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ANNUAL LIPID CYCLES IN HIBERNATORS: Integration of Physiology and Behavior

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■ **Abstract** Mammalian hibernation is a temporary suspension of euthermia allowing endotherms to undergo reversible hypothermia and generate a marked savings in energy expenditure. In most fat-storing hibernator species, seasonal changes in food intake, triacylglycerol deposition, metabolism, and reproductive development are controlled by a circannual clock. In ground-dwelling sciurid rodents (ground squirrels and marmots), for example, energy intake increases during a summer body mass gain phase, and toward the end of this phase metabolic rate also begins to decrease, resulting in a profound increase in lipid deposition as fat. Increased activity of lipogenic hormones and enzymes correspond with this increase. The hibernation mass loss phase begins after the body mass peak in the fall and ends in spring. During this phase, stored lipids are slowly utilized in a programmed manner by undergoing deep torpor or hibernation during which the hypothalamic setpoint for body temperature is typically reduced to just above 0°C. Throughout the hibernation season, bouts of deep torpor are punctuated by periodic arousals in which brown adipose tissue thermogenesis plays a critical role. Lipid oxidation nearly exclusively fuels deep torpor and most of the rewarming process. The fatty acid composition of stored lipids can affect the depth and duration of deep torpor, and saturated fatty acids may be preferentially used during hibernation, whereas polyunsaturated fatty acids may be preferentially retained. Female and underweight male hibernators terminate hibernation in spring when aboveground food becomes available; in contrast, heavier males with sufficient lipid reserves spontaneously terminate hibernation several weeks before females and independent of food availability. Mating occurs shortly after emergence from hibernation, and the lipid cycle begins again with the completion of reproduction. Lipid deposition and mobilization, temperature regulation, reproduction, and circannual timing are intimately interdependent. The unique manner in which they are controlled during the annual cycle, especially lipid reserves, makes hibernators valuable and promising models for research into the mechanisms underlying these processes in all mammals.

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INTRODUCTION

> It was the best of times, it was the worst of times . . . it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us. . ..
>
> (Charles Dickens, *A Tale of Two Cities*)

These familiar lines at the beginning of the Dickens classic could just as well be the first lines of a text on environmental physiology entitled *A Tale of Two Seasons.* For animals living in temperate or more extreme latitudes, summer is a period of plenty, with moderate temperatures and a bounty of food. Winter, on the other hand, can be bitter, with reduced temperatures and decreased food availability and/or quality. Organisms living in such an environment are faced with the challenge of how to take advantage of a summer of abundance yet survive or, better still, avoid the harsh demands of winter.

BACKGROUND

Definition of Hibernation

Most mammalian species are homeothermic, i.e., they more or less always remain euthermic (i.e., body temperature = 35°C–37°C). Hibernating mammals periodically abandon homeothermy in favor of heterothermy, in which body temperature (T_b) may be reduced to nearly 0°C, then returned to euthermia without ill effect—a degree of hypothermia lethal to most mammals, including humans. T_b decreases to close to T_a, typically as low as 2°C–3°C in ground squirrels and marmots (107, but see 11). T_b, heart rate, breathing, and metabolic rate (MR) are all profoundly reduced during hibernation (30, 69, 107). Fat-storing hibernators generally remain in their burrows or dens throughout winter, and hibernation has been estimated to provide a savings of ~85% over the energetic cost of remaining euthermic during the entire hibernation season (see, e.g., 3).

Reversible hypothermia most likely evolved independently in several mammalian groups (see, e.g., 21, 69) as a de novo adaptation and represents an extension of temperature regulation around a new low T_b setpoint, possibly an extension of the circadian decrease in T_b during deep, slow-wave sleep (for a review, see 82). The apparent central regulation of T_b during hibernation suggests that mammalian hibernation is not the re-emergence of a more primitive or reptilian form of thermoregulation (105).

Most fat-storing hibernators continue to undergo cycles in body mass, food consumption, and reproduction despite being housed in the laboratory under constant conditions of light, temperature, etc. (reviewed in 151). The annual changes persist, but their timing only approximates a year; the duration or period of each animal's circannual rhythm (e.g., days from peak body mass to peak body mass) is unique. One animal may have a period of 320 days, and the animal in the adjacent cage a 370-day cycle. After two years in the laboratory, the circannual cycles of these two hibernators will be ~100 days, or more than three months, out of phase. The fact that 20 animals in the same room express circannual cycles with 20 different periods implies generation by an endogenous circannual clock. The evidence for an endogenous circannual clock is overwhelming; nevertheless, its neural substrate has yet to be identified (see 151). Although the rhythm persists in constant conditions, its period is adjusted to ~365 days by the natural progression of daylight duration in the spring and summer after constant darkness during winter (103).

Scope

This review focuses primarily on annual lipid cycles in fat-storing ground squirrels and marmots because of my own bias and their prominence in the literature (for a recent review on food-storing hibernators, see 90).

ANNUAL CYCLES IN FOOD INGESTION, FAT STORAGE, HIBERNATION, AND REPRODUCTION

It is practically impossible to discuss one of these four functions without considering its interaction with the others. The annual cycle of a typical hibernator, such as the golden-mantled ground squirrel (*Spermophilus lateralis*), has several characteristic phases: (*a*) a body mass gain phase typically is initiated during the spring or summer after reproductive activity is completed; (*b*) a body mass loss phase begins in late summer or fall, depending on latitude or altitude, and is characterized by the animals' immergence into burrows and onset of hibernation; and (*c*) a reproductive phase, which generally produces a single litter each year, begins with emergence from hibernation in the spring. Hibernation invariably is accompanied by body mass loss.

BODY MASS GAIN PHASE

Body Mass Gain is not Simply a Consequence of Increased Food Intake

A neural mechanism appears to control or regulate the deposition and mobilization of lipid stores in white adipose tissue (WAT) around a preferred level (see 38, 108, 122). This in turn presumes the availability of a feedback signal from WAT to the brain sufficiently accurate to reflect total body fat reserves. Regulation of WAT mass has been a point of contention in research on obesity and ingestive behavior, as well as hibernation, for decades. Some of the most convincing evidence for centrally mediated body fat regulation comes from heterothermic species in which WAT mass progressively changes over the course of the annual cycle (see 109).

The increases in fat stores during the body mass gain phase can be quite remarkable. Golden-mantled ground squirrels double their body mass and triple their fat mass (97). Despite its larger size, the arctic ground squirrel also doubles body mass and in a much shorter active season (four months active, eight months hibernating) (67). Plasma lipids mimic the changes in fat reserves during the body mass gain phase (88). Plasma lipids in both males and females are decreasing at emergence from hibernation and continue to decrease until fattening begins. Males start fattening after mating and plasma lipids and fatty acid (FA) synthesis coincidently increase (20, 88). Although female body mass increases during pregnancy and lactation, FA synthesis and plasma lipids continue to decrease until the young are weaned and fat deposition commences, up to seven weeks later than males (88).

