AMER. ZOOL., 38:331–340 (1998)

# Lipid Metabolism in Hibernators: The Importance of Essential Fatty Acids[1]

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SYNOPSIS. **Two polyunsaturated essential fatty acids, linoleic acid and linolenic acid, are important for their inherent energy during lipid oxidation. In addition, they influence the length of hibernation bouts and the metabolic rates of mammals that hibernate. Hibernators that lack linoleic acid in their diet or that are fed a diet high in saturated fatty acids have significantly shorter bouts of hibernation and have a higher mass specific metabolic rate. The decrease in the length of a bout of hibernation is significant because the animal arouses from hibernation more frequently, using more of its energy stores. This could result in a decreased chance of survival. How the essential fatty acids exert their actions in hibernators is just beginning to be elucidated. Essential fatty acids are the sole precursors for the eicosanoids that influence thermoregulation. Thus, studies of eicosanoid function during hibernation are warranted. The recent discovery and characterization of the protein leptin, which can regulate energy balance and may be regulated by polyunsaturated fatty acids, may prove to be important to hibernation and the regulation of body mass. Future investigations of the regulation of body mass during hibernation should consider the fatty acid composition of the diet and the effect of the essential fatty acids on gene transcription.**

## INTRODUCTION

> "To summarize, until 1940 science had succeeded in establishing what might have been guessed by an intelligent savage: that since many hibernators get fat in the autumn and thin by spring, they are mainly utilizing fat reserves during the winter. Modern biochemistry has added little to this conclusion" (Willis, 1982).

Mammals that hibernate (*i.e.,* hibernators) have the unenviable task of surviving through winter when environmental temperatures are low and food supplies are virtually nonexistent. Consequently, it is not surprising that hibernators either put on mass in the form of lipid prior to winter and/or may store food to sustain them till spring. Throughout the summer and early fall, hibernators lower their metabolism, increase their food consumption, and convert much of their ingested food to fat (Ward and Armitage, 1981). Because lipid in the form of triacylglycerols has a high caloric density, triacylglyerol is a preferred storage fuel for future energy demands. The fatty acids that occupy the three positions on the glycerol molecule can vary, but a long-chain polyunsaturated fatty acid (*e.g.,* linolenic acid) frequently occupies the middle (sn-2) position (Brockerhoff *et al.,* 1966), thereby preventing the formation of a triacylglycerol with saturated fatty acids at all three positions. As such, the melting point of the triacylglycerol molecule is usually very low (<10°C) (Gunstone *et al.,* 1986).

All mammals can synthesize many fatty acids in the liver and possibly in white adipose tissue. Certain unsaturated fatty acids, however, cannot be synthesized: linoleic acid (18:2 (n-6)) and alpha-linolenic acid (18:3 (n-3)) must be obtained from the diet and are considered to be essential fatty acids (Cook, 1985).

The role of lipids and fatty acids in hibernation and torpor was investigated throughout the early 1950s and 1960s. Fawcett and Lyman (1954) suggested the polyunsaturated fatty acids were critical for hibernation in hamsters (*Mesocricatus anur-*

[1] From the Symposium *The Biology of Lipids: Integration of Structure and Function* presented at the Annual Meeting of the Society for Integrative and Comparative Biology, 26–30 December 1996, Albuquerque, New Mexico.

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*atus*). However, this study did not focus on which polyunsaturated fatty acids may be most important for hibernation. Kayser (1961) reported a R.Q. of near 0.7 and he suggested that lipid metabolism is the primary source of energy during deep hibernation. Upon arousal from hibernation, hibernators have a R.Q. close to 1.0 indicating that carbohydrate reserves are also used for cellular metabolism. The fact that liver glycogen reserves are metabolized during the arousal from hibernation supported this hypothesis. Further, Spencer *et al.* (1966) reported the simple lipid composition for a hibernator (*Spermophilus lateralis*), but no inference was made about lipid composition and the ability to hibernate.

