Compositional correlates of metabolic depression in the mitochondrial membranes of estivating snails

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Stuart, J. A., T. E. Gillis, and J. S. Ballantyne. Compositional correlates of metabolic depression in the mitochondrial membranes of estivating snails. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R1977–R1982, 1998.—The phospholipid and protein compositions of mitochondrial membranes from hepatopancreas of active and estivating terrestrial snails (*Cepaea nemoralis*) were compared. Mitochondria from estivating snails contained 82.7% less cardiolipin, and this was associated with an 83.9% reduction in cytochrome-*c* oxidase activity. Substantial changes also occurred in the proportional amounts of other individual phospholipid classes and their constituent fatty acids, including a 72% loss of total mitochondrial phospholipids, a 37% increase in monoenes, and 49% fewer n–3 fatty acids in membranes of estivating snails. These changes are consistent with those correlated with lowered metabolic rate and lower rates of proton leak in other animal models. Estivating snail hepatopancreas showed no change in total phospholipid content, indicating that the phospholipids lost from mitochondrial membranes may be sequestered elsewhere within the cell. We suggest that estivating snails remodel mitochondrial membranes as part of a coordinated, reversible suppression of mitochondrial membrane-associated processes, which may include a concomitant reduction in rates of proton pumping and leaking.

mitochondria; membrane; cytochrome-*c* oxidase; phospholipid

SLOWING OF METABOLIC RATE is a defining characteristic of such physiological states as facultative anaerobiosis, hibernation, torpor, dormancy, and estivation (16). Estivation occurs in a number of fish and amphibian species, but is perhaps best characterized in terrestrial snails, where it occurs in response to desiccating environmental conditions. In estivating land snails, standard metabolic rate (SMR) is depressed to ~16% of "normal" resting rates (17). In this state, the snails can survive for periods of months or years until a return of favorable environmental conditions (29).

Metabolism in estivating snails is aerobic, with minimal recruitment of anaerobic pathways (30). Thus mitochondrial oxidative metabolism continues, but at a significantly reduced rate. Metabolic rate reduction in estivating snails must, therefore, involve a controlled suppression of mitochondrial aerobic metabolism, although this has not been directly studied.

The function of the mitochondrion is largely organized within and around the mitochondrial membranes, which provide the milieu within which the electron transport chain (ETC) and related enzymes and transporters operate. Specific membrane phospholipids, such as cardiolipin, are required for the optimal function of a wide variety of mitochondrial membrane-bound transporters and enzymes (32, 19). Physical properties of the bulk bilayer also directly affect the function of many membrane-bound proteins (8, 12, 15). Modulation and control of the activities of mitochondrial membrane-bound proteins may thus be achieved through modification of the membrane phospholipid composition.

Estivation offers a unique opportunity to study such compositional modifications as they relate to metabolic depression. Unlike during hibernation and torpor, the depression of SMR in estivators occurs in the absence of temperature change. Unlike in facultative anaerobes, anaerobic pathways do not appear to play a significant role in maintaining ATP levels (30).

We have tested the hypothesis that mitochondrial function and phospholipid composition are altered during estivation in the terrestrial snail *Cepaea nemoralis* in a manner consistent with a reduction in mitochondrial oxidative metabolism and the observed lowering of whole animal SMR (16–18, 25, 30).

MATERIALS AND METHODS

Cepaea nemoralis were collected in early summer, kept in terraria in the laboratory for ~6 wk, and fed a diet of lettuce. A group of these snails was removed to a dry terrarium, and food was withheld to induce estivation. Estivation, identified by the presence of a calcareous epiphragm at the shell aperture, lasted for 6 wk. Hepatopancreas from active or estivating snails were excised, pooled into groups of two, weighed, and immersed in 10 volumes of mitochondrial isolation buffer (HEPES, 0.5% BSA, pH 7.5) and homogenized by three passes with a Potter-Elvejhem homogenizer. Homogenates were centrifuged at 150 *g* for 10 min, and the pellet was discarded. The remaining supernatant was centrifuged at 5,000 *g* for 10 min, and the pellet was "washed" by repeating this step twice. The mitochondrial pellets were immediately frozen at −20°C. They were thawed before lipid extraction ~24 h later.

