Analysis of the hibernation cycle using LC-MS-based metabolomics in ground squirrel liver

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hibernation is a form of metabolic depression that conserves energy during seasonal periods of high energy demand coupled with reduced energy availability in the environment. In mammals, the hibernation season is composed of extended bouts of torpor [body temperature (Tb) ~5°C] lasting days to weeks that are interrupted by brief (<24 h) periods of euthermia (Tb ~37°C), called interbout arousals. The purpose of interbout arousals is not entirely clear, but possibilities include normalizing of neuronal processes, synthesis of molecules required for torpor or survival during hibernation, and general cellular maintenance (2, 7). Understanding the mechanisms by which hibernating mammals safely and reversibly undergo metabolic depression and survive the physiological extremes of the hibernation season could lead to new approaches for disease treatment and prevention in humans and other animals. By its nature, hibernation is a highly integrative process that is well suited for a systems biology approach that incorporates technologies at multiple levels, from molecules to behavior. The molecular basis of hibernation is being increasingly explored using genomic and proteomic technologies. High-throughput transcriptomic analyses have revealed state-specific fluctuations in mRNAs for genes involved in a variety of processes including fatty acid oxidation, gluconeogenesis, apoptosis, and circadian rhythm (5, 56, 60, 61). Proteomic screens of hibernator tissues have also revealed candidate molecules that characterize the hibernation phenotype and may play roles in hibernation-induced protection from the extreme changes in physiology that occur during torpor-arousal cycles (14, 33, 39). Although transcriptomic and proteomic approaches have had some success, in general the number of differentially expressed genes is low and the degree of change is relatively small given the dramatic changes in phenotype that occur seasonally and during torpor-arousal cycles. Furthermore, both transcriptomic and proteomic approaches are limited by the lack of a high-coverage, well-annotated genome for the hibernating species under study.

Metabolomics is the newest systems biology tool being applied to the analysis of the hibernation phenotype. Three commonly applied techniques in metabolomic investigations are nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) based approaches (23). Henry and colleagues (25) applied NMR spectroscopy in vivo to assess metabolite changes in the brain of torpid and aroused 13-lined ground squirrels. Levels of some metabolites were stable between the two states, but others, including the neurotransmitters GABA and glutamate, were significantly altered. Recently, NMR spectroscopy was used to identify metabolites in liver that serve as biomarkers for the two phenotypic switches, summer-winter and torpor-arousal, that characterize hibernation in ground squirrels (42). Results of this screen were consistent with the well-known shift from carbohydrate to lipid metabolism in hibernation and also revealed novel metabolite changes including accumulation of glutamine and betaine late in the torpor bout. While NMR-based metabolomics is convenient due to minimal sample preparation and absolute identification, this technique is less sensitive than MS and therefore accesses a smaller collection of compounds.

The aim of the present study was to use an LC-MS-based approach to compare metabolite levels in ground squirrel liver during the circannual hibernation cycle. As the metabolic hub of the body, the liver performs several key roles that directly influence hibernation including gluconeogenesis, ketogenesis, protein synthesis, and removal of metabolic byproducts. Our analysis identified multiple liver metabolites that vary seasonally and among four hibernation states. Some results are consistent with current understanding of mammalian hiberna-
tion, and others revealed novel patterns that provide new insight into the metabolic effects of torpor-arousal cycles in hibernators.

METHODS AND MATERIALS

Reagents and syntheses. Methanol, acetonitrile, and trifluoroacetic acid were purchased from Fisher Scientific. Sphingosine, propionyl chloride, butyryl chloride, isovaleryl chloride, inosine, phenylalanine, and tyrosine were purchased from Arcos Chemicals. Uridine, hexadecanediolic acid, isobutyryl chloride, and biliverdin were purchased from MP Biomedical. 5-(Trifluoromethyl)-2-pyridinol, oxygen-fluorine, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), 2-methylbutyryl chloride, tryptophan, and 1-octoeyllspophatidylcholine were purchased from Sigma Aldrich. Cholesterol sulfate was purchased from Alexis Biochemicals.

Short chain acyl carnitine esters were synthesized as described previously (4).

