowed (16). This finding was supported by the finding of increased half life of injected [^{14}C]alanine with considerable reduction in $^{14}CO_2$ loss in expired air during hibernation (1).

It was apparent that reciprocal changes in protein turnover and amino acid degradation and urea formation had occurred. Under conditions of no dietary intake of amino acids, when entry of leucine and alanine from blood into protein anabolic pathways increased, their rate of entry into the urea cycle decreased and urea formation decreased. Analogous situations are found in human beings fed low protein diets (25); in protein calorie-deficient children (22); in newborn animals, which show intense protein synthesis but limited degradation of circulating amino acids (12); and in protein calorie-deficient rats, whose essential amino acids are incorporated at increased rates into liver proteins, whereas their oxidative degradation is markedly decreased (10). Thus, such reciprocal changes are not unique to hibernating bears. Usually, in mammalian tissue, a change in one route of disposal of amino acids is accompanied by reciprocal changes in others (12). There are three metabolic outlets for amino acids in the body: protein synthesis, synthesis of compounds of low molecular weight such as creatine and nonessential amino acids, and degradation through pathways of amino acid catabolism (12).

In the bear, there must be increased demands for enzymes necessary for its vitality as it adapts to conditions demanded by hibernation. Surely there must be an increased synthesis of lipolytic enzymes during hibernation because the bear relies mostly on fat as an energy source. Also, protein synthesis is required to provide the proteolytic and synthetic enzymes responsible for the increased protein turnover in the dormant bear.

A by-product of increased protein turnover is thermogenesis. Heat produced during protein metabolism in the hibernating bear may be partly responsible for preventing the profound drop in body temperature during hibernation that is a common feature of the classic deep hibernators such as the 13-lined ground squirrel, woodchuck, and marmot.

Although catabolism of amino acids was greatly reduced in winter, it was a well-defined and distinct process. Carbon dioxide and ammonia were produced (1). The ammonia mu have entered the urea cycle becau urea was constantly formed, althou its rate of formation was also grea slowed in hibernation (16). Howeve urea was also constantly hydrolyz (16), most likely due to diffusion urea across the gut epithelium in the lumen where it can be degrade by bacterial urease. The ammon and carbon dioxide produced di fused back into the blood, and carbo dioxide was lost in expired air. Sinc both urea and alanine were catab lized and no net changes in nitroge containing substances were detecte during hibernation, the nitroge produced must have been reinco porated into lean body mass. A non protein source of carbon, therefor was necessary to combine with th nitrogen thus freed, replacing carbo atoms of amino acids and urea los through catabolic pathways.

FAT METABOLISM

An important—perhaps the mos important—nonprotein source of ca bon that combines with nitrogen wa found to be glycerol released durin lipolysis. Injection of ^{14}C-labeled glyc erol during hibernation demonstrate rapid conversion to alanine and othe amino acids. They, in turn, were incorporated into plasma proteins (1). Thus, nitrogen released from amino acid and urea catabolism was bound with glycerol and ultimately incor porated into protein. This process must operate at a slightly faster rate than the formation of urea, since blood urea gradually decreases as hibernation progresses (1, 4, 16, 17).

Ketosis does not develop in hibernating bears. Only slight increases were found in β-hydroxybutyrate and acetoacetate, increasing from 20 $\pm$ 7 and 9 $\pm$ 2 μM/liter, respectively, before hibernation to 163 $\pm$ 70 and 81 $\pm$ 32 μM/liter during hibernation (multiple values determined on three bears by Dr. T. T. Aoki, Joslin Research Laboratory, Department of Medicine, Harvard Medical School). Human beings, after 5 weeks of starvation with free access to water, showed increases in β-hydroxybutyrate and acetoacetate from 60 $\pm$ 10 and 30 $\pm$ 10 μM/liter to 5850 $\pm$ 380 and 1340 $\pm$ 140 μM/liter, respectively (20).

