Meeting Program

15th International Hibernation Symposium
Sunday, July 31st through Thursday, August 4th, 2016
at the Suncoast Hotel in Las Vegas, NV, USA

Time	Monday, August 1st	Tuesday, August 2nd	Wednesday, August 3rd	Thursday, August 4th
8:00 AM to 8:50 AM	Breakfast (St. Tropez Buffet)	Breakfast (St. Tropez Buffet)	Breakfast (St. Tropez Buffet)	Breakfast (St. Tropez Buffet)
9:00 AM to 10:40 AM	**Oral Presentations: Hypoxia, Hypothermia & Torpor (Madrid Room)**	**Oral Presentations: BAT & Mitochondrial Metabolism (Madrid Room)**	**Oral Presentations: Molecular Genetics (Madrid Room)**	**Oral Presentations: Biomedical Aspects (Madrid Room)**
10:40 AM to 11:10 AM	Morning Coffee Break (Madrid Room)	Morning Coffee Break (Madrid Room)	Morning Coffee Break (Madrid Room)	Morning Coffee Break (Madrid Room)
11:10 AM to 12:50 PM	**Oral Presentations: Immune System Function & Blood (Madrid Room)**	**Oral Presentations: Musculoskeletal Function (Madrid Room)**	**Oral Presentations: Austral Organisms (Madrid Room)**	**Oral Presentations: Bats (Madrid Room)**
1:00 PM to 2:15 PM	Lunch (St. Tropez Buffet)	Lunch (St. Tropez Buffet)	Lunch (St. Tropez Buffet)	Lunch (St. Tropez Buffet)
2:20 PM to 4:00 PM	**Oral Presentations: Evolutionary Aspects (Madrid Room)**	**Oral Presentations: Metabolism (Madrid Room)**	**Oral Presentations: Nervous System Function & Neural Control of Torpor (Madrid Room)**	**Oral Presentations: Diversity of Organismal Models (Madrid Room)**
4:00 PM to 4:30 PM	Afternoon Coffee Break (Madrid Room)	Afternoon Coffee Break (Madrid Room)	Afternoon Coffee Break (Madrid Room)	Afternoon Coffee Break (Sunrise Room)
4:30 PM to 6:30 PM	**Oral Presentations: Timing (Madrid Room)**	**Oral Presentations: Organism-Environment Interactions (Madrid Room)**	**Oral Presentations: Feeding, Digestive System Function & the Microbiome (Madrid Room)**	Planning for the 2020 Symposium (Sunrise Room)
6:30 PM to 8:00 PM	Dinner (St. Tropez Buffet)	Dinner (St. Tropez Buffet)	Dinner (St. Tropez Buffet)	Formal Banquet Dinner (Madrid Room) 7:00 to 9:30 PM Speaker: Geoff Schumacher Writer and Nevada Historian
8:00 PM to 9:00 PM		**Poster Reception (Sunrise Room)**	**Poster Reception (Sunrise Room)**	

Registration Table (Madrid Room)
Sunday 3:00 PM to 7:00 PM

Monday – Thursday 9:00 AM to 6:00 PM

Welcome Reception (Madrid Room)
Sunday, July 31st 7:00 to 9:00 PM

Group Outing
Friday, August 5th 9:00 AM to 5:00 PM
Meet outside the east (front) entrance to board the bus promptly at 9:00 AM

Welcome Message

Dear Participants,

Welcome to Living in a Seasonal World: the 15th International Hibernation Symposium! Since 1959, researchers from around the globe have met to discuss how animals overcome the hardships associated with cold and seasonal environments. The work encompasses evolutionary perspectives, elucidation of mechanisms, and biomedical applications of the findings.

This year (2016), we ventured to Las Vegas, Nevada, USA. While one does not usually think of Las Vegas as a place to study hibernation, just a few minutes away exists a fascinating habitat referred to as a Sky Island. Nevada is the most mountainous state in the USA. These mountains serve as refugia for alpine species. The optional outing on Friday will visit just such a Sky Island where we will find golden-mantled ground squirrels, a very common model for hibernation research.

Our venue is the Suncoast Casino. We hope you enjoy the amenities of the resort and its views of Red Rock. At the banquet, we will learn a little of the colorful history of Las Vegas. For those of us participating on the Friday outing, there will be opportunity to reinforce these lessons at the Mob Museum.

We thank all of you for making this meeting happen. We have an amazing and diverse line up of speakers and poster presenters. We thank the session organizers in advance for ensuring we stay on schedule! The University of Nevada Las Vegas helped support this meeting and we are particularly indebted to the efforts of Tim Fishburn and Pat Hunt from the School of Life Sciences. Shane Bevell from the College of Sciences at UNLV provided amazing web support. We thank Georgina Puentedura for an amazing painting that we used as our logo.

We thank Sable Systems International and in particular John Lighton and Robbin Turner for helping to sponsor the meeting. The financial support was well timed and greatly appreciated.

Las Vegas is just a few hours from 13 National Parks! We hope you enjoy the natural beauty that surrounds Las Vegas before or after the meeting. Please don't hesitate to ask us any questions!

Frank van Breukelen and Jenifer Utz
IHS 2016 Organizers
School of Life Sciences
University of Nevada Las Vegas

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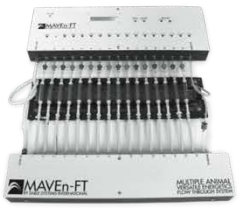

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3:00 PM to 7:00 PM	Meeting Registration (Madrid Room)
7:00 PM to 9:00 PM	Welcome Reception (Madrid Room)

Presenting Author*

Monday, August 1st, 2016	
9:00 AM to 6:00 PM	Meeting Registration (Madrid Room)
8:00 AM to 8:50 AM	Breakfast (St. Tropez Buffet)
9:00 AM to 10:40 AM	**Oral Presentations** (Madrid Room) **Session 1: Hypoxia, Hypothermia, and Torpor** Session Chair: Steven Swoap
9:00 AM to 9:20 AM	GROWING UP IS SO OVERRATED: HIBERNATING AND BURROWING MAMMALS TOLERATE LOW ENVIRONMENTAL OXYGEN THROUGH THE RETENTION OF NEONATAL TRAITS Authors: Yvonne A. Dzal*, Julia M. York, Matthew E. Pamenter, Paul A. Faure, and William K. Milsom
9:20 AM to 9:40 AM	BENIGN NEONATAL DEEP HYPOTHERMIA: WHERE IS IT SEEN? IS IT BENIGN? HOW DOES IT COMPARE TO HIBERNATION? Author: Richard W. Hill*
9:40 AM to 10:00 AM	TORPOR PHENOTYPE IN INBRED MICE Authors: Genshiro A. Sunagawa* and Masayo Takahashi
10:00 AM to 10:20 AM	ADENOSINE AND DAILY TORPOR IN MICE Authors: Steven Swoap*, Maria Vicent Allende, Amelia Hidalgo, and Ethan Borre
10:20 AM to 10:40 AM	A NOVEL, PARADOXICAL "THERMOREGULATORY INVERSION" FOR THE INDUCTION OF A TORPOR-LIKE STATE Authors: Domenico Tupone* and Shaun F. Morrison
10:40 AM to 11:10 AM	Morning Coffee Break (Madrid Room)
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11:10 AM to 11:30 AM	REGULATION OF OXYGEN TRANSPORT IN HIBERNATING MAMMALS Authors: Inge G. Revsbech, Alina L. Evans, Ole Fröbert, Hans Malte, Danielle M. Tufts, Jay F. Storz, and Angela Fago*
11:30 AM to 11:50 AM	IMMUNOLOGIC ASPECT OF HIBERNATION AS LEADS IN THE PREVENTION OF ACUTE ORGAN INJURY Authors: Hjalmar R. Bouma*, Vera R. Reitsema, and Robert H. Henning

11:50 AM to 12:10 PM	IS HIBERNATION GOOD FOR YOUR HEALTH? LEUKOCYTE NUMBERS IN A SMALL HIBERNATING MAMMAL, THE EDIBLE DORMOUSE (*GLIS GLIS*) Authors: Joanna Fietz* and Nadine Havenstein
12:10 PM to 12:30 PM	COAGULATION IN HIBERNATION Authors: Edwin L. de Vrij*, René Mulder, Hjalmar R. Bouma, Ton Lisman, Michaël V. Lukens, and Robert H. Henning
12:30 PM to 12:50 PM	Poster Previews Brief presentations from authors presenting work in poster format
1:00 PM to 2:15 PM	Lunch (St. Tropez Buffet)
2:20 PM to 4:00 PM	**Oral Presentations** (Madrid Room) **Session 3: Evolutionary Aspects** Session Chair: Barry Lovegrove
2:20 PM to 2:40 PM	EMBRACING HETEROTHERMIC DIVERSITY: AN ANALYTICAL APPROACH FOR COMPARING AND CATEGORIZING PATTERNS OF TEMPERATURE VARIATION IN ENDOTHERMS Authors: Danielle L. Levesque*, Brian J. McGill, Manuelle Landry-Cuerrier, Allyson Menzies, and Murray M. Humphries
2:40 PM to 3:00 PM	HIBERNATION IN THE PROTOENDOTHERMIC TENREC (*TENREC ECADUATUS*): AN EARLY FORM OF HIBERNATION? Author: Frank van Breukelen*
3:00 PM to 3:20 PM	FIRES OF LIFE: THE ANTIQUITY OF HIBERNATION Author: Barry Lovegrove*
3:20 PM to 3:40 PM	Poster Previews Brief presentations from authors presenting work in poster format
3:40 PM to 4:00 PM	Group Photo
4:00 PM to 4:30 PM	Afternoon Coffee Break (Madrid Room)
4:30 PM to 6:30 PM	**Oral Presentations** (Madrid Room) **Session 4: Timing** Session Chair: C. Loren Buck
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4:50 PM to 5:10 PM	PLASTIC HIBERNATION: LATE SPRING SNOWSTORMS DRIVE A PHENOLOGICAL MISMATCH BETWEEN THE SEXES IN ARCTIC GROUND SQUIRRELS Authors: Cory T. Williams*, Brian M. Barnes, and C. Loren Buck
5:10 PM to 5:30 PM	AGE-RELATED VARIATION IN HIBERNATION PHENOLOGY OF WILD COLUMBIAN GROUND SQUIRRELS Author: Jeffrey E. Lane*

5:30 PM to 5:50 PM	BEING BORN LATE IN THE ACTIVE SEASON: IMPLICATIONS FOR GROWTH, PRE-HIBERNATION FATTENING AND ENERGETIC STRATEGIES Authors: Sylvain Giroud*, Stéphane Blanc, Hanno Gerritsmann, Gabrielle Stalder, and Thomas Ruf
6:30 PM to 8:00 PM	Dinner (St. Tropez Buffet)

Tuesday, August 2nd, 2016	
9:00 AM to 6:00 PM	Meeting Registration (Madrid Room)
9:00 AM to 6:00 PM	Poster Presentations on display (Sunrise Room)
8:00 AM to 8:50 AM	Breakfast (St. Tropez Buffet)
9:00 AM to 10:40 AM	**Oral Presentations** (Madrid Room) **Session 5: BAT and Mitochondrial Metabolism** Session Chair: Martin Jastroch
9:00 AM to 9:20 AM	ADAPTATION TO THE SEASONALITY OF FOOD ACCESS: THE SIGNIFICANCE OF UCP1 AND BROWN ADIPOSE TISSUE Authors: Jan Nedergaard* and Barbara Cannon
9:20 AM to 9:40 AM	TEMPERATURE EFFECT ON SEASONAL MITOCHONDRIAL METABOLISM IN A MAMMALIAN HIBERNATOR Authors: Clair Hess*, Mallory A. Ballinger, Max W. Napolitano, James A. Bjork, and Matthew T. Andrews
9:40 AM to 10:00 AM	REGULATION OF MITOCHONDRIAL METABOLISM DURING HIBERNATION BY POST-TRANSLATIONAL MODIFICATION Authors: Kate Mathers*, Sarah McFarlane, Amanda MacCannell, and James Staples
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10:20 AM to 10:40 AM	EVOLUTION OF UCP1 AND BROWN ADIPOSE TISSUE Authors: Martin Jastroch* and Susanne Keipert
10:40 AM to 11:10 AM	Morning Coffee Break (Madrid Room)

11:10 AM to 12:50 PM	**Oral Presentations** (Madrid Room) **Session 6: Musculoskeletal Function** Session Chair: Jenifer Utz
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11:30 AM to 11:50 AM	PRESERVATION OF CALCIUM HOMEOSTASIS AND INHIBITION OF APOPTOSIS CONTRIBUTE TO ANTI-MUSCLE ATROPHY EFFECT IN HIBERNATING GROUND SQUIRRELS Authors: Weiwei Fu, Huanxin Hu, Kai Dang, Hui Chang, Bei Du, Xue Wu, and Yunfang Gao*
11:50 AM to 12:10 PM	ITRAQ-BASED ANALYSIS OF MYOFIBRILLAR CONTENTS AND SYNTHESIS AND PROTEOLYTIC PROTEINS IN SOLEUS MUSCLE OF DAURIAN GROUND SQUIRRELS Authors: Hui Chang*, Shan-Feng Jiang, Kai Dang, Hui-Ping Wang, and Yunfang Gao
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12:30 PM to 12:50 PM	Poster Previews Brief presentations from authors presenting work in poster format
1:00 PM to 2:15 PM	Lunch (St. Tropez Buffet)
2:20 PM to 4:00 PM	**Oral Presentations** (Madrid Room) **Session 7: Metabolism** Session Chair: Brian Barnes
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2:40 PM to 3:00 PM	FASTING-INDUCED DAILY TORPOR IN DESERT HAMSTERS (PHODOPUS ROBOROVSKII) Authors: Qingsheng Chi, Xinrong Wan, Fritz Geiser, and Dehua Wang*
3:00 PM to 3:20 PM	EFFECTS OF TEMPERATURE ON BREATHING PATTERN AND VENTILATORY RESPONSES DURING HIBERNATION IN THE GOLDEN-MANTLED GROUND SQUIRREL Authors: Cheryl L. Webb and William K. Milsom*
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3:40 PM to 4:00 PM	Poster Previews Brief presentations from authors presenting work in poster format
4:00 PM to 4:30 PM	Afternoon Coffee Break (Madrid Room)

4:30 PM to 6:30 PM	**Oral Presentations** (Madrid Room) **Session 8: Organism-Environment Interactions** Session Chair: Christopher Turbill
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4:50 PM to 5:10 PM	THE COSTS OF FORAGING – MAXIMUM BODY TEMPERATURES AND USE OF TORPOR DURING THE ACTIVE SEASON IN EDIBLE DORMICE Authors: Claudia Bieber*, Jessica Cornils, Franz Hoelzl, and Thomas Ruf
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5:30 PM to 5:50 PM	BASKING HAMSTERS? Authors: Fritz Geiser*, Kristina Gasch, Claudia Bieber, Gabrielle L. Stalder, Hanno Gerritsmann, and Thomas Ruf
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6:10 PM to 6:30 PM	SEASONAL PREVALENCE OF LYME DISEASE SPIROCHETES IN A HETEROTHERMIC MAMMAL, THE EDIBLE DORMOUSE (GLIS GLIS) Authors: Dania Richter* and Joanna Fietz
6:45 PM to 8:00 PM	Dinner (St. Tropez Buffet)
8:00 PM to 9:00 PM	Poster Reception Authors of even-numbered posters will be available for discussion

Wednesday, August 3rd, 2016	
9:00 AM to 6:00 PM	Meeting Registration (Madrid Room)
9:00 AM to 6:00 PM	Poster Presentations on display (Sunrise Room)
8:00 AM to 8:50 AM	Breakfast (St. Tropez Buffet)
9:00 AM to 10:40 AM	**Oral Presentations** (Madrid Room) **Session 9: Molecular Genetics** Session Chair: Sandy Martin
9:00 AM to 9:20 AM	COMPARATIVE TISSUE TRANSCRIPTOMICS HIGHLIGHT METABOLIC TRANSCRIPT PRIORITIZATION IN PREPARATION FOR AROUSAL FROM TORPOR Authors: Lori K. Bogren*, Katharine R. Grabek, Greg Barsh, and Sandra L. Martin
9:20 AM to 9:40 AM	SYSTEMIC PRE-HIBERNATION REMODELING PRIOR TO HIBERNATION IN SYRIAN HAMSTERS (*MESOCRICETUS AURATUS*) Authors: Yoshifumi Yamaguchi*, Yuichi Chayama, Lisa Ando, Daisuke Anegawa, Shuji Shigenobu, Takayuki Fujimoto, Hiroki Taii, Yutaka Tamura, and Masayuki Miura

9:40 AM to 10:00 AM	HIBERNATION, SOMATIC MAINTENANCE, AND AGING: TELOMERE DYNAMICS IN FREE-LIVING EDIBLE DORMICE Authors: Thomas Ruf*, Franz Hoelzl, Jessica Cornils, Claudia Bieber, and Steve Smith
10:00 AM to 10:20 AM	PHOTOPERIOD AND HIBERNATION: LESSONS FROM JUMPING MOUSE GENOMES Authors: Qian Cong, Alyssa D. McNulty, and William J. Israelsen*
10:20 AM to 10:40 AM	BUILDING A GENETICS FRAMEWORK FOR THE STUDY OF HIBERNATION IN THE 13-LINED GROUND SQUIRREL Authors: Katharine R. Grabek* and Carlos D. Bustamante
10:40 AM to 11:10 AM	Morning Coffee Break (Madrid Room)
11:10 AM to 12:50 PM	**Oral Presentations** (Madrid Room) **Session 10: Austral Organisms** Session Chair: Fritz Geiser
11:10 AM to 11:30 AM	POSSIBLE CAUSES AND CONSEQUENCES OF DIFFERENT HIBERNATION PATTERNS IN *CHEIROGALEUS* SPECIES – MITOVY FATSY SAHALA Authors: Kathrin H. Dausmann* and Marina M. Blanco
11:30 AM to 11:50 AM	SEASONAL ADAPTATIONS AND INTER- SPECIFIC DIFFERENCES OF THE ENERGY BUDGETS OF TWO PRIMATES (*L. LEUCOPUS & L. RUFICAUDATUS*) Authors: Janina Bethge*, Bianca Wist, Eleanor Stalenberg, and Kathrin Dausmann
11:50 AM to 12:10 PM	DOES HIBERNATION INCREASE SEXUAL CONFLICT IN TASMANIAN ECHIDNAS? Authors: Stewart C. Nicol*, Gemma E. Morrow, and Rachel L. Harris
12:10 PM to 12:30 PM	CHARCOAL AND ASH – NEW CUES FOR TORPOR ENHANCEMENT Authors: Clare Stawski*, Julia Nowack, Gerhard Körtner, and Fritz Geiser
12:30 PM to 12:50 PM	RESPONSES OF AUSTRALIAN HETEROTHERMIC MAMMALS TO FIRE Authors: Anna C. Doty*, Jaya K. Matthews, Brad Law, Clare Stawski, and Fritz Geiser
1:00 PM to 2:15 PM	Lunch (St. Tropez Buffet)
2:20 PM to 4:00 PM	**Oral Presentations** (Madrid Room) **Session 11: Nervous System Function & Neural Control of Torpor** Session Chair: Barbara Horwitz
2:20 PM to 2:40 PM	CHARACTERIZING VARIATION IN THE BRAIN TRANSCRIPTOME AS A FUNCTION OF HIBERNATION PHENOTYPE IN 13-LINED GROUND SQUIRRELS Authors: Lori Bogren and Sandy Martin*
2:40 PM to 3:00 PM	UNDERSTANDING THE MOLECULAR MECHANISM OF COLD THERMOSENSATION Authors: Vanessa Matos-Cruz*, Eve R. Schneider, Marco Mastrotto, Dana K . Merriman, Slav N. Bagriantsev, Elena O. Gracheva
3:00 PM to 3:20 PM	HISTAMINERGIC NEUROMODULATION OF SYRIAN HAMSTER CENTRAL NERVOUS SYSTEM ACTIVITY AND BOUT DURATION Authors: John Horowitz*, Jock Hamilton, Kevin Malins, Ekaterina A. Tangog, and Barbara Horwitz