Animals. All procedures were approved by the University of Wisconsin Madison School of Veterinary Medicine Institutional Animal Care and Use Committee. Thirteen-lined ground squirrels (Spermophilus tridecemlineatus) of both sexes were trapped in the vicinity of Madison, WI. Other squirrels were obtained as pups born in captivity from pregnant females trapped in spring. Squirrels were housed individually at 22°C with a 12:12 h light-dark cycle with free access to water and food (Purina rodent chow 7001, supplemented with sunflower seeds) except for pups born in captivity, which were food-restricted after weaning (12 g chow/day). Prior experience indicated this level of daily intake prevents excessive weight gain, which is common in ground squirrel pups born in captivity, yet allows them to gain weight at a rate similar to wild-caught animals. Squirrels were held in these conditions for at least 1 mo before use in experiments. Summer-active squirrel (SUM) squirrels were collected from the wild in early summer and, based on body mass, were judged to be at least 1 yr old (and therefore had hibernated in the wild for at least one winter). In August, other squirrels were implanted with temperature-sensitive radio teleimeters (VitalView S3000; Minimitter, Bend, OR), and Tis was monitored every 2 min during the hibernation season. In September-October, squirrels were transferred to a room maintained at 4°C. The room was dark except for brief periods (<20 min) of low lighting once per day to check activity state. Water and food were removed after squirrels began regular bouts of torpor.

SUM animals were killed in late July/early August when Ta ∼37°C after an overnight fast. Hibernating squirrels were killed in one of four states: entering torpor (EN) (Ta = 20–25°C); late torpor (LT), ≥7 d in torpor (Ta = 5–7°C); arousing from torpor (AR) (Ta = 20–25°C); and interbout arousal (IBA) (≥2 h at Ta >34°C). Summer squirrels and hibernators in EN, AR, and IBA were killed by decapitation after isoflurane anesthesia, and LT squirrels were decapitated without prior anesthetic. After laparotomy liver tissue was harvested and immediately frozen in liquid nitrogen.

Sample preparation. Frozen liver samples were ground with a mortar and pestle in liquid nitrogen while on dry ice. Methanol was selected as the extraction solvent because it has proven to be an effective broad-spectrum solvent for metabolite extraction (36, 54, 55). Approximately 50 mg of liver tissue was extracted three times with 20 μl of methanol (chilled on dry ice) per mg. Methanol contained spiked-in oxygen-fluorine acid and 5-(trifluoromethyl)-2-pyridinol at 0.8 μg/ml to correct for extraction efficiency and instrument variability. The three extractions were combined and dried at room temperature using a rotary evaporator (Savant). Samples were then stored at −80°C until analysis when they were resuspended in 9:5 [double distilled (dd) H2O:acetonitrile] at 6 μl per mg of tissue extract and filtered through a 0.2 μm spin filter prior to analysis.

MS analysis. Filtered samples were analyzed in the positive and negative mode by electrospray ionization liquid chromatography mass spectrometry (ESI-LC/MS). For positive mode analyses, 12 μl of sample was loaded onto a (2.1 mm × 5 cm) C18-reverse phase 1.8 μm particle size column (Agilent) using an Agilent 1100 Capillary HPLC. Buffer A consisted of 0.1% formic acid in ddH2O, and buffer B consisted of 0.1% formic acid in acetonitrile. A gradient was conducted from 5 to 95% buffer B in 30 min at a flow rate of 200 nml/min to elute samples into an LC/MSD TOF mass spectrometer (Agilent). Data were collected from 50–1,200 m/z, a defragmenter voltage of 185 V, a capillary voltage of 3,200 V, and a skimmer voltage of 60 V. For negative mode analyses, 30 μl of sample was analyzed using the same chromatographic conditions with modified buffers [buffer A: ddH2O, 15 mM ammonium formate (pH 5.0); buffer B: acetonitrile].

For LC MS/MS analyses, samples were separated using the same chromatographic conditions and buffers with an Agilent 1100 Capillary HPLC in line with an Applied Biosystems 3200 QTRAP mass spectrometer. The instrument was operated in enhanced product ion mode with the following settings: collision energy of 25 or 30 V, collision energy spread 10 V, declustering potential 20 V, and a high curtain gas pressure. The capillary voltage was 5,500 and 4,500 V for positive and negative modes, respectively.

Data analysis. The WIFF data files were converted to .d format using the Agilent Translator (v.B.01.02) utility. The .d files were then centroided, deisotoped, and converted to mzData xml files using the MassHunter Qualitative Analysis Program (v.B.01.02) (Agilent). Following conversion, xml files were analyzed using the open source XCMS package (v1.9.3) (http://metlin.scripps.edu), which runs in the statistical package R (v. 2.5.0) (http://www.r-project.org), to pick, align, and quantify features (chromatographic events corresponding to specific m/z values and elution times). The software was used with default settings as described (http://metlin.scripps.edu) except for xset(bw = 5) and retcor(plottype = “m”, family = “s”, span = 0.2).