The prevention of ketosis in the

bear appeared to be due, in part, to glycerol metabolism. Injection of [^{14}C]glycerol revealed that the labeled molecule appeared in lipid esters, including triglycerides, at an increased rate in hibernation (1). Our present hypothesis is that increased triglyceride turnover in winter sufficiently inhibits fatty acid entry into metabolic pathways for ketone production to effectively prevent ketosis. If ketosis developed it would predictably affect acid-base balance in an animal burning 4000 kilocalories of fat daily without urinating. Data in support of this hypothesis are found for the marmot, a deep hibernator, which gradually develops ketonemia in hibernation until arousal occurs. Ketonemia is considered a "trigger" that induces the periodic arousals in this mammal (3).

COMPARATIVE PHYSIOLOGY

Considering the changes in protein and fat metabolism that occur in hibernation, it is interesting that the mechanisms employed by the bear appear to be no different from mechanisms found in both hibernating and nonhibernating mammals, with the bear having the best of two worlds. For instance, human beings with anorexia nervosa show a decrease in body temperature, heart rate, and basal metabolic rate (15). These people also have hypothalamic hypothyroidism (7), a condition that our recent studies have shown is also present in the hibernating bear (2). Starving obese human beings demonstrate increased utilization of body fat, decreased urea production, and marked reduction in lean body mass utilization (20). The dog shows resorption of water and solute through the bladder wall when urine flow is low (8). Small hibernators, like the 13-lined ground squirrel, show no change in blood urea while hibernating (21). However, neither human beings, dogs, ground squirrels, nor any other mammal for that matter can accomplish the fine tuning of these adaptative mechanisms that characterizes the state of hibernation in the bear.

INDUCTION AND CONTROL OF THE STATE OF HIBERNATION

In early studies with bears, it was suggested that special mechanisms associated with hibernation may not be involved in its winter control of protein metabolism. That is, when the bear stopped eating, perhaps inhibition of urea formation occurred sooner and was complete, rather than incomplete, as it is in human beings and some other nonhibernating mammals. If no urea were synthesized, no urine need be excreted. However, in summer, when starved outside at ambient temperature or housed in darkness and cold without food or water, the bear responded as do other warm-blooded animals, including humans, utilizing muscle continuously as a source of energy and becoming dehydrated and azotemic when denied water (16).

Further, there are bears who die in winter in the wilds from unknown causes. One explanation is that they must have failed to achieve the state of hibernation. Two such animals who died in winter highlighted the remarkable difference between true hibernation and starvation. These two bears had lost 30% of their body weight in less than 3 weeks (more than the usual amount lost in 3 months of dormancy), had urinated in their dens, and postmortem blood samples revealed urea concentrations of 650 and 690 mg/dl (14). These findings are similar to those of summer starvation experiments (16). Thus, it was reasoned that the winter state of the bear, which permits independence from food and water, was a result of a specific controlling mechanism or mechanisms.

Studies of deep hibernators have supported this conclusion. A dialyzable small molecular weight substance called "trigger," found in the blood of hibernating ground squirrels, woodchucks, and arctic marmots, will induce hibernation in ground squirrels in summer (5, 19, 23, 24). Urine from hibernating ground squirrels will also induce hibernation in ground squirrels in summer (23). The substance has been likened to insulin in that it is a substance biologically identical, or nearly so, across phylogenetic lines (24). In fact, there is evidence that the "trigger" substance not only crosses phylogenetic lines of different deep hibernators, it crosses over to mammals who do not hibernate. Rats develop hypothermia, to 5 C, lasting 1 to 30 hours when injected with a decorticate brain extract obtained from hibernating ground squirrels. Similar extracts from nonhibernating squirrels do not have this effect (26).