Annual Changes in Lipids of Hibernators are Generally Circannual

Peak body mass of juvenile ground squirrels is only two-thirds that of adults (91), exemplifying the challenge for young to both grow and lay down adequate fat

reserves. Although their body mass is lower than that of adults before the initial hibernation season, linear or skeletal growth is completed during the first body mass gain phase (32, 94). The initial body mass peak is significantly lower than the second peak, even under presumably optimal laboratory conditions (43). Increased second-year body mass represents a programmed increase in fat deposition (12%) and fat-free dry mass (15%) (43). During the second mass gain phase, neither total subcutaneous WAT mass nor subcutaneous adipocyte number increases. Both WAT mass and fat cell number increase in abdominal fat from the first to second body mass peak. Perhaps because of its insulative value in low T_as, subcutaneous WAT achieves its full mass and cellularity during the first mass gain phase, whereas final maturity of abdominal WAT depots is delayed one year.

Circannual Interaction of Food Intake and Metabolic Rate

Peak body and fat mass and peak food intake do not occur concomitantly, as is also the case for minimum body and fat mass and food intake (see 35). Food consumption peaks and begins to decrease well before body mass maxima are achieved. This counterintuitive outcome derives from the systematic decreases in metabolic rate during the body mass gain phase. Over the course of the year, the oxygen (O_2) consumption, food intake, and body mass cycles are slightly out of phase (5, 145). In spring, O_2 consumption increases before the spring increase in food intake, accounting for continued decreases in body mass after food intake begins to increase. Similarly, MR begins decreasing by midsummer, several weeks before food intake declines, thereby enhancing late lipid deposition by shifting the energy balance equation toward energy surfeit. Although all three processes are ultimately controlled by the endogenous circannual clock, body fat mass control is the critical function and the other two are likely subservient to its maintenance at a prescribed level.

Metabolic rate of European hedgehogs housed at constant T_a progressively decreases from June to October, but respiratory quotient (RQ) remains steady at a value indicative of the mixed oxidation of carbohydrate and FAs (132). Similarly, from midwinter to spring, hedgehogs housed at a low T_a increase their minimum MR during deep torpor; again RQ remains constant but at 0.72, a value indicating nearly 100% FA oxidation. Marmots and ground squirrels undergo a comparable decrease in euthermic MR during mass gain, decreasing MR by nearly half at the end of the weight-gain phase (8, 135); insectivorous bats even undergo daily torpor to enhance fat deposition before the hibernation season (131). Circannual changes in MR explain the discrepancies between circannual changes in food intake and body mass, and reduced MR at the end of mass gain may function to exaggerate the critical deposition of stored lipids for hibernation.

Changes in Lipogenic Factors During Body Mass Gain

Male ground squirrels emerge from hibernation with residual fat stores that are lost during the first few weeks of aboveground activity, whereas females emerge at their

body mass nadir (see, e.g., 22, 93). In the field, maximum mass occurs in both sexes in late summer–early fall, when male plasma insulin concentrations also peak (22). Female insulin concentrations peak during pregnancy and lactation and remain elevated until peak body mass is achieved. Adequate glucocorticoids provide a necessary permissive anabolic effect for insulin's lipogenic actions (see 22); adrenal glucocorticoids also increase over the mass gain phase, peaking with body mass. In the laboratory, plasma insulin concentrations in fasted yellow-bellied marmots (*Marmota flaviventris*) change little during the annual cycle except during the final weeks of mass gain, when fasting plasma insulin concentrations nearly double (56). Insulin responsiveness to exogenous dextrose, on the other hand, progressively increases throughout the mass gain phase, peaking as body mass peaks; dextrose-induced insulin release remains consistently low during mass loss (56, 137). Interestingly, when insulin concentrations are highest at peak mass, glucose absorption is lowest, indicating adipocyte insensitivity to insulin at the body mass maximum. Insulin concentrations and insulin responsiveness progressively increase during lipid deposition in hibernators and correspond to a shift toward lipogenesis until peak WAT lipid stores are attained and adipocytes become insulin resistant.

Whether adipocytes are lipogenic or lipolytic is a product of shifts in the balance between those factors that promote fat storage (e.g., insulin) versus those that promote energy liberation (e.g., norepinephrine). This balance is then reflected in the ratio of lipogenic enzymes [e.g., lipoprotein lipase (LPL)] to lipolytic enzymes [e.g., hormone-sensitive lipase (HSL)]. A shift to a preponderance of lipogenic factors generally occurs when energy intake exceeds energy expenditure and lipolytic factors usually prevail during negative energy balance. During mass gain in marmots, the ratio of HSL mRNA concentrations (and presumably HSL activity) to LPL mRNA concentrations favors lipogenesis, coinciding with profound fat deposition (147). Fatty acid synthase (FAS), and presumably FA synthesis, increases in WAT, brown adipose tissue (BAT), and liver during body mass gain, peaking at peak mass; FAS activity was highest in liver, moderate in WAT, and lowest in BAT (118, 144). WAT fatty acid CoA ligase, representing the first step in triacylglycerol (TG) synthesis from FA, and another enzyme in TG synthesis, diacylglycerol acetyltransferase (DGAT), follow the same pattern as FAS, increasing in WAT from minimal values in hibernation to maximal values during mass gain. Although hepatic and BAT CoA ligase are moderately high during mass gain, they are much higher during hibernation (see below) (118), possibly reflecting reconversion into TG of the excess FAs liberated by the marked sympathetic activity during arousal from hibernation (see below). When fat deposition is at its greatest, factors for hepatic and WAT FA synthesis and TG synthesis markedly increase.

Although marmot monoacylglycerol acetyltransferase (MGAT) activity—another enzyme involved in TG synthesis—is comparatively low in WAT and BAT relative to liver, it increases significantly during mass gain (118). MGAT activity was 80 times higher in liver than in WAT or BAT and evidenced no annual variation. Because of MGAT's unique properties that specifically retain polyunsaturated fatty acids in TGs, this enzyme is discussed in detail in the "Hibernation" section below.

Programmed Changes in Lipid Mass

The prodigious fat deposition during the mass gain phase is not simply the product of hyperphagia and/or decreased MR, but rather represents a programmed change in the preferred level of WAT lipid reserves. Several lines of evidence support this contention. Temporary restricted access to food does not prevent projected increases in body mass. Ground squirrels food-deprived for 2–3 days overeat when food is returned and accumulate weight at a more rapid rate than previously until they catch up to the body mass extrapolated from their previous rate of increase (15). Trapping ground squirrels in the field regularly and holding them for part of each day (without food) limits their foraging time and they lose body mass (7). This mass loss represents a decrease in lean mass, but fat mass remains unchanged from that of unrestricted squirrels. In the laboratory, ground squirrels fed a daily ration of food comparable to their daily intake at their spring nadir (which produces marked mass loss at one time of year) undergo a significant mass gain phase even though peak mass is less than that of freely feeding squirrels (35, 38). Although peak body mass is reduced, total abdominal WAT mass is not different from that of freely feeding squirrels. In other words, caloric intake that results in negative energy balance and mass loss during one phase is sufficient for positive energy balance and fat deposition comparable to controls during another phase. Presumably, MR is sufficiently changed to compensate for reduced caloric intake. Circannual increases in preferred WAT mass level appear paramount and circannual changes in ingestion and metabolism more labile.