3:20 PM to 3:40 PM	THE INFLUENCE OF THYROID HORMONES ON SPONTANEOUS DAILY TORPOR IN DJUNGARIAN HAMSTERS (*PHODOPUS SUNGORUS*) Authors: Jonathan Bank* and Annika Herwig
3:40 PM to 4:00 PM	THE NEURAL CONTROL OF TORPOR IN MICE Authors: Stefano Bastianini, Chiara Berteotti, Alessia Di Cristoforo, Timna Hitrec, Viviana Lo Martire, Marco Luppi, and Matteo Cerri*
4:00 PM to 4:30 PM	Afternoon Coffee Break (Madrid Room)
4:30 PM to 6:30 PM	**Oral Presentations** (Madrid Room) **Session 12: Feeding, Digestive System Function & the Microbiome** Session Chair: Hannah Carey
4:30 PM to 4:50 PM	HIGH FAT DIET AFFECTS ENERGY BALANCE IN PREHIBERNATORY GOLDEN-MANTLED GROUND SQUIRRELS Authors: Jessica E. Healy*, Isaac A. Groover, Siena R. Krueger, and Gregory L. Florant
4:50 PM to 5:10 PM	EFFECTS OF CROPLAND-BASED DIETS ON THE HIBERNATION OF A FOOD-STORING RODENT Authors: Mathilde L. Tissier*, Yves Handrich, and Caroline Habold
5:10 PM to 5:30 PM	MEASURING STRESS IN THE WILD – EFFECTS OF FLUCTUATING FOOD AVAILABILITY ON THE EDIBLE DORMOUSE (*GLIS GLIS*) Authors: Jessica S. Cornils*, Franz Hoelzl, Franz Schwarzenberger, Claudia Bieber, and Thomas Ruf
5:30 PM to 5:50 PM	FEEDING WHILE HIBERNATING Authors: Mathieu Weitten, Mathilde Tissier , Jean-Patrice Robin, and Caroline Habold*
5:50 PM to 6:10 PM	COLD ADAPTED MICROORGANISMS FROM THE CECUM OF HIBERNATING THIRTEEN-LINED GROUND SQUIRRELS Authors: Jessica R. Sieber*, James A. Bjork, and Matthew T. Andrews
6:10 PM to 6:30 PM	HIBERNATION AND THE GUT MICROBIOME: SYMBIOSIS THROUGH THE SEASONS Authors: Hannah V. Carey*, Sadie Gugel, and Fariba Assadi-Porter
6:45 PM to 8:00 PM	Dinner (St. Tropez Buffet)
8:00 PM to 9:00 PM	Poster Reception (Sunrise Room) Authors of odd-numbered posters will be available for discussion

Thursday, August 4th, 2016	
9:00 AM to 6:00 PM	Meeting Registration (Madrid Room)
8:00 AM to 8:50 AM	Breakfast (St. Tropez Buffet)

9:00 AM to 10:40 AM	**Oral Presentations** (Madrid Room) **Session 13: Biomedical Aspects** Session Chair: Matthew Andrews
9:00 AM to 9:20 AM	LIPID EMULSION IMPROVES CARDIOPROTECTION AGAINST ISCHEMIA-REPERFUSION INJURY IN NORMOTHERMIC ARCTIC GROUND SQUIRREL ISOLATED HEARTS Authors: Michele M. Salzman*, Anna E. Dikalova, Hunter F. Douglas, Dorothee Weihrauch, Brian M. Barnes, and Matthias L. Riess
9:20 AM to 9:40 AM	NONINVASIVE ASSESSMENT OF THE HIBERNATING GROUND SQUIRREL EYE Authors: Benjamin Sajdak*, Brent Bell, Dana K. Merriman, Wei Li, Joseph Carroll, and Alfredo Dubra
9:40 AM to 10:00 AM	HIBERNATION IN A DISH?: INDUCED PLURIPOTENT STEM CELLS FROM THE THIRTEEN-LINED GROUND SQUIRREL. Authors: Jingxing Ou*, Barbara Mallon, Kiyoharu Miyagishima, and Wei Li
10:00 AM to 10:20 AM	ROLES OF SULFIDE AND THIOL METABOLITES IN BROWN BEAR HIBERNATION Authors: Angela Fago*, Inge G. Revsbech, Xinggui Shen, Christopher G. Kevil, Alina L. Evans, and Ole Fröbert
10:20 AM to 10:40 AM	THE ROLE OF H_2S IN PROTECTING FROM ORGAN DAMAGE DURING HIBERNATION IN THE SYRIAN HAMSTER Authors: Robert H. Henning*, Jojanneke Bruintjes, George J. Dugbartey, Arjen M. Strijkstra, Ate S. Boerema, and Hjalmar R. Bouma
10:40 AM to 11:10 AM	Morning Coffee Break (Madrid Room)
11:10 AM to 12:50 PM	**Oral Presentations** (Madrid Room) **Session 14: Bats** Session Chair: Thomas Tomasi
11:10 AM to 11:30 AM	COLD AND ALONE? ROOST CHOICE AND SEASON IMPACT TORPOR PATTERNS OF A FREE RANGING NEW ZEALAND BAT (MYSTACINA TUBERCULATA) Authors: Zenon J. Czenze*, R. Mark Brigham, Anthony J.R. Hickey, and Stuart Parsons
11:30 AM to 11:50 AM	HUNG OUT TO DRY? ARID ADAPTATION IN HIBERNATING BIG BROWN BATS (*EPTESICUS FUSCUS*) Authors: Brandon J. Klüg-Baerwald* and R. Mark Brigham
11:50 AM to 12:10 PM	ADAPTATIONS FOR EXTREME WINTER ENDURANCE IN TEMPERATE BATS Authors: Craig Willis*, Alana Wilcox, Quinn Webber, Kaleigh Norquay, Kristina Muise, Heather Mayberry, Kristin Jonasson, Mary-Anne Collis, Zenon Czenze, and Dylan Baloun
12:10 PM to 12:30 PM	CONSPECIFIC DISTURBANCE CONTRIBUTES TO ALTERED HIBERNATION PATTERNS IN BATS WITH WHITE-NOSE SYNDROME Authors: James M. Turner*, Lisa Warnecke, Alana Wilcox, Dylan Baloun, Trent K. Bollinger, Vikram Misra, and Craig K.R. Willis
12:30 PM to 12:50 PM	THE ROLE OF EPIDERMAL LIPIDS IN THE RESISTANCE TO WHITE-NOSE SYNDROME Authors: Craig L. Frank*, Melissa R. Ingala, and Rebecca E. Ravenelle

1:00 PM to 2:15 PM	Lunch (St. Tropez Buffet)
2:20 PM to 4:00 PM	**Oral Presentations** (Madrid Room) **Session 15: Diversity of Organismal Models** Session Chair: Glenn Tattersall
2:20 PM to 2:40 PM	HETEROTHERMY IN COLUMBIFORM AND PICIFORM BIRDS: INSIGHTS FROM THE AFROTROPICS Authors: Andrew E. McKechnie*, Matthew J. Noakes, Ryno Kemp, and Ben Smit
2:40 PM to 3:00 PM	TORPOR DYNAMICS IN HIGH ANDEAN HUMMINGBIRDS Authors: Blair O. Wolf*, Jonathan Schmitt, Andy Johnson, and Christopher Witt
3:00 PM to 3:20 PM	EXTERNAL ENERGY RESERVES AND HIBERNATION PATTERNS IN COMMON HAMSTERS Authors: Eva Millesi* and Carina Siutz
3:20 PM to 3:40 PM	METABOLIC DEPRESSION DURING WINTER COULD MITIGATE IMPACTS OF CLIMATE CHANGE ON LIZARDS Authors: Carolina Noronha, Ofir Levy, Rory S. Telemeco, and Michael J. Angilletta Jr.*
3:40 PM to 4:00 PM	FORCED HIBERNATION: A TECHNIQUE TO TEST SURVIVAL OF TEMPERATE SNAKES IN A PEATLAND LIFE ZONE Authors: Anne R. Yagi and Glenn J. Tattersall*
4:00 PM to 4:30 PM	Afternoon Coffee Break (Sunrise Room)
4:30 PM to 5:30 PM	Planning for the 2020 International Hibernation Symposium (Sunrise Room)
7:00 PM to 9:30 PM	Formal Banquet Dinner (Madrid Room) Guest Speaker: Geoff Schumacher Writer and Nevada Historian

Friday, August 4th, 2016	
Time	Event
9:00 AM to 5:00 PM	Group Outing Meet **promptly** at 9:00 AM on steps outside east (front) entrance

Abstracts are listed according to the order in which the presentations were given. The institutional affiliation of the Corresponding Author* is also listed.

Session 1: Hypoxia, Hypothermia, and Torpor

GROWING UP IS SO OVERRATED: HIBERNATING AND BURROWING MAMMALS TOLERATE LOW ENVIRONMENTAL OXYGEN THROUGH THE RETENTION OF NEONATAL TRAITS

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There are striking parallels in physiological traits between all newborn mammals and adults of species capable of hibernation. Tolerance to low environmental oxygen (hypoxia) is one of these. Most mammals are not hypoxia tolerant, however, hibernating and burrowing mammals are exceptional in this regard, unfortunately the basis of this tolerance is not well understood. We investigated whether differences in hypoxia tolerance of adult hibernators (13-lined ground squirrel, *Ictidomys tridecemlineatus*; and golden hamster, *Mesocricetus auratus*) and adult non-hibernators (Norway rat, *Rattus norvegicus*; common mouse, *Mus musculus*; and little yellow-shouldered bat, *Sturnira lilium*) reflect developmental changes in the way oxygen demand and supply are matched. Additionally, we compared the strategies of a burrowing non-hibernator (naked mole rat, *Heterocephalus glaber*), and a non-burrowing hibernator (big brown bat, *Eptesicus fuscus*) to decipher whether enhanced hypoxia tolerance of hibernating mammals is due to a burrowing lifestyle, or the ability to hibernate. To test these questions, we exposed newborn and adult mammals to hypoxia and measured their metabolic, thermoregulatory, and ventilatory responses. Our study yielded three important findings. First, the most hypoxia tolerant mammals (bats and mole rats) matched oxygen supply to demand by increasing the amount of oxygen they extracted from each breath (up to 85%). Second, severe hypoxia (7% O_2) led to an active depression of oxygen demand in burrowing non-hibernators (>60%) and non-burrowing hibernators (>40%), independent of decreases in body temperature. Third, strategies of newborns and adult hibernators were indistinguishable. Non-hibernators decreased oxygen demand as newborns (>45%) but increased oxygen supply as adults (>80%), while newborn and adult hibernators responded by reducing oxygen demand (>35%; hamsters and hibernating bats) or by increasing ventilation (>80%; ground squirrels). Overall our results support the hypothesis that the ability to hibernate evolved through the retention of neonatal traits. This research was supported by a NSERC Discovery Grant.

BENIGN NEONATAL DEEP HYPOTHERMIA: WHERE IS IT SEEN? IS IT BENIGN? HOW DOES IT COMPARE TO HIBERNATION?

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Extensive data, to be presented, suggest that Old World mice (*Mus*) and New World mice (*Peromyscus*) have diverged in neonatal tolerance of deep hypothermia (i.e., $T_b \leq 8°C$); *Peromyscus* are far more tolerant than *Mus*. However, there is little plasticity within either group; feral *Mus*, for example, are closely similar to laboratory strains of *Mus* (e.g., C3H), despite centuries of separation. A key question for hibernation-oriented biologists is whether neonatal deep hypothermia provides a second model system for study of questions addressed in hibernation research. Arguments for and against will be reviewed. One of the central questions for comparing neonatal deep hypothermia and hibernation is whether the former is benign. I have addressed the question in lab and semi-natural experiments measuring whether neonatal near-freezing hypothermia has effects on the performance of young white-

footed mice (*Peromyscus leucopus*) tested as early in life as possible. Experiments on avoidance of screech owl (*Megascops asio*) predation will be emphasized in this report. Results are compatible with the hypothesis that multiple neonatal experiences of near-freezing hypothermia have no effect on postweaning avoidance capability.

TORPOR PHENOTYPE IN INBRED MICE

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Some mammals leverage active hypometabolism to survive severe environmental stress such as cold climate or food shortage. The reduced basal metabolic rate during active hypometabolism is very attractive for implementing hypometabolic medicine such as patient transportation or organ stock, although little is known about the mechanism of torpor/hibernation. To investigate the mechanism of torpor, we are using mice, which can enter daily torpor when exposed to a certain ambient temperature (T_A) and food restriction. Although past studies report several methods to induce torpor in mice, they differ in strains, ambient temperature, light condition, and in the timing of food restriction. To carry torpor research forward, we are probing the optimal method for stable and efficient induction of torpor. When torpor is induced by food restriction from the beginning of light phase under TA=16C, in C57BL/6NJcl (B6N), the minimal metabolic rate (oxygen consumption or VO_2) during active phase was reduced from 4.45 ± 0.16 ml/g/h to 2.30 ± 0.38 ml/g/h. Interestingly, in C57BL/6J (B6J), the minimal VO_2 during normal condition was 4.32 ± 0.16 ml/g/h while during torpor, it dropped to 1.64 ± 0.33 ml/g/h which is significantly lower (p = 0.010, unpaired t-test) than that of B6N mice. We are now testing the different torpor phenotypes in different inbred strains.

ADENOSINE AND DAILY TORPOR IN MICE

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Clinical advantages of forced hypothermia (FH) are mitigated by homeostatic mechanisms invoked to prevent core cooling. Torpor lowers body temperature (T_b) without eliciting such mechanisms. It was hypothesized that torpor is vastly different from FH at multiple levels, including autonomic balance, aerobic/anaerobic metabolism, and hypoxia related gene expression in heart and liver. Further, central administration of an adenosine receptor agonist (CHA) was hypothesized to recapitulate many aspects of daily torpor in the mouse. To test these hypotheses, female C57Bl/6 mice were implanted with ECG/ T_b telemeters and subjected to three conditions, 1) FH, 2) CHA, and 3) naturally induced torpor. During cooling and at a T_b of 30°C, mice showed a heart rate (HR) of 185 ± 18 bpm during torpor, 484 ± 20 bpm during FH, and an intermediate value of 305 ± 18 bpm with CHA. Analysis of hysteresis curves of T_b vs. HR showed parasympathetic (PNS) influence during entrance and maintenance of torpor, which shifted to sympathetic (SNS) influence during recovery. Mice undergoing FH showed consistent and maximal SNS activity, while CHA administration initiated PNS activity followed quickly by SNS activation. Circulating lactate levels were significantly elevated in FH relative to torpor and to CHA administration (3.9 ± 0.9 vs. 0.6 ± 0.2 mM vs. 0.5 ± 0.2 mM respectively), suggesting an increased reliance on anaerobic metabolism only during forced hypothermia. Steady state mRNA levels in the heart and liver from torpid mice, as assessed by qRTPCR, showed a unique pattern of expression compared to euthermic tissues, forced hypothermic tissues, and vastly different in tissues from CHA-injected mice. In conclusion, data in this study support the hypothesis that adenosine-induced hypothermia and natural torpor share a few attributes, while they have greatly different sympathetic, shivering, and hepatic/cardiac gene expression profiles.

A NOVEL, PARADOXICAL "THERMOREGULATORY INVERSION" FOR THE INDUCTION OF A TORPOR-LIKE STATE

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The CNS thermoregulatory network normally maintains body core temperature (T_{CORE}) through a balance of cold defensive and heat defensive responses: skin cooling elicits heat retention through cutaneous vasoconstriction (CVC) and heat production (thermogenesis) through shivering and sympathetic neural activation of brown adipose tissue (BAT), while skin or core warming results in the inhibition of CVC, BAT and shivering.

However, there are several conditions (e.g., hibernation, torpor, REM sleep) in which these "normal" homeostatic thermoregulatory responses to protect T_{CORE} appear to be a superseded by a paradoxical "thermoregulatory inversion" in which cold exposure inhibits thermogenesis, and warm exposure stimulates it. Here we describe two experimental conditions leading to the induction of a "thermoregulatory inversion".

First is the thermoregulatory inverted state following central administration of the A1 adenosine receptor agonist, N6-Cyclohexyladenosine (CHA). In rats exposed to an ambient temperature (T_{AMB}) of 15°C, ICV injection of CHA (10µl, 1mM) produced a "torpor-like state" consisting of a reduced BAT temperature (T_{BAT}) and a deep hypothermia. Increasing T_{AMB} to 28°C, still below the rats' minimum T_{CORE}, caused a rapid increase in T_{BAT}, consistent with an activation of BAT thermogenesis.

Second is the thermoregulatory inverted state following brain transection just rostral to the dorsomedial hypothalamus (DMH). In rats with a warm (>36°C) skin and a low level of BAT thermogenesis, brain transection just rostral to DMH caused a prompt increase in BAT sympathetic nerve activity (SNA) and BAT thermogenesis, and these increases in BAT SNA and thermogenesis were reversed by subsequent skin cooling.

In conclusion, thermoregulatory inversion is a unique state of the central thermoregulatory network that allows a deep, stable and reversible hypothermia similar to that in torpor and hibernation, and may represent a paradigm for the induction of therapeutic hypothermia.

Session 2: Immune System Function & Blood

REGULATION OF OXYGEN TRANSPORT IN HIBERNATING MAMMALS

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Hibernation entails a drastic reduction in metabolism and thus in oxygen (O_2) consumption. Key to avoid free O_2 toxicity and excess production of reactive O_2 species is to adjust delivery to consumption. One way this can be achieved is by elevating blood O_2 affinity, thus decreasing O_2 unloading to tissues. In mammalian hibernators, blood O_2 affinity is potentially regulated via two major factors: change in body temperature and change in the concentration of red cell 2,3-diphosphoglycerate (DPG), that would normally stabilize the low- affinity tense state of the hemoglobin (Hb), the blood O_2 carrier.

We have investigated the effects of temperature and DPG on the oxygen affinity of the Hb in the golden mantled and thirteen-lined ground squirrels, as well as in the large hibernator, the brown bear. We found that in hibernating brown bears, blood O_2 affinity was elevated compared to that of summer active bears due to a decrease in both body temperature (by ~7˚C) and in red cell DPG, that was found reduced to around half summer values. In contrast, the ground squirrel Hbs were largely unresponsive to changes in DPG concentration. This suggests that in the colder small hibernators, a mere fall in body temperatures (>30 ˚C) is sufficient to induce the increase in blood O_2 affinity during hibernation reported in earlier studies.

In a hibernator an increase in blood O_2 affinity may be crucial to balance O_2 delivery to consumption. Indeed, our modeling of the brown bear O_2 transport supports the conclusion that seasonal adjustments in the blood O_2 affinity may be instrumental to prevent a high venous O_2 tension that could lead to an excess of reactive O_2 species in the tissues.

IMMUNOLOGIC ASPECT OF HIBERNATION AS LEADS IN THE PREVENTION OF ACUTE ORGAN INJURY

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Hibernators are able to survive long periods of low blood flow and body temperature followed by rewarming and reperfusion without overt signs of organ injury, which makes these animals excellent models for application of natural protective mechanisms to human medicine. Specific alterations are believed to conserve energy sources, prevent cell death and limit organ injury during periods with extremely low body temperature and during rewarming. In addition, the function of the innate and adaptive immune system is strongly reduced. During torpor, numbers of leukocytes are decreased more than 10-fold and both the innate and adaptive immune systems are severely depressed in functioning. Although observed changes in the innate immune system are reversed upon arousal, parts of the adaptive immune system remain depressed throughout hibernation (e.g. lymphocyte proliferation). Previously, we revealed the mechanisms underlying reversible clearance of lymphocytes and neutrophils during torpor. Additionally, we recently demonstrated a reduced phagocytic capacity of neutrophils during torpor, which may (at least in part) be explained by lowered levels of complement. The reduced immune function during torpor may render animals susceptible to psychrophilic infections, such as the white nose syndrome in hibernating bats. Unraveling the biochemical signaling pathways underlying hibernation-associated immunosuppression will not only enhance our understanding of fundamental immunological aspects, but might also lead to the discovery of new pharmacological targets for clinical disorders that are characterized by acute immunometabolic disturbances, such as sepsis, shock, major surgery and transplantation.