The median mass for each feature defined by XCMS was then compared with masses for metabolites downloaded from the Kyoto Encyclopedia for Genes and Genomes (http://www.genome.jp). Metlin (http://metlin.scripps.edu), and the Human Metabolome Database (http://www.hmdb.ca) (20, 43, 57). Candidate metabolites were considered further if experimental mass differed <4 ppm from the theoretical mass. Integrated peak areas for candidate metabolites were corrected using the compounds spiked into the methanol to account for variability in sample handling and instrument response. For metabolites that were observed and validated in both positive and negative modes (FMN, FAD, phenylalanine), the integrated peak areas from each mode were normalized to the median value for the given mode (positive or negative) and averaged. These values were then log transformed and a one-way ANOVA conducted assuming equal variance using R. A false discovery correction was applied to post hoc intergroup comparisons using the Agilent Translator (v.B.01.02) utility. Two additional metabolites (uridine and cholesterol sulfate) were validated using retention time and mass accuracy only. A list of the other...
candidate metabolites for which standards were not available is presented in Supplementary Fig. S1.1

**Carnitine esters.** We validated several short chain fatty acid (SCFA) esters that varied dramatically among the different activity states. All were very low in LT and rose upon arousal to euthermia (AR and IBA). Two five-carbon esters, isovaleryl-carnitine (Fig. 3A) and 2-methylbutyrylcarnitine (Fig. 3B), as well as a feature isobaric to butyrylcarnitine and isobutyrylcarnitine (Fig. 3C), were reduced in all hibernation states relative to SUM. In contrast, propionylcarnitine (Fig. 3D) increased during AR and IBA to levels similar to SUM.

**Amino acids.** Multiple amino acids were observed; however, due to limitations of our chromatographic conditions most were not effectively retained on the column and so could not be reliably quantified. Three that chromatographed well were the aromatic amino acids tryptophan, phenylalanine, and tyrosine (Fig. 4), and these varied significantly among activity states. All three tended to be lowest during torpor (EN and LT) and increased in IBA.

**Purines and pyrimidines.** The purine metabolite inosine was lowest in LT squirrels and rose significantly during AR and IBA states (Fig. 5A). The pyrimidine nucleoside uridine, identified by retention time and accurate mass only, showed a similar pattern with levels reduced in EN and LT relative to AR, IBA, and SUM animals (Fig. 5B).

**Redox cofactors and catabolites.** The bioactive forms of riboflavin, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), were lowest in EN and LT states and rose ~4-fold during arousal (Fig. 6, A and B). For FAD, levels in AR were greater than in SUM (Fig. 6B). Biliverdin, a heme catabolite, was elevated 15–20 fold in AR and IBA compared with EN and LT (Fig. 6C).

**Lipids.** Several lysophosphatidylcholines were identified by database searches using accurate mass measurement as well as retention time information. These putative metabolites were enriched in SUM and EN compared with other hibernation states. We validated one of these, 1-oleoyllysophosphatidylcholine, which results from phospholipase A2 cleavage of a fatty acid from the sn-2 position of this phospholipid (Fig. 7A). Other putative lysophosphatidylcholine compounds that were unique in mass were observed (Supplemental Fig. S1), but standards were not available for validation. Hexadecanedioic acid, a dicarboxylic acid, was elevated in AR and LT compared with IBA and EN animals (Fig. 7B). Sphingosine, a primary component of sphingolipids, was reduced in LT relative to EN, IBA, and SUM (Fig. 7C). Cholesterol sulfate, identified by retention time and accurate mass only, was elevated in EN relative to LT and AR (Fig. 7D).

**DISCUSSION**

The biochemical mechanisms by which mammalian hibernators reversibly depress their metabolism and survive prolonged periods at low T\textsubscript{b} are poorly understood. There is great interest in elucidating the molecular basis of these mechanisms, both from a fundamental science perspective and for potential biomedical applications. In this study we used an unbiased metabolomic approach to identify changes in small molecules in liver of ground squirrels during defined states in the circannual activity cycle.