Purity of the mitochondrial preparation. This protocol for isolation of intact mitochondria is similar to those used in other studies of *C. nemoralis* (22). This procedure consistently gives a recovery of ~85% of mitochondria. Equal recovery of mitochondria from hepatopancreas of active snails and estivators is indicated by similar recoveries of cytochrome-*c* oxidase (CCO) activity [82.4 ± 5.1 and 88.8 ± 4.0% (mean ± SE), respectively, of whole homogenate activity; $n = 4$; $P > 0.05$]. Peroxisomal contamination was assessed with the use of measurements of peroxidase activity. Only 3.5 ± 1.0% of homogenate peroxidase activity was recovered in the mitochondrial fraction from active snails, and 3.8 ± 0.8% was recovered in estivating snails. These values were not significantly different ($P > 0.05$; $n = 4$). The amount of sphingomyelin in the mitochondrial fraction can be used to indicate the presence of nonmitochondrial membrane fragments, because mitochondria contain only trace amounts of sphingomyelin, which is localized largely in the outer membrane (10). Nuclear membranes, endoplasmic reticulum, and lysosomal and plasma membranes all have much higher proportional con-

tents of sphingomyelin (10). The proportional content of sphingomyelin in the mitochondrial fraction was 2.4 ± 1.5% in active snails and 4.2 ± 1.5% in estivators. These values were not significantly different ($P > 0.05$; $n = 7$ or 8).

Lipid extraction and analysis. Total mitochondrial lipids were extracted by the method of Bligh and Dyer (1). Phospholipids were separated from neutral lipids and each other by thin-layer chromatography as in Holub and Skeaff (22). Fatty acids were methylated by scraping individual phospholipid bands into glass tubes containing 2 ml of 6% H_2SO_4 in methanol and 10 µg of heptadecanoic acid. The tubes were tightly sealed with Teflon-lined caps and vortexed for 60 s and then maintained at 80°C for 2 h. Petroleum ether (2 ml) was then added to the cooled tubes, and each tube was vortexed for 60 s. Double-distilled H_2O (1 ml) was added, and the tubes were vortexed again for 30 s. The upper petroleum ether phases were transferred to glass minivials. Individual fatty acids from each phospholipid fraction were separated by gas chromatography as described by Glémet and Ballantyne (14). Petroleum ether samples containing fatty acid methyl esters were dried under oxygen-free nitrogen gas and redissolved in 25 µl carbon disulfide (CS_2). CS_2 samples (2 µl) were injected into a gas chromatograph (Hewlett-Packard, HP5890 series II) fitted with a flame ionization detector and an automatic injector (Hewlett-Packard, 7673A). Fatty acid methyl esters were analyzed on a DB 225 megabore fused silica column (Chromatographic Specialties, Brockville, ON, Canada) at 210°C for 30 min, which included an initial ramping from 150 to 210°C over the first 1 min. Fatty acids were identified by comparison of retention times from a known standard containing all fatty acids of interest. Chain lengths shorter than C:14 were not resolved under these conditions and are therefore not reported.

Total amounts of each phospholipid were determined from the summed amounts of fatty acids in each phospholipid. Absolute amounts of individual fatty acids were determined by comparison with a known concentration of an internal standard, heptadecanoic acid (17:0), added to the samples before the methylation process.