IS HIBERNATION GOOD FOR YOUR HEALTH? LEUKOCYTE NUMBERS IN A SMALL HIBERNATING MAMMAL, THE EDIBLE DORMOUSE (*GLIS GLIS*)

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In vertebrates, immune defense is essential for survival and varies remarkably among individuals and in time. Extreme physiological changes occurring during hibernation are known to affect various components of the immune system. To determine the effects of hibernation on immune parameters and their relevance for fitness, we investigated leukocyte profiles during the active season of edible dormice (*Glis glis*) in the field. Edible dormice are small arboreal rodents characterized by an extraordinarily long hibernation period of up to 8 months. Previous studies on other hibernators showed a dramatic decrease (~90%) in circulating leukocytes during torpor, applying to all subtypes, which is reversed within a few hours after torpor is terminated. In contrast to these findings, our study revealed that in edible dormice hibernation results in depleted phagocyte (neutrophils and monocytes) stores upon emergence that recovered only slowly. As the phenomenon of low phagocyte counts was even more pronounced at the beginning of a low-food year and primarily immature neutrophils were present in the blood upon emergence, preparatory mechanisms seem to determine the regeneration of phagocytes before hibernation is terminated. Recovery of phagocytes thereafter took several weeks, presumably due to energetic restrictions. This impaired first line of defense coincides with lowest survival probabilities in the annual cycle of this species. Summarized, results of our study clearly reveal that the leukocyte

picture of active edible dormice responds sensitively to physiological conditions associated with hibernation, and can further be linked to fitness parameters such as survival.

COAGULATION IN HIBERNATION

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Hibernators are imposed to increased risk of thromboembolism during their torpor bouts, which include long immobility, increased viscosity and low blood flow. Despite these procoagulant risk factors, no signs of thrombosis are present on arousal. We explored the anticoagulant strategies deployed by hibernating Syrian hamster to prevent thromboembolism during torpor and their reversal during arousal to prevent bleeding. To this end, both primary and secondary hemostasis were analyzed. Primary hemostasis is reduced by a massive reduction of platelet count in torpor, as well as through reduction of the amount and function of von Willebrand factor (VWF). Secondary hemostasis shows increased clotting times (PT, APTT). Clotting time seems prolonged due to reduced levels of procoagulant factors (VIII, IX, XI) despite that anticoagulant factors (antithrombin III, Protein C) are also reduced.

Platelet count as well as procoagulant factors quickly return to normal values during arousal. Reversibility of platelet count relies on platelet storage-and-release particularly involving liver rather than on clearance and *de novo* synthesis. Platelet storage seems driven by decreased body temperature as non-hibernating rats demonstrate platelet storage-and-release in induced hypothermia-rewarming. The mechanism of reversibility of levels of procoagulant factors has to yet to be determined.

Hibernators reduce the risk of thromboembolism during torpor through rapidly reversible changes in the coagulation system, suppressing both primary and secondary hemostasis in torpor. Platelet storage-and-release is the key mechanism alternating primary hemostasis. While components of secondary hemostasis are suppressed as well, its mechanism of suppression and recovery has yet to be elucidated. Intriguingly, similar changes found in the hibernator's coagulation system are also induced in non-hibernators during hypothermia, which reverse during rewarming.

Session 3: Evolutionary Aspects

EMBRACING HETEROTHERMIC DIVERSITY: AN ANALYTICAL APPROACH FOR COMPARING AND CATEGORIZING PATTERNS OF TEMPERATURE VARIATION IN ENDOTHERMS

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Recent research is revealing incredible diversity in the thermoregulatory patterns of wild and captive endotherms. Classic thermoregulatory categories of 'homeothermy', 'daily heterothermy', and 'hibernation' are becoming harder to delineate and understanding the physiological and evolutionary significance of variation within and around these categories is becoming more important. But we lack a generalized analytical approach for categorizing and comparing the nature of temperature variation expressed by individuals, populations, and species. Here we propose a new approach that decomposes body temperature time series into three inherent properties – wave form, amplitude, and period – using a non-stationary technique that accommodates temporal variation in form, amplitude and period. This approach quantifies circadian and seasonal variation in thermoregulatory patterns, and uses the distribution of observed thermoregulatory patterns as a basis for intra- and inter-specific comparisons. We analyze body temperature time series from multiple species, including classical hibernators, tropical heterotherms, and homeotherms, to highlight the approach's general usefulness and the major axes of thermoregulatory variation that it reveals.

HIBERNATION IN THE PROTOENDOTHERMIC TENREC (*TENREC ECADUATUS*): AN EARLY FORM OF HIBERNATION?

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Many view our early mammalian ancestors as having been nocturnal, insectivorous, with variable body temperature (T_b) and the capacity for metabolic depression or torpor. Protoendothermic mammals have much more variable T_b (e.g. > 5 °C diel fluctuations). Examples include monotremes, some marsupials, and tenrecs. Tenrecs are Afrotherians and related to elephants, manatees, elephant shrews, and hyraxes. There is tremendous diversity amongst the tenrecs in niche, morphology, and the use of torpor. *Tenrec ecaudatus* may be the most 'primitive' of the tenrecs. *T. ecaudatus* uses a previously unidentified form of hibernation. These tenrecs are consistently torpid during the hibernation season even when housed at 28 °C or disturbed. However, animals may be 'more or less' torpid independent of T_b. There are remarkable mismatches between heart rate, oxygen consumption, and T_b. We suggest these mismatches may reflect a more plesiomorphic and perhaps even, poor, ability to use torpor. Such torpor use may be connected to the extreme metabolic plasticity of active season tenrecs. In the active season, tenrecs oftentimes experience large oscillations of T_b. Tenrecs may have low T_bs that are just above ambient and be fully active (e.g. T_b of 14 °C when housed at 12 °C). Alternatively, tenrecs may be facultatively torpid with depressed O_2 consumption rates. We speculate the large plasticity exhibited by these animals may have allowed for these animals to survive but that selection allowed for a refining of the hibernation phenotype in other extant hibernators.

FIRES OF LIFE: THE ANTIQUITY OF HIBERNATION

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A model for the evolution of endothermy in birds (sauropsids) and mammals (synapsids) has proposed that it occurred in three distinct phases; an initial land-conquering phase (Permian – Middle Triassic), a thermoregulatory phase associated with extreme body size miniaturization (Late Triassic – Jurassic), and a locomotor and climate adaptation phase (Cretaceous – Cenozoic). The model argues that endothermy increased iteratively over this period of more than 250 million years. In terms of hibernation, the model also proposes that the capacity to abandon endothermy, that is, to enter a state of hibernation – effectively a temporal return to the ectothermic state – was retained in small-sized insulated endotherms from the inception of endothermy to the present. Following this model, hibernation is as old as the origin of endothermy. Data for modern mammals, *Tenrec ecaudatus,* and the Early Triassic therapsid *Thrinaxodon,* are used to support the hypothesis of the antiquity of hibernation.

Session 4: Timing

CIRCADIAN CLOCK FUNCTION ACROSS THE POLAR DAY AND POLAR NIGHT: FIELD AND LABORATORY INVESTIGATIONS IN THE ARCTIC GROUND SQUIRREL

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Organisms that inhabit the Arctic are exposed to extreme environmental conditions that are rare or absent at lower latitudes. In addition to a protracted season of sub-zero temperatures, the Arctic is characterized by profound seasonal changes in photoperiod. Animals that reside north of the Arctic Circle experience weeks to months of either continuous daylight or darkness, respectively. Given that the light–dark cycle of day and night serves as the primary *Zeitgeber* for entraining the daily physiological, hormonal, and behavioral rhythms of most organisms, the question is how circadian systems operate in animals exposed to these conditions. In addition, because the master circadian clock

of the suprachiasmatic nucleus keeps time through rhythmic transcriptional-translational feedback loops, processes that are thought to be inhibited by low tissue temperature characteristic of hibernation, the status of the clock and function in keeping time during hibernation is intriguing. Using a combination of field and laboratory approaches, we set out to investigate the expression of circadian rhythms across the annual cycle of the arctic ground squirrel (*Urocitellus parryii*). We found that free-living squirrels do not express circadian rhythms of body temperature during the heterothermic and pre-emergent euthermic intervals while sequestered in their dark hibernacula. From captive animal work, our data suggests that clock gene expression in the SCN is either arrested or desynchronized during deep torpor and arousal. Further, we found that light exposure at emergence from hibernation in the field or exposing captive animals to a 5 sec light pulse was sufficient to initiate daily rhythms of core body temperature that persisted across their active season. Although a light pulse in captive animals is sufficient to initiate circadian rhythms in body temperature and activity, it is not necessary as rhythms will spontaneously appear within two weeks of ending heterothermy in the laboratory.

PLASTIC HIBERNATION: LATE SPRING SNOWSTORMS DRIVE A PHENOLOGICAL MISMATCH BETWEEN THE SEXES IN ARCTIC GROUND SQUIRRELS

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Shifts in the timing of seasonal events are among the most commonly reported responses of vertebrates to climate change. However, the mechanistic underpinnings of phenological shifts in hibernators are unclear and the potential for sex-dependent responses has not been examined. Here, we describe sex-dependent plasticity in the hibernation physiology and phenology of free-living arctic ground squirrels in response to late spring snowstorms, which are predicted to increase under climate change. Female and non-reproductive male arctic ground squirrels responded to the >1month delay in snow melt by either extending hibernation or re-entering hibernation following several days of euthermia. The pattern of heterothermy differed between a typical hibernation season and one that included extended hibernation; body temperature during deep torpor was several degrees higher and torpor bout length was much shorter (2-3 days) during extended hibernation. Reproductive males, in contrast, were not plastic and did not re-enter hibernation, presumably because high testosterone associated with seasonal gonadal recrudescence prevents torpor. Our results suggest that climate-driven delays in spring combined with differences in sex-dependent plasticity could lead to a seasonal mismatch between the sexes.

AGE-RELATED VARIATION IN HIBERNATION PHENOLOGY OF WILD COLUMBIAN GROUND SQUIRRELS

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Phenologies represent the annual timing of life cycle events, and have received growing attention in recent decades. Hibernation phenologies, however, have received considerably less attention than analogous traits (e.g., migration). Consequently, our understanding of the eco-evolutionary causes and consequences of natural phenological variation in wild hibernators is limited. I have been conducting comprehensive observations of the hibernation emergence dates of individuals in a wild population of Columbian ground squirrels (*Urocitellus columbianus*) for the past nine years (with additional records dating back to 2003) in southeastern Alberta, Canada so as to investigate age-related variation in hibernation phenologies. Such variation in life history traits is now appreciated as commonplace, and often includes a late-life deterioration referred to as senescence. I hypothesized that hibernation phenology would show similar variation (i.e., a gradual advance with age until a peak in 'prime-aged' individuals and a subsequent delay during senescence). I knew the ages of all individuals born into the

study population with certainty and monitored their phenologies across their full lifetime (Range: 1-12 years). I determined hibernation emergence dates for all females (N ≈ 50-75, depending on population density) through comprehensive visual surveys and live-trapping over the 0.13 ha field site. I will present the results of analyses on these data to test my primary hypothesis of age-related variation in hibernation phenology. Interestingly the link between hibernation and senescence has previously been drawn, with the former proposed to delay the onset of the latter (with the tremendously long lifespans of bats used as a classic example). Rarely, however, has the reverse been investigated (i.e., the relevance of age to hibernation expression). In Columbian ground squirrels, hibernation phenology is a fitness-relevant trait and previous studies have shown late-life declines in fitness. Age-related variation in phenology thus represents a plausible explanation for these senescent declines.

BEING BORN LATE IN THE ACTIVE SEASON: IMPLICATIONS FOR GROWTH, PRE-HIBERNATION FATTENING AND ENERGETIC STRATEGIES

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Prior to winter, juvenile hibernators have to allocate energy to both growth and fattening, to survive winter hibernation and to avoid possible disadvantages during their first reproductive season. Being born late in the active season may have important effects on growth and fattening. Torpor is thought to sustain these processes in young individuals. We tested the hypothesis that late-born ('LB') female juvenile garden dormice (*Eliomys quercinus*) (i) grow and fatten at higher rates, (ii) reach similar pre-hibernation levels of body size and fat reserves due to (iii) an increased use of torpor, and (iv) show similar hibernating patterns than early born ('EB') individuals. We found that, during the pre-weaning phase, young from early and late litters mainly grew structurally and gained body mass at similar rates. However, at early post-weaning phase, LB juveniles were smaller but fatter, then grew and gained mass twice as fast as EB individuals during their post-weaning period. Accelerated growth was sustained by an increased food intake and reduced activity duration in LB juveniles, leading to body sizes similar to EB individuals prior to hibernation. Torpor use was low during intensive growth (*i.e.* the first weeks of body mass gain), but increased after the termination of growth, during pre-hibernation fattening. LB juveniles increased their torpor use to a greater extent and showed 10%-lower pre-hibernation fat reserves, compared to EB individuals. Intermittent fasting had no effect on fat stores prior to hibernation, neither in EB nor in LB juveniles. Apart from a 4-week difference in their hibernation duration, EB and LB juveniles showed similar hibernating patterns. Hence, our results suggest that torpor is incompatible with structural growth, but instead promotes pre-hibernation fattening late in the season. This study further supports evidence for a seasonal setpoint for a threshold of body fat reserves in juveniles prior to hibernation.

Session 5: BAT and Mitochondrial Metabolism

ADAPTATION TO THE SEASONALITY OF FOOD ACCESS: THE SIGNIFICANCE OF UCP1 AND BROWN ADIPOSE TISSUE

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Animals in the wild may experience large variations in the availability of food during the year. We have examined in laboratory animals (mice) to which degree uncoupling-protein-1 (UCP1) in brown and brite/beige adipose tissues is involved in the response and adaptation to certain of these variations. The variations may include food scarcity or plentifulness, the presence of different amounts of protein in the diet, the palatability of the diets (including fat and sugar content) and the presence of substances, natural or anthropogenic, that may affect the adipose tissues and the activity of UCP1. Additionally, we have examined the effect of the probable hormonal mediators of feeding status, such as leptin. We have

particularly examined to what extent these variations affect the total amount of UCP1 in the animals (and thus their capacity for nonshivering thermogenesis) and directly the activity of UCP1 (in isolated brown-fat mitochondria). We have examined the essentiality of UCP1 by making parallel investigations in UCP1 KO mice. We have found that some anthropogenic substances such as PFOA may directly activate UCP1, that certain natural agents such as amorfrutins may be discussed to affect PPARg activity and thus influence tissue recruitment state, and that UCP1 is essential for mediation of the facultative part of the acute thermogenic response to high-fat feeding. Unexpectedly, we found that a major candidate for being the mediator of feeding status, leptin, does not acutely stimulate thermogenesis but rather acts as a pyrogen, i.e. it increases the "set-point" for body temperature (or rather shifts the threshold for initiating thermogenesis). We conclude that in many adaptation circumstances UCP1 has an essential role but that its essentiality must be examined for each such condition.

TEMPERATURE EFFECT ON SEASONAL MITOCHONDRIAL METABOLISM IN A MAMMALIAN HIBERNATOR

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Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) enter a torpid state during the winter months to combat low food availability and higher energy demands required to maintain a constant body temperature. While in torpor, body temperature, heart rate, oxygen consumption, and other physiological processes are decreased for a few days to a couple weeks. Torpor is interrupted by periodic, interbout arousals (IBA) characterized by a return to normothermic rates within a few hours. Re-warming from torpor is facilitated by thermogenic brown adipose tissue (BAT) as energy is dissipated and heat is produced via uncoupling protein 1 (UCP-1) in BAT mitochondria. Since BAT functions across a range of temperatures, and generates heat while other tissues are inactive, we hypothesized that mitochondrial respiration in thermogenic BAT may differ from that in a non-thermogenic tissue and that respiration rates would be higher in BAT mitochondria across a range of temperatures. To address this, we examined mitochondrial bioenergetics of BAT and liver across five seasonal time points: spring, summer, fall, torpor, and IBA. Using an oxygen electrode, succinate fueled respiration rates of isolated BAT and liver mitochondria were measured at five temperatures (5, 13, 21, 29, and 37^{o}C). Based on a two-way ANOVA, when comparing mitochondrial respiration across the seasons at each temperature, less significant differences were found in BAT than in the liver. These data imply that if BAT mitochondria have a mechanism that allows it to maintain function across the circannual cycle and a range of temperatures then it is less effective in liver.

REGULATION OF MITOCHONDRIAL METABOLISM DURING HIBERNATION BY POST-TRANSLATIONAL MODIFICATION

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Seasonal changes in liver metabolism has been well studied, including the role of post-translational modification (PTM) on metabolic control. Less is understood about the regulation of liver metabolism between as animals cycle between hibernation states. In 13-lined ground squirrels, liver mitochondrial metabolism is suppressed by 70% in torpor, and this suppression is rapidly reversed during interbout euthermia (IBE). This suppression of liver mitochondrial metabolism corresponds with suppression of maximal activity of electron transport system protein complexes I and II. Due to the rapid and reversible nature of this transition, we hypothesized that the changes in mitochondrial metabolism between torpor and IBE are regulated by PTMs of mitochondrial electron transport system proteins. We investigated the role of PTMs using 2D differential gel electrophoresis to compare mitochondrial protein from animals sampled during torpor and IBE. We selected protein spots that differed between these two states in

either molecular weight or isoelectric point, and identified the proteins via mass spectrometry. Our data suggest that PTMs play an important role in regulating rapid changes in mitochondrial metabolism during hibernation.

REVERSIBLE TEMPERATURE-DEPENDENT DIFFERENCES OF BROWN ADIPOSE TISSUE MITOCHONDRIAL RESPIRATION DURING TORPOR IN A MAMMALIAN HIBERNATOR

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While seasonal modifications of brown adipose tissue (BAT) in hibernators are well documented, we know little about the functional regulation of this tissue in different phases of hibernation. In the 13-lined ground squirrel, liver mitochondrial respiration is suppressed by up to 70% during torpor but this suppression is reversed during arousal and interbout euthermia (IBE). In BAT, respiration is regulated by sympathetic activation, so we hypothesized that further regulation at the level of the mitochondria would be of little further advantage. We predicted, therefore, that neither mitochondrial respiration rates nor electron transport system (ETS) enzyme activities would differ between torpor and IBE. Contrary to our predictions, mitochondrial respiration rates from torpid individuals were significantly suppressed compared to rates from IBE individuals when measured at 37°C. When assayed at 10°C, however, mitochondrial respiration rates were greater in torpor than IBE. The data suggest that BAT mitochondria become less sensitive to temperature during torpor, with respiration Q_{10} values that are less than 2 and significantly lower than values in IBE. Contrary to these results, maximal activities of ETS enzymes did not differ between torpor and IBE, when measured at either 37°C or 10°C. Whole, intact BAT adipocyte respiration and response to norepinephrine also did not differ between torpor and IBE at either experimental temperature. The aforementioned differential temperature sensitivity between torpor and IBE in mitochondrial respiration is not reflected in ETS enzyme activities or whole BAT adipocyte respiration. We suggest the remodeling of BAT mitochondrial membrane lipids between torpor and IBE may contribute to the shift in temperature sensitivity and that the adipocyte isolation process may have reversed these subtle, temperature-dependent changes. Such a shift in temperature sensitivity might facilitate BAT uncoupled thermogenesis even at the low body temperatures experienced in transitions from torpor to arousal.

EVOLUTION OF UCP1 AND BROWN ADIPOSE TISSUE

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Brown adipose tissue (BAT) is a unique evolutionary outcome enabling adaptive thermoregulation in eutherian mammals through heat production catalyzed by mitochondrial uncoupling protein 1 (UCP1). Despite the presence of UCP1 orthologs prior to the divergence of teleost fish and mammalian lineages, UCP1's significance for thermogenic adipose tissue emerged at later evolutionary stages.

In marsupials, which diverged from eutherian mammals about 140 MYA, UCP1 is expressed in adipose tissue of 'brownish' appearance but noradrenaline does not trigger the classical adaptive excitation of thermogenesis (2, 3). Here, we show progressively increasing UCP1 gene expression during juvenile development of the grey short-tailed opossum, *Monodelphis domestica*. To determine the molecular identity of UCP1-positive adipose tissue in marsupials, we assessed the global transcriptome of the interscapular adipose tissue and characterized gene program changes associating with UCP1 gene expression. We annotated gene products to the respective modern eutherian orthologs to answer the question which pathways were already wiring UCP1 biology in adipocytes about 140 MYA. Although UCP1-positive marsupial adipose tissue resembles eutherian brown fat-like molecular signatures, ectopically expressed marsupial UCP1 does not catalyze proton conductance. Current work

in our laboratory aims to unravel the functional origin of thermogenic UCP1 by determining specific metabolite transport prior to thermogenic uncoupling.