By the late 1970s and early 1980s investigators focused on the importance of membrane lipids and their possible role in maintaining membrane fluidity at low tissue temperatures (see Aloia and Raison, 1989; Wang, 1989). The lipids referred to in these early studies are membrane phospholipids (*i.e.*, phosphatidylcholine), not the neutral lipids associated with triacylglycerol stored in white adipose tissue and brown adipose tissue. Although cell membranes should be fluid in order to function at low tissue temperature, the exact biochemical mechanisms that lead to correct cellular function at low tissue temperatures are unclear. The tissues (*e.g.*, brain) of some hibernators are composed of cell membranes that are relatively more unsaturated compared to the same tissues of non-hibernators. No consistent change in tissue membrane fatty acid composition has been reported prior to or during hibernation, however (Aloia and Raison, 1989; Wang, 1989).

The purpose of this paper is to review the most recent work regarding the effect of dietary lipids on hibernation. How dietary lipids change the fatty acid composition of the white adipose tissue, liver, and brown adipose tissue in hibernators will be presented. Future research directions that underscore the importance of essential fatty acids for hibernation will also be discussed.

## LONG-CHAIN POLYUNSATURATED FATTY ACIDS AND HIBERNATION

Geiser and Kenagy (1987) addressed the hypothesis that a diet high in polyunsaturated fatty acids would alter the thermoregulatory behavior of a mammalian hibernator. They studied the yellow-pine chipmunk (*Eutamias amoenus*) which gains mass in the form of body fat and probably stores food in its burrow during the winter. By varying the amount of saturated or polyunsaturated fatty acids in the diet, they demonstrated that chipmunks on a diet high in polyunsaturated fatty acids had longer bouts of hibernation than either animals fed a control diet or animals fed the diet containing additional saturated fatty acids (Fig. 1). In addition, the animals fed a diet high in polyunsaturated fatty acids also had a lower minimum body temperature during hibernation. Thus, polyunsaturated fatty acids appear to alter the bout length and metabolic rate during hibernation in the chipmunk (Geiser and Kenagy, 1987).

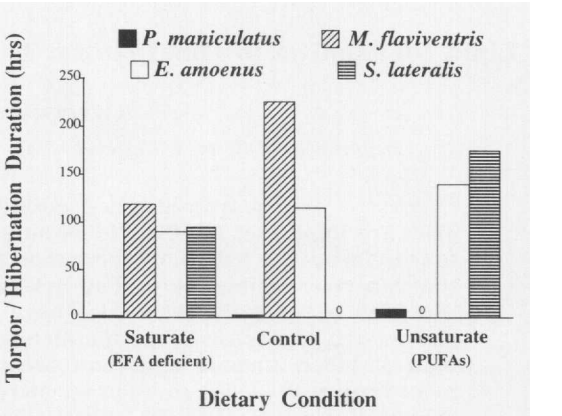


FIG. 1. Bout lengths of hibernation/torpor in four species of mammals under various dietary conditions. The data presented in the figure were taken from Geiser and Kenagy (1987), Geiser (1991), Geiser *et al.* (1994), and Thorp *et al.* (1994).

During the same year, our laboratory approached the question of how fatty acids influence hibernation patterns in marmots (*Marmota flaviventris*) from a different point of view. We found that the relative percentage of the essential fatty acids in marmot white adipose tissue rose slightly during winter (Florant *et al.*, 1990). Furthermore, earlier work on marmots had demonstrated that marmots increase their home range for particular plant species (Armitage, 1979). We hypothesized that in order for marmots to maintain a high com-

position of essential fatty acids in depot fat, animals would expand their territory to include plants that were high in essential fatty acids and would have biochemical mechanisms for decreasing the oxidation of the essential fatty acids. We demonstrated that linoleic and linolenic acids in white adipose tissue are not metabolized as quickly as other fatty acids during the hibernation period when marmots do not feed. Our studies (Florant *et al.,* 1989, 1990) demonstrated that marmots do not metabolize the essential fatty acids as rapidly as other polyunsaturated fatty acids during hibernation and animals extend their home range for cow parsnip (*Heracleum lanatum*), a plant that has high amounts of linoleic acid and linolenic acid (Florant *et al.,* 1990). This led us to hypothesize that the essential fatty acids, and not just any unsaturated fatty acid, were very important for hibernation. In a later study, we demonstrated that the duration of a bout of hibernation was significantly shorter when essential fatty acids were removed from the diet (Fig. 1), body temperatures were higher than controls, and metabolic rates were higher during deep hibernation (Thorp *et al.,* 1994). The increase in the frequency of arousal from hibernation is detrimental for these animals. Nearly 90% of the energy used during the hibernation period is expended during arousal (Wang, 1989). Thus, frequent arousals could put the animal into a position of depleting all of its endogenous lipid stores prior to the end of winter, hence decreasing its chances of survival.