Measurements of enzyme activities and protein. CCO was measured as described by Stuart and Ballantyne (31). Briefly, oxidation of fully reduced cytochrome *c* (50 µM) by homogenate in 50 mM imidazole (pH 8.0) buffer was followed at 550 nm with the use of a Hewlett Packard HP8452 diode array spectrophotometer (Hewlett Packard, Mississauga, ON, Canada) equipped with a thermostatted cell changer maintained at 20°C with a Haake D8 circulating water bath (Haake Buchler Instruments, Saddlebrook, NJ). Peroxidase activity was measured at 240 nm after addition of 30 mM peroxide (H_2O_2) to diluted homogenate in 50 mM imidazole (pH 7.0).

Protein was determined on aliquots of thawed samples using the Bio-Rad (Bio-Rad Laboratories, Hercules, CA) protein microassay.

Determination of total hepatopancreas phospholipids. Total hepatopancreas membrane phospholipids in active and estivating individuals were measured in a second group of snails kept under the same laboratory conditions as above. For these snails, whole hepatopancreas from individual snails (not pooled) were homogenized in 2 ml of 50 mM imidazole, pH 7.5, using three 10-s bursts of a Polytron PT10 unit (Kinematica Lucerne, Switzerland). Phospholipid extraction was as described above, with the exception that the phospholipid fraction was separated only into cardiolipin and all other phospholipids. Further analysis was carried out by gas chromatography as in the first experiment.

Statistical analyses. Absolute data values were compared with the use of Student's *t*-tests. Proportional phospholipid and fatty acid compositional data were arcsine transformed and then compared with the use of Student's *t*-tests. For comparisons of individual fatty acids, *P* values were adjusted to compensate for the use of multiple *t*-tests (21).

RESULTS

No significant differences were observed between active and estivating snails in hepatopancreas weight or protein content (Table 1). Similarly, the protein content of the mitochondrial fraction isolated from hepatopancreas tissue was unchanged after 6 wk of estivation. Although the lack of change in protein content suggests that the absolute amounts of most enzymes may have remained constant in estivating snails, the activity, but not necessarily the amount (see DISCUSSION), of the respiratory chain enzyme CCO was reduced by 83.9%.

In contrast to the unchanged protein levels, mitochondrial membrane phospholipid composition was altered significantly in estivators. Perhaps the most dramatic changes were a 71.7% decrease in total mitochondrial phospholipid content of hepatopancreas from estivating *Cepaea* and an 82.7% reduction in cardiolipin content (Table 1). Despite the loss of phospholipid from the mitochondrial fraction, there were no changes in the total phospholipid content of whole hepatopancreas tissue with estivation (Table 1). Similarly, no significant difference between groups was observed for whole tissue cardiolipin content.

Relative proportions of mitochondrial phospholipids were also altered in hepatopancreas of estivators (Table 2). Reductions occurred in proportional content of the hexagonal phase-preferring phospholipids, cardiolipin and phosphatidylethanolamine, of 35 and 14%, respectively. These were accompanied by an 89% increase in

Table 1. *Morphological and biochemical parameters from hepatopancreas of active and estivating snails*

Morphological and Biochemical Measurements	Active	Estivating	%Change
Hepatopancreas wt, mg	275 ± 26	238 ± 5	NS
Mitochondrial protein, mg/g hepatopancreas	8.9 ± 1.0	8.1 ± 0.3	NS
Total hepatopancreas protein, mg/g hepatopancreas	26.4 ± 2.2	25.6 ± 1.3	NS
Mitochondrial phospholipid, nmol/mg mitochondrial protein	89.1 ± 8.0	25.2 ± 2.7*	−71.7
Mitochondrial phospholipid, nmol/mg cellular protein	30.0 ± 2.7	8.0 ± 0.9*	−73.3
Total hepatopancreas phospholipid, nmol/mg cellular protein	68.5 ± 7.7	59.3 ± 6.4	NS
Cytochrome *c* oxidase activity, $\mu mol \cdot min^{-1} \cdot g\ wet\ tissue\ wt^{-1}$	1.9 ± 0.2	0.3 ± 0.1*	−83.9
Mitochondrial cardiolipin content, nmol/mg cellular protein	3.4 ± 0.1	0.6 ± 0.1*	−82.7
Tissue cardiolipin content, nmol/mg cellular protein	3.8 ± 0.7	2.6 ± 0.4	NS

Values are means ± SE of 7 or 8 measurements. *Significantly different from active snails ($P < 0.05$). NS, not significant.

the proportion of phosphatidylinositol in the mitochondrial membranes (Table 2).