Session 6: Musculoskeletal Function

THERMOGENESIS AND THE SEASONAL EXPRESSION OF SARCOLIPIN IN A HIBERNATING MAMMAL

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Skeletal muscle is the single most abundant tissue in the mammalian body, accounting for about 40% of total body mass and 20-30% of resting metabolic rate. A significant amount of this energy is for the maintenance of a high calcium gradient across the sarcoplasmic reticular membrane, necessary for contraction. Calcium pumping by sarco-endoplasmic reticulum calcium ATPase (SERCA) is responsible for 40-50% of resting muscle metabolism, or as much as 15% of whole-body resting metabolism. Multiple high-throughput 'omics analyses from our lab have shown significant circannual fluctuations in the expression of genes and proteins involved in maintaining calcium homeostasis in the muscles of thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*). Of particular interest, expression of the gene sarcolipin is dramatically reduced during the hibernation season. Sarcolipin is a small protein shown to uncouple SERCA calcium transport from ATP hydrolysis, thus reducing its pumping efficiency. This reduced efficiency results in greater energy expenditure and heat production, and has recently been demonstrated to play a significant role in mammalian thermogenesis and body composition. The large reduction in SLN expression during hibernation likely indicates a mechanism by which these hibernators are regulating their muscle-based metabolism and heat production. To test this mechanism, we have developed an assay to measure SERCA efficiency using isolated sarcoplasmic reticular vesicles and a calcium electrode system. Using this system, we have assayed seasonal SERCA activity at points during the circannual cycle that represent a nearly 10-fold difference in sarcolipin gene expression.

PRESERVATION OF CALCIUM HOMEOSTASIS AND INHIBITION OF APOPTOSIS CONTRIBUTE TO ANTI-MUSCLE ATROPHY EFFECT IN HIBERNATING GROUND SQUIRRELS

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The underlying mechanisms that hibernators deviated from muscle atrophy during prolonged hibernating inactivity remain elusive. This study tested the hypothesis that the maintenance of intracellular Ca^{2+} homeostasis and inhibition of apoptosis would be responsible for preventing muscle atrophy in hibernating Daurian ground squirrels. The results showed that intracellular Ca^{2+} homeostasis was maintained in soleus and extensor digitorum longus (EDL) in hibernation and post-hibernation, while cytosolic Ca^{2+} was overloaded in gastrocnemius (GAS) in hibernation with a recovery in post-hibernation. The Ca^{2+} overload was also observed in interbout arousals in all three type muscles. Besides, the Bax/Bcl-2 ratio was unchanged in transcriptional level among pre-hibernation, hibernation and interbout arousals, and reduced to a minimum in post-hibernation. Furthermore, the Bax/Bcl-2 ratio in protein level was reduced in hibernation but recovered in interbout arousals. Although cytochrome C was increased in GAS and EDL in post-hibernation, no apoptosis was observed by TUNEL assay. These findings suggested that the intracellular Ca^{2+} homeostasis in hibernation might be regulated by the cytosolic Ca^{2+} overload during interbout arousals, which were likely responsible for preventing muscle atrophy via inhibition of apoptosis. Moreover, the muscle-specificity indicated that the different mechanisms against disuse-induced atrophy might be involved in different muscles in hibernation.

ITRAQ-BASED ANALYSIS OF MYOFIBRILLAR CONTENTS AND SYNTHESIS AND PROTEOLYTIC PROTEINS IN SOLEUS MUSCLE OF DAURIAN GROUND SQUIRRELS

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Daurian ground squirrels (*Spermophilus dauricus*) deviate from significant increase of protein catabolism and loss of myofibrillar contents during long period of hibernation inactivity. Here we use iTRAQ based quantitative analysis to examine proteomic changes in myofibrillar contents and proteolytic pathways from the soleus of squirrels in pre-hibernation and hibernation states. We found the levels of most contractile and regulatory myofibrillar proteins examined were maintained through hibernation period, with 1 contractile protein (myosin-2) upregulated while 3 contractile proteins (myosin-3, myosin-13 and actin) downregulated in hibernation compared to pre-hibernation group. Besides, regulatory proteins such as troponin C and tropomodulin-1 were dysregulated in hibernation compared to pre-hibernation group. Moreover, some proteins with synthesis and proteolytic function were also dysregulated in hibernation compared to pre-hibernation group. These findings suggest that myofibrillar protective remodeling and partial suppression of myofibrillar proteolysis was likely responsible for preventing skeletal muscle atrophy during prolonged disuse in hibernation.

Significance: This is the first work that the myofibrillar contents and relevant synthesis and proteolytic proteins in slow soleus was discussed basic on proteomic investigation performed on wild Daurian ground squirrels. Our results lay the foundation for further research in preventing disuse-induced skeletal muscle atrophy in mammals.

SCIATIC LESION DOES NOT INDUCE BONE DISUSE ATROPHY INDEPENDENT OF HIBERNATION IN GROUND SQUIRRELS

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Bone loss is evident in as little as 2 weeks in common rodent models of disuse atrophy. Yet hibernating mammals are inactive for 6-9 months per year. We previously demonstrated the maintenance of bone strength following the hibernation season in ground squirrels. Here, we investigated if active season squirrels naturally resist bone disuse atrophy. Unilateral sciatic lesions were performed on squirrels collected from the wild in June and September. Following a 6-week period of disuse, leg bones were collected and the following parameters were evaluated: bone morphometry, break force, flexural strength, flexural modulus, and ultrastructure (visualized through micro computed tomography). Despite sciatic denervation and disuse for 6 weeks, squirrels experienced no bone disuse atrophy. These results indicate resistance to bone disuse atrophy is not restricted to the hibernation season in ground squirrels.

Session 7: Metabolism

COLD-HEARTED BATS: CARDIAC FUNCTION AND METABOLISM DURING TORPOR AT SUBZERO TEMPERATURES

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Extremely low ambient temperatures (T_a) create an energetic hurdle for many small bats as they must markedly increase internal heat production to remain normothermic when exposed to such conditions. Torpor is therefore critical for these animals with regard to managing daily energy budgets and saving energy during inclement conditions. During torpor, most bats thermoconform and body temperature (T_b) approximates ambient temperature (T_a). However, in conditions where T_a falls below

freezing, torpid bats must thermoregulate to maintain T_b at a specific set point temperature (T_{set}), which involves considerable alterations in physiology. We investigated the T_{set} for a small southern hemisphere bat (*Chalinolobus gouldii*, 12 g) that is often exposed to subzero temperatures during winter in parts of its range. We simultaneously measured heart rate (HR) and oxygen consumption ($\dot{V}O_2$) in thermoconforming bats at mild T_a (5 - 25°C) compared to bats exposed to progressively lower temperatures down to -2°C. T_{set} for this species was determined at T_a 0.7 ± 0.4°C. Below this temperature animals began to thermoregulate and there was a substantial increase in both HR and $\dot{V}O_2$. However, the maximum increase in HR was only 4-fold greater than the thermoconforming minimum at T_a of 2°C, compared to a 46-fold increase in $\dot{V}O_2$. When T_a fell below -1.5°C the majority of animals rewarmed spontaneously and only one animal remained torpid down to T_a of -2°C before arousal. Our data are the first to quantify HR and $\dot{V}O_2$ during steady-state torpor in a southern hemisphere bat below 0°C. We also show that there is a significant physiological difference between thermoconforming torpor and thermoregulating torpor with regard to the relationship between HR and $\dot{V}O_2$, suggesting that different aspects of the cardiovascular system may be altered while HR remains low.

FASTING-INDUCED DAILY TORPOR IN DESERT HAMSTERS (*PHODOPUS ROBOROVSKII*)

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Daily torpor is frequently expressed in small rodents when facing energetically unfavorable ambient conditions. Desert hamsters (*Phodopus roborovskii*, ~20g) appear to be an exception as they have been described as homeothermic. However, we hypothesized that they can use torpor because we observed reversible decreases of body temperature (Tb) in fasted hamsters. To test this hypothesis we (i) randomly exposed fasted summer-acclimated hamsters to ambient temperatures (Tas) ranging from 5 to 30°C or (ii) supplied them with different rations of food at Ta 23°C. All desert hamsters showed heterothermy with the lowest mean Tb of 31.4±1.9°C (Minimum, 29.0°C) and 31.8±2.0°C (Minimum, 29.0°C) when fasted at Ta of 23°C and 19°C, respectively. Below Ta 19°C, the lowest Tb and metabolic rate increased and the proportion of hamsters using heterothermy declined. At Ta 5°C, nearly all hamsters remained normothermic by increasing heat production, suggesting the heterothermy only occurs in moderately cold conditions, perhaps to avoid freezing at extremely low Tas. During heterothermy, Tbs below 31°C with metabolic rates below 25% of those during normothermia were detected in four individuals at Ta of 19°C and 23°C. Consequently, by definition, our observations confirm that fasted desert hamsters are capable of shallow daily torpor. The negative correlation between the lowest Tbs and amount of food supply shows that heterothermy was mainly triggered by food shortage. Our data indicate that summer-acclimated desert hamsters can express fasting-induced shallow daily torpor, which may be of significance for energy conservation and survival in the wild.

EFFECTS OF TEMPERATURE ON BREATHING PATTERN AND VENTILATORY RESPONSES DURING HIBERNATION IN THE GOLDEN-MANTLED GROUND SQUIRREL

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During entrance into hibernation in golden-mantled ground squirrels (*Callospermophilus lateralis*), ventilation decreases as metabolic rate and body temperature fall. Two patterns of respiration occur during deep hibernation. At 6 – 10°C a breathing pattern characterized by bursts of multiple breaths (20.6 ± 1.9) separated by long apneas or non-ventilatory periods (T_{NVP}; 11.1 ± 1.2 min) occurs, while at 2-4°C a pattern in which breaths are evenly distributed separated by a relatively short T_{NVP} (0.5 ± 0.05 min) occurs. Squirrels exhibiting each pattern have similar metabolic rates and levels of total ventilation (0.2 and 0.23 ml O_2/hr/kg and 0.11 and 0.16 ml air/min/kg respectively). Squirrels at 6-10°C exhibit a

significant hypoxic ventilatory response and when the decrease in metabolic rate during hibernation is taken into account, their hypoxic sensitivity is similar to that in awake squirrels. Squirrels at 2-4°C do not respond to hypoxia at any level tested. Squirrels at both temperatures exhibit a significant hypercapnic ventilatory response that is greater in the 6-10°C squirrels. When ventilation is standardized for the reductions in metabolic rate during hibernation both groups exhibit a greater hypercapnic sensitivity than that seen in awake squirrels. Carotid body denervation has little effect on the breathing patterns or on the hypercapnic ventilatory responses. It does reduce the magnitude and threshold for the hypoxic ventilatory response. Taken together the data suggest that 1) the fundamental rhythm generator remains functional at low temperatures; 2) the hypercapnic ventilatory response arises from central chemoreceptors that remain functional at very low temperatures; 3) the episodic breathing pattern is generated centrally by neuron pools that are silenced at the lower temperatures; and 4) the hypoxic ventilatory response arises from both carotid body and aortic chemoreceptors that are silenced at the lower temperatures. This research was funded by the NSERC of Canada.

COMPONENTS OF METABOLIC SUPPRESSION IN HIBERNATING BLACK BEARS

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While metabolic suppression in small hibernators is dominated by temperature effects on metabolic rate, with increased body size comes the capacity to carry more energy stores compared to scaling of basal metabolic rate (BMR), thus the metabolic rate suppression can be less pronounced. We have shown that minimum metabolism of black bears is suppressed to 25% of basal rates in mid-hibernation. At emergence from dens in spring, core body temperature in bears (T_b) has increased to normal, euthermic levels, while there is still a temperature-independent suppression of metabolic rate of about 50% of summer BMR. Prying apart the different components of patterns of metabolic rate within hibernation is complicated. Due to their suppressed metabolic rate, below a den temperature (T_{den}) of about 0°C black bears thermoregulate by shivering and also benefit from heat from sporadic activity. Then there are 3-5°C multiday body temperature cycles that appear unique to hibernating bears at low T_{den}. At increasing T_b during a cycle, shivering is most intense, while it is mostly absent when T_b decreases at the fastest rate. This allows calculation of Q_{10} (the temperature coefficient normalized to a 10°C interval) from correlates of metabolic rate and T_b during the decreasing phase of temperature cycles. Q_{10} can then be used to temperature correct a time course of minimum metabolic rates during non-thermoregulating conditions to calculate the temperature-independent metabolic suppression component throughout hibernation. Preliminary data indicates that temperature-independent metabolic suppression is more pronounced than previously thought and recovery starts weeks before bears emerge from their dens. Knowing the time course can be important for understanding regulatory mechanisms at organ, cell, and gene expression level. Harnessing the regulation of metabolic suppression could have wide applications to decrease oxygen demands in patients as well as allowing safer long duration space flights in the future.

Session 8: Organism-Environment Interactions

MORE FUNCTIONS OF TORPOR

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In the past decade it has become evident that torpor has many more functions than just energy conservation in winter. The list of "the other functions of torpor" seems to be continuously growing. Major recent findings in torpor research are that animals employ torpor to survive and cope with adverse and unpredictable conditions during and after natural disasters, in particular fires and storms. Such data support the hypothesis that heterotherms with their flexible energy requirements have an adaptive

advantage over homeotherms in response to unpredictable conditions. As climatologists predict that climate change will cause increases to the frequency and intensity of extreme weather events, the opportunistic use of torpor to survive these events will therefore likely enhance survival.

However, it is unclear whether torpor use may also impose a risk during such conditions as it may delay perception of stimuli, such as smoke, and because it hinders locomotor performance. We will present new data on behaviour and physiology of heterotherms responding to extreme environmental events and evaluate the risks and advantages of torpor use in the context of climate change.

THE COSTS OF FORAGING – MAXIMUM BODY TEMPERATURES AND USE OF TORPOR DURING THE ACTIVE SEASON IN EDIBLE DORMICE

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The costs of foraging are difficult to determine, especially in arboreal species. Although not an exact measure of activity, high core body temperatures are expected to reflect periods with increased activity levels. On the other hand, the occurrence of short phases of torpor indicates the necessity to reduce energy expenditure and may therefore be used more frequently in periods with restricted food supply. We analyzed data on core body temperatures in edible dormice, *Glis glis*, recorded during the active season in free-ranging and captive dormice. We compared temperature patterns at different phases within the active season and between years with and without beech mast, as beechnuts are an important food source of dormice. Further we tested whether enclosure housed animals, provided with food *ad libitum*, showed lower peak core temperatures and/or less occurrence of torpor throughout the active season.

The maximum recorded core body temperature was 41,5°C. High core temperatures (> 40°C) occurred throughout the active season in both free-ranging and enclosure-housed dormice. Short phases of torpor were more frequently observed in mast failure years. However, irrespective of the beechnut availability periods of torpor were significantly less frequent during the pre-hibernation fattening phase. Our results indicate that dormice showed high core temperatures most likely due to increased activity and high costs of arboreal foraging. On the other hand short bouts of torpor are an intensively used mechanism to decrease energy expenditure in years with lower food availability.

SURVIVAL BENEFITS OF TORPOR-FACILTATED INACTIVITY

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The use of torpor by mammals and birds provides a mechanism to reduce their minimum daily energy requirements. At one extreme, prolonged deep torpor bouts combined with food or fat storage allows hibernating mammals to remain dormant over the entire winter season. At the other, even short shallow torpor bouts provide sufficient energy savings to allow reductions in minimum daily foraging effort. We propose that an important ecological function of torpor-facilitated energy savings is to permit small endothermic animals to reduce their activity (at daily and annual temporal scales) and therefore also their exposure to environmental-caused mortality risk. Hence, torpor could be used to modulate a relationship between environmental conditions and the relative benefits and mortality costs of activity. We review published studies and highlight results of our own recent experiments and comparative studies to evaluate support for this hypothesis.

BASKING HAMSTERS?

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Basking can substantially reduce thermoregulatory energy expenditure of mammals. We tested the hypothesis that the change of fur color from brown in summer to white in winter in hamsters (*Phodopus sungorus*), originating from Asian steppes, is related to camouflage to permit sun basking on or near snow for energy conservation. Winter-acclimated hamsters in our study were largely white and had a high proclivity to bask when resting and torpid. Resting hamsters reduced metabolic rate (MR) significantly (>30%) when basking at ambient temperatures (T_a) of ~15 and 0°C. Interestingly, body temperature (T_b) also was significantly reduced from ~35°C (T_a 15°C not basking) to ~30°C (T_a 0°C basking); this in turn resulted in an extremely low (<50% of predicted) apparent thermal conductance. Induced torpor during respirometry at T_a 15°C occurred on 83% of days and the minimum torpor MR was 36% of basal MR at an average T_b of 22°C; movement to the basking lamp was observed at T_b<20.0°C. Energetic costs of rewarming were significantly reduced (~50%) during radiant heat-assisted rewarming, however, radiant heat *per se* without an endogenous contribution by the animals did not strongly affect metabolism and T_b during torpor. Our data show that basking substantially modifies thermal energetics in hamsters, with a drop of resting T_b and MR not previously observed and a reduction of rewarming costs. The energy savings afforded by basking in hamsters suggest that this behavior is of energetic significance not only for mammals living in deserts where basking is common, but also for *P. sungorus* and likely other cold-climate mammals.

SEASONALLY-RELEVANT ACUTE WARMING MODIFIES METABOLISM IN FISH FROM THE MITOCHONDRION TO THE WHOLE ORGANISM

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Temperature is well recognized as a major influence of metabolic capacity and energetic demand in fishes. At temperate and higher latitudes there is marked seasonality for most fish habitats during summer, causing areas of potential resource exploitation to often exceed the thermal optima for cool water fish species. As such, when fish sojourn into high temperature environments to feed they are exposed to short-term (scale of minutes) acute thermal challenges where their cells may increase in temperature by 5-10°C, potentially having consequences at the level of metabolism. We have been exploring the potential impact of acute warming on fish using experiments ranging in scale from in vitro experiments on isolated mitochondria to whole animal studies of oxidative metabolism. For isolated heart mitochondria (rainbow trout and lake sturgeon), a warming of as little as 5°C can lead to increased potential to produce reactive oxygen species (ROS). This elevation in potential ROS burden may lead to altered cellular redox environment and elevated oxidative damage of macromolecules. In wild sampled lake trout from small lakes that stratify during summer, we find the glutathione pool is more oxidized in fish that have to travel through a greater distance of warm water to reach energy rich prey in the shallows. This implicates that short foraging bouts into waters with elevated temperatures may lead to physiological consequences at the cellular level in the wild. To evaluate the impact of a mimicked single daily foraging bout laboratory raised lake trout have been exposed to a short (~ 10 minute) increase of approximately 5 or 10°C over two months. This led to altered aerobic scope and may also influence food conversion efficiency and appetite. Overall, our findings suggest that there are marked consequences at many levels of metabolism from a brief heat challenge in temperate climate fishes.

SEASONAL PREVALENCE OF LYME DISEASE SPIROCHETES IN A HETEROTHERMIC MAMMAL, THE EDIBLE DORMOUSE (*GLIS GLIS*)

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In Europe, dormice serve as competent reservoir hosts for particular genospecies of the tick-borne agent of Lyme disease (LD) and seem to support them more efficiently than do mice or voles. The longevity of edible dormice (*Glis glis*) and their attractiveness for ticks may result in a predominance of LD spirochetes in ticks questing in dormouse habitats. To examine the role of edible dormice in the transmission cycle of LD spirochetes, we sampled skin tissue from the ear pinnae of dormice inhabiting five different study sites in southwestern Germany. Of 501 edible dormice, 12.6% harbored DNA of LD spirochetes. Edible dormice were infected most frequently with the pathogenic LD spirochete *Borrelia afzelii*. No spirochetal DNA was detectable in the skin of edible dormice until July, 6 weeks after they generally start to emerge from their obligate hibernation. Thereafter, prevalence of spirochetal DNA in edible dormice increased during the remaining period of their 4 to 5 months of activity, reaching nearly 40% in September. Males were more than four times more likely to harbor LD spirochetes than females, and yearlings were almost twice more likely to be infected than adults. The seasonality of the prevalence of LD spirochetes in edible dormice was pronounced and may affect their role as a reservoir host in respect to other hosts.