One of the most striking patterns we observed was the severe reduction in levels of short-chain acyl carnitine esters in torpid squirrels. Carnitine readily esterifies with carboxylic acids and...
is best known for its role in movement of palmitate and other long chain fatty acids into mitochondria via carnitine palmitoyltransferase. However, carnitine performs at least three other roles in tissues including maintenance of adequate levels of free CoA, removal of excessively abundant acyl groups to prevent cytotoxicity, and shuttling of acyl moieties between different organelles (9). All of the SCFAs we validated (in their carnitine ester forms) can be generated by amino acid catabolism. Thus, the pattern we observed for SCFA carnitine esters in liver likely reflects reduced protein and amino acid catabolism during torpor and its resumption during periodic arousals to euthermia.

In contrast to the other carnitine esters we observed, levels of propionylcarnitine were very low during torpor and increased significantly during arousal to levels similar to SUM animals. In addition to amino acid catabolism, two other sources of propionate in mammals are metabolism of odd-chain length fatty acids and fermentation by gut microbes. In humans, these other two sources can each contribute ~25% of total propionate levels in the body (31). The extent to which these sources contribute to changes in liver propionate levels in hibernators is unknown, but propionate is produced in the ground squirrel cecum during interbout arousals (H. V. Carey, unpublished observations). Propionate produced during arousals could serve as a substrate for gluconeogenesis, which occurs in liver of aroused hibernators to restore glucose and glycogen stores depleted during the torpor bout (6, 18, 22, 38).

The pattern for butyryl/isobutyryl-, isovaleryl-, and 2-methylbutyrylcarnitine was suppression relative to SUM squirrels in all of the other hibernation states including IBA. The CoA esters of isobutyrate, isovalerate, and 2-methylbutyryl carnitine was suppression relative to SUM squirrels in all of the other hibernation states including IBA. The CoA esters of isobutyrate, isovalerate, and 2-methylbutyryl carnitine were products of the branched chain 2-oxo acid dehydrogenase complex. Expression of one of this complex’s subunits, dihydrolipoamide branched-chain transacylase, is lower in liver of hibernating compared with SUM golden-mantled ground squirrels (14), suggesting a general conservation of branched-chained amino acids during the hibernation season.

The aromatic amino acids tryptophan, tyrosine, and phenylalanine were low in EN and LT squirrels and increased upon arousal to levels similar to SUM. Tyrosine and phenylalanine increase in plasma upon arousal in hibernating ground squirrels (28). In our NMR-based liver metabolomic study (42) levels of aromatic amino acids were similar in EN, LT, and SUM squirrels (IBA was not examined), which differs somewhat
from our results. In the present study SUM squirrels were
fasted overnight prior to tissue collection but were not in the
NMR-based study, which may contribute to this disparity.
Phenylalanine and tryptophan are essential amino acids,
whereas tyrosine can be made from phenylalanine by phenyl-
alanine hydroxylase. This enzyme is reduced 1.4-fold in liver
of hibernating golden-mantled ground squirrels, which was
proposed to be a mechanism to spare amino acids (14).

The pyrimidine metabolite uridine was reduced in EN and
LT squirrels and increased upon arousal. The liver is a central
site for uridine synthesis and degradation and thus plays a key
role in maintaining circulating levels of uridine throughout the

![Graphs showing carnitine esters and amino acids](image)

**Fig. 3.** Carnitine esters in liver of SUM and in EN, in LT, AR, and during IBA. A: isovalerylcaritine; B: 2-methylbutyrylcarnitine; C: butyrylcaritine/isobutyrylcarnitine; D: propionylcaritine. Shown are means ± SE calculated from peak areas of extracted ion chromatograms using the XCMS package. Data are expressed relative to the mean value for LT group. Means with different letters are significantly different, P ≤ 0.05; n = 5 animals per group.

![Graphs showing amino acids](image)

**Fig. 4.** Amino acids that varied significantly in ground squirrel liver of SUM and in EN, in LT, AR, and during IBA. A: phenylalanine; B: tryptophan; C: tyrosine. Shown are means ± SE calculated from peak areas of extracted ion chromatograms using the XCMS package. Data are expressed relative to the mean value for LT group. Means with different letters are significantly different, P ≤ 0.05; n = 5 animals per group.
body (8). Uridine can be generated by the action of 5'-nucleotidase on uridine monophosphate (UMP) or alternatively cytidine deaminase acting on cytidine. Uridine can be recycled to UMP by uridine kinase or catabolized to uracil by uridine nucleosidase. Although these pathways have not been studied in hibernators, uridine incorporation into RNA in liver and other tissues is reduced during torpor relative to euthermia and might influence uridine levels (37, 52). To our knowledge ours is the first report of changes in tissue uridine levels during the hibernation cycle.