Although brain lesions that produce obesity in laboratory rats also substantially increase body mass in hibernators, circannual changes in body mass persist. For example, ablation of the ventromedial nucleus of the hypothalamus (VMH) produces a significant increase in peak body mass; however, trough body mass is essentially unaffected (15, 119, 120). VMH ablation shifts body fat mass at its peak upward without otherwise affecting its programmed circannual changes. Ablation of the paraventricular nucleus of the hypothalamus, which can also produce obesity, altered neither peak body mass nor circannual body mass changes (44).

Direct manipulation of WAT mass also supports the concept of programmed changes in preferred WAT mass. Surgical excision of a substantial quantity of WAT ($\sim$30 g) from the inguinal subcutaneous depot and retroperitoneal and gonadal abdominal fat depots during mass gain (or mass loss) is quickly recovered (37). Regardless of whether WAT is ablated during mass gain or loss, body mass is quickly restored to a value appropriate to that extrapolated from its previous pattern of mass change. Peak body mass was unaffected when as much inguinal, retroperitoneal, and gonadal fat as feasible was extirpated four months earlier (36). Total lipid mass was identical to that of intact squirrels and was restored without compensatory increases in food intake. Ablated gonadal fat was not recovered; gonadal WAT mass was only $\sim$15% that of normal and total gonadal adipocyte number was reduced by $\sim$85%. Retroperitoneal WAT, on the other hand, differed in neither mass, adipocyte number, nor adipocyte size between treatments; presumably, excised adipocytes completely regenerated and matured in this depot. Total

subcutaneous fat (measures of individual subcutaneous depots are not feasible at peak mass because all subcutaneous fat becomes contiguous) was significantly heavier in lipectomized squirrels. Although total subcutaneous fat cell number was comparable in lipectomized and intact animals, fat cell size from the inguinal WAT region was 25% greater and from the dorsal WAT region was 19% greater than corresponding adipocytes of intact squirrels (36). Ground squirrels quickly recover WAT mass after lipectomy, without increasing food intake, by complete regeneration of retroperitoneal adipocytes and a mix of adipocyte hyperplasia and hypertrophy in subcutaneous WAT (cf. 36, 38). It appears that the programmed peak lipid mass is still achieved after lipectomy despite lack of recovery in gonadal WAT, presumably by compensatory changes in MR and regionally specific changes in adipocyte cellularity.

Leptin and Hibernators

A requirement for regulating WAT mass, especially at an annually changing level, is a feedback signal accurately reflecting total body lipid stores. A sufficiently accurate feedback signal(s) from WAT to the CNS allowing subtle changes in energy balance (food intake and/or MR) to appropriately maintain WAT mass has not yet been identified in hibernators; however, the peptide, leptin, may provide such feedback. Chronic leptin levels provide an accurate WAT feedback signal to the CNS in a number of species (for reviews, see 1, 127, 142). Exogenous leptin decreases food intake and body mass in ground squirrels (23, 124) as in laboratory animals. The few studies of leptin concentrations and lipid mass have yielded contradictory results. During prehibernatory body mass gain of the little brown bat, the usual relation between increased WAT mass and increased leptin does not appear to hold. Circulating leptin levels increase before body mass and decrease as body mass increases to its peak (100). Leptin may be dissociated from WAT mass during hibernation because leptin increases energy expenditure; therefore, leptin may be dissociated to permit decreased MR during maximum fat deposition and, subsequently, deep torpor (100). Indeed, high leptin titers inhibit shallow, daily torpor expression in several species (64, 74). Leptin concentrations, on the other hand, closely follow circannual changes in body mass in woodchucks held at a room temperature (20°C–23°C) that prevents hibernation (34). The relation between leptin and the annual lipid cycle and hibernation remains an open question.

BODY MASS LOSS PHASE

Because the annual mass loss phase is coincident with hibernation, programmed changes in lipid metabolism are discussed primarily with relation to hibernation.

Little is known of the programmed progression of WAT changes during mass loss in the field because the animals are inaccessible after immergence into their

burrows. Nevertheless, mass loss is apparent upon emergence in the spring. For example, golden-mantled ground squirrels appear aboveground with little or no fat reserves in males and females (see, e.g., 24, 93), whereas in the larger Belding's ground squirrel both males and females emerge with considerable lipid reserves (88, 116). Larger hibernators emerge from hibernation with more substantial fat reserves, presumably their reduced rate of conductance and heat loss resulting from their decreased surface-to-volume ratio also reduces their rate of mass loss (see, e.g., 67, 118). Typically, food consumption in nonhibernating animals during body mass loss phase progressively decreases, reaching minimum intake 2–4 weeks before trough body mass (5, 35, 145); i.e., body and lipid mass continue decreasing even after food intake begins to increase. During body mass loss under optimal conditions of food availability and a T_a of $\sim$23°C, nearly all loss represents decreased lipid mass ($\sim$85%); neither water, protein, nor ash mass decrease during mass loss (43). The mass of individual fat depots successively decrease at the same rate, and these decreases in lipid mass are entirely mirrored in proportional decreases in adipocyte size (43). Hibernators do not have access to water in their hibernacula throughout winter; for the arctic ground squirrel, at least, lipid catabolism liberates sufficient water to maintain proper hydration (67).

Changes in Lipolytic Factors During Body Mass Loss

Pancreatic content of the lipolytic hormone glucagon, unlike insulin, does not decrease during hibernation (18), possibly reflecting glucagon's lipolytic function during periodic arousals from hibernation. During the body mass loss phase, it is presumed that lipolytic enzyme activity increases, shifting the balance between lipolytic and lipogenic enzymes toward lipolysis. Indeed, marmots increase the ratio of HSL mRNA to LPL mRNA concentrations during mass loss (147). Sensitivity of marmot adipocytes to norepinephrine, a norepinephrine agonist, and glucagon, however, were comparable during mass gain and hibernation (33).

Evidence for Programmed Decreases in Body Mass

Body mass forced below its programmed level by food deprivation or by preventing deep torpor rapidly recovers when animals are allowed to eat. Recovery is not to previous levels but instead to a mass appropriate to the stage of the cycle (15, 81, 121). Although time spent torpid was not measured during recovery, it is likely that either food intake or time spent torpid increased, but not both, because digestion is precluded at low T_bs (see 30). Alternatively, a highly palatable diet resets maintained body mass upward slightly, and body mass increases somewhat before resuming its previous loss rate (15). Similarly, obesity-producing VMH lesions during the loss phase produce an immediate small increase in body mass before returning to the same pattern of loss (119). Both treatments shift preferred body mass upward without otherwise affecting programmed circannual changes in body mass.