Another study investigated the role of essential fatty acids on the hibernation patterns of golden-mantled ground squirrels (*Spermophilus lateralis*) maintained on a high polyunsaturated fatty acids diet (Frank, 1992). This study has been supported by the work of Florant *et al.,* (1993) and Geiser and Heldmaier (1995). Animals fed a diet high in polyunsaturated fatty acids, such as linoleic acid, hibernated more frequently and maintained lower body temperatures than animals on a diet high in saturated fats (Fig. 1). Maintaining a lower body temperature when ambient temperature is low will enable a hibernator to use less energy over the course of the winter. Frank (1992) suggested that changes in fatty acids of membrane phospholipids may also be important for normal hibernation patterns. A study on hamsters (*M. brandti*) did not find an increase in the length of a bout of hibernation with dietary manipulation (*e.g.,* polyunsaturated fatty acids *vs.* control). However, the concentration of polyunsaturated fatty acids in the experimental diets was not significantly different from controls and this may explain why no affect was observed (Bartness *et al.,* 1991).

Most of the above studies were performed on hibernators maintained in laboratories on defined diets. In a more recent study, it was suggested that ground squirrels in the wild select diets that are high in polyunsaturated fatty acids. The fatty acid composition of stomach contents from ground squirrels was investigated and found to have significantly more essential fatty acids during the late summer and fall (Frank, 1994). This is not totally unexpected because these animals cache seeds and nuts that are high in all polyunsaturated fatty acids at this time of year.

To understand the mechanism(s) by which hibernators might reduce the metabolism of essential fatty acids during torpor, we investigated the position of essential fatty acids on the triacylglycerol backbone in the white adipose tissue depot fat of marmots. We found that long-chain polyunsaturated fatty acids occupy the middle position (*i.e.,* sn-2) of triacylglycerol isolated from marmot white adipose tissue about 70% of the time (unpublished data). This is significant because this middle fatty acid is not hydrolyzed by hormone-sensitive lipase, the major lipase in white adipose tissue, but rather is released by a monoglyceride lipase. Selective retention of essential fatty acids was also observed in fasting adult rats (Cunnane, 1988), and very recent work on rats by Raclot *et al.,* (1995) reveals that certain long-chain polyunsaturated fatty acids are not metabolized as rapidly as other fatty acids. How fast they are released from the glycerol backbone depends on chain-length and on the number of double bonds (Raclot *et al.,* 1995). We also demonstrated that the enzyme monoacylglycerol acyltransferase could be respon-

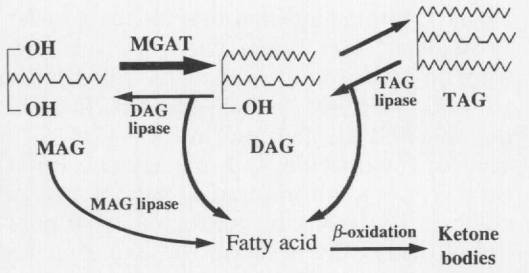


FIG. 2. The monoacylglyerol pathway of glycerol-lipid synthesis during lipolyis in fasting mammals. Monoacylglycerol acyltransferase (MCAT) reesterifies fatty acids to the sn-1 position of a sn-2-monoacylglycerol. Triacylglycerol (TAG); Diacylglycerol (DAG). This figure is adapted from Xia *et al.* (1993).

sible for maintaining a polyunsaturated fatty acids in the middle position of stored triacylglycerol (Fig. 2) in white adipose tissue (Xia *et al.*, 1993).