Hepatic inosine levels were lowest in LT squirrels and increased ~50-fold upon arousal. This purine is produced by conversion of AMP to adenosine by a 5'-nucleotidase followed by catabolism to inosine by adenosine deaminase. Alternatively, inosine can be produced by conversion of AMP to IMP via AMP deaminase (AMPD) and subsequent degradation of IMP to inosine by 5'-nucleotidase. By action of purine-nucleoside phosphorylase, inosine is catabolized to hypoxanthine and ultimately degraded to urate for excretion. Plasma membrane-bound 5'-nucleotidase activity was greater in livers from hibernating than in nonhibernating hamsters, which might alter adenylate catabolism in the extracellular compartment (26). Results from prairie dog skeletal muscle suggest that AMPD activity is reduced under physiological conditions at low T_b (13). If the same holds for liver, this would be consistent with lower inosine levels in torpid squirrels relative to aroused animals. However, these pathways must be considered in light of any changes in relevant metabolites (e.g., ATP) in the different activity states. Studies that have examined liver adenylate levels (ATP, ADP) in torpid and euthermic states have yielded variable results (19, 30, 42, 44, 62). Nucleotides of inosine or adenosine or adenosine itself were not measurable in our analysis. Our data for inosine suggest a reduction in adenylate catabolism for squirrels entering torpor and during the torpor bout, which is consistent with our NMR data and other studies indicating elevated ATP during torpor (30, 42, 62). However, this is not consistent with other studies that did not observe elevated liver ATP levels during torpor (19, 44). The pattern we observed for inosine levels in 13-lined ground squirrels is consistent with the increase in liver urate concentrations during arousal in arctic ground squirrels (48).

The bioactive forms of riboflavin, FMN and FAD, were low in EN and LT squirrels and increased significantly during AR and IBA. These cofactors are involved in cellular redox reactions and are required for the functioning of many enzymes, including those involved in the Krebs cycle and electron transport chain and several acyl CoA dehydrogenases. Upon entering the cell, riboflavin is converted to FMN via flavokinase and FMN is then converted to FAD by the action of FAD synthetase. Under normal conditions there is little free FAD or...
FMN in liver cells (59); rather, most is bound as soluble and membranous enzyme prosthetic groups. Disruption of membranes by the methanol extraction we used would be expected to release FMN and FAD from both soluble and membranous protein complexes.

The liver is the largest and most dynamic pool of riboflavin vitamers. In rats, riboflavin deficiency reduces hepatic riboflavin levels by >50%, and levels return quickly to normal upon riboflavin refeeding (34). Because mammals do not make riboflavin or store it to any significant degree, the increase in FMN and FAD in liver of AR and IBA squirrels could result from their release from catabolized tissues upon arousal analogous to the bolus produced upon feeding. Protein degradation is inhibited during torpor and resumes upon arousal (51, 53). During arousal, riboflavin would be released from catabolized tissues into the general circulation, arrive at the liver, and be converted to its bioactive forms. Alternatively or in combination, the surge in hepatic FMN and FAD in liver of AR and IBA squirrels could result from their release from catabolized tissues upon arousal analogous to the bolus produced upon feeding. Protein degradation is inhibited during torpor and resumes upon arousal (51, 53). During arousal, riboflavin would be released from catabolized tissues into the general circulation, arrive at the liver, and be converted to its bioactive forms. Alternatively or in combination, the surge in hepatic FMN and FAD in liver of AR and IBA squirrels could result from their release from catabolized tissues upon arousal analogous to the bolus produced upon feeding. Protein degradation is inhibited during torpor and resumes upon arousal (51, 53).

Biliverdin levels were low in EN and LT squirrels and increased ~20-fold during arousal, with levels in IBA exceeding those in SUM animals. Biliverdin is produced by catabolism of heme via heme oxygenase (HO), which is expressed in Kupffer cells and hepatocytes, and is then further catabolized to bilirubin via bilirubin oxidase (46). The rise in hepatic biliverdin levels in IBA squirrels may therefore reflect a burst of heme degradation as the liver is reperfused with blood upon arousal. Although there is little information on heme metabolism in hibernators, plasma bilirubin levels are elevated in torpid jerboas (35). Increased biliverdin during arousal may have functional significance beyond just heme catabolism. Biliverdin exerts antioxidant properties in a variety of tissues (40), and exogenous administration of biliverdin reduces liver cold and warm ischemia-reperfusion injury (17, 47). We showed previously that livers harvested from hibernating squirrels are more resistant to injury induced by cold ischemia followed by ex vivo reperfusion than are livers from summer squirrels or rats (32), making biliverdin an attractive candidate molecule responsible for endogenous protection in that model.