HIBERNATION

Hibernators Markedly Reduce Metabolic Rate

The reduction in MR during deep torpor is remarkable. O_2 consumption at T_bs approaching 0°C is typically ~5% of euthermic values but can be as low as ~1% (e.g., 30, 69). Hibernation generally provides an energy savings of 80%–85% over expenditures if remaining euthermic (see, e.g., 3, 6, 69). A recent review (69) thoroughly described the several mechanisms proposed to underlie the decrease in metabolism and its energy-saving function. Hibernation is an active process and its initiation is under neuroendocrine control. Because euthermia isolates mammals from thermal effects on cellular metabolism, any mechanism eliciting hibernation must begin with the CNS lowering hypothalamic setpoint for T_b and eliminating thermoregulatory heat production. The primary evidence for the CNS's role in hibernation expression is that T_b is regulated during hibernation but at a very low setpoint (see, e.g., 26, 53, 79, 80, 107); nevertheless, CNS involvement in the initiation, maintenance, and termination of hibernation consistently receives little attention.

A rapid decrease in hypothalamic setpoint for T_b initiates two mechanisms that suppress MR during hibernation: (*a*) a neuroendocrine mechanism inhibiting thermoregulatory thermogenesis and (*b*) a separate mechanism, most likely also under neuroendocrine control, that suppresses cellular metabolism. Hibernation onset occurs during sleep and its concomitant decrease in T_b; however, T_b continues to progressively decrease, rapidly reaching a new lowered setpoint (53, 79, 80), often near 0°C (11, 26, 70, 72). The primacy of the change in hypothalamic setpoint is evident from the decrease in MR prior to any observable decrease in T_b (3, 69, 104, 125).

The sympathetic branch of the autonomic nervous system activates the organism. Increasing sympathetic activity enhances heart rate, respiration, blood flow, etc., which are counterproductive to hibernation; it was proposed to be an important function in hibernation nearly 30 years ago (140). Sympathetic neural activity also is the primary mediator of BAT nonshivering thermogenesis (NST) as well as WAT lipolysis (e.g., 9, 16, 29). Sympathetic activity suppression remains an important step in hibernation onset, especially as a means for "turning off" thermogenic heat production.

Lipid is the Primary Metabolic Fuel During Deep Torpor

Plasma metabolic fuel concentrations provide circumstantial evidence as to which fuel(s) may be the source of cellular oxidation during hibernation, reduced as it may be. Glucose is a major source of cellular energy in nonfasted animals; however, in the absence of food intake, the only sources of glucose are liver and muscle glycogen stores and glucose produced via gluconeogenesis. Because glycogen stores are very limited and gluconeogenesis from glycerol is inefficient, the required gluconeogenesis from amino acids would result in overwhelming muscle wasting if

glucose were the primary metabolic fuel. Although capacity for gluconeogenesis from amino acids is increased during hibernation (28), there is little evidence of muscle wasting during hibernation (see, e.g., 107). It is likely, therefore, that noncarbohydrate fuels comprise most of the energy utilized during hibernation. Plasma glucose concentrations in ground squirrels and marmots remain remarkably stable at unchanged or elevated levels throughout a hibernation bout (55, 99, 123, 134, 139). In hibernating hedgehogs and dormice, glucose concentrations also are stable but at somewhat reduced levels (31, 98). Radiolabeled glucose incorporation into CO_2 is markedly suppressed during hibernation (134), and plasma lactate concentrations are correspondingly decreased during deep torpor (123, 150); both are indications of reduced glucose oxidation. Glycogen reserves may actually increase during deep torpor, which suggests that they may be preserved during deep torpor for a possibly critical role during arousal (107, 150). Additionally, a number of enzymes critical for glycolytic activity (e.g., pyruvate dehydrogenase and pyruvate phosphofructokinase) appear to be actively suppressed during hibernation (25, 27), a finding that supports this contention. Although glycolysis may be actively inhibited, this effect is reversible because carbohydrate oxidation can increase during deep torpor if an animal is energetically challenged (26). In addition, ketone bodies ($\beta-$hydroxybutyrate and acetoacetate) are a byproduct of hepatic oxidation of FA and can be utilized as metabolic fuel in several organs, especially the brain, during energetic challenge. Ketone concentrations are substantially elevated during deep torpor (99, 123, 126) and appear to contribute to glucose sparing during deep torpor by inhibiting glucose uptake by muscle (99). Ketones also may be actively preserved during hibernation.

The fact that most hibernators deposit considerable quantities of fat during the summer mass-gain phase and that this fat is subsequently lost during the hibernation phase is a priori evidence that stored lipids are the primary fuel during hibernation. Nevertheless, this is not evident from plasma FA concentrations during hibernation, which in ground squirrels do not appear to differ from euthermic values (99, 123), but are reduced during hibernation in hedgehogs (98). Triacylglycerol concentrations, in contrast, markedly decrease during deep torpor (123). Measures of metabolite concentrations during hibernation support the conclusion that lipids are the primary fuel.

RQ values calculated from measurements of O_2 consumption and CO_2 production during hibernation provide convincing evidence that FA oxidation nearly exclusively fuels deep torpor. RQ values vary from 0.7, representing nearly 100% FA oxidation, to 1.0, representing nearly 100% carbohydrate oxidation. Invariably, RQ during deep torpor is $\sim$0.7, indicative of nearly pure FA oxidation (see, e.g., 106, 114, 130, 133). The limited cellular metabolism during deep torpor, therefore, is fueled by FA oxidation.

T_b is regulated during deep torpor, and if T_a is reduced sufficiently below the T_b setpoint, MR must increase to maintain T_b. Increased MR at T_as $< \sim 0^\circ$C increases RQ from 0.7, indicating an increase in carbohydrate (i.e., glucose) utilization (26). There may be a limit on FA availability for oxidation at low temperatures such

that increased O_2 consumption necessitates carbohydrate oxidation. If there are limits to FA availability at low temperatures, then FAs may not be mobilized by lipolysis during deep torpor. The enzyme pancreatic triacylglycerol lipase, found in pancreas of nonhibernators, is also expressed in heart and WAT of hibernating ground squirrels (17, 132). This enzyme may be able to mediate lipolysis during hibernation because it appears to continue functioning at very low temperatures without hormonal control. In this case, pancreatic triacylglycerol lipase–mediated lipolysis may be a slow source of FAs during deep torpor. This would seem to suggest that FAs can be mobilized during hibernation, but it does not address whether FAs actually are mobilized or whether their rate of mobilization is limited during hibernation.

There is little or no unstimulated glycerol release from hibernating jerboa or ground squirrel WAT incubated in vitro at 5°C–7°C, indicating that unstimulated lipolysis at T_bs characteristic of deep torpor is markedly suppressed (40, 115). Norepinephrine-stimulated glycerol release from WAT adipocytes in vitro also is nearly nonexistent at low tissue temperatures. Glycerol release from WAT continues to be suppressed until temperature exceeds ~15°C, then increases as temperature increases (40, 115). In addition, norepinephrine-stimulated glycerol release from BAT tissue of hibernating ground squirrels in vitro is nearly nil at 5°C (40). Although this suggests that BAT lipolysis may be suppressed at low temperatures, it has no bearing on the efficacy of norepinephrine to induce BAT NST, which presumably is unaffected at these same temperatures. Epinephrine-stimulated glycerol release of WAT in situ, as measured by microdialysis, also is markedly reduced during hibernation (52). It appears that spontaneous as well as neuroendocrine-stimulated lipolysis is inhibited at low temperatures characteristic of hibernation. A mechanism may exist capable of mediating lipolysis in WAT at very low temperatures, but any lipolysis that occurs at these T_bs is minimal because at $T_a < 0$°C the increased MR necessary to maintain T_b is supported by an increase in carbohydrate, not lipid, oxidation (26).