## EFFECTS OF LINOLEIC ACID AND LINOLENIC ACID IN DIET

The results from the studies cited above suggest that polyunsaturated fatty acids, and especially the essential fatty acids are needed for normal hibernation. However, the fatty acid composition of the depot fat from laboratory fed hibernators and from field animals is very different. Linolenic acid content is high in field animals and very low in laboratory animals, while linoleic acid content is similar in field and laboratory animals. This difference in linoleic acid and linolenic acid is also true for membrane phospholipids; these contain more linoleic acid than linolenic acid during hibernation. A review of all available data indicates that linolenic acid is rapidly lost from the fat storage depots and cell membranes of animals once they are brought into the laboratory, regardless of diet. Interestingly, most laboratory diets contain some omega-3 linolenic acid, but the percentage of linolenic acid in the depot fat of laboratory animals is still much lower than that observed in field animals. Further, the length of a bout of hibernation in field animals may not be significantly different from a bout length in laboratory animals. Thus, the function of linolenic acid in hibernation remains unclear.

Free-ranging hibernators consume plants that are high in linolenic acid all summer and, thus, may maintain a higher percentage of linolenic acid in their body tissues. Whether hibernation and minimum body temperatures would change if only linolenic acid were present in their diet is unknown. In marmots fed a control diet, the triacylglycerol classes (*e.g.*, the kinds of fatty acids attached to the glycerol backbone of triacylglycerol) in white adipose tissue depot fat change after only a few months on the laboratory diet. Very few studies have attempted to determine the changes in triacylglycerol classes (*i.e.*, triolein, which contains 3 mono-oleic acids) during the hibernation period. Florant *et al.*, (1991) determined the triacylglycerol classes in the white adipose tissue of marmots from the field and after several months in the laboratory. Animals taken recently from the field had substantial amounts of trienoic fatty acid in white adipose tissue compared to laboratory animals. However, within three months of capture, triacylglycerol that had three linolenic fatty acids attached to the glycerol backbone rapidly disappeared from the white adipose tissue in favor of triacylglycerol with mostly linoleic fatty acids attached; triacylglycerol esterified with all saturated fatty acids were very uncommon. This result suggests that the triacylglycerol in the white adipose tissue of marmots changes with diet, and that the laboratory diet produces white adipose tissue with more triacylglycerol containing polyunsaturated fatty acids.

The effect of double bonds in dietary fatty acids on hibernation patterns was investigated by Geiser *et al.* (1994) using chipmunks (*E. amoenus*) fed diets that varied in the amount of steric (18:0), oleic (18:1 (n-9), and linoleic acids. They determined that 50% of the identifiable fatty acids in white adipose tissue depot fat were significantly different between dietary groups. The animals receiving sterate and oleate had shorter bout of hibernation at an ambient temperature of 4°C and had higher minimum body temperatures. These results must be interpreted with some caution, however, because some of the identified fatty acids are extremely uncommon and other fatty acids

TABLE 1. *Fatty acid composition of neutral lipid in brown adipose tissue of control and essential fatty acid deficient animals.*

| Fatty acid | Control | | Fatty acid deficient | |
|---|---|---|---|---|
| | Summer | Hibernation | Summer | Hibernation |
| 14:0 | 2.9 | 0.9† | 2.0 | 1.0 |
| 16:0 | 26.5 | 18.7 | 22.5 | 18.4 |
| 16:1 | 4.6* | 3.2 | 7.5 | 3.8† |
| 18:0 | 5.4 | 1.7*† | 5.6 | 4.5 |
| 18:1 | 32.9* | 57.4† | 56.4 | 66.1 |
| 18:2 | 18.6* | 14.6* | 3.0 | 2.6 |
| 18:3 | 3.9* | — | — | — |
| 20:4 | 0.6 | — | 0.3 | — |
| 20:5 | 0.2 | — | — | 0.2 |
| 22:5 | 1.2* | 0.3 | — | — |
| 22:6 | 1.5 | 0.8 | 0.2 | 0.5 |
| Other | 1.2 | 1.4 | 1.6 | 1.5 |

† Significantly different ($P < 0.01$) between hibernation and summer seasons.