Several classes of lipids changed significantly during the hibernation cycle in squirrel livers. Lysophosphatidylcholines, including one we validated (1-oleoyllysophosphatidylcholine), displayed a pattern of high levels in EN (similar to SUM) and lower levels in LT, AR, and IBA. Lysophosphatidylcholines are formed by the cleavage of a fatty acid from the glycerol backbone of phospholipids, catalyzed by phospholipase A. Woods and Storey (58) reported reduced activity of calcium-
dependent phospholipase A2 in livers of torpid ground squirrels relative to euthemic controls, although no measurements were made during arousal from or entrance into torpor (58). Our NMR-based study (42) also indicated higher levels of liver phospholipids including phosphatidylcholine in EN compared with LT squirrels, suggesting catabolism of membrane phospholipids during torpor bouts. However, this pattern has not been observed in all studies (1).

The sphingoid base sphingosine was reduced in LT squirrels relative to the other activity states except EN. Sphingosine is formed from ceramide breakdown by the action of ceramidases, and sphingosine is further catabolized to sphingosine 1-phosphate through sphingosine kinase catalysis. Sphingolipids not only serve as building blocks for cell membranes but also are potent signaling molecules, regulating cellular events including cell cycle, apoptosis, endocytosis, and cytoskeletal dynamics (24). Although sphingolipids have not been well studied in hibernators, recent reports indicated that sphingomyelins are reduced in cerebral cortex of Yakuian ground squirrels during hibernation (29).

Another lipid, cholesterol sulfate was lowest in LT and AR and highest in AR animals, reaching levels comparable to SUM. Cholesterol sulfate is generated by a cholesterol-specific sulfotransferase and is catabolized by steroid sulfatases. Cholesterol sulfate is involved in the regulation of cholesterol synthesis and membrane stability (45). Although there is no information on cholesterol sulfate or its related enzymes in hibernators, our NMR study indicated hepatic cholesterol levels are elevated in LT squirrels relative to SUM with intermediate levels in EN animals (42). Cholesterol sulfate is a putative ligand for the retinoic acid receptor-related orphan receptor-α (RORα), a transcription factor involved in lipid metabolism, inflammatory responses and coupling between the circadian clock and metabolism (11).

Hexadecanedioic acid, a dicarboxylic acid, was highest in LT and AR among the hibernation states and lowest during IBA and EN. We also identified the carnitine ester of hexadecanedioic acid, which was elevated ~ 4-fold in LT over all other activity states, although it was not validated (see Supplemental Fig. S1). Long chain dicarboxylic acids are preferentially catabolized via β-oxidation in peroxisomes as opposed to mitochondria (15). Dicarboxylate levels generally increase in the presence of excess fatty acids; thus, the elevated hexadecanedioic acid levels observed here would be consistent with the accumulation of fatty acids during torpor, a trend also observed in our previous NMR study (42). Indeed, several enzymes involved in metabolism of dicarboxylic fatty acids are seasonally regulated in hibernators, including aldehyde dehydrogenase 1A1 (14), acetyl-coenzyme A acyltransferase 1 (60), and acyl CoA oxidase (27, 56), with the latter two being peroxisomal enzymes. Despite the importance of fatty acid oxidation in fuel utilization during the hibernation season peroxisomes have not been well studied, other than in jerboas (12, 27). These results suggest an important role for peroxisomes in the hibernation phenotype, especially given the number of peroxisomal enzymes whose transcripts are elevated during hibernation (12, 56, 60).

In conclusion, we report the first LC-MS-based assessment of metabolite changes in a mammalian hibernator. Some of the patterns we observed are consistent with known metabolic changes associated with hibernation, whereas others provide new insight into the hibernation phenotype and form a basis for further experimentation. As more information on the molecular changes associated with hibernation emerges, it may be worthwhile to compare metabolic oscillations in hibernators with those in other systems such as the circadian cycle which is closely linked to energy metabolism (21), particularly since changes in genes that control circadian rhythms fluctuate during torpor-arousal cycles of at least one hibernating species (60). Comparisons with simpler model systems that undergo periodic fattening and metabolic depression, such as the Caenorhabditis elegans dauer state (16), may also prove fruitful to identify common patterns that may be shared between the metabolic cycles of diverse organisms (49, 50).

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GRANTS

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REFERENCES

METABOLOMICS ANALYSIS IN HIBERNATING SQUIRRELS