Lipid Composition Affects Hibernation

Metabolic energy during hibernation is derived primarily from lipid stored in WAT in the form of TGs, which are composed of a single glycerol molecule and three FA molecules. The nature of the FAs comprising TG in WAT is variable and somewhat dependent upon diet. Although mammals can synthesize saturated fatty acids (SFAs) and monounsaturated (MUFAs) fatty acids de novo, they cannot synthesize polyunsaturated fatty acids (PUFAs). Many plants synthesize PUFAs, thus providing a readily available source of essential fatty acids, i.e., PUFAs that are necessary but not synthesized in the body. The presence of PUFAs in the WAT TG of hibernating ground-dwelling squirrels must come through dietary intake of plants containing PUFAs (57, 59). The most prevalent PUFA in plants consumed by hibernating ground-dwelling squirrels is linoleic fatty acid (see, e.g., 57, 60). Because T_bs during deep torpor are frequently as low as 1°C–3°C, or 20°C–25°C

below the melting point of typical mammalian lipids, the relation between low T_bs and loss of lipid fluidity in WAT, as well as cellular membranes, is a long-standing issue (see 2, 68, 107). Lipid fluidity depends on degree of saturation of its composite FAs, therefore TG's melting point decreases as PUFA content increases (68). Increasing PUFA content of WAT in ground squirrels and marmots typically reduces WAT's melting point to 0°C to −5°C (63). Cell membranes are known to undergo structural changes prior to hibernation, presumably to maintain fluidity (see, e.g., 2, 107), but WAT TGs also would have to retain fluidity or lipolysis and mobilization of energy from stored lipids would be negatively affected at low T_bs (see, e.g., 63, 68), even if lipolysis is restricted to T_bs > 15°C.

Experiments on hibernating yellow pine chipmunks illustrate pronounced effects of lipid PUFA content on deep torpor (68, 71, 75). Diets supplemented with sunflower seed oil (high in PUFAs) consistently increase chipmunk hibernation bout duration over a range of low T_as (and T_bs), especially relative to those fed a diet supplemented with sheep fat (high in SFAs). On all measures, hibernation bouts of chipmunks fed the standard diet were at intermediate values at each T_a. The SFA diet increased O_2 consumption nearly twofold over that of PUFA-fed chipmunks; decreased metabolism was a consequence of the lower T_b in high-PUFA-fed animals. Slowly lowering T_a to determine minimum T_b ($T_{b\ min}$) before animals require increased MR to maintain T_b results in a $T_{b\ min}$ of SFA-fed chipmunks nearly fourfold higher than that of PUFA-fed chipmunks. PUFAs, in other words, lower T_b setpoint during hibernation, which decreases MR, thereby increasing torpor bout duration and ultimately reducing total energy expenditure.

In both the field and laboratory, three FAs comprise ∼95% of the FAs found in ground-dwelling squirrel diets and their WAT lipids: palmitic, oleic, and linoleic FAs—an SFA, an MUFA, and a PUFA, respectively (see, e.g., 57, 60). MUFA and PUFA contents in WAT are comparable and proportionally greater than SFA content. PUFA intake occurs primarily in the final month prior to hibernation (see, e.g., 61). Diets high in PUFAs similarly affect hibernation in golden-mantled ground squirrels, yellow-bellied marmots, and black- and white-tailed prairie dogs (54, 59–61, 63, 73, 77). The most prevalent PUFA in plants available to hibernators is linoleic acid, and a diet increased in only linoleic acid content is sufficient to generate increases in torpor bout duration and reduced T_b (59). When offered diets varying in PUFA content, ground squirrels choose diets higher in PUFA content. Dietary preference for PUFAs, however, is controlled. Hibernators prefer a diet moderately high in PUFAs nearly threefold to one very high in PUFAs; if offered two diets, one very high in PUFAs and one very low in PUFA content, they consume approximately equal quantities of each. Total PUFA intake was equivalent in the two experiments and nearly comparable to PUFA content of animals in the field (62).

In contrast to diets reduced in PUFAs or higher in SFAs, diets higher in PUFAs enhance deep torpor. Surprisingly, hibernators fed a diet lacking PUFAs continue to hibernate, although $T_{b\ min}$ is increased and consequently torpor duration is nearly halved (54, 135). MR during deep torpor was significantly increased in PUFA-deficient animals, concomitantly increasing total energy expenditure

during hibernation. Shortened torpor durations in PUFA-deficient animals negatively affect fat reserves, causing an accelerated rate of mass loss during the hibernation season. In other words, a hibernator whose diet is deficient in PUFAs during mass gain may maintain T_b only somewhat higher than normal animals, but will arouse from deep torpor nearly twice as often. Periodic arousals consume ~85%–90% of energy expended during the hibernation season; therefore, doubling the number of arousals is costly, increasing total energy utilization and jeopardizing overwinter survival, especially in males (see below).

Unlimited increases in WAT PUFA content do not necessarily translate into increases in depth and duration of torpor. Excessive dietary PUFA content contrarily increases $T_{b\ min}$ and decreases hibernation bout duration in the same manner as insufficient PUFAs (63). There appears to be an optimal level of dietary PUFAs, and animals will modify their dietary PUFA intake to achieve it. When offered diets varying in PUFA content, ground squirrels proportionally consume the diets such that total PUFA intake is within an optimal window (see 62). In addition, a rose may still be a rose, but a PUFA is not necessarily a PUFA. The predominant PUFA obtained from plants is linoleic acid (a diene, 18:2); however, hibernators in the field also typically possess measurable quantities of linolenic acid (a triene, 18:3). Supplementing diet with linseed oil, which increases WAT linolenic acid and concomitantly decreases WAT linoleic acid content, eliminates hibernation (83). Marmots remain euthermic and continue to feed throughout winter. Presumably, increased linolenic acids, and not reduced linoleic acid, somehow prevent hibernation because PUFA-deficient diets do not eliminate hibernation.

Increased linoleic acid content may be sufficient to improve hibernation parameters, but it may not be necessary. Increasing dietary MUFA content, for example, produces improvements in torpor duration and $T_{b\ min}$ comparable to a diet increased in linoleic acid content (75). Unsaturation of fatty acids is the key to lowering lipid melting point; thus, MUFA as well as PUFA content may affect hypothalamic setpoint for T_b, lowering $T_{b\ min}$s and improving chances for overwinter survival and successful reproduction. In fact, some propose that MUFAs sustain most of the improved hibernation characteristics; PUFAs only provide minor improvements (see, e.g., 51). Utilization of PUFAs during deep torpor has a downside, which would seem to jibe with this proposal. PUFAs have a greater propensity for peroxidation (auto-oxidation) than do either SFAs or MUFAs, and increasing PUFA content increases peroxidation rate. Additionally, deep torpor increases the rate of peroxidation, and the peroxides formed by this self-sustaining process are cellular toxins (see, e.g., 62). Although hibernators appear to markedly increase cellular antioxidants (e.g., ascorbate) during hibernation (48, 136), hibernators increase PUFA intake in a limited fashion, presumably in a trade-off of its beneficial versus detrimental consequences during hibernation (see 62). Peroxidation also may explain the reduced torpor bout duration after animals ingest diets containing excessive PUFAs, especially those with high linolenic acid content.