Studies of food intake in bears have indicated that central controlling mechanisms must be intimately involved with the hibernation state. The annual cycles of food intake behavior show that in the spring, after leaving dens, bears are anorectic. This phenomenon has been observed in the wilds and in captive bears taken out of their hibernaculum (14). Caged animals, upon emergence from their dens, do not assume normal food intake until 10 days to 2 weeks have elapsed, although they are allowed normal rations during this time. After 2 to 3 weeks, they eat normally throughout the summer. In the fall, however, bears demonstrate hyperphagia, usually more than doubling summertime rations. Bears consuming 8000 kilocalories per day in summer will consume 15,000 to 20,000 kilocalories per day in the fall. Grizzly bears observed in the wilds feed 20 hours per day in the late fall to prepare for hibernation and they too consume upwards of 20,000 kilocalories per day. When the bear enters hibernation, it again becomes anorectic (14).

These changes in food intake behavior and subsequent studies have indicated that changes in hypothalamic and pituitary function are a necessary feature of hibernation. During hibernation, circulating thyroxine (T4) and triiodothyronine (T3) gradually decrease as hibernation proceeds. Thyroid stimulating hormone (TSH) in serum falls below the detectability range of bioassay. If synthetic thyrotropin-releasing hormone (TRH) is injected into hibernating bears, a delayed, prolonged release of TSH from the pituitary gland occurs and release of T4 and T3 from the thyroid gland is detected. Quantities of T4 and T3 released are identical with the amount released in similar studies of active bears. The low T4, T3, and TSH levels and the delayed, but prolonged, response to TRH suggest a state of hypothalamic hypothyroidism in hibernation (2).

Testosterone levels increase during hibernation yet the animals are anorectic (11). This must be a rare instance indeed, in which increased appetite is not associated with increased levels of testosterone. Our

most recent studies have shown that testosterone is important for the maintenance of hibernation. Castration of hibernating bears caused arousal from hibernation and produced urination and defecation, findings that had not occurred in 9 previous years of study of these animals (18). Two weeks later, while the bears were still in the hibernaculum, intramuscular injection of testosterone produced a profound drop in blood urea concentration in both animals. In one bear it decreased to 0.5 mg/dl. Thus testosterone, or some form of androgen, appears to be involved in the control of blood urea and maintenance of the state of hibernation in male bears. No comparable data are available from female bears. However, it is possible that estrogen and progesterone and/or an adrenal androgen could effect similar responses.

It may well be that the state of hypothalamic hypothyroidism reduces catabolic metabolism, and increased levels of testosterone or other anabolic hormones may support anabolic metabolism in an animal whose food-seeking behavior has been effectively shut off. At the present writing it appears that these changes are a result of the hibernation state and are necessary to bring about the perfect regulation of protein and fat metabolism that occurs in hibernating bears. FP