Session 9: Molecular Genetics

COMPARATIVE TISSUE TRANSCRIPTOMICS HIGHLIGHT METABOLIC TRANSCRIPT PRIORITIZATION IN PREPARATION FOR AROUSAL FROM TORPOR

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Animals cycle between torpor and arousal throughout the hibernation season. However, the molecular drivers of the drastic physiological changes that occur during arousal from torpor remain poorly understood. Although transcription effectively ceases during torpor, previous work has demonstrated that a subset of transcripts are protected from bulk degradation in brown adipose tissue, presumably for their necessary role in arousal from torpor, causing them to increase in relative abundance as the torpor bout progresses. In the current study, we examine the transcriptome of skeletal muscle, heart, and liver to identify transcripts that increase in relative abundance during torpor in these tissues. EDGE-tag transcriptomics was performed on samples from five distinct physiological states in the circannual cycle of thirteen-lined ground squirrels. Supervised clustering with Random Forest based on transcript abundance separated animals entering torpor from those late in a torpor bout in all three tissues, indicating that the transcriptome is dynamic across the torpor period. We identified 6784, 3347, and 2433 differentially expressed transcripts among all sampling points in skeletal muscle, heart, and liver, respectively. Using DIANA clustering of the differentially expressed transcripts, cohorts that increased during torpor were identified in each tissue. DAVID pathway analysis of these subsets highlighted protein and amino acid catabolism and glucose metabolism in all three tissues. Additional pathways included the TCA cycle in skeletal muscle, muscle cell development and cardiac muscle morphogenesis in the heart, and cholesterol biosynthesis in the liver. The observed increase in the relative abundance of transcripts associated with metabolic pathways during torpor across multiple tissues is consistent with a larger role for these processes in the progression or necessity of arousal from torpor.

SYSTEMIC PRE-HIBERNATION REMODELING PRIOR TO HIBERNATION IN SYRIAN HAMSTERS (*MESOCRICETUS AURATUS*)

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Hibernation is an adaptive strategy for surviving during periods with little or no food availability. It is suggested that hibernators remodel their bodies to become adapted to hibernation prior to hibernation season. However, the inductive and regulatory mechanisms of the pre-hibernation remodeling remain to be elucidated. We consider that the Syrian hamster (*Mesocricetus auratus*) is one of good animals for addressing these points; they can enter hibernation throughout the year when exposed to a short day photoperiod and cold ambient temperatures (SD-Cold) for a long period (typically 2–3 months) under laboratory conditions. Despite its seasonal independence of hibernation, the requirement of a long pre-hibernation period before the induction of hibernation in Syrian hamsters implies that these animals undergo pre-hibernation remodeling prior to hibernation, which occurs in autumn in seasonal hibernators. Continuous measurements of core body temperature (Tb) revealed that the Tb set-point was remodeled during the pre-hibernation and hibernating periods in this species. In addition, there was a body mass threshold for hibernation induction. We then conducted exhaustive gene expression analyses using a next-generation sequencer to identify adaptive remodelings for hibernation in various organs. Hundreds of genes were significantly up-regulated or down-regulated in the hibernation period in liver, white adipose tissue, and kidney. Time-course analyses further revealed that the up-regulation of many genes, for instance, ones involved in lipid metabolisms, occurred prior to hibernating period, and were cancelled in post-hibernation period even when the animals were still maintained in SD-Cold. These results suggest that regulations of those genes were coupled with the ability to hibernate in Syrian hamsters, and support the idea that Syrian hamsters remodel their bodies in the pre-hibernation period to prepare for hibernation.

HIBERNATION, SOMATIC MAINTENANCE, AND AGING: TELOMERE DYNAMICS IN FREE-LIVING EDIBLE DORMICE

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Hibernators have higher chances of survival during winter than during the active season, higher annual survival rates, and larger lifespans than non-hibernators. Life history theory predicts that this should select for high investment into somatic maintenance and enhanced cellular repair mechanisms. One marker of cellular damage and repair is the length of telomeres, the end-caps of chromosomes. Telomeres shorten during mitosis and due to oxidative stress, but can be re-elongated by the enzyme telomerase and other mechanisms. We investigated telomere-dynamics determined in mucosa cells of free-living edible dormice (*Glis glis*). This hibernating rodent has a maximum lifespan of 13 years, which exceeds the expected lifespan for a non-hibernator of its size (8 years) by 60%. We found that telomeres significantly shorten over the hibernation season. The single best predictor of telomere shortening was the number of arousals from deep torpor, which can reach 28 in dormice hibernating up to 11.4 months. This finding points to increased oxidative stress during arousals. We also found that telomeres can be re-elongated during the active season. However, a supplemental feeding experiment in a year of low natural food abundance indicated that telomere elongation requires high food supply. Thus, we conclude that cellular repair in terms of telomere restoration may be energetically costly. Telomere length was related to the age of the animals. Surprisingly, both telomere length as such as well as the rate of telomere elongation increased up to an age of 9 years. This contrasts with findings from most other vertebrates, including humans, which show telomere shortening with increasing age. We attribute this unusual pattern to preparation for increased oxidative stress during reproduction, because

in edible dormice the probability of reproduction also continually increases with progressing age. We propose that hibernators may be interesting models for the study of aging.

PHOTOPERIOD AND HIBERNATION: LESSONS FROM JUMPING MOUSE GENOMES

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Jumping mice (*Zapus* spp.) are small hibernating rodents found throughout North America. Differences between the strategies employed by meadow jumping mice (*Z. hudsonius*) and western jumping mice (*Z. princeps*) for reproduction and hibernation provide an opportunity to study the genetic basis of these phenotypes. Meadow jumping mice produce litters from June to August; both offspring and adults use short autumn photoperiod as the primary cue for fattening and entry into hibernation. In contrast, western jumping mice breed once following spring emergence, then fatten when food availability peaks at the end of summer regardless of photoperiod. We sequenced, *de novo* assembled, and annotated a meadow jumping mouse reference genome, which allowed us to obtain the complete genomes of 7 individuals of each jumping mouse species. Comparative genomic analysis using these genomes provides information about phylogeny and evolution in jumping mice and reveals insights into genes important for speciation and photoperiod response in these species.

BUILDING A GENETICS FRAMEWORK FOR THE STUDY OF HIBERNATION IN THE 13-LINED GROUND SQUIRREL

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Genetic approaches can provide critical insight into the evolutionary and molecular mechanisms that underlie phenotype. One such approach is quantitative genetics, which can be used to identify genes that explain variation in the expression of phenotypic traits. Despite extensive studies at the ecological, anatomical and physiological levels, a genetic basis of hibernation has yet to be established. While hibernation studies report biological variance at all levels ranging from gene-expression to behavioral measurements, almost nothing is known about genetic variation within these mammals and the effect that it exerts on phenotype. Our goal is to establish a genetic basis of the hibernating phenotype for the 13-lined ground squirrel, *Ictidomys tridecemlineatus*. To this end, we are first developing a high-quality genomics resource for this species, which includes bringing the current genome assembly to chromosome scale and building a linkage map. We have added long-range sequence data to increase the contiguity of assembly, nearly tripling the scaffold N50 from 8Mb to approximately 23Mb. We are constructing a linkage map via a genotype-by-sequencing strategy applied to DNA samples collected from individuals in full-sibling families. Once complete, this linkage map will be used to orient and order the genome assembly scaffolds into chromosomes. Finally, we are applying a genotype-by-sequencing strategy to DNA samples collected from a cohort of 155 13-lined ground squirrels. We recover several hundred thousand SNPs located genome-wide, enabling us to characterize the underlying genetic architecture of this species and to precisely estimate the heritability of several hibernation-related traits. For those detected as significantly heritable, we will perform a quantitative trait loci analysis using our improved genomic resource to identify candidate genes underlying traits of hibernation.

POSSIBLE CAUSES AND CONSEQUENCES OF DIFFERENT HIBERNATION PATTERNS IN *CHEIROGALEUS* SPECIES – MITOVY FATSY SAHALA

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All dwarf lemur species of Madagascar (*Cheirogaleus spp.*) hibernate in their natural habitats, decreasing energy and water demands drastically to survive the harsh seasons of the Malagasy winter, from the eastern rainforests to the western dry forests. However, the different species are very flexible in the *modus operandi* and timing of hibernation, and even within species differences are considerable, depending on habitat and climatic parameters. Our presentation will compare hibernation aspects of the different dwarf lemur species and discuss possible causes of consequences of different hibernation patterns. Whereas dry forest species hibernate in tree hollows of various insulation properties, rainforest species retreat underground for hibernation. These different strategies of hibernaculum choice have extensive consequences on hibernation parameters and patterns. In underground hibernacula and in well-insulated hollows in large trees energy expenditure and body temperature (T_b) during the hibernation bouts are almost constant and spontaneous arousals occur regularly. In contrast, in thinner trees, insulation capacities are decreased, and energy expenditure and T_b are fluctuating with the ambient temperature (T_a) to various degrees. When these passive fluctuations are pronounced T_b reaches values above 30°C passively during the day, arousals become dispensable. As expected, the colder the habitat is during winter, the greater the energy savings by hibernation are. There is evidence suggesting that more stable environments in the hibernacula are preferred and thus possibly represent the ancestral condition, indicating that the hibernation machinery was originally adapted to and runs best at mostly constant T_a. However, it is also conceivable that hibernation in tree hollows could have been the original *modus operandi* in the ancestral *Cheirogaleus*, implying that hypometabolism in some species is truly independent of T_a, and fluctuating T_a might even be advantageous, allowing for extended, continuous bouts of hypometabolism by avoiding interruptions by active arousals.

SEASONAL ADAPTATIONS AND INTER-SPECIFIC DIFFERENCES OF THE ENERGY BUDGETS OF TWO PRIMATES (*L. LEUCOPUS & L. RUFICAUDATUS*)

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Animals experience seasonal changes in terms of environmental and ecological conditions in most of their habitats. Fluctuations in ambient temperature have a strong influence, particularly on small endothermic mammals, and are therefore an important source of functional constraints. However, different mammalian species react differently to these temperature changes. It is important to understand the physiological responses of organisms to different seasons and to analyze the mechanisms that account for intra- and interspecific physiological traits and the ecological consequences of these variations. Through the use of open-flow respiratory and measurement of skin temperature in the field, we sought to identify adaptive changes in the energy budgeting of the small Malagasy folivorous lemur, *Lepilemur leucopus*. We focused on this species as it appears to be particularly sensitive to climatic variation. Furthermore, we compared the inter-specific adaptions of two *Lepilemur* species (*L. leucopus* & *L. ruficaudatus*) to the harsh dry season in South- and Southwest-Madagascar. Our study did not detect any signs of heterothermic episodes in either species, despite the fact that such episodes have been observed in other lemurs of similar size and life style. The metabolic rate of *L. leucopus* was consistently higher in the cooler dry than in the wet season. Surprisingly, the thermoneutral zone in the wet season was lower (25°C to 30°C) than in the dry season (29°C to 32°C). *L. leucopus* and *L. ruficaudatus* demonstrate one of the lowest weight-specific metabolic rates measured so far for mammals - particularly notable in the case of the heavier *L. ruficaudatus*. However, the magnitude of

divergence is highly dependent on the season, and the traditional assumption that the genus *Lepilemur* has considerably lower metabolic rates than other folivorous mammals has thus to be more carefully differentiated. The findings of our data highlight the importance of studying physiological parameters, in different seasons, and considering the differences between various species - even if they are closely related.

DOES HIBERNATION INCREASE SEXUAL CONFLICT IN TASMANIAN ECHIDNAS?

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Echidnas have a promiscuous mating system with intense competition between males. In Tasmania, hibernation reduces the active period and appears to intensify competition.

We studied a population of echidnas over the period 1996-2013. Tracking transmitters were attached to 105 echidnas (60 F, 34 M, 11 juvenile). We also made opportunistic observations on an additional 179 echidnas (64F, 72M, 43 juvenile or burrow young). 16 echidnas (10F, 6M) were implanted with temperature loggers, and external temperature loggers attached to a further 45 (30F, 15M). In the field we recorded location, and body mass, and took 313 cloacal swabs from 43 females to check for the presence of sperm.

Mating males emerged from hibernation between May 7 and August 5, with males in the best condition emerging the earliest. For females the pattern of arousal was more complex: very few emerged from hibernation spontaneously but instead were disturbed by males. Matings occurred from June 6 to October 27, with a peak in mid-July. We recorded 185 mating groups of 1 to 4 males with a female. Females were found in up to 7 mating groups over 3 weeks, with up to 4 different males, but nearly all females became pregnant at the first mating. Females that became pregnant before July 27 re-entered hibernation, but the length of this torpor depended on the amount of subsequent harassment by males.

CHARCOAL AND ASH – NEW CUES FOR TORPOR ENHANCEMENT

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Recent studies have revealed some physiological and behavioural responses that mammals employ to survive and cope with the aftermath of fires. These data suggest that the post-fire reduction in food resources and ground cover are the primary triggers for an increase in torpor use, likely to deal with energetic constraints and heightened predation pressure. However, it is possible that there are currently unappreciated cues that enhance torpor use following fires. To identify these we aimed to provide new experimental data on activity and torpor use in a small marsupial, the yellow-footed antechinus (*Antechinus flavipes*), housed in outdoor aviaries in response to a combination of food restriction, smoke exposure and a charcoal-ash substrate. Throughout the study period all individuals were nocturnal; diurnal activity was only observed as a direct response to smoke exposure. As expected, antechinus increased torpor duration in response to food restriction. However, while torpor duration in response to the combination of food restriction and smoke exposure was similar to food restriction alone, interestingly the addition of a charcoal-ash substrate to these variables resulted in significantly longer torpor duration. This suggests that the combination of food restriction, smoke and the charcoal-ash substrate provides a stronger torpor cue than food shortage on its own. Our study provides significant new information on how a small mammal responds behaviourally and physiologically to survive during and immediately after a fire. Most importantly it reveals a novel cue for torpor use, a charcoal-ash substrate that would be present immediately after a fire and likely signalling long-term energetic and foraging challenges.

RESPONSES OF AUSTRALIAN HETEROTHERMIC MAMMALS TO FIRE

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The number of forest fires is increasing as a result of climate change and therefore fires are becoming a progressively pressing global issue. However, how small mammals deal physiologically with changes in landscape and food availability due to fire remains largely unknown, although recent studies on antechinus, small terrestrial marsupials, have shown a post-fire increase in torpor for energy conservation. To fully understand the impact of large-scale environmental changes on small mammals and the role of torpor in enhancing survival, it is essential to also investigate the response of flying animals. Therefore, we determined the post-fire thermal biology of lesser long-eared bats (*Nyctophilus geoffroyi*) using temperature-telemetry in Warrumbungle National Park, NSW, which experienced a devastating wildfire in 2013 that destroyed about 88% of the park. Physiological data was gathered 4 months after the fire and then again 2 years post-fire to determine any shift in torpor use with a recovering landscape. Data on these small, insectivorous and aerial mammals were compared with those collected for the terrestrial antechinus. Post-fire survival strategies widely differed between bats and antechinus. Bats demonstrated a decrease in torpor use in response to wildfire when insect abundance increased, and no change in torpor use following prescribed fire. This is in contrast to antechinus, which used significantly more torpor both after the wildfire and prescribed fire. Although heterothermic mammals can reduce energy via a decrease in metabolic rate and body temperature, torpor use after a disaster may not always be the ideal response. It is likely that these differences in heterothermy in terrestrial and volant mammals are due to a suite of factors, such as perceived predation risk, an increase in aerial resource abundance and easier foraging for bats resulting from a decrease in vegetative clutter.

Session 11: Nervous System Function & Neural Control of Torpor

CHARACTERIZING VARIATION IN THE BRAIN TRANSCRIPTOME AS A FUNCTION OF HIBERNATION PHENOTYPE IN 13-LINED GROUND SQUIRRELS

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The 13-lined ground squirrel, like many obligate hibernators, divides each year into homeothermic (active) and heterothermic (hibernating) phases. The heterothermy that characterizes hibernation is itself a cycle between extended (~2 weeks) periods of torpor and short (<24 hrs) arousals to euthermia. During torpor, core body temperature approaches freezing and metabolic, heart and respiratory rates plummet to a few percent of those observed during either the homeothermic season or the brief euthermic periods of hibernation. Because gene expression determines phenotype, it is reasonable to expect that differential gene expression underlies both seasonal and torpor-arousal phenotypic transitions. We interrogated three regions of the brain, medulla, hypothalamus and forebrain-hypothalamus (hereafter forebrain), for changes in gene expression. The first two regions harbor neurons that maintain activity throughout the torpor bout, in contrast to the forebrain which is inactive. Moreover, the brain is transcriptionally complex and the site of numerous post-transcriptional events, including alternative splicing, 3' end processing and RNA editing. Transcriptome-wide data from these three brain regions were collected using RNA-seq and polyA-seq protocols from six (n=5), precisely-timed sample groups, representing the homeo- and heterothermic seasons. Specifically, these groups are post-emergence in spring, pre-immergence in summer, and spontaneously aroused, entering torpor, late in torpor and early in the process of arousing from torpor during winter hibernation. Although previous studies have used RNA-seq to evaluate gene expression changes in the forebrain and hypothalamus of this species, this work extends those findings to the medulla, adds critical physiological states, and

examines qualitative as well as quantitative variation. The effects of both season and body temperature on these brain transcriptomes will be discussed.

UNDERSTANDING THE MOLECULAR MECHANISM OF COLD THERMOSENSATION

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The ability for an organism to sense and respond to the environment is key for its survival. Harsh winter conditions are detrimental for nonhibernating species, while prolonged exposure to cold has no adverse effect on hibernating animals. We are currently using the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*, as a model to study cold sensitivity and tolerance. During summer, active squirrels must cross a temperature-threshold barrier to enter into the torpor state. We hypothesize that active squirrels are more resistant to cold than nonhibernating rodents, allowing them to overcome the temperature barrier. In support of this, two-plate temperature preference tests revealed that in comparison with mice, squirrels fail to exhibit robust cold avoidance at temperatures below 20°C. Radiometric calcium imaging of dissociated somatosensory neurons showed a significantly diminished Ca^{2+} influx in the squirrel neurons upon cooling. These data suggest that squirrels are less responsive to cold when compared to mice.

In vertebrate somatosensory neurons, the detection of cold is mediated by TRPM8, a Ca^{2+} permeable, nonselective cation channel activated by temperatures below 26 °C and its agonists, menthol and icilin. We found, using RNA in situ hybridization that the proportion of trpm8-expressing cells does not differ between squirrels and mice. However, unlike the murine channel, squirrel TRPM8 fails to respond to cooling at temperatures below 20°C when expressed in Xenopus oocytes. Thus, the apparent cold resistant phenotype exhibited by active squirrels in behavioral tests is explained, at least in part, by diminished cold sensitivity of TRPM8 in the somatosensory neurons.

HISTAMINERGIC NEUROMODULATION OF SYRIAN HAMSTER CENTRAL NERVOUS SYSTEM ACTIVITY AND BOUT DURATION

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In vivo studies showed that histamine (HA) infused into ground squirrel hippocampi lengthened hibernation (torpor) bouts by ~50%. However, it is unclear if HA acted directly on hippocampal pyramidal neurons and/or acted on downstream brain regions via HA spillover into lateral ventricles. To clarify this, we determined if HA would modulate CA1 pyramidal neurons at low levels of synaptic activity (as occurs during hibernation). Specifically, we tested the hypotheses that HA modulation of Syrian hamster CA1 pyramidal neurons: (a) increased their excitability [population spike amplitude (PSAs)] at low stimulation voltages; (b) this increase, persisted at temperatures below 37°C; and (c) this increase involved histaminergic H2 receptors. PSAs were recorded in hippocampal slices following Schaffer collateral stimulation from subthreshold levels to a maximum response plateau (max) at 30°C (n=14) and 20°C (n=17). Taking max response before HA as 100%, near-threshold PSAs increased from 2.7% (no HA) to 12.9% (10μM HA) of max at 30°C ($P<0.01$) and from 2.5% to 20.7% at 20°C ($P<0.001$). Cimetidine (an H2 antagonist) blocked HA-mediated PSA increases in 8 of 8 slices, but pyrilamine (an H1 antagonist) had no effect in 7 of 8 slices. HA enhancement of PSA was observed in slices from both hibernating and non-hibernating hamsters. Results support our hypotheses and are consistent with the proposal that HA enhances hippocampal pyramidal cell firing and that these neurons, in turn, send signals that inhibit the brainstem ascending arousal system and prolong torpor.