Hibernators appear to retain PUFAs specifically, preferentially oxidizing SFAs and MUFAs during hibernation. WAT composition changes across the hibernation

season based upon FA saturation: The proportion of SFAs decreases, the proportion of MUFAs remains constant, and most interestingly, the proportion of PUFAs increases (54, 57). Venous blood exiting gonadal WAT contains proportionally more SFAs than found in gonadal WAT at that time, and proportionally less linoleic acid is in gonadal venous blood than is found in gonadal WAT (57). There is a primary utilization of SFAs, secondary utilization of MUFAs, and specific retention of PUFAs. Whether this occurs to retain the hibernation-enhancing effects of PUFAs or to exclude the negative effects of PUFA peroxidation is unknown. Animals undergoing exclusive FA oxidation (e.g., neonatal rats, chick embryos, and hibernators) evidence a secondary pathway for TG synthesis involving monoacylglycerol acetyltransferase (MGAT) primarily in the liver (118, 149). Hepatic MGAT concentrations in marmots, for example, are 80-fold greater than concentrations found in either WAT or BAT. MGAT acylates and accumulates monoacylglycerols containing PUFAs, specifically resulting in the retention of PUFAs in TG. MGAT therefore serves to retain PUFAs selectively in the face of increased lipolytic activity over prolonged periods. Regardless of the mechanism responsible for its original development, in hibernators this pathway retains PUFAs for their hibernation-facilitating effect while simultaneously reducing PUFA availability for possible toxic peroxide formation.

Arousal from Hibernation

One minimum requirement for successful hibernation is that the animal must be able to return to euthermia (i.e., rewarm). Mammalian hibernators arouse not just in spring as ectothermic hibernators do, but also periodically throughout the hibernation season. The occurrence of these periodic rewarmings probably represents the public's most common misunderstanding about hibernation. This erroneous concept evidences itself frequently in popular entertainment, everything from the spectacular *2001: A Space Odyssey* to the campy *Planet of the Apes.* In a favorite moment from the original *Star Trek* television series, Captain Kirk awakens a naughty Ricardo Montalban from centuries of continuous deep hibernation only to have him raise hell everywhere. This example is whimsical; nevertheless, it raises several relevant questions. For instance, if we ever can place humans into deep torpor for space travel or, more likely, for medical reasons (e.g., while they await a suitable organ for transplant), will they need to periodically arouse?

The duration of hibernation before a spontaneous arousal varies regularly from several days to several weeks (70), and the duration of the period of euthermia in fat-storing species is consistent and fairly short, typically $<$24 hours (see 30, 69, 107). Periodic arousals are energetically expensive; $\sim$85%–90% of a hibernator's energy budget during the hibernation season is used to fuel these periodic rewarmings to euthermia (see, e.g., 92, 143). Because it is so energetically costly to rewarm, frequent periodic arousals from hibernation appear contradictory and suggest that their occurrence is mandatory. Presumably, critical processes or functions that must be periodically restored at euthermic temperatures for the organism's

survival necessitate spontaneous arousals. Functions that have been proposed to underlie spontaneous rewarming include renal function, restorative sleep, and most recently, neural memory functions (see 90 for a review). In light of the myriad critical processes that are suppressed at T_bs $< \sim 15^\circ$C, including immune responsiveness, transcription, translation, renal function, and even norepinephrine-induced lipolysis, it is more than likely that no single cause is responsible for spontaneous arousals during the hibernation season (see, e.g., 90). There is a long-standing relation, nonetheless, that suggests that the lower the T_a and T_b (up to a point), the longer the hibernation bout (see, e.g., 70, 72, 138, 141). The proposed advantage from consuming adequate dietary PUFAs most likely is the basis for this relation. The resulting reduced $T_{b\ min}$ decreases MR and increases torpor bout duration, and this energy savings is compounded over the course of the hibernation season.

Metabolic fuel utilization during deep torpor represents almost 100% oxidation of FAs as indicated by its characteristic RQ of 0.7. Interestingly, O_2 consumption and CO_2 production during the earliest stage of arousal reveal a shift in RQ and a dramatic shift in metabolic fuel usage. During the initial stage of arousal until T_b increases to $\sim 12^\circ$C–16°C, RQ is increased to $\sim$1.0, indicating that carbohydrates comprise the primary fuel for cellular oxidation (see, e.g., 114, 130). At this point, RQ returns to $\sim$0.7 and oxidation primarily of fat for the remainder of arousal. Although some of this most likely represents glycolysis of muscle and liver glycogen reserves, glucose oxidation increases as well. From $T_b = 6^\circ$C to $T_b = 15^\circ$C, disappearance of radiolabeled glucose and appearance of labeled CO_2 increase 15-fold over hibernation values, suggesting a marked increase in glucose oxidation (134). Concomitantly, lactate production that was suppressed during hibernation increases eightfold during arousal, also indicating increased glucose oxidation (123). Anti-insulin treatment at the initiation of rewarming produces marked hyperglycemia, signifying that endogenous insulin normally is facilitating glucose uptake and utilization (85). BAT's thermogenic contribution to rewarming is proposed to be paramount during initiation of arousal (see below) and to be dependent on increased insulin concentrations (45, 84, 89, 101). Because norepinephrine-stimulated lipolysis is suppressed at these T_bs (40), insulin-stimulated glucose oxidation may be critical for BAT NST. Indeed, glucose and acetate oxidation in BAT are markedly increased until T_b exceeds $\sim 15^\circ$C (95).

Although protein loss is not programmed during the mass loss phase (43), there is some protein loss during hibernation (see, e.g., 67). Plasma free amino acids are not increased during hibernation, but do increase considerably during the later stages of arousal, possibly supporting increased gluconeogenesis for restoration of plasma glucose and muscle and hepatic glycogen reserves utilized during arousal (96, 146). Plasma ketones increase during hibernation (19, 99, 123) and subsequently decrease during arousal (99, 123), especially from $T_b = 6^\circ$C to $T_b = 15^\circ$C (19). A few tissues can utilize ketones as metabolic fuel, the most notable being the brain, which may rely on ketones as a substitute fuel during the energy demanding process of rewarming to euthermia. Glucose (including glycogen) and ketone oxidation may markedly increase during torpor initiation; nevertheless, the

bulk of arousal remains dependent upon FA oxidation (see, e.g., 130). For example, despite glycerol concentrations increasing nearly fivefold during arousal, ground squirrel plasma FA concentrations actually decrease by 20%, demonstrating both increased lipolysis and increased fatty acid utilization (123). Rewarming to euthermia appears to utilize, if not require, combined glucose, ketone, and fatty acid oxidation.