* Significantly different ($P < 0.01$) between control and essential fatty acid deficient diets. (—) indicates the fatty acids were not detected. Values represent the percent of total fat and each is the mean of 4 or 5 different samples. Statistical differences were determined using an ANOVA with pair-wise comparisons and Bonferroni adjustments.

constituted a very small percentage and their function is unclear.

We investigated how the fatty acid composition of brown adipose tissue, liver, and white adipose tissue in marmots changes with dietary manipulation. The fatty acid composition in the brown adipose tissue of ten animals from summer and winter is reported in Table 1; five animals were maintained on a control diet (Purina Rodent Chow #5001) and five were fed a diet deficient in essential fatty acids. Summer samples were obtained while animals were still eating but hibernation samples were collected from animals in January when they were not eating. Animals on the control diet showed a significant decrease in the saturated fatty acids (*e.g.,* 16:0, 18:0) from brown adipose tissue from summer to winter, while unsaturated fatty acids (*e.g.,* 18:1 and 16:1) showed either no change or an increase. Interestingly, the essential fatty acids decreased: linoleic acid decreased slightly but significantly and linolenic acid was not detectable in the hibernation season. Similar results have been reported for the Djungarian hamster (*Phodopus sungorus*) maintained on different fatty acid diets and in various ambient temperatures (Geiser and Heldmaier, 1995).

Further, the total lipid content of the tissue sample (micromoles of fat) decreased in control and animals fed a diet deficient in essential fatty acids in winter. This was expected because brown adipose tissue and muscle use lipids from white adipose tissue for heat production during hibernation and arousal from hibernation. Therefore, we expected to find lower lipid concentrations in this tissue during the hibernation period. Animals fed an essential fatty acid deficient diet had a lower percentage of saturated fatty acid in brown adipose tissue by winter while a compensatory increase occurred in oleic acid due to the lack of essential fatty acids in the diet. The concentration of lipid decreased with winter hibernation as expected, and the relative percent of long-chain polyunsaturated fatty acids (*e.g.,* 22:6) was low and not significantly different in animals fed either a control or essential fatty acid deficient diet, regardless of season.

Lipid composition changes in the liver of animals fed a control and essential fatty acid deficient diet during different seasons were very different (Table 2). Unlike brown adipose tissue, the percent of linoleic acid in liver rose significantly during hibernation as did some of the other polyunsaturated fatty acids. In animals deficient in essential fatty acids, the relative percent of linoleic acid increased although the percentage in liver was extremely low. This supports our

TABLE 2. *Fatty acid composition in liver of control and essential fatty acid deficient animals.*

| Fatty acid | Control | | Fatty acid deficient | |
|---|---|---|---|---|
| | Summer | Hibernation | Summer | Hibernation |
| 14:0 | 1.4 | 2.1 | 2.2 | 2.2 |
| 16:0 | 19.1 | 18.0 | 18.2 | 21.6 |
| 16:1 | 4.6* | 4.8* | 7.7 | 6.7 |
| 18:0 | 4.6 | 0.8*† | 3.6 | 3.8 |
| 18:1 | 55.8* | 56.5 | 64.6 | 61.6 |
| 18:2 | 7.7* | 13.9*† | 1.2 | 1.7 |
| 18:3 | 1.2 | — | 0.4 | — |
| 20:4 | 0.8 | 0.2 | 0.5 | — |
| 20:5 | 1.0* | — | — | — |
| 22:5 | 0.4* | 0.2* | — | — |
| 22:6 | 1.0* | 1.3* | — | — |
| Other | 1.6 | 1.5 | 0.6 | 1.1 |

The legend is the same as Figure 1.

previous hypothesis that the sparing of linoleic acid is predominantly occurring in liver, where monoacylglycerol acyltransferase concentration and activity are highest (Mostafa *et al.,* 1993). Another interesting result is that the concentration of lipid rose in liver with season. This suggests that the liver maybe storing lipid during the winter hibernation period.