THE INFLUENCE OF THYROID HORMONES ON SPONTANEOUS DAILY TORPOR IN DJUNGARIAN HAMSTERS (*PHODOPUS SUNGORUS*)

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Djungarian hamsters are well adapted to changes in ambient temperature and food availability in a seasonally changing environment and express daily torpor in winter. Daily torpor bouts in this species are spontaneous phases with depressed metabolism and body temperatures between 13 and 32°C. In the last decade availability of the active thyroid hormone (TH) triiodothyronine (T3) to the hypothalamus has been demonstrated to be a key signal in seasonal adaptations of body weight and reproduction. In our studies we investigated the effect of TH on torpor by manipulating TH concentrations centrally in the hypothalamus via microdialysis or systemically via drinking water. Body temperature recordings revealed pronounced effects of thyroid hormone status on daily torpor. Elevated systemic as well as hypothalamic T3 concentrations immediately reduced torpor frequency and torpor duration. Torpor was completely blocked after few days of treatment. Systemic hypothyroidism induced by methimazole had the opposite effect with increased torpor frequency and longer torpor bouts. Gene expression, analysed by qPCR in hypothalamus, brown adipose tissue (BAT) and muscle, revealed decreased transcription of deiodinase type 2, the T3 activating enzyme, during torpor. This suggests lower intracellular T3 activation and reduced T3 concentrations might be required for torpor. Also uncoupling proteins, target genes of T3, were down regulated during torpor in BAT and muscle and might contribute to switching-off thermogenesis during hypothermia. Altogether we provide new evidence that T3 and TH-related genes play an important role in the regulation of torpor.

THE NEURAL CONTROL OF TORPOR IN MICE

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At the moment, the neural pathways responsible for the metabolic suppression that characterizes torpor are not known. Mice are facultative heterotherms and can be a good model to unravel such circuits. Torpor in mice can be reliably triggered by changing environmental conditions, consenting therefore to the use of an anatomical marker, such as cfos, to identify groups of neurons activated at torpor onset.

The metabolic suppression that characterizes torpor requires a reduction in the activity of metabolically active organs. Most of these organs, such as the brown adipose tissue, are controlled by the putative sympathetic premotor neurons located within the Raphe Pallidus (RPa). To enter torpor, these neurons have to be necessary inhibited. To pinpoint the neural circuits actively inducing the metabolic suppression, a retrograde tracer was injected within the Rpa. The main aim was to identify populations of inhibitory neurons with torpor-related activity.

Here we will present preliminary results showing the neural circuits with torpor-related activity and projections to the sympathetic premotor neurons within the RPa.

Session 12: Feeding, Digestive System Function & the Microbiome

HIGH FAT DIET AFFECTS ENERGY BALANCE IN PREHIBERNATORY GOLDEN-MANTLED GROUND SQUIRRELS

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Golden-mantled ground squirrels were placed on either a high-fat (chow + 10% olive oil + sunflower seeds) or a control (chow) diet during the pre-hibernation fattening period. Food intake was measured

daily, and body mass, body composition, and circulating hormones related to energy balance (the food intake-inducing hormone ghrelin and the anorexigenic sex hormone estradiol) were measured on a weekly basis prior to hibernation. Temperature data loggers (iButtons) were implanted abdominally and recorded core body temperature (Tb) every 2 hours from November to May. Within a week of final arousal from hibernation in May, animals were euthanized, and tissues (liver, brown adipose tissue (BAT), white adipose tissue (WAT), muscle, and brain) removed for Western blot analysis of the energy-sensing enzyme AMPK. Animals fed a high-fat diet had significantly higher ghrelin and estradiol concentrations than control animals during the pre-hibernation fattening period. High-fat diet animals ate significantly less than control animals, but diet type did not affect body mass or composition during this time period. Females decreased food intake and entered into hibernation earlier than males. Animals fed a high-fat diet prior to hibernation spent somewhat less time in torpor than control animals. These results indicate that a pre-hibernation high-fat diet may alter hormonal controls of energy balance in a sex-dependent manner.

EFFECTS OF CROPLAND-BASED DIETS ON THE HIBERNATION OF A FOOD-STORING RODENT

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With agriculture intensification, farmland species are often constrained by a lack of food diversity. The European hamster (*Cricetus cricetus*) is particularly threatened by the expansion of wheat and maize monocultures in Western-Europe. This food-storing hibernator relies on the food hoarded in its burrow in the late summer to hibernate and to reproduce in spring. In a previous study, we have shown that hamsters fed on maize-rich diets display high rates of infanticide (75%) and that an experimental supplementation of nicotinamide (a vitamin deficient in maize) restored a good reproductive success.

With the aim of understanding how food stores might affect hibernation and subsequent reproduction, we have investigated the effects of 6 new crops-based diets on the hibernation of 42 pairs of captive hamsters: three maize-based diets (maize-radish, maize-soybean and maize-sunflower) and three wheat-based diets (wheat-radish, wheat-soybean and wheat-sunflower). Hamsters were kept at 10°C on a winter photoperiod (8:16 LD). We found a strong effect of the diet on the torpor patterns and body mass loss. Hamsters fed on wheat-soybean, maize-radish and wheat-sunflower were more active than hamsters from the three other diets. Moreover, individuals fed on wheat-sunflower wheat-soybean and maize-sunflower lost twice less body mass than individuals fed on the other diets. Sunflower thus appears to be favorable whilst radish appears to be unfavorable, independently of whether they are associated to maize or wheat. Surprisingly, the diet wheat-soybean is highly favorable but soybean appears unfavorable when associated to maize.

We will now investigate for the effects of these diets on the first reproductive event of these hamsters. Given that sunflower and soybean are good sources of nicotinamide, proteins and fatty acid, we expect that they will guarantee a good reproductive success. Ultimately, crops that appear to be nutritionally favorable both for hibernation and reproduction of the European hamster will be included in the agricultural schemes in its French area of distribution.

MEASURING STRESS IN THE WILD – EFFECTS OF FLUCTUATING FOOD AVAILABILITY ON THE EDIBLE DORMOUSE (*GLIS GLIS*)

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Fluctuating environments require physiological adaptations to cope with unpredictable food sources or climatic challenges. Beech mast seeding is a prime example for unpredictably fluctuating food sources. The edible dormouse is a small arboreal rodent that is well adapted to this fluctuation. Dormice

reproduce only in years with intermediate or high mast, and the litters are born very late in the season (End of July-August) to time weaning with the peak of the mast season. We addressed the question whether differences in food availability and hence foraging requirements affect stress, as determined by cortisol levels. Specifically, we hypothesized that low food abundance may suppress reproduction via effects of cortisol on gonadal function. Cortisol levels were determined in feces via enzyme immunoassay analysis (EIA).

The study years 2012 to 2014 were characterized by very different levels of food availability: 2012 was a non-mast year with no access to high caloric food and no reproduction; 2013 was a full mast year, when most females reproduced. In 2014 we conducted a supplemental feeding experiment in half of the population, in the control area there was a low intermediate mast situation. Surprisingly, our results show that cortisol levels were reduced during non-mast years, compared with full mast years or with animals that were supplementary fed. These findings suggest that mating and reproduction, which occurred only when high caloric food was easily available, are associated with elevated stress. Vice versa, during non-mast years, dormice may avoid increased foraging effort and the associated exposure to predators. They instead save energy by the use of short torpor, which was significantly more frequent in the non-mast year. Thus, our findings contradict the hypothesis that skipping of reproduction in years of low food abundance is mediated via chronic stress effects on the reproductive axis of dormice.

FEEDING WHILE HIBERNATING

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Hibernation is an energy-saving strategy that enables some endotherm species to survive food scarcity. Most hibernators rely on body fat to overcome the hibernation period (fat-storing strategy), but some others feed during interbout euthermia (IBE, food-storing strategy). This intermittent fasting/feeding behaviour should imply specific metabolic and digestive adaptations. Also, the hibernation pattern might be significantly modified according to the nature of energy supplies (body or food reserves). To investigate these questions, we measured food consumption, plasma metabolites and hormones, and digestive function throughout the annual lifecycle in two food-storing hamster species: the golden (*Mesocricetus auratus*) and the common hamster (*Cricetus cricetus*). We also investigated the link between energy availability and hibernation pattern in common hamsters.

Both hamster species store very large amounts of food prior to hibernation and feed during IBE. For example, golden hamsters feed even two times more during IBE than during summer (9.6±1.0 versus 4.9±0.8 $g.day^{-1}$). Contrasting with fat-storing species, intestinal absorption capacities of hamsters are thus maintained and even increased (for glucose) during hibernation in order to optimize nutrient assimilation during IBE. Refeeding then results in decreased free fatty acid plasma concentration and increased glycaemia and triglyceridaemia, and plasma incretin concentration, characteristic of a postprandial state. Finally, we observed that the time spend by hamsters in torpor ($Bt<32°C$) is inversely correlated with body mass just before the entry into hibernation, or as seen elsewhere, with the amount of hoarded food. These data suggest that since torpor is experienced to face food scarcity, enough energy reserves (internal and/or external) might render the strategy of torpor less useful in food- than in fat-storing hibernators. It will be therefore interesting to further compare the energetic costs and benefits of both hibernating strategies.

COLD ADAPTED MICROORGANISMS FROM THE CECUM OF HIBERNATING THIRTEEN-LINED GROUND SQUIRRELS

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The intestinal microbiome is increasingly being considered an essential organ, unto itself, in mammalian organisms. The microbiome influences the host organism's health in a variety of ways including fat storage rates and carbohydrate sensitivity. It also contributes significantly to host energy requirements. The thirteen-lined ground squirrel is a model organism of fat storing hibernators. Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) are obligate hibernators, seasonally entering a period of depressed metabolism to survive periods of little to no food availability. This adaptive fasting eliminates the degradable substrates that are commonly available to the intestinal microbiome during the active portion of the year. Previous research has shown that there is also no increase in the host-derived nutrient, mucin, during hibernation. Previous community analyses have shown that the cecal microbiome undergoes an expected decrease in phylogenetic diversity during hibernation. These decreases are predominately in microorganisms that rely on dietary polysaccharides. There is also a decrease in total community abundance, yet there is still a wide variety of microorganisms present during hibernation. We hypothesize that members of this microbial community must still be active during this long season of cold temperatures and low food availability. It was previously thought that the microbiome is inactive during hibernation. Using a variety of cultivation techniques and conditions, we have isolated multiple cold adapted microorganisms from the cecum of thirteen-lined ground squirrels in torpor. These anaerobic microorganisms are active and grow quickly at 4°C. These microorganisms are largely fermenters, providing essential short-chain fatty acids to intestinal epithelial cells. These fatty acids are not only a major source of energy for the epithelial cells but also play a role in regulating overall host health.

HIBERNATION AND THE GUT MICROBIOME: SYMBIOSIS THROUGH THE SEASONS

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Bacteria that reside in the vertebrate gut are part of a complex consortium that greatly expands the metabolic capabilities of the host, providing biochemical functions such as conversion of indigestible dietary components into usable forms, and modulation of host development, immunology, physiology and behavior. Hibernation provides a unique platform to understand the interplay among host biology, diet and the commensal microbiota, due the extended absence of dietary substrates that many gut bacteria rely on for metabolic needs. The annual hibernation cycle modifies the gut microbiota of 13-lined ground squirrels: it increases relative abundance of taxa that can degrade host glycans including Akkermansia, a dedicated mucin-degrader, and reduces abundance of many taxa that prefer plant glycans. To determine functional significance of these seasonal changes we gavage active season squirrels and aroused hibernators with ^{13}C-labeled substrates including inulin, a plant-derived fiber, and mannitol, a simple sugar alcohol, neither of which can be metabolized by mammalian enzymes. Subsequent measurement of $\delta^{13}CO_2$ in breath is used an index of bacterial degradation of the substrates in vivo. Results suggest that as hibernation progresses the capacity to degrade complex plant-derived glycans, but not simpler sugars, diminishes. Antibiotic manipulations of bacterial communities can reveal whether the microbiota affects seasonal cycles in hibernators. Administration of low dose penicillin (Pen) to pregnant squirrels during gestation and lactation has modest effects on total bacterial abundance of their pups but substantial effects on community composition, including greatly increased abundance of Akkermansia which persists through the hibernation season. Pen exposure in early life increases adiposity by midsummer and increases gut serotonin content. Ongoing studies using NMR-

based metabolomics and ^{13}C-isotope-assisted labelling studies track bacterially-derived molecules in plasma and tissues to identify mechanisms that link gut microbes with their hibernator hosts.

Session 13: Biomedical Aspects

LIPID EMULSION IMPROVES CARDIOPROTECTION AGAINST ISCHEMIA-REPERFUSION INJURY IN NORMOTHERMIC ARCTIC GROUND SQUIRREL ISOLATED HEARTS

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Introduction: Myocardial ischemia/reperfusion (IR) injury is species-dependent. Hibernating mammals such as the Arctic Ground Squirrel (AGS) have shown a profound resistance to IR injury. Because substrate preference may play an important role, we hypothesized that perfusion with lipid emulsion protects isolated hearts from summer-active AGS better against IR injury than Brown Norway (BN) rat hearts.

Methods: AGS (n = 11) and BN hearts (n = 12) were perfused (80 mmHg perfusion pressure, 37°C) with Krebs solution containing 7.5 mM glucose ± 1% Intralipid™ (a lipid emulsion). Following 20 min stabilization and 5 min cardioplegia, hearts underwent 45 min global ischemia and 60 min reperfusion. Isovolumetric left ventricular pressure (LVP) and its derivatives were measured via a saline-filled latex balloon. Statistics: two-way-ANOVA and SNK; alpha = 0.05 (two-tailed).

Results: Glucose-only perfused AGS hearts displayed significantly better systolic and developed LVP, contractility/relaxation, and coronary flow on reperfusion than BN hearts. Lipid emulsion resulted in further improvement of all functional indices, especially diastolic contracture, in AGS hearts compared to glucose only and to BN hearts.

Discussion: Even under non-hibernating conditions AGS hearts are better protected against IR injury than the best IR-protected rat strain (BN). This, however, appears to strongly depend on substrate metabolism: perfusion with lipid emulsion leads to a remarkable improvement in return of function in AGS, more so than in BN rats. This suggests that year-round endogenous mechanisms are involved in myocardial lipid utilization and contribute to cardioprotection independent of decreased metabolism during hypothermia and hibernation. The concept of metabolic fuel switching in the AGS heart with increased fatty acid oxidation challenges the current paradigm that increased glucose and decreased lipid metabolism are favorable during myocardial IR.

Supported by Department of Veterans Affairs (IK2BX001278), NSF, NIH (R01 HL095122), and institutional funds.

NONINVASIVE ASSESSMENT OF THE HIBERNATING GROUND SQUIRREL EYE

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Thirteen-lined ground squirrels (13LGS), which rely on vision as their primary sense for survival, seem to avoid any permanent functional or structural impairment despite the extraordinary down-regulation of metabolism during hibernation. This is quite remarkable, given that the retina is one of the most energy demanding tissues in the entire body, and many visual disorders stem from consequences of ischemia and hypoxia. Little is known about the molecular mechanisms mediating retinal homeostasis and reversible remodeling during hibernation. Understanding these natural processes could have potential application in preventing permanent consequences of hypoxia-ischemia and photoreceptor degenerative disease. Thus, to better understand these changes, we utilized optical coherence tomography (OCT) and reflectance adaptive optics scanning light ophthalmoscopy (AOSLO) to noninvasively and longitudinally examine the living 13LGS eye. These techniques reveal choroidal

thinning and reduced retinal reflectivity during torpor, both reversible during forced arousal to euthermia. In addition, we observed torpor-mediated corneal thickening that is most likely due to stromal edema. We hypothesize this altered corneal thickness may be caused by endothelial water pump inactivation during torpor. Collectively, our findings provide novel evidence that 13LGS hibernation may be a natural model for studying retinal homeostatic and mediating corneal homeostasis mechanisms.

HIBERNATION IN A DISH?: INDUCED PLURIPOTENT STEM CELLS FROM THE THIRTEEN-LINED GROUND SQUIRREL

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In winter, thirteen-lined ground squirrels (GS) cease food consumption and hibernate in a rhythm of days of deep torpor interspersed with short interbout arousals. Accordingly, the animal's body temperature oscillates from 2-8 degrees Celsius during deep torpor, to 37 degrees Celsius in interbout arousals. In most mammals, including human, the neuronal microtubules become destabilized at such hypothermic condition and die, while the nervous system in hibernating GS maintains its functionality, although profound neural morphological changes have been noted. Hibernation research covers a broad spectrum of subjects that continues to grow, but is often limited by the lack of transgenic hibernators and powerful cell models. Here we report the first establishment of induced pluripotent stem cell (iPSC) lines from the thirteen-lined GS, and that GS primary or iPSC-derived cultured neurons maintain their vital features under cold stress. Based on this cell culture platform, we used RNA-seq and other techniques to find profound and distinct differences between GS and human in genetic pathways that respond to cold stress. Subsequently, we used genetic and/or pharmacological strategies to temporarily, but remarkably, reverse the cold-lability of the neuronal microtubule in human iPSC-derived neurons and rat primary cortical neurons. Hence, acquiring full neural resilience to cold stress or injury in non-hibernating species is hopeful given that the GS iPSC models may facilitate a better understanding of cold-adapting mechanisms. Moreover, we anticipate that the GS iPSCs could serve as a versatile platform that leads to discovery of novel mechanisms in metabolism regulation, autophagy, wound healing, cell cycle/fate regulation and beyond.

ROLES OF SULFIDE AND THIOL METABOLITES IN BROWN BEAR HIBERNATION

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During winter, mammalian hibernators enter a hypometabolic state while maintaining organ function. The biochemical mechanisms underlying metabolic suppression and tissue protection during hibernation are largely unknown and are likely to be complex. Because of its ability to reversibly inhibit mitochondrial oxygen consumption and induce artificial suspended animation in mice, it has long been proposed that endogenous hydrogen sulfide (H_2S) could be a key signaling molecule involved in the suppression and reconfiguration of metabolism associated with hibernation. However, an important part of endogenous hydrogen sulfide is enzymatically produced from thiols and in particular cysteine (Cys), which is also used for synthesis of a major cellular antioxidant, glutathione (GSH). To better understand the interplay between pathways controlling sulfide and thiol levels in hibernation, we measured sulfide metabolites and thiol compounds in blood samples taken from winter hibernating and summer active anesthetized, free-ranging Scandinavian brown bears. Measurements of sulfide metabolites showed significant seasonal changes in their composition, suggesting that in hibernating bears H_2S may be recycled from one or more of its oxidation products rather than produced de novo from Cys. Remarkably, free H_2S did not increase during hibernation. Among thiols, GSH was particularly abundant in the red blood cell of hibernating bears and correlated with free Cys levels found in plasma

and red blood cells. This finding suggests that during hibernation Cys may be preferentially used for GSH synthesis. Different changes in sulfide metabolites composition were found in skeletal muscle and adipose tissue biopsies, indicating tissue-specific effects. Taken together, our data indicate a major remodeling of sulfide metabolism, whose functional role in hibernation has yet to be fully uncovered, and enhanced GSH, most likely involved in the protection against oxidative stress when normal basal metabolic rate is resumed.

THE ROLE OF H_2S IN PROTECTING FROM ORGAN DAMAGE DURING HIBERNATION IN THE SYRIAN HAMSTER

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Entrance into torpor features active metabolic suppression, but the underlying mechanism(s) is unknown. We previously coined a role of hydrogen sulfide (H_2S) in hibernation and found that changes in the production of tissue H_2S is involved in the adaptation of lung tissue during the torpor-arousal cycle in Syrian Hamster. Moreover, blood levels of H_2S are increased in hibernating bear and inhalation of H_2S induces a torpid-like state in mice. Thus, we explored the role of H_2S in orchestrating the torpor-arousal cycle during natural hibernation.

Blood and organs were harvested at early and late torpor (24 h and >72 h after onset) and early and late arousal (1.5 h and 8 h after onset) from hibernating Syrian Hamster that underwent at least 8 torpor bouts. Summer euthermic and winter euthermic animals served as controls. Serum H_2S and kidney protein levels of the three major H_2S-producing enzymes (CBS, CSE, 3-MST) increase substantially during torpor and normalized rapidly upon arousal. In contrast, maximal kidney H_2S production capacity at optimal availability of substrates in homogenized tissue is unchanged throughout hibernation. Blockade of overall H_2S production by continuous infusion of amino-oxyacetic acid from early arousal onwards lowers blood H_2S levels, precludes hibernating animals from entering torpor and precipitates kidney injury. These results suggest that H_2S contributes significantly both to entrance into torpor and to organ protection during hibernation, which may reflect the differential metabolic and cytoprotective effects of H_2S, depending on the redox status of the tissue.