Neither disruption of glucose oxidation with a low dose of nonmetabolizable glucose analogue, 2-deoxy-D-glucose (2DG), nor interference with FA oxidation by a low dose of mercaptoacetate (MA) affects time to rewarm (41). Injecting the same doses of 2DG and MA simultaneously, on the other hand, increases time required to reach euthermia; a minimum level of total metabolic fuel appears necessary for arousal to proceed at its normal pace. A large dose of MA at torpor initiation does not slow rewarming, but it reduces T_b during subsequent euthermia and often inordinately lengthens periodic arousal (39). If glucose metabolism is critical for the earliest stage of arousal, as appears to be the case, then disrupting glucose oxidation at this time should have a profound effect on the initiation of rewarming. This, indeed, is the case. A large dose of 2DG injected at the time of torpor initiation increases the time required to raise T_b to 15°C by >200% (39).

Time spent torpid throughout the hibernation season was increased by reducing ground squirrel lipid reserves by food restriction at hibernation outset (58). Increased time spent in deep torpor reduced rate of body mass loss and resulted in a total lipid mass in spring comparable to that of unrestricted animals. Surgically reducing total lipid mass by WAT lipectomy resulted in equivocal changes in torpor that were intermediate to that displayed by the food-restricted and normal-mass animals (58). Although lipectomy somewhat increased time in hibernation, body mass decreased at the same rate as that of normal squirrels and total lipid mass in spring was only ~50% of control values. Food restriction reduces lipid mass by decreasing adipocyte size, whereas lipectomy decreases adipocyte number. At room temperatures, lipid mass recovers following lipectomy by pronounced adipocyte hyperplasia in several WAT depots (36); T_bs characteristic of deep torpor preclude cellular proliferation (see 30), presumably including adipocyte hyperplasia and recovery after lipectomy. Increasing lipid mass of juvenile ground squirrels above its regulated level before the hibernation season by access to a high-fat diet produces no compensatory change in time spent torpid between high-fat-fed and chow-fed squirrels; thus, body mass and lipid mass of high-fat-fed juveniles remain elevated in spring (42). The ability of hibernators to undergo compensatory increases (or decreases) in time spent torpid to accommodate energetic challenges detrimental to programmed WAT mass remains little understood.

At some point, a hibernator rewarms from deep torpor and fails to re-enter hibernation. This defines the terminal arousal and the return to homeothermy. Although PUFAs are specifically retained throughout hibernation, resumption of continuous euthermy renders them superfluous and they are mobilized upon terminal arousal (57). The terminal arousal in the spring is followed by resumption of reproduction.

Timing of terminal arousal is shaped by sex and fat reserves, a topic that is discussed below.

Brown Adipose Tissue and Hibernation

Because of its pronounced development in hibernators, there has been a long-standing belief that BAT must be important during hibernation (e.g., 107, 129). In fact, BAT was often referred to as the "hibernating gland." Its importance for hibernation was not realized, however, until BAT's thermogenic mechanism and function as a major heat-producing organ were identified. There are numerous thorough reviews of the thermogenic mechanism underlying NST in BAT (see 29, 97). Briefly, BAT mitochondria have the unique ability to dissociate fuel oxidation from ATP production via uncoupling protein (UCP 1), resulting solely in the generation of heat. UCP 1 concentrations reflect BAT's thermogenic capacity; however, thermogenic activity is more accurately represented by guanosine 5′-diphosphate (GDP) binding in BAT. Surprisingly, UCP 1 content is constant across seasons in ground squirrel BAT; however, both BAT mass and BAT mitochondrial concentration increase during the hibernation season (113). During a deep torpor bout, UCP 1 levels remain elevated but GDP binding is markedly reduced (86, 113, 123). Because UCP 1 concentrations do not differ between deep torpor and arousal, the marked increase in GDP binding during arousal represents an unmasking of binding sites with the onset of arousal (113, 123).

Hibernation appears dependent upon a pronounced suppression of autonomic activity (see 140), and rewarming is characterized by exaggerated sympathetic activity, especially to BAT. BAT's influence during the initial stage of rewarming (until T_b exceeds ~15°C–16°C) is evident from the primacy of increases in BAT temperature over increases in T_b. During initiation of rewarming, BAT temperature increases markedly before T_b increases; in fact, BAT temperature exceeds 30°C before T_b reaches 15°C (87). Although shivering thermogenesis contributes significantly to rewarming from hibernation, shivering is not initiated until T_b exceeds 15°C–16°C (see, e.g., 78, 109, 129). What is not clear is whether neural input to skeletal muscle increases before T_b is $> \sim$15°C and muscle is nonresponsive, or whether neural input to muscle is delayed until T_b exceeds this temperature. Even in the absence of muscle contractions, an increased rate of depolarizations at the neuromuscular junction would likely increase cellular metabolism (see, e.g., 49). BAT NST is critical for the initial stage of rewarming until T_b reaches ~15°C, when shivering and other functions that are suppressed during deep torpor resume. Preventing sympathetic activation (and BAT NST) during torpor initiation with a noradrenergic receptor blocker prevents rewarming (50), whereas preventing shivering thermogenesis with curare slows but does not prevent rewarming (129). Similarly, after surgically removing ~50% of BAT, hedgehogs cannot rewarm from hibernation (129). The juxtaposition of BAT near major blood vessels (see 129) suggests BAT heats circulating blood, thereby serving as a central heating system and indirectly rewarming peripheral tissues, specifically muscle, until they escape suppressive low temperatures.

HIBERNATION, FAT RESERVES, AND REPRODUCTION

Fat-storing hibernators are making a physiological bet with their lives in the balance. They are wagering that they have sufficient energy stored as lipids to survive within their burrows until aboveground conditions allow emergence and access to food resources. There are numerous reports of the dire consequences visited by late-season storms that delay emergence from hibernation (see, e.g., 4, 117, 128). Similarly, reduced food availability in the summer reduces fat deposition, which in turn increases overwinter mortality during hibernation (see, e.g., 47, 128).

Terminal arousal in fat-storing hibernators generally falls into two categories: (*a*) facultative terminators of hibernation that terminate dormancy when aboveground conditions (e.g., food availability) improve, and (*b*) obligate terminators of hibernation in which an endogenous circannual clock induces the terminal arousal, regardless of aboveground conditions.

Female Hibernators Facultatively Terminate Hibernation

Females typically emerge from hibernation several weeks later than males (see, e.g., 20, 24, 46, 65, 88, 91, 112, 116). For example, male Richardson's ground squirrels typically emerge in March, females emerge several weeks later, and by April 90% of females are pregnant (20). Female hibernators opportunistically terminate hibernation when the burrow mouth is free of snow and food availability returns. Providing hibernating females with food near the end of the hibernation phase induces terminal arousal, and delaying food availability prolongs hibernation (see, e.g., 65). Both pregnancy and lactation require marked increases in energy intake; intake is often doubled during lactation. Because of limits on energy intake and assimilation, the primary challenge for females is to be impregnated as soon as possible. The reproductive female must bear and wean her litter before she can begin to lay down sufficient WAT stores for the subsequent hibernation season. Similarly, the time that young of the year have to grow as well as deposit adequate adipose tissue for winter survival also is limited by the timing of weaning. Terminal arousal in females, therefore, appears to be opportunistic and keyed to the availability of adequate food resources.