The fatty acid composition of mitochondrial phospholipids (pooled) was also altered in estivators (Table 3). Mitochondrial membranes of estivating snails contained greater proportions of monoenes, due primarily to a large increase in the proportional amount of 16:1. They also contained fewer polyenes. This difference was reflected in the monoenes-to-polyenes ratio, which increased by 68%. The proportional content of n–3 polyenes was reduced in estivating snails, primarily through reductions in 18:3(n–3) and 18:4(n–3) content. As a result, the ratio of n–3 to n–6 was lowered by one-half in estivators. No significant differences between active and estivating snails were observed for proportions of saturated fatty acids, unsaturation index, or average fatty acid chain length.

DISCUSSION

These data indicate that substantial changes to mitochondrial structure and function occur in estivating *C. nemoralis*. The general suppression of oxidative metabolism that accompanies estivation in terrestrial snails is reflected by a significant reduction in CCO activity. The changes in membrane phospholipid composition are also consistent with a reduced mitochondrial function.

Reductions in CCO activity in hepatopancreas of estivating snails could be explained by a reduction in the number of mitochondria in hepatopancreas. However, both whole tissue and mitochondrial protein levels were similar in active and estivating snails. Thus, although the protein content of mitochondria appears to be unchanged during estivation, substantial reductions occur in mitochondrial phospholipid content. This suggests that the downregulation of metabolic flux in mitochondria does not occur through wholesale reductions in number of mitochondria or enzyme concentrations. In estivating snails, posttranslational modifications, such as reversible phosphorylation, have been shown to be a particularly common means of regulating activities of soluble enzymes during metabolic depression (16, 17, 30). However, because many of the enzymes involved in oxidative metabolism are not soluble but membrane-bound, an alternative regulatory strategy is available for their regulation.

Table 2. *Proportions of phospholipid species in mitochondrial membranes of hepatopancreas from active and estivating snails*

Phospholipid	Active, mole %	Estivating, mole %	%Change
Cardiolipin	11.4 ± 0.1	7.4 ± 1.4*	−35
Phosphatidylcholine	37.7 ± 2.7	39.6 ± 1.9	NS
Phosphatidylethanolamine	35.2 ± 1.5	30.1 ± 0.7*	−14
Phosphatidylinositol	8.0 ± 0.6	15.1 ± 1.8*	+89
Phosphatidylserine	7.7 ± 0.3	7.9 ± 1.7	NS

Values are means ± SE of 7 or 8 individual measurements. *Significantly different from active snails ($P < 0.05$).

Table 3. *Proportions of individual fatty acids in hepatopancreas mitochondrial phospholipids from active and estivating C. nemoralis*