Session 14: Bats

COLD AND ALONE? ROOST CHOICE AND SEASON IMPACT TORPOR PATTERNS OF A FREE RANGING NEW ZEALAND BAT (*MYSTACINA TUBERCULATA*)

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Seasonal changes in weather and food availability differentially impact energy budgets of small mammals such as bats. While most thermal physiological research has focused on species that experience extreme seasonal temperature variations, knowledge is lacking for bats from less variable temperate to subtropical climates. We quantified ambient temperatures (T_a), and skin temperature (T_{sk}) responses by individuals from a population of New Zealand lesser short-tailed bats (*Mystacina tuberculata)* during summer and winter using temperature telemetry. Ambient temperatures differed significantly between seasons. During summer, communal roosts were more thermally stable than T_a. During winter, solitary roosts were significantly warmer than T_a and buffered against sub-zero T_a, indicating significant thermal buffering. Communal roost trees were used on 83% of observation days during summer, and individuals occupying them never entered torpor. Solitary roosts were occupied on 93% of observation days during winter, and 100% of individuals occupying them used torpor. During summer and winter bats employed torpor on 11% and 95% of observation days respectively. Winter torpor bout duration correlated negatively with mean T_a. Minimum T_{sk} were positively correlated with

T_a during winter but not summer. Torpor bout duration did not differ between sexes, though female minimum T_{sk} was significantly lower than males. Summer Heterothermy Index varied, and was also significantly affected by T_a. Mean arousal time was correlated with sunset time and arousals occurred most frequently on significantly warmer evenings likely associated with an increased probability of foraging success. We provide the first evidence that torpor is used flexibly throughout the year by *M. tuberculata*, demonstrating that roost choice and season impact torpor patterns.

HUNG OUT TO DRY? ARID ADAPTATION IN HIBERNATING BIG BROWN BATS (*EPTESICUS FUSCUS*)

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Hibernation is typically a period of water deficit. Thus, humidity within hibernacula strongly influences hibernation patterns of small mammals. Drier conditions reduce the length and depth of torpor bouts, stimulate arousals, and can lead to decreased overwinter survival. To mitigate these effects and avoid dehydration, many small mammals hibernate in humid areas that are often near saturated (100% RH). However, big brown bats (*Eptesicus fuscus*) hibernate in a wide variety of conditions and tolerate lower humidity than most other bat hibernators. To assess arid adaptation in this species, we compared torpid metabolic rate (TMR) and total evaporative water loss (TEWL) between two populations of *E. fuscus* with differing winter ecologies. We sampled individuals that overwinter in a humid (>98% RH) karst cave in Wood Buffalo National Park (WBNP), Alberta, and those that hibernate in dry (ca. 65% RH) rock-crevices in Dinosaur Provincial park, AB. We used flow-through respirometry to measure TMR and TEWL of bats of each population in relatively dry (<10% RH) and humid (ca. 85% RH) conditions. Torpid metabolic rate did not differ between populations or with humidity. However, TEWL was 3.3 fold lower in bats from DPP than those from WBNP. Our results suggest that *E. fuscus* that hibernate in arid environments have adaptations that mitigate water loss, but such adaptations are not evident in bats that typically hibernate in humid areas. This adaptation complements the sedentary nature of *E. fuscus*, allowing them to tolerate more variable microclimate during hibernation and ultimately increasing the amount of overwintering habitat available to them.

ADAPTATIONS FOR EXTREME WINTER ENDURANCE IN TEMPERATE BATS

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Hibernation energetics can be considered in terms of three phases: a phase of positive energy balance during autumn; a period of negative energy balance during hibernation proper; and an emergence/recovery phase when positive energy balance is restored in spring. Most of what we know about all three phases is based on rodents (e.g., ground squirrels, chipmunks) which differ from bats in important ways. Rodent hibernators construct their own burrows, hibernate solitarily or in small groups, use nesting material for insulation and often store food. In recent years, our group has been studying little brown (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*), two species which hibernate in caves and mines, to test the broad hypothesis that similar selection pressures influence all three phases of hibernation for rodents and bats despite dramatic differences in ecology and behaviour. We have used temperature telemetry, infrared video and passive transponders (PIT tags) to understand how population and individual characteristics (e.g., sex, personality), and environmental factors (e.g., weather, winter duration) affect hibernation energetics and phenology. Sex differences in the timing of reproduction have a pronounced influence on winter energy expenditure and the timing of spring emergence, while individual behavioural traits (i.e., personality) appear to influence pre-hibernation fattening and hibernation energetics. However, despite similarity to rodents, bats perform unique hibernation

behaviours for extreme energy savings in their large, open-air hibernacula. These include what we term heterothermic arousals (i.e., expression of shallow torpor in the midst of periodic arousals) and cold arousals (i.e., periods during which bats exhibit pronounced behavioural activity at deeply torpid body temperatures). Our findings are important for understanding the ecology of temperate-zone bats in general and have implications for understanding and potentially mitigating white-nose syndrome, a recently emerged disease heavily impacting populations of hibernating bats in North America.

CONSPECIFIC DISTURBANCE CONTRIBUTES TO ALTERED HIBERNATION PATTERNS IN BATS WITH WHITE-NOSE SYNDROME

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The emerging wildlife disease white-nose syndrome (WNS) affects both physiology and behaviour of hibernating bats. Infection with the fungal pathogen *Pseudogymnoascus destructans* (*Pd*), the first pathogen known to target torpid animals, causes an increase in arousal frequency during hibernation, and therefore premature depletion of energy stores. Infected bats also show a dramatic decrease in clustering behaviour over the winter. To investigate the interaction between disease progression and torpor expression we quantified physiological (i.e., timing of arousal, rewarming rate) and behavioural (i.e., arousal synchronisation, clustering) aspects of rewarming events over four months in little brown bats (*Myotis lucifugus*) experimentally inoculated with *Pd*. We tested two competing hypotheses: 1) Bats adjust arousal physiology adaptively to help compensate for an increase in energetically expensive arousals. This hypothesis predicts that infected bats should increase synchronisation of arousals with colony mates to benefit from social thermoregulation and/or that solitary bats will exhibit faster rewarming rates than clustered individuals because rewarming costs fall as rewarming rate increases. 2) As for the increase in arousal frequency, changes in arousal physiology and clustering behaviour are maladaptive consequences of infection. This hypothesis predicts no effect of infection or clustering behaviour on rewarming rate and that disturbance by normothermic bats contributes to the overall increase in arousal frequency. We found that arousals of infected bats became more synchronised than those of controls as hibernation progressed but the pattern was not consistent with social thermoregulation. When a bat rewarmed from torpor, it was often followed in sequence by up to seven other bats in an arousal "cascade". Moreover, rewarming rate did not differ between infected and uninfected bats, was not affected by clustering and did not change over time. Our results support our second hypothesis and suggest that disturbance, not social thermoregulation, explains the increased synchronisation of arousals. Negative pathophysiological effects of WNS on energy conservation may therefore be compounded by maladaptive changes in behaviour of the bats, accelerating fat depletion and starvation.

THE ROLE OF EPIDERMAL LIPIDS IN THE RESISTANCE TO WHITE-NOSE SYNDROME

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White Nose Syndrome (WNS) greatly increases the over-winter mortality of little brown (*Myotis lucifugus*), Indiana (*Myotis sodalis*), northern (*Myotis septentrionalis*), and tricolored (*Perimyotis subflavus*) bats. It is caused by a cutaneous infection with the fungus *Pseudogymnoascus destructans* (*Pd*). Big brown bats (*Eptesicus fuscus*) are much more resistant to cutaneous infection with *Pd*, however. We thus conducted analyses of wing epidermis from hibernating *E. fuscus* and *M. lucifugus* to determine their fatty acid compositions, and laboratory *Pd* culture experiments at 4.0 – 13.4°C to determine the effects of these fatty acids on *Pd* growth. Our analyses revealed that the epidermis of both bat species contain the same 7 fatty acid types (14:0, 15:0, 16:0. 16:1, 18:0, 18:1, & 18:2), but the

epidermis of *M. lucifugus* contains: a) more stearic(18:0) acid, b) less palmitoleic (16:1) acid, c) less myristic (14:0) acid, and, d) less oleic(18:1) acid than that of *E. fuscus*. The growth of *Pd* was inhibited by: a) myristic and stearic acids at 10.5-13.4°C, but not at 4.0-5.0°C, b) oleic acid at 5.0 -10.6°C, c) palmitoleic acid, and, d) linoleic (18:2) acid at 5.0 – 10.6°C. One set of factors that enables *E. fuscus* to better resist cutaneous *P. destructans* infections (and thus WNS) is therefore the relatively higher myristic, palmitoleic, and oleic acid contents of the epidermis.

Session 15: Diversity of Organismal Models

HETEROTHERMY IN COLUMBIFORM AND PICIFORM BIRDS: INSIGHTS FROM THE AFROTROPICS

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Investigations of avian heterothermy have tended to focus on a small subset of taxa, most notably hummingbirds, caprimulgids, mousebirds and passerines. Consequently, the phylogenetic distribution of avian heterothermy remains far from clear. The order Columbiformes (pigeons and doves) is of particular interest, since at least one species is capable of torpor and this order clusters with several highly heterothermic taxa in the avian phylogeny. We investigated heterothermy in African green-pigeons (*Treron calvus*), a frugivorous species from mainland Africa. Relationships between body temperature (T_b), resting metabolic rate (RMR) and air temperature (T_a) differed from those associated with classic endothermic homeothermy, and restricted feeding led to reductions in rest-phase T_b. The lowest T_b we recorded was 33.1°C, and there was no evidence of torpor. We argue that the absence of torpor in *T. calvus*, but presence thereof of in an ecologically similar fruit pigeon from the South Pacific, reflects differences in selection pressure arising from predation risk. A second order in which we have been investigating heterothermy is the Piciformes (woodpeckers, barbets, toucans and allies). Two species of African barbets (Lybiidae) showed slight reductions in rest-phase T_b in response to restricted feeding in captivity, and acacia pied barbets reduced cloacal T_b to ~32 °C in a field laboratory in the Kalahari Desert. We are currently investigating T_b patterns in free-ranging ground woodpeckers (*Geocolaptes olivaceus*), one of just three ground-dwelling woodpecker species worldwide, during the austral winter in the Drakensberg mountains.

TORPOR DYNAMICS IN HIGH ANDEAN HUMMINGBIRDS

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The energetic costs of life in the high Andes of Peru is great for small endotherms such as hummingbirds. As a consequence, the use of torpor by hummingbirds living in these regions has been of significant interest. We studied the use of torpor and the dynamics of body temperature and mass loss in a community of Andean hummingbirds (*Metallura phoebe*, 5.4g, *Aglaeactis cupripennis*, 7.0g, *Oreotrochilus melanogaster*, 8.0g, *Colibri corascans*, 9.0g, and *Patagona gigas*, 22g) during the wet spring at an elevation of 3755M. We captured hummingbirds during the day, habituated them to feeders in a large screen tent, and then measured body temperature dynamics through the night using cloacal thermocouples and a data logger. Torpor dynamics varied greatly among species with some species directly tracking air temperatures and achieving body temperatures as low as 2.9 °C. In other species body temperature set point were several degrees higher then air temperatures. Maximum heat up rates during the rewarming phase of torpor also varied with small species rewarming a rates > 1°C/ minute and large species showing much lower rates. Torpor duration also varied with body size. Mass loss data suggest that birds may be able to reduce overnight energy expenditure by 50% or more. Overall, torpor use appeared to be ubiquitous among Andean species where afternoon rains and cold temperatures produce significant challenges.

EXTERNAL ENERGY RESERVES AND HIBERNATION PATTERNS IN COMMON HAMSTERS

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In free-ranging Common hamsters (*Cricetus cricetus*) unique sex differences in hibernation patterns have been documented. Females hibernate for shorter periods than males although autumnal immergence and vernal emergence sequences of sex and age groups resemble those of most hibernators. Adult females delayed hibernation onset until up to early January and consequently spent extended periods at normal body temperatures in their hibernacula. These results indicate that females had to rely on sufficient food hoards whereas males showed a more pronounced energy-saving strategy by hibernating for longer periods. In field studies, however, the information on the quantity and quality of food stores is lacking. We therefore manipulated the quantity and quality of food stores in female hamsters kept in constant condition chambers and provided focal individuals with food supplements in the field. Captive females used artificial burrows, built of tubes and chambers equipped with accessible lids. Body temperature was recorded with subcutaneously implanted data loggers. In the first experiment female hamsters had either access to unlimited food reserves for hoarding or received only daily portions and could not build up food stores. Almost all females without food hoards hibernated whereas less than half of the other group showed deep torpor bouts. The timing and duration of the hibernation period did not differ significantly between the hibernating individuals in both groups. Hamsters with access to food stores, however, spent less time in torpor and had higher minimum body temperatures during deep torpor bouts than those without. Nevertheless, in spring, body mass and the proportion of body fat did not differ between the groups. We conclude that female Common hamsters adjust the use of torpor in relation to external energy reserves. Preliminary data of field experiments support this assumption.

METABOLIC DEPRESSION DURING WINTER COULD MITIGATE IMPACTS OF CLIMATE CHANGE ON LIZARDS

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Climate warming will affect not only the potential for activity but also the conditions experienced during inactivity. Warmer conditions during winter would raise energy demands and decrease surplus energy for growth or reproduction. Will thermal acclimation of metabolism enable animals to save energy during warm winters? We answer this question for a widespread group of lizards, *Sceloporus undulatus* complex, by combining an experimental study of metabolic rates with a model of seasonal energetics. In the lab, lizards from four populations were exposed to either a constant 12°C, a constant 2°C, or a linear decrease in temperature from 12°C to 2°C. After three weeks, we compared metabolic rates of these lizards to their rates prior to acclimation. For all populations, lizards exposed to 12°C reduced their metabolic rate during the three weeks. In three of the four populations, metabolic rate at 12°C dropped by more than 50%! By contrast, lizards exposed to 2°C failed to acclimate metabolically and suffered a high rate of mortality. Lizards in the gradually cooling group showed some degree of metabolic depression in two of the four populations. We see whether acclimation can mitigate impacts of global warming by simulating energetics of lizards in climates projected for the past and the future.

FORCED HIBERNATION: A TECHNIQUE TO TEST SURVIVAL OF TEMPERATE SNAKES IN A PEATLAND LIFE ZONE

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Temperate snakes spend more than half their life underground within a hypothetical space known as the "Life Zone". The Life Zone is a vertical subterranean space that remains aerobic, frost and flood free throughout winter. To test this hypothesis, fifteen years of life zone data (snow, frost, groundwater temperature and dissolved oxygen) were collected from a known hibernation area within a peatland. These data were used to establish minimum Life Zone criteria for artificial burrow design. In the fall of 2014, Eastern gartersnakes (*Thamnophis s. sirtalis*) (4 neonatal, 7 juvenile) were collected and temporarily held in captivity to ensure stomachs were empty, then placed into artificial burrows and monitored for movement abilities. They were placed into the Life Zone on Oct 11, 2014 and removed April 11, 2015 (182 days). All snakes survived the winter, except for one juvenile snake that was eaten by three Mask shrews (*Sorex cinereus*) that had chewed through the nylon top screening and also perished. The artificial burrow design was modified and the experiment repeated for a second winter 2015-16, (n= 10; 5 neonates; 5 juveniles), with results pending. Winter 2014-15 was relatively harsh by surface weather values (total snow and number of days mean temperature < -5˚C), whereas the winter of 2015-16 is considered relatively mild. For the 10 surviving snakes from 2014, half overwintered in a moist terrestrial environment above the groundwater table and half overwintered where groundwater levels changed (moist to wet) but did not flood completely (max 64.5 cm decreased to 31.5 cm). This supports the Life Zone hypothesis that snakes survive within a vertical space that does not freeze or flood completely, although the amount of space may change. Forced hibernation into the Life Zone is a useful method to test habitat suitability for supporting overwinter survival of temperate snakes.

Abstracts are listed alphabetically by the last name of the Presenting Author*; the institutional affiliation is also listed. Posters will be displayed on the board labeled with the corresponding number.

1. INVESTIGATION OF MECHANISMS FOR THE RESISTANCE TO ISCHEMIA-REPERFUSION STRESS DURING HIBERNATION PERIOD IN SYRIAN HAMSTER

Daisuke Anegawa*, Yuichi Chayama, Lisa Ando, Takayuki Fujimoto, Hiroki Taii, Shuji Shigenobu, Masayuki Miura, and Yoshifumi Yamaguchi

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Several mammalian hibernators, such as squirrels and hamsters, exhibit heterothermy throughout the hibernation period. They undergo periods of low body temperature and minimal heart rate during deep torpor (DT), followed by periodic arousal (PA) when body temperature and heart rate are elevated to normal level. Because of such drastic physiological changes, hibernator's tissue can be exposed to a lot of stress including Ischemia-Reperfusion (I/R) stress, which may result from rapid restoration of blood flow at PA after lowered oxygen supply at DT. Indeed, it is already shown that hibernating ground squirrels have the resistance to experimental I/R stress in gut and liver. However, the molecular mechanisms for the resistance to I/R have not been elucidated.

Syrian hamster (*Mesocricetus auratus*) is one of good animals to investigate such molecular mechanisms, since their hibernation can be induced in laboratory condition throughout the year and genetic manipulation has been reported. We first established the system for I/R model experiment in the hamster kidney to confirm that hibernating Syrian hamsters also have the resistance to I/R. In addition, to identify genes involved in the resistant mechanisms, we have conducted RNA-seq analysis of different tissues using next generation sequencer. Given that hibernators are known to have the resistance to I/R in several organs, we have been searching the genes which were differentially expressed in multiple tissues. The data will be discussed.

2. EXTREME RESISTANCE TO OXYGEN GLUCOSE DEPRIVATION IN BRAIN TISSUE FROM THE ARCTIC GROUND SQUIRREL

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Cerebral ischemia/reperfusion (I/R) triggers a cascade of uncontrolled cellular processes that perturb normal cell homeostasis. Arctic ground squirrel (AGS) tolerates brain damage following cerebral I/R during cardiac arrest and resuscitation. In vivo studies show no signs of neuropathology one week after 10 min of asphyxial cardiac arrest; however, the limit of this tolerance has not been well established. Here, we aim to decipher if tolerance to I/R injury is intrinsic to the species that evades the excitotoxic cascade of I/R events and if the level of tolerance depends on the hibernation season. We hypothesized that "tolerance to I/R injury in AGS is modulated downstream of NMDAR activation and is independent of the hibernation season". Hippocampal brain slices (400 micron) from AGS were subjected to oxygen glucose deprivation (OGD) that mimicked I/R in vivo. Each events of the ischemic cascade such as ATP depletion, tissue acidosis, neurotransmitter efflux (glutamate and aspartate), and neuronal injury (LDH release) were monitored. Role of hibernation season in tolerance was evaluated by sampling the AGS hippocampus to OGD during summer and winter season (hibernators and interbout arousal) and injury were monitored by the amount of LDH released. All groups were compared to Sprague Dawley rat, as a positive control species vulnerable to I/R injury. Result shows that despite disruption in energy balance, neurotransmitter release, AGS show tolerance to OGD injury marked by LDH release. Tissue acidosis showed no signs of injury when compared to rats. In response to OGD, the smallest LDH response was observed in slices from both hibernating and summer euthermic AGS, a slightly greater LDH response

was observed during interbout arousal but significantly less than rats. Result of this study indicates that AGS exhibit an intrinsic tolerance ability that is modulated downstream of NMDAR activation and the tolerance is independent of hibernation season.

3. H_2S PRODUCTION IN LIVER AND KIDNEY OF HIBERNATING SYRIAN HAMSTER

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H_2S has previously been implicated in lung remodeling during hibernation, with increased levels of H_2S and expression of the H_2S synthesizing enzyme cystathionine-beta-synthase (CBS) in torpor. Here, we investigated H_2S production capacity in liver and kidney tissue from Syrian hamster (*Mesocricetus auratus*).

Snap frozen tissue samples originated from summer euthermic (SE, Ta=20 ^{0}C, L:D 14:10) and winter euthermic animals (WE, Ta=4 ^{0}C, L:D 0:24). Hibernating animals were euthanized at 16-24 h (TE) or >72 h of torpor (TL), and at 90 min (AE) or 8 h after arousal (AL). H_2S production capacity was assessed by lead acetate: protein lysate (500-1000ug) in PBS supplemented with cysteine (10mM) and PLP (2mM) was incubated in a 96 wells plate covered with lead acetate soaked filter paper for 24 h at 37 ^{0}C followed by quantification of lead darkening. Kidney H_2S levels were visualized using 2-photon confocal microscopy after incubation (10 min) with a fluorescent probe (3-aryl-3-oxoprop-1-enyl)benzaldehyde type) of cryosections followed by tissue fixation.