As noted previously, changes occurred during hibernation in the relative percent of certain fatty acids in white adipose tissue (Table 3). We found that linoleic acid decreased significantly from summer to winter, but this was expected because animals in summer were feeding and those in winter had stopped. Like in brown adipose tissue, the relative percent of saturated fatty acids in white adipose tissue decreased from summer to winter in both dietary groups. This finding suggests that saturated fatty acids are used preferentially or that polyunsaturated fatty acids are spared oxidation in winter. Although polyunsaturated fatty acids were not found in a relatively large percentage, the concentration of lipid in our samples was much larger than that found in liver or brown adipose tissue; again this was predictable because white adipose tissue is primarily a lipid storage depot. Because the control animals hibernated, we were again puzzled by the lack of linolenic acid in any of the tissues. We believe that this essential fatty acid plays some as yet undefined role in the hibernation process. As shown in Fig. 3, linoleic acid is the precursor for several polyunsaturated fatty acids which were not found in large percent-

TABLE 3. *Fatty acid composition of neutral lipid in white adipose tissue of control and essential fatty deficient animals.*

| Fatty acid | Control | | Fatty acid deficient | |
|---|---|---|---|---|
| | Summer | Hibernation | Summer | Hibernation |
| 14:0 | 2.7 | 2.4 | 2.7 | 2.3 |
| 16:0 | 25.2 | 21.4 | 26.5 | 20.9† |
| 16:1 | 6.6 | 5.7* | 8.5 | 8.2 |
| 18:0 | 2.8 | 1.0*† | 4.4 | 2.6† |
| 18:1 | 39.7* | 54.1† | 53.4 | 62.0† |
| 18:2 | 18.0* | 12.5*† | 2.5 | 1.3 |
| 18:3 | 2.1* | — | — | — |
| 20:4 | — | — | — | 0.8 |
| 20:5 | 0.4 | — | — | 0.3 |
| 22:5 | 0.3 | — | — | — |
| 22:6 | 0.7 | 0.5 | — | — |
| Other | 0.5 | 1.7 | 1.0 | 0.6 |

Legend is the same as Figure 1.

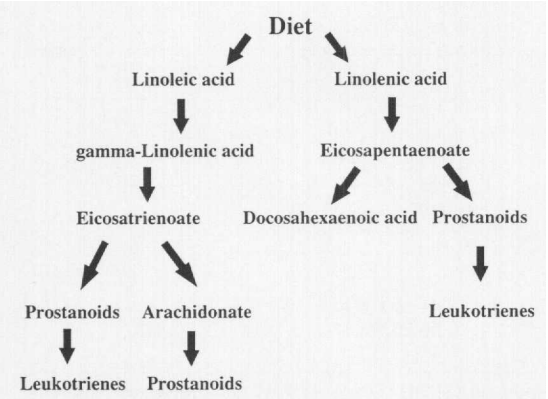


FIG. 3. The production of leukotrienes and eicosanoids from dietary linoleic and linolenic acid. The pathway illustrates that linoleic and linolenic acids produce different eicosanoids and prostaglandins.

age as a neutral lipid in any of the tissues examined, suggesting that linoleic acid is quickly converted to a long chain fatty acid not stored in white adipose tissue.

From Figure 3, we conclude that different physiologically important eicosanoids are produced from linoleic and linolenic acids. Linoleic acid is converted to arachidonate (20:4;n-6) which is very important for membrane function and a vital precursor for certain prostaglandins. Linolenic acid does not produce arachidonate, but instead produces equally important molecules, such as eicosapentaenoate (20:5;n-3) and docosahexaenoic acid (22:6;n-3). These polyunsaturated fatty acids are important in membrane function and are precursors for a different prostaglandin series (Smith and Borgeat, 1985). Perhaps the importance of linolenic acid is its ability to be a precursor for prostaglandins or some other physiologically important molecule.

## THE EFFECT OF DIET ON METABOLIC RATE

No study has yet determined the effect of saturated fatty acids, unsaturated fatty acids, polyunsaturated fatty acids, or lack of just essential fatty acids on metabolic rate in the same animals during summer, normothermia in winter, and deep hibernation. A few studies have been performed during the winter, but usually just on hibernators during deep hibernation or torpor when body and ambient temperatures are low.