Adult Male Hibernators Typically are Obligate Terminators of Hibernation

Male hibernators participating in reproduction terminate hibernation early, prior to reproductive females (20, 24, 46, 65, 88, 91, 112, 116). Often males will emerge from hibernation when snow is present and food is either unavailable or remains very limited (see, e.g., 46). Commitment to normothermy and subsequent testicular development occur before the possibility of emergence because testes are full grown, spermatogenesis is complete, and testosterone is maximal at first appearance aboveground (24, 93, 110, 111). As a result, only heavy males terminate hibernation early and possess large testes (see, e.g., 10, 42, 65, 93) because males

committing to reproduction must rely on lipids remaining in adipose tissue reserves. Males spend less time torpid than females over the hibernation season, but this is mostly due to the male's early terminal arousal before emergence is possible (see, e.g., 112). The male ground squirrel's early termination of hibernation has serious ramifications; remaining euthermic for up to four weeks without food is costly, often equaling the entire cost of the preceding hibernation season (cf. 12).

Because testicular recrudescence and spermatogenesis require prolonged euthermia, a fascinating interplay takes place between energy reserves, the circannual clock, and the hypothalamic-pituitary-gonadal axis when males possess sufficient WAT reserves. Testis mass of golden-mantled ground squirrels increases from ~500 mg at hibernation outset to ~1100 mg at terminal arousal, but there is no spermatogenic development (12). The circannual clock increases hypothalamic sensitivity to gonadal steroid feedback such that very low testosterone titers are sufficient to inhibit gonadotropin release throughout most of the year (152). As the reproductive season approaches, the circannual clock markedly decreases hypothalamic sensitivity in normal weight males, resulting in increased gonadotropin release despite increasing plasma testosterone concentrations (112). Testosterone release and spermatogenesis require euthermic tissue temperatures because testicular tissue is unresponsive to the stimulatory effects of luteinizing hormone (LH) at T_bs characteristic of hibernation. Testosterone release from testicular tissue in vitro is nonexistent at 5° and 9°C, and testosterone release at 20°C is only 25% of the maximal value at 32°C (14). Plasma testosterone, LH, and follicle-stimulating hormone values remain at basal concentrations throughout the hibernation season, until approximately three weeks before terminal arousal, when testosterone and LH concentrations begin to rise, but only during periodic arousals, not during deep torpor (13). Hormone concentrations reach maximum values after approximately six hours of euthermia, then decrease to basal levels before deep torpor resumes. Maximal testosterone release during successive periodic arousals progressively increases, and during the rewarming that becomes the terminal arousal, testosterone and LH increase as before but this time remain elevated (cf. 13). Presumably, hypothalamic sensitivity to steroid feedback has decreased to the extent that testosterone concentrations rise sufficiently within six hours to prevent resumption of deep torpor. A negative relation between testosterone and hibernation has been presumed for some time, based on the inference that reproduction and hibernation are incompatible (see 148); exogenous testosterone treatment prevents hibernation (76, 102).

Only males with normal body mass generally terminate hibernation early in the field (see, e.g., 24, 65, 66, 116) because commitment to reproduction in males is dependent on adequate energy reserves. Ground squirrels fail to terminate hibernation spontaneously when starting hibernation underweight, whereas heavier males terminate hibernation early (see, e.g., 65). Both fasting- and lipectomy-induced WAT reduction immediately before the onset of hibernation inhibit male reproductive development in spring; testicular and accessory sex organ development are positively correlated with body mass (58). Although fasted squirrels spend

more time hibernating than do either control or lipectomized animals, and thereby reduce the rate of body mass loss, reproductive development remains inhibited. It is not clear whether decreased hypothalamic sensitivity to testosterone feedback and terminal arousal in spring are dependent upon adequate fat reserves at hibernation outset in fall or on those present at the time of the terminal arousal in spring. Chronic leptin concentrations would provide an ideal feedback signal reflecting total body lipid stores; however, elevated leptin concentrations appear incompatible with reversible hypothermia (cf. 64, 74) and most likely leptin release may have to be suppressed during the hibernation phase to permit deep torpor (see 100). In this case, commitment to early terminal arousal would have to occur near peak body mass, when leptin concentrations may more accurately reflect total lipid mass. The relation between WAT mass, feedback from WAT (e.g., leptin), and reproductive development in male ground squirrels obviously is an area rife for future research.

Juvenile Male Hibernators Generally are Facultative Terminators of Hibernation

In the majority of fat-storing hibernators, juvenile males do not undergo an obligate terminal arousal, but await food availability in spring before terminating hibernation in the same manner as females (see 65). Although many juvenile female ground squirrels reproduce at the end of their first hibernation season, juvenile males generally are reproductively quiescent. In the limited time from weaning to winter's outset, juvenile energy intake must supply the requirements for growth as well as fat deposition. Insufficient lipid reserves in juvenile males somehow negate the circannual decrease in hypothalamic sensitivity to gonadal steroid feedback, which results in persistently low testosterone levels and a facultative termination of hibernation. Juvenile males initiating hibernation with lipid stores comparable to those of normal mass adults spontaneously terminate hibernation in spring (42, 65). Adult and juvenile male ground squirrels were provided either standard laboratory chow or a high-fat diet during the body mass gain phase before hibernating without food available. All adult males, regardless of diet, and 90% of high-fat-fed juvenile males spontaneously terminated hibernation (42). Body mass, lipid mass, testis mass, and testosterone concentrations of high-fat juveniles were greater than were those of chow-fed juveniles, but not different from values in adult males, regardless of diet. Lack of an obligate terminal arousal and accelerated reproductive development in juvenile ground squirrels is not a function of age, but a direct consequence of insufficient WAT mass.

SUMMARY

Hibernating mammals undergo remarkable annual cycles in body lipid content. During the summer and early fall, circannual changes in food intake, energy expenditure, and lipogenic factors combine to assure deposition of large reserves of white adipose tissue before the onset of winter. Attainment of a peak body

mass precedes immergence and the onset of a body mass loss/hibernation phase. Profound metabolic suppression during deep torpor allows a slow utilization of stored lipids over winter and avoidance of the harsh climatic conditions aboveground. Not surprisingly, lipid oxidation nearly exclusively fuels deep torpor and the major portion of the brief periodic arousals to euthermia that regularly interrupt hibernation. T_b during deep torpor affects duration of hibernation bouts, and the degree of saturation of fatty acids comprising stored lipids affects T_b during deep torpor. Thus, a diet higher in unsaturated fatty acids during fattening decreases frequency of periodic arousals and may increase likelihood of overwinter survival. Females and juvenile males terminate hibernation in the spring when aboveground food becomes available. Males with sufficient WAT reserves, however, terminate hibernation early, before aboveground food is available, relying solely on WAT stores to complete reproductive development before emergence in spring. Mating occurs immediately upon emergence of females, and completion of reproductive duties marks the beginning of the next lipid deposition cycle.

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