REFERENCES

1. **Ahlquist, D. A.; Nelson, R. A.; Jones, J. D.; Ellefson, R. D.** Glycerol and alanine metabolism in the hibernating black bear. *Physiologist* 19: 107; 1976.
2. **Azizi, F.; Mannix, J.; Howard, D.; Nelson, R. A.** Effect of winter sleep on pituitary-thyroid axis in American black bear. *Am. J. Physiol.* 237: E227–E230; 1979.
3. **Baumber, J.; South, F. E.; Ferren, L.; Zatman, M. L.** A possible basis for periodic arousal during hibernation: accumulation of ketone bodies. *Life Sci.* 10: 463–467; 1971.
4. **Brown, D. C.; Mulhausen, R. O.; Andrew, D. J.; Seal, U. S.** Renal function in anesthetized dormant and active bears. *Am. J. Physiol.* 220: 293–298; 1971.
5. **Dawe, A. R.; Spurrier, W. A.** The blood borne "trigger" for natural mammalian hibernation in the 13-lined ground squirrel and the woodchuck. *Cryobiology* 9: 163–172; 1972.
6. **Folk, G. E., Jr.** Hibernation. *Textbook of environmental physiology.* Philadelphia: Lea & Febiger; 1974: 280–309.
7. **Hurd, H. P.; Palumbo, P. J.; Garib, H.** Hypothalamic endocrine dysfunction in anorexia nervosa. *Mayo Clin. Proc.* 52: 711–716; 1977.
8. **Levinsky, N. G.; Berliner, R. W.** Changes in composition of urine in ureter and bladder at low urine flow. *Am. J. Physiol.* 196: 549–553; 1959.
9. **Lundberg, D. A.; Nelson, R. A.; Wahner, H. W.; Jones, J. D.** Protein metabolism in the black bear before and during hibernation. *Mayo Clin. Proc.* 51: 716–722; 1976.
10. **McFarlane, I. G.; Von Hold, C.** Metabolism of amino acids in protein-calorie-deficient rats. *Biochem. J.* 111: 557–563; 1969.
11. **McMillin, J. M.; Seal, U. S.; Rogers, L.; Erickson, A. W.** Annual testosterone rhythm in the black bear. *Biol. Reprod.* 15: 163–167; 1976.
12. **Munro, H. N.** *Mammalian protein metabolism*, Vol. 4. New York: Academic Press; 1970. 299–386.
13. **Nelson, R. A.** Winter sleep in the black bear: a physiologic and metabolic marvel. *Mayo Clin. Proc.* 48: 733–737; 1973.
14. **Nelson, R. A.** Urea metabolism in the hibernating black bear. *Kidney Int.* 13 Suppl. 8: S177–179; 1978.
15. **Nelson, R. A.; Hayles, A. B.; Wahner, H. W.** Exercise and urinary nitrogen excretion in two chronically malnourished subjects. *Mayo Clin. Proc.* 48: 549–555; 1973.
16. **Nelson, R. A.; Jones, J. D.; Wahner, H. W.; McGill, D. B.; Code, C. F.** Nitrogen metabolism in bears: urea metabolism in summer starvation and in winter sleep and role of urinary bladder in water and nitrogen conservation. *Mayo Clin. Proc.* 50: 141–146; 1975.
17. **Nelson, R. A.; Wahner, H. W.; Jones, J. D.; Ellefson, R. D.; Zollman, P. E.** Metabolism of bears before, during, and after winter sleep. *Am. J. Physiol.* 224: 491–496; 1973.
18. **Nelson, R. A.; Wellik, D. L.; McMillin, J. M.; Palumbo, P. J.** Role of testosterone in hibernating black bears. *Physiologist* 21: 84; 1978.
19. **Oeltgen, P. R.; Bergmann, L. C.; Spurrier, W. A.; Jones, S. B.** Isolation of a hibernation inducing trigger(s) from the plasma of hibernating woodchucks. *Prep. Biochem.* 8: 171–188; 1978.
20. **Owen, O. E.; Felig, P.; Morgan, A. P.; Wahren, J.; Cahill, G. F., Jr.** Liver and kidney metabolism during prolonged starvation. *J. Clin. Invest.* 48: 574–583; 1969.
21. **Pengelley, E. T.; Asmundson, S. J.; Uhlman, C.** Homeostasis during hibernation in the golden-mantled ground squirrel *Citellus lateralis*. *Comp. Biochem. Physiol.* 38A: 645–653; 1971.
22. **Picow, D.; Taylor Young, T.** The measurement of total protein synthesis and catabolism and nitrogen turnover in infants in different nutritional states and receiving different amounts of dietary protein. *Clin. Sci.* 36: 283–296; 1969.
23. **Rosser, S. P.; Bruce, D. S.** Induction of summer hibernation in the 13-lined ground squirrel. *Cryobiology* 15: 113–116; 1978.
24. **Spurrier, W. A.; Folk, G. E., Jr.; Dawe, A. R.** Induction of summer hibernation in the 13-lined ground squirrel shown by comparative serum transfusions from artic mammals. *Cryobiology* 13: 368–374; 1976.
25. **Steffee, W. P.; Goldsmith, R. S.; Pencharz, P. B.; Scrimshaw, N. S.; Young, V. R.** Dietary protein intake and dynamic aspects of whole body nitrogen metabolism in adult humans. *Metabolism* 25: 281–297; 1976.
26. **Swan, H.** Antimetabolic extract from the brain of the hibernating ground squirrel, *Citellus tridecemlineatus*. *Science* 195: 84–85; 1977.

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