	Active, mole %	Estivating, mole %	%Change
	Fatty acid		
14:0	0.97 ± 0.40	2.41 ± 0.62	NS
14:1	1.50 ± 0.52	1.57 ± 0.47	NS
16:0	6.22 ± 0.25	8.38 ± 0.92	NS
16:1	1.66 ± 0.35	5.49 ± 0.85*	+331
18:0	10.32 ± 0.74	10.73 ± 0.62	NS
18:1	7.89 ± 0.37	8.02 ± 0.45	NS
18:2(n–6)	18.28 ± 0.74	7.29 ± 0.57*	−60
18:3(n–3)	7.87 ± 0.24	3.06 ± 0.15*	−61
18:4(n–3)	2.72 ± 0.36	1.01 ± 0.14*	−63
20:0	0.43 ± 0.10	0.66 ± 0.04	NS
20:1	3.48 ± 0.23	4.76 ± 0.835	NS
20:2(n–6)	7.99 ± 0.25	9.87 ± 0.62	NS
20:3(n–6)	2.69 ± 0.55	4.11 ± 0.46	NS
20:4(n–6)	18.26 ± 0.76	21.08 ± 1.30	NS
20:3(n–3)	2.87 ± 0.40	1.09 ± 0.18*	−62
20:4(n–3)	0.06 ± 0.06	0.27 ± 0.10	NS
20:5(n–3)	1.44 ± 0.15	1.17 ± 0.20	NS
22:0	ND	ND	NS
22:1	ND	ND	NS
22:2(n–6)	ND	ND	NS
23:0	0.43 ± 0.10	0.83 ± 0.20	NS
22:4(n–6)	0.92 ± 0.04	1.19 ± 0.08*	+29
22:5(n–6)	3.18 ± 0.18	5.40 ± 0.36*	+70
22:5(n–3)	0.12 ± 0.03	0.12 ± 0.04	NS
22:6(n–3)	0.62 ± 0.09	1.27 ± 0.29	NS
24:0	ND	ND	NS
24:1	ND	ND	NS
	Total		
Total saturates	18.46 ± 1.00	23.01 ± 2.00	NS
Total monoenes	14.52 ± 0.75	19.84 ± 1.35*	+37
Total polyenes	67.02 ± 0.91	56.94 ± 3.30*	−15
n-3 Polyenes	15.68 ± 0.81	7.99 ± 0.62*	−49
n-6 Polyenes	51.32 ± 0.88	48.93 ± 2.74	NS
n-3/n-6	0.31 ± 0.02	0.16 ± 0.01*	−48
Monoenes/polyenes	0.22 ± 0.01	0.37 ± 0.05*	NS
Unsaturation index	222.45 ± 3.86	213.78 ± 10.21	NS
Chain length	18.36 ± 0.05	18.52 ± 0.16	NS

Values are presented as means ± SE of 7 or 8 individual measurements. Total mole % of active and estivating fatty acids is 100. ND, not detectable. *Significantly different from active values, $\alpha = 0.0034$. Unsaturation index, $= \Sigma m_i \cdot n_i$; where m_i is the mole percentage and n_i is the number of C—C double bonds in fatty acid i. Mean chain length, $\Sigma f_i \cdot c_i$; where f_i is the mole fraction and c_i is the number of carbon atoms in fatty acid i.

Modulation of the lipid environment of membrane-bound proteins may be one mechanism for their regulation during metabolic depression. The activities of many membrane-bound proteins are responsive to changes in their phospholipid environment (19, 26, 32). Cardiolipin, in particular, is found in close association with a number of mitochondrial inner membrane proteins and in some cases has been shown to directly affect activity (19). For example, CCO; the mono-, di-, and tricarboxylic acid transporters; carnitine acyltransferases; ATPase; ADP/ATP exchanger; and P_i transporter all have absolute or partial requirements for cardiolipin to achieve maximal activities (19). The dramatic reduction in cardiolipin content of estivating *Cepaea* mitochondria (82.7%), which occurs in conjunc-

tion with a nearly identical (83.9%) reduction in CCO activity and corresponds closely to the ~84% reduction in SMR observed in several species of estivating pulmonate snails (16–18, 25, 30), is therefore an interesting result. The similarity of these values may be somewhat fortuitous, but the large changes occurring are undeniable. Differences in contamination of the two mitochondrial membrane preparations could have an effect on the observed reduction in cardiolipin content in estivating snails, but the maximal effect of such contamination would be only to lower slightly the change in cardiolipin content from 82.7%. Regardless of the magnitude of the change, the activities of many enzymes and transporters in the mitochondrial inner membrane could be reduced in a coordinated fashion simply by removing cardiolipin from the membrane. For instance, the observed reduction in CCO activity in estivating snails could be mediated through the removal of cardiolipin from the mitochondrial inner membrane. A number of studies have demonstrated the direct dependence of CCO on cardiolipin for activity (Ref. 26, reviewed in Ref. 19).