Thorp *et al.* (1994) determined that metabolic rate of marmots during normothermia in summer/fall was not significantly different between animals fed a control diet and animals fed a diet deficient in essential fatty acids. The mean metabolic rate of summer marmots on a control diet was 7.63 ml $O_2$/kg min compared to 6.70 ml $O_2$/kg min for essential fatty acid deficient animals of similar mass. The mean metabolic rate of normothermic marmots in winter was not significantly different between the two dietary groups and Geiser, (1991) also found no significant difference between the metabolic rates of *Peromyscus maniculatus* fed a diet high in saturated fats, high in polyunsaturated fats, or control as long as the animals were normothermic during the winter. Hibernators lacking essential fatty acids in their diet, or on a diet high in saturated fat, had significantly higher metabolic rates than hibernators fed a control or fed a diet high in polyunsaturated fatty acids while in deep hibernation (Table 4).

In all hibernators studied thus far, the lowest metabolic rate during hibernation was recorded in animals that were fed a high polyunsaturated fatty acid diet or control diet, regardless of low ambient temperature (Table 4). How polyunsaturated fatty acids influence thermoregulation and/or metabolism just during deep hibernation is unknown. The significance of this finding is provocative, however, because one might hypothesize that polyunsaturated fatty acids change in the hypothalamic thermoregulatory set-point of animals fed different diets. One might test this hypothesis by manipulating hypothalamic temperature manipulations in animals maintained on different diets. Perhaps the polyunsaturated fatty acids are exchanged with phospholipids in the membranes of the hypothalamus, and this in some way alters fluidity and the set-point for thermoregulation and metabolism. Another possibility may be that the overall tissue membrane composition is altered thereby producing more "leaky" membranes. This has been suggested as a possible difference between ectotherms and endotherms in general (Pan *et al.*, 1994).

Regardless of what mechanisms are involved, this effect should be reproduced in

TABLE 4. *The effect of diet on metabolic rate in four species of hibernators.*

| Species | Diet | Hibernation | Normothermia (winter) |
|---|---|---|---|
| *Marmota flaviventris* | EFA | 1.99 ± 0.6 | 10.2 ± 0.3 |
| (ml $O_2$/kg min) | control | 0.65 ± 0.1 | 9.6 ± 0.7 |
| *Spermophilus saturatus* | saturate | 0.043 ± 0.009 | |
| (ml $O_2$/g hr) | control | 0.034 ± 0.007 | — |
| | unsaturate | 0.029 ± 0.002 | |
| *Eutamias amoenus* | saturate | 64 ± 18 | — |
| (ml $O_2$/kg hr) | control | 47 ± 13 | |
| | unsaturate | 34 ± 9 | |
| *Peromyscus maniculatus* | saturate | 1.21 ± 0.6 | |
| (ml $O_2$/g hr) | control | 0.55 ± 0.1 | NS |
| | unsaturate | 0.45 ± 0.1 | |

Data were taken from Geiser and Kenagy (1987), Thorp *et al.* (1994), Geiser (1991) and Geiser and Kenagy (1993). EFA represents a diet low in essential fatty acids. Saturate represents a diet high in 16:0 (>20%) and 18:0 (>24%), unsaturate represents a diet high in 18:2 (>60%), and control is the purina rodent chow diet (#5001). NS means there was no significant difference in metabolic rate between the three dietary groups.

other hibernators under all conditions. The ground squirrel (*S. lateralis*), for example, has not been investigated and would be an excellent hibernator to study under all of the conditions cited above. In non-hibernating species (*e.g.*, rat), dietary manipulations such as these alter not only metabolic rate but also lipid composition and the metabolism of particular lipids. Rats that are deficient in essential fatty acids die during even moderate cold stress (Rafael *et al.*, 1988). Thus, polyunsaturated fatty acids affect metabolic rate even in non-hibernators, but the mechanism by which they affect metabolic rate remains to be determined.