Inhibition of ETC activity through modifications of phospholipid composition is also suggested by studies of mitochondria from hibernating squirrels (6). Electron transport in hibernating squirrels is inhibited by 70–80% at ubiquinol-cytochrome. Two results suggest that this inhibition is phospholipid mediated. When phospholipase A_2 is activated (by hypoosmotic swelling), ETC activity is recovered (6). Also, pharmacological inhibition of phospholipase A_2 activity during swelling prevents the recovery of electron transfer (6). Thus general adjustments to membrane fluidity may be involved in mediating the reduction of rates of electron transfer. Our results indicate some of the specific compositional changes that could occur to accomplish this.

In addition to the specific effects of cardiolipin on protein activities, properties of the bulk membrane, such as phase behavior and fluidity, have also been shown to modulate protein function. The mitochondrial membranes of estivating *Cepaea* contain lower proportions of the hexagonal phase-preferring phospholipids cardiolipin and phosphatidylethanolamine and unchanged or greater proportions of more bilayer-stable phospholipids, including phosphatidylcholine, phosphatidylserine, and phosphatidylinositol. Hexagonal phase-preferring lipids are associated with a number of active processes (reviewed in Ref. 12), including transmembrane trafficking, mitochondrial contact sites, and stimulation of some enzyme activities (8). Thus the lower proportion of these phospholipids suggests reductions in these processes during estivation.

The observed changes in mitochondrial phospholipid composition may also be related to reductions in the permeability of the inner membrane to protons. As mitochondria from all animals are semipermeable to protons, a futile cycle of outward proton pumping and inward proton leak exists across the inner membrane (2). Leakage of protons from the cytosol to the mitochondrial matrix accounts for a considerable energy expenditure in animal cells. In mammals, the cost of this proton leak is estimated at between 20 (28) and 25% (24) of SMR, and this proportion appears to be similar in ectotherms, based on similarities in the properties of lizard and mammalian hepatocytes (3). Estivating land snails consistently achieve SMR reductions of 84%, which occur in the absence of anaerobic metabolism (25). Thus on the assumption that mitochondrial proton leak incurs a similar cost as a proportion of SMR in snails as in other ectotherms, for SMR to be lowered to 16% of normal during estivation a concomitant and significant reduction in the rate of proton leak must occur.

The mechanism of proton leak in most tissues remains unknown. Nonetheless, reductions in proton leak correlate well with some mitochondrial phospholipid composition parameters, suggesting a role for specific phospholipid fatty acids in determining proton permeability of the intact membrane. Specifically, two properties of the inner membrane, a decrease in the total amount of phospholipids, and therefore surface area, and reduced inner membrane leakiness per unit membrane surface area, are correlates of less proton-permeable mitochondrial membranes (2–4, 24). The modifications to fatty acid composition of mitochondrial phospholipids of estivating snails are consistent with both of these mechanisms of leak reduction.

Decreased mitochondrial inner membrane surface area correlates with reduced proton leak in hypothyroidism (4). Similarly, in allometric studies of mammalian mitochondria, differences in inner membrane surface area explain two-thirds of the large differences in proton leak between mitochondria from mammals of different sizes (24). Other studies (4) have demonstrated that total phospholipid content is approximately proportional to membrane surface area. As the convoluted inner membrane accounts for a much greater proportion of total mitochondrial phospholipids than the outer membrane, the reduction in total phospholipids in *Cepaea* mitochondria should reflect a decrease in the surface area of the inner membrane. Thus the observed 72% reduction in total mitochondrial phospholipids in estivating *Cepaea* may reflect a similar reduction in membrane surface area and thus a corresponding reduction in proton leak.

Two phospholipid fatty acid parameters are also modified in manners that suggest decreased proton leak per unit membrane surface. The proportion of monounsaturated fatty acids, which tends to be higher in organisms with lower metabolic rates and less leaky membranes (2, 3), increases 37% in mitochondrial membrane phospholipids of estivating snails relative to active ones. Similarly, the proportion of n–3 polyunsaturates correlates positively with membrane leakiness and metabolic rate (3, 24), and a 49% decrease in the proportion of n–3 fatty acids in mitochondrial membranes is observed in estivating snails. These observations are both consistent with a decreased leakiness per unit surface area of membranes from estivators. Thus both the reduction in mitochondrial membrane surface area and an altered fatty acyl composition of the

remaining membrane phospholipids are consistent with a reduction of ion leak in these membranes.

The substantial reduction observed in CCO activity and the adoption of membrane characteristics associated with reduced proton leak, suggest that a coordinated reduction in proton pumping and leaking occurs in estivating *Cepaea*, which is likely a key component of the total energy savings necessary to depress SMR by 84%. Such coordinated reductions would be similar in principle to the "channel arrest" mechanism by which Na^+ and K^+ gradients are maintained across the plasma membranes of metabolically depressed organisms (7, 9, 13, 23). Reductions in Na^+ leak across plasma membranes can be achieved through the downregulation of specific ion channels (23). Similarly, it may be possible to reduce mitochondrial proton leak through removal of membrane components that are associated with increased leak.

In this context, our finding that the total phospholipid content of hepatopancreas tissue does not change during estivation is interesting. This suggests that those phospholipids that are lost from mitochondrial membranes remain within the cell. A possible mechanism for sequestering these phospholipids is that which has been suggested to reduce plasma membrane Na^+ leak, i.e., the "blebbing off" of channel-containing membrane fragments, which are then stored elsewhere in the cell (7, 23). Such reversible reductions in membrane surface area have been demonstrated in the alga *Dunaliella salina* (11), in which exposure to hyperosmotic conditions results in the transfer of membrane phospholipids from mitochondria and other organelles to the endoplasmic reticulum, which serves as a temporary reservoir for membrane until a return to isosmotic conditions. Such a strategy could allow snails emerging from metabolically depressed states to restore a mitochondrial phospholipid composition typical of active metabolism through fusion with vesicles of stored phospholipid, so that phospholipid-mediated reductions in SMR might be rapidly reversed. Snails, typical of many estivators, enter into and emerge from metabolically depressed states quite rapidly (i.e., minutes to hours) (18).

Perspectives

Strategies for achieving metabolic depression (e.g., enzyme phosphorylation, intracellular acidification, and association/dissociation of enzymes with the cellular structural elements) are characterized, in part, by their ready reversibility. Similarly, alterations to membrane structural elements (e.g., phospholipids) that may be rapidly reversed on resumption of normal activity may provide a mechanism for the regulation of membrane-bound protein activities during metabolic depression. Phospholipid-dependence has been demonstrated for many membrane-bound protein activities, and this characteristic may be exploited in metabolically depressed organisms. Those membrane-bound proteins (e.g., pumps and channels) that play a role in the creation and maintenance of transmembrane ion gradients linked to various metabolic functions are likely important regulatory targets during metabolic depression. The energy consumed by the maintenance of H^+, Na^+, K^+, and Ca^{2+} gradients across cellular and organellar membranes appears to be substantial, representing perhaps 25–50% of the overall cellular energy budget. This must be reduced in organisms that achieve a sustained metabolic depression in excess of 75% of normal SMR. We have proposed that a temporary reorganization of structural elements (phospholipids) within the cell could represent one powerful and readily reversible means of effecting a suppression of H^+ pumping and leaking in mitochondria. We suggest that adaptations at the level of cellular and organellar membranes could play a causative role in reducing both the rates of ion pumping and their transmembrane leak during states of metabolic depression.

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