## FUTURE RESEARCH

The essential fatty acids are important precursors for many biologically active molecules like the eicosanoids (Fig. 3). These molecules in turn are very important for processes such as reproduction, water balance, retinal function, and cell-signaling in normothermic animals (Serhan *et al.*, 1996). The role of prostaglandins or eicosanoids in hibernation is unknown. Prostaglandins alter thermoregulatory behavior in hibernators like they do in non-hibernators in summer. However, whether prostaglandins alter thermoregulatory patterns during hibernation is unclear. Because essential fatty acids are the precusors for prostaglandins, modifying the essential fatty acids in the diet of hibernators may alter the prostaglandin concentrations in tissues that regulate thermoregulation during winter. Another possibility is that changes in dietary essential fatty acids modify cAMP levels in tissues that are important for thermoregulation. A recent study on mouse thyroid cells found that the thyroid cells produced less cAMP if mice had fed a diet high in saturated fat. Whereas, the thyroid cells from control mice, which were given 4% safflower oil in addition to the diet high in saturated fat, produced normal amounts of cAMP (Siddhanti *et al.*, 1990). Furthermore, lethal hypothermia was observed in mice fed a diet high in saturated fat and the toxicity was greatly reduced if essential fatty acids were added back to the diet. Further analysis of plasma lipid fractions indicated that the only differences between mice fed the high saturated fat diet and mice fed a diet supplemented with safflower oil was in the fatty acid composition of the cholesterol esters. The plasma of mice fed a diet high in saturated fatty acids had 3% cholesterol linoleic acid while the plasma of mice receiving the supplemented diet was 32%. Siddhanti *et al.* (1990) suggest that this difference in the plasma lipid composition may in part be due to particular gastrointestinal hormones that are regulated by the balance between unsaturated and saturated fats.

Thyroid function in hibernators varies with season and perhaps species (Tomasi and Stribling, 1996), so the fatty acids ingested by an animal may influence thyroid

function. Furthermore, we have demonstrated that the proportion of cholesterol esters in the plasma lipids of marmots resists changes in lipid composition to a significant degree: Cholesterol esters remain high in linoleic acid despite a decrease in dietary linoleic acid within tissues (unpublished observations). Linoleic acid appears to be important not only for normal hibernation behavior, but also for proper hypothalamic function in non-hibernators. Release of pituitary hormones, particularly prolactin and thyroxin, is influenced by a lack of linoleic acid. As far as linolenic acid is concerned, the only direct effect documented to date is on retina formation and thrombosis (Lands, 1992). Perhaps linolenic acid competes with linoleic acid for eicosanoid receptors in specific tissues (Lands, 1992), although this has yet to be demonstrated in a hibernator.

Lastly, the recent characterization of the rodent ob-gene and its product, leptin, has stimulated many interesting questions regarding food intake, energy balance, and fattening that may be best answered by studying hibernators. For instance, leptin may inhibit prehibernation fattening in Arctic ground squirrels (*Spermophilus parryi*) when given in late summer (Ormseth *et al.*, 1996). This result needs to be confirmed and extended because seasonal and species variation may influence the ability of leptin to act on food intake and energy balance. In addition, the ob-gene appears to be partially regulated by transcription factors, such as peroxisome proliferator-activated receptors found in white adipose tissue and brown adipose tissue. These peroxisome proliferator-activated receptors are ligand-activated transcription factors that are stimulated by several molecules, including long-chain fatty acids, and they are capable of altering cell differentiation (De Vos *et al.*, 1996). Further, peroxisome proliferator-activated receptors are activated by the prostaglandin $J_2$ which is derived from an essential fatty acid (linoleic acid).

In summary, long-chain polyunsaturated fatty acids, especially the essential fatty acids, are very important as cell signals in food intake, energy balance, and cell differentiation pathways. The recent advances in lipid metabolism using transgenic animals and molecular cloning techniques will help to further our understanding of how animals gain and lose mass in the form of fat. Using these new discoveries, I believe we can now address hypotheses regarding regulation of body mass and fat metabolism in hibernators that probably could not have been guessed by an intelligent savage.

## ACKNOWLEDGMENTS

I thank Drs. P. K. Ram and David A. Rintoul for technical assistance and for reading a draft of the manuscript. Nancy McIntyre helped with the preparation of the figures. I also thank Drs. Allen Gibbs and Elizabeth Crockett for organizing this symposium and the National Science Foundation for supporting my research (IBN #9630683).

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