Liver H_2S production capacity of SE and WE were similar, and increased 4-6 fold in TE and TL, followed by a subsequent decrease during AE and AL to 75% of torpid values. In contrast, kidney H_2S production capacity was nearly similar in all groups. Imaging H_2S levels in kidney proved feasible with signals detected both in glomeruli and tubuli, with lower intensity in TL compared to SE and AL. Expression of CBS was mainly found in glomeruli, while another H_2S producing enzyme, 3-MST, was detected in glomeruli and tubuli. Enzyme expression and H_2S production overlays showed good correlation in all phases.

H_2S production capacity in liver increases in hibernation - with topor exceeding arousal. Detection of H_2S levels employing a fluorescent probe proved feasible. These data warrant further investigation of H_2S production changes in various organs throughout the hibernation cycle.

4. THERMOREGULATORY EFFECTS INDUCED BY THE ACTIVATION OF NEURONS WITHIN THE RAPHE PALLIDUS IN SWINE

Mino Zucchelli, Stefano Bastianini, Domenico Ventrella, Francesca Barone, Alberto Elmi, Noemi Romagnoli, Timna Hitrec, Chiara Berteotti, Roberto Amici, Maria L. Bacci, and Matteo Cerri*
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The Raphe Pallidus (RPa) is a region within the brainstem where the putative sympathetic premotor neurons to many metabolically active organs are located. In several species, such as the rat, and the rabbit, the activation of RPa neurons by injection of a GABA-A antagonist was shown to activate the sympathetic outflow, producing activation of the brown adipose tissue, increase heart rate (HR) and CO_2 production, and induce cutaneous vasoconstriction. On the other hand, the inhibition of RPa neurons was shown to induce a state of undefended hypothermia resembling torpor in a non-hibernating species like the rat, suggesting the possibility to use this approach to induce therapeutic hypothermia in humans. To explore such possibility, experiments were conducted on a larger non-hibernator such as the pig. Here we show preliminary observations from such experiments, showing that the effects induced by the activation of RPa neurons in the pig (n=2) are consistent with those described in other animals. Microinjection of the GABA-A antagonist GABAzine within the RPa region of anaesthetized pig induced an average (± SEM) increase in HR from 88 ± 6 to 138 ± 23 bpm, an increase in systolic arterial pressure from 91 ± 8 to 153 ± 17 mmHg, and an increase in expired CO_2 from 49 ± 0.6 to 64 ±

2.3 (%). In conclusion, the activation of RPa neurons appear to produce the same effects in pigs as described in other species thus sustaining the possibility to use induced hypothermia as therapy in humans.

5. MOLECULAR REMODELLING OF INGUINAL ADIPOSE TISSUE PRECEDES HIBERNATION PERIOD IN SYRIAN GOLDEN HAMSTER

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Hibernation is an adaptive strategy for winter survival with little or no food availability by remarkably reducing the metabolism and the core body temperature. Corresponding to dramatic changes of metabolism during hibernation, individual organ systems undergo molecular and cellular remodeling in season from fall to winter; however, the molecular mechanisms responsible for the remodeling have not been fully elucidated. In this study, we aim to reveal the mechanism of molecular remodeling in white adipose tissue (WAT) of Syrian golden hamster (*Mesocricetus auratus*), an opportunistic hibernator. Global gene expression analysis of inguinal WAT indicated that the targets of peroxisome proliferator-activated receptor signaling pathway were differentially expressed between non-hibernating and hibernating animals. Both lipid catabolism and anabolism genes were highly up-regulated during hibernation period. Furthermore, time course analysis by qRT-PCR showed that the lipid metabolism genes were significantly induced at the late stage of pre-hibernation period prior to hibernation. Our data suggests that Syrian golden hamster undergoes remodeling of lipid metabolism in inguinal WAT during the period of pre-hibernation euthermy to prepare for hibernation.

6. NEUROINFLAMMATORY RESPONSE IN THE HIBERNATING SYRIAN HAMSTER BRAIN

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Hibernators tolerate a reduced cerebral blood flow and hypothermia during torpor without noticeable neuronal or synaptic dysfunction in arousals. Previous studies have shown extensive changes in brain during torpor, including synaptic rearrangements, documented morphologically and molecularly, and hyperphosphorylation of neuronal *tau* protein. We reasoned that if such adaptations represent a form of organ damage, a neuroinflammatory response might occur in the brain during specific hibernation phases. Thus we examined the expression of cytokines and morphology of microglia in the brain from Syrian Hamster (*Mesocricetus Auratus*).

Neuroinflammatory response was quantified in summer euthermic (SE, Ta=20 ^{0}C, L:D 14:10) and winter euthermic hamsters (WE, Ta=4 ^{0}C, L:D 0:24). Animals in hibernation were housed under similar conditions as WE and were euthanized at 16-24 h of torpor (TE), >72 h of torpor (TL5), after 90 min of arousal (AE) and 8 h after arousal (AL), with all groups n=4-7. Expression levels of the pro-inflammatory cytokines TNF-α, IL-6, IL-1β were measured by qPCR. Further, microglia activation was quantified by calculation of cell body size to total cell size ratio ("SizeRatio").

Expression of IL-1β, TNF-α and IL-6 was upregulated in AE compared to SE and WE, followed by a normalization to euthermic levels in AL. SizeRatio increased from 5% in SE and WE to 10-12% in TE, TL and AE and decreased to euthermic levels in AL.

These data indicate activation of a neuroinflammatory response throughout the torpor bout, which disappears by late arousal. Activation of microglia is likely not caused by hypothermia as forced hypothermic conditions inhibit microglia activation. Possibly, the potential deleterious neuroinflammatory response of microglia is counteracted by its secretion of neurotrophic factors to

ensure maintenance of brain homeostasis during the extreme physiological changes that occur during the bouts of torpor.

7. PLATELET VWF AND COLLAGEN BINDING ARE DECREASED IN HIBERNATING 13-LINED GROUND SQUIRRELS

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The objective of this study is to determine how a hibernating mammal avoids the formation of blood clots under periods of low blood flow, in part by reducing platelet levels. To differentiate the effects of temperature, flow rate, and the nature of the platelets on their ability to bind to collagen, a microfluidic flow assay was performed. Human and ground squirrel whole blood was incubated at 15 or 37°C and then passed through a microfluidic chamber over a 250 μm strip of type I fibrillar collagen at that temperature and shear rates of 50/sec or 300/sec to simulate torpid and aroused conditions. At 15°C, both human and ground squirrel platelets showed a 90-95% decrease in binding to collagen. At 37°C, a decrease in flow rate reduced human platelet binding by 50%, while ground squirrel platelet binding dropped by 80%. When compared to platelets from non-hibernating animals, platelets from animals collected after arousal from torpor showed a 60% decrease in binding at 37°C and 300/sec, but a 2.5-fold increase in binding at 15°C and 50/sec. Fibrinogen binding could be stimulated after storage at 4°C or 25°C for two days, while human platelets decreased in activity. Storing human platelets at 4°C increased vWF binding, while vWF binding in platelets from ground squirrels that had been hibernating were decreased by 50%. The source of the plasma the platelets were stored in did not affect the results indicating that the decreased binding was a property of the platelets. In conclusion, it appears that ground squirrel platelets have reduced adhesion to vWF and thus collagen under low flow and after storage at cold temperatures, while still being activated by external agonists. This would protect the animals from DVT during torpor but allow them to restore platelet function upon arousal.

8. NO INSULATING EFFECT OF OBESITY

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The development of obesity may be aggravated if obesity itself insulates against heat loss and thus diminishes the amount of food burnt for body temperature control. This would be particularly important under normal animal house conditions where mice experience a chronic cold stress (at ≈20 °C). We used Scholander plots (energy expenditure plotted versus ambient temperature) to examine the insulation (thermal conductance) of mice, defined as the slope of the Scholander curve at subthermoneutral temperatures. We verified the method by demonstrating that shaved mice possessed only half the insulation of nonshaved mice. We examined a series of obesity models (mice fed high-fat diets and kept at different temperatures, classical diet-induced obesity mice, ob/ob mice and obesity-prone (C57Bl/6) versus obesity-resistant (129S) mice). We found that neither acclimation temperature, nor any kind or degree of obesity affected the thermal insulation of the mice, when analyzed at the whole mouse level on as energy expenditure per lean weight. Calculation per body weight erroneously implied increased insulation in obese mice. We conclude that in contrast to what would be expected, obesity of any kind does not increase thermal insulation in mice and it does therefore not in itself aggravate the development of obesity. It may be discussed whether even in humans, excess adipose tissue is without insulation effect and thus does not promote further obesity in this way.

9. ALTERED GENE EXPRESSION PATTERN IN THE HYPOTHALAMUS OF DJUNGARIAN HAMSTER (*PHODOPUS SUNGORUS*) DURING SPONTANEOUS DAILY TORPOR

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Djungarian hamsters (*Phodopus sungorus*) exhibit multiple physiological adaptions to their seasonally changing environment. The most extreme adaption of this animal to maintain energy balance during winter is the expression of spontaneous daily torpor. Daily torpor is a voluntary hypometabolism that reduces energy requirements by up to 70%. The hypothalamus has been proposed to play a role in the regulation of daily torpor. However, neuronal signals, which initiate the metabolic depression during the entrance into torpor, are still unknown.

To uncover new molecular mechanisms which are involved in the regulation of daily torpor we investigated the hypothalamic transcriptomes of hamsters during the entrance into torpor using Illumina sequencing. Identified candidate genes were verified by quantitative analyses (qPCR) in different stages of torpor.

The expression of 284 genes was significantly altered during entrance into the torpid state (ZT 1, $T_b \leq 31$ °C). Analysis of most up-regulated transcripts showed significant changes in 5 collagenous genes, which are major extracellular matrix structural constitutes. Additionally dnha2 and myo15a, components of the cytoskeleton, rank among the most up-regulated genes. Quantitative analyses of col17a1, myo15a and dnah2 showed an up-regulation in gene expression during entrance into torpor in comparison to hamsters which remain euthermic. Gene regulations display similar fold changes like NGS approach. During deep torpor (ZT4), arousal (ZT7) and at night after a day with torpor (ZT 16) they were down-regulated. These genes seem to be exclusively involved in torpor induction mechanisms and play no role in maintenance of torpor or arousal. Our Results support the hypothesis that the hypothalamus plays a key role in the regulation of this extreme physiological phenomenon and provide evidence of cellular remodeling and plasticity during torpor entrance.

10. ANALYSIS OF CARDIAC ARRHYTHMIAS IN N-6 CYCLOHEXYLADENOSINE-ASSISTED COOLING OF RATS

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Drugs are needed to facilitate cooling in people for treatment of cardiac arrest and stroke. A1 adenosine receptor (A1AR) agonist N6-cyclohexyladenosine (CHA) works within the brain to inhibit thermogenesis and induces a torpor-like state in rats. CHA is in development as a novel thermolytic to facilitate cooling in people, and to negate shivering response during cooling. The drug also has side-effects on the heart that have not been well characterized. A1AR agonists are known to produce bradycardia; hypothermia produces bradycardia and arrhythmias. The purpose of this project is to test the hypothesis that CHA assisted hypothermia produces arrhythmias and to characterize the types of arrhythmias during CHA-induced hypothermia. Rats were administered CHA or vehicle and placed on a surface set to 17°C. EKG was acquired with CTA F40 transmitters (DSI) and Ponemah software. EKG was reviewed from the first 10min of every hour beginning 2h prior to onset of CHA (0.25mg/kg/h, IV) or vehicle (25% 8-hydroxypropyl-β-cyclodextrin), for 24h of hypothermia and during rewarming to 37°C. Arrhythmias were identified and quantified. Types of arrhythmias that can be identified include premature ventricular contractions, premature atrial contractions, sinus block, and type I, II, and III atrioventricular blocks. Shivering can also be identified as artifact on EKG. Preliminary results show that CHA-assisted cooling with surface temperature controlled cooling and rewarming produces core body temperatures between 30-32°C for up to 24h and animals rewarm without incidence, and do not exhibit shivering artifact. Preliminary analysis of EKG shows evidence of premature atrial and

ventricular contractions in CHA treated animals during cooling. CHA-assisted cooling to core body temperature between 30-32°C increases the frequency of non-life threatening arrhythmias.

11. SURFACE TEMPERATURE CONTROL COMPENSATES FOR THE VARIABLE THERMOLYTIC RESPONSE TO AN A_1 ADENOSINE RECEPTOR AGONIST DURING TORPOR-LIKE TARGETED TEMPERATURE MANAGEMENT IN RATS

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Managing core body temperature (T_b) between 32-36°C improves neurological outcome after cardiac arrest and cooling to 32-34°C is in clinical trials for stroke. Cooling, however, is compromised by shivering. Central adenosine receptor signaling is necessary for hibernation in ground squirrels and hamsters and for daily torpor in mice. N^6 cyclohexyl adenosine (CHA) is an A_1 adenosine receptor (A_1AR) agonist that induces hibernation-like effects in ground squirrels and torpor-like effects in rats, largely through the inhibition of thermogenesis. Although CHA inhibits thermogenesis and decreases core body temperature, efficacy varies between individuals. Here we tested the hypothesis that the variable, thermolytic response of CHA could be normalized by dietary restriction, by doubling the dose of CHA or by controlling T_b via surface temperature. We further hypothesized that CHA-assisted cooling would not produce metabolic stress often seen during cooling by other means. Male Sprague-Dawley rats (3-4 months old) were instrumented with iButton dataloggers (Maxim Integrated, Sunnyvale, CA) or CTA-F40 transmitters (DSI Inc.) to monitor T_b or IPTT transponders to monitor subcutaneous temperature. One group of rats was fed ever-other-day, a paradigm of dietary restriction. CHA (0.5 or 1.0 mg/kg) was delivered IP at an ambient temperature of 17°C. Dietary restriction was found to enhance and normalize the thermolytic efficacy of CHA based on a decrease in the rate of oxygen consumption as well as a decrease in T_b. Increasing the dose of CHA produced greater increases in T_b, but did not normalize the response. A Peltier controlled, radiant heated and cooled surface prevented over-cooling and regulated T_b after CHA. ^{1}H-NMR metabolomic analysis on liver and forebrain showed that cooling did not increase lactate levels, a common marker of metabolic stress. These analyses, did, however reveal an association between minimal T_b and brain tissue levels of trimethyl glycine (betaine). CHA with surface temperature control is the most effective and feasible means to manage a specific target T_b with CHA.

12. EFFECT OF DIET ON THE ARCTIC GROUND SQUIRREL GUT MICROBIOTA AND PRE-HIBERNATION FATTENING

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Arctic ground squirrels (*Urocitellus parryii*) exhibit extreme fluctuations in body fat annually, increasing from 5% body fat to 45% body fat during a 3-week pre-hibernation fattening phase. In non-hibernating mammals, fat accumulation and storage has been linked to the gut microbiota, and obesity in humans, mice, and rats is correlated with reduced gut microbial diversity and a characteristic shift in relative abundance of core gut microbial community members compared to lean individuals. Our objective was to examine the effect of diet on pre-hibernation fattening and the gut microbiota of captive arctic ground squirrels. We measured body composition across time (deuterium dilution) and gut microbiota density (flow cytometry), diversity (16S rRNA), and metabolic activity (short chain fatty acid production and metatranscriptomics) prior to and after five-weeks on high-fat (40% energy from fat), low-fat (10% energy from fat), restricted calorie (50% of control diet calories) or control diets (18% energy from fat). We found that squirrels fattened at the same rate and to the same degree despite differences in caloric and dietary fat consumption. Additionally, our results showed no significant

differences in gut microbial community diversity or short chain fatty acid production across time, or with diet. However, analysis of the gut microbial transcriptome indicated differences in community function among diet groups, but not across time, and revealed shifts in the relative contribution of activity at a taxonomic level. Our results clearly demonstrate that pre-hibernation fattening of arctic ground squirrels is robust to changes in diet and indicate it is accomplished by more than increased energy uptake. Furthermore, analysis of the gut microbiota suggests that arctic ground squirrels evolved a gut microbial community structure that is unaffected by dietary changes and maintains efficiency at energy extraction by altering gene expression.

13. NUTRITIONAL SIGNIFICANCE OF DAILY TORPOR: THE VARIABLE RESPONSE TO DIETARY PROTEIN DEFICIENCY

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Daily heterotherms employ daily torpor to cope with food shortage. Although it is to be expected that food shortages in the wild impact not only on energy budgets also on other aspects of nutritional status, it is not known whether a deficiency in dietary nutrients directly induces daily torpor. In addition to serving as an energy substrate, dietary protein also provides a variety of other vital nutrients, not least the essential amino acids which endotherms are variously unable to synthesize *de novo*. We used the African woodland dormouse (*Graphiurus murinus*) to evaluate the hypothesis that dietary protein deficiency may induce daily torpor as a means of maintaining protein metabolism. *G. murinus* consumes a protein-rich diet and lacks a cecum, limiting the potential for hindgut fermentation and coprophagy. Twenty dormice were fed two isocalorific diets for two weeks in turn under controlled laboratory conditions. One diet lacked protein, the other contained adequate protein as a control. Dormice were maintained at an ambient temperature (T_a) of ca. 30°C or 27°C and body temperatures were logged throughout the experiment. While no dormice expressed daily torpor during the control period, eleven dormice expressed daily torpor under protein deficient conditions. The incidence of torpor was affected by body conditions, but not affected by T_a, sex, or age in days. Among dormice expressing torpor, some individuals exhibited reduced caloric intake due to spontaneously reduced consumption or expression of daily torpor, while others maintained a calorific intake comparable to that achieved on the control diet. These results suggest that daily torpor can be induced directly or indirectly by dietary protein deficiency even in the absence of energy constraints, although responses vary between individuals. Daily torpor may therefore contribute to adjustments in protein metabolism as well as the maintenance of energy budgets.

14. BRAIN PATHWAY INVOLVED IN ADENOSINE A1 RECEPTOR AGONIST-INDUCED HIBERNATION

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The adenosine A1 receptor agonist $N^{(6)}$-cyclohexyladenosine (CHA) has been previously shown to induce hibernation in the arctic ground squirrel (AGS). Even under constant environmental conditions (light cycle 4:20 L:D and 2ºC ambient temperature) CHA-induced hibernation is seasonal dependent. What regulates the seasonal effect of the agonist is still unknown. The objective of this study is to identify the central process which modulates the seasonal response to CHA. Our hypothesis is that a central process in the CNS within the hypothalamus alters the thermogenic response to CHA-induced hibernation.

Physiological response to CHA has been monitored by abdominal temperature logger and open flow-respirometry as a measure of metabolism. CHA injected IP lowers body temperature and suppresses metabolism inducing hibernation in winter animals. Summer animals (AGS not displaying

any torpor bouts for at least one month) show a transient response to the drug and within an hour from injection AGS restore their euthermic temperature and metabolism. When both groups (winter and summer) have been treated with vehicle, no changes in body temperature or metabolism have been detected. Three hours after CHA or vehicle, AGS have been intracardially perfused with 4% PFA and brains have been collected for immunohistochemistry analysis. cFos expression (mouse anti-cFos 1:20,000,) has been used as a marker for neuronal activation to identify the neuron groups regulating CHA-induced hibernation. Results show intense cFos- immunoreactivity in the paraventricular nuclei (PVN) and in the medial hypothalamus. These regions are characterized by brain nuclei controlling sleep and thermoregulation. Work is in progress to stain cFos+ neurons with cell markers characteristic of the hypothalamus as galanin, orexin, vasopressin and thyrotropin-releasing hormone.

Support: NIH R15NS070779 and Alaska INBRE P20GM103395

15. DIETARY LIPIDS AFFECT THE ONSET OF HIBERNATION IN THE GARDEN DORMOUSE (*ELIOMYS QUERCINUS*)

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Polyunsaturated fatty acids (PUFA) have strong effects on hibernation and daily torpor. Increased dietary uptake of n-6 PUFA, particularly of Linoleic acid (LA, C18:2 n-6), lengthens torpor bout duration and enables animals to reach lower body temperatures (T_b). Conversely, dietary n-3 PUFA have negative impacts on hibernation propensity. High proportions of LA and low content of Docosahexaenoic acid (DHA, 22:6 n-3) in sarcoplasmic reticulum (SR) phospholipids (PL) of Syrian hamster's heart increased SR Ca^{2+} ATPase 2a (SERCA) activity and lowered minimum T_b during torpor. We therefore tested the effects of diets contrasting in their content of either LA or DHA on hibernation ability and SERCA activity levels during torpor in garden dormice. Prior to hibernation, individuals fed a high-DHA diet showed 9-fold higher DHA levels and 1.4-fold lower LA amounts in their white adipose tissue (WAT) compared to animals fed a high-LA diet. When fed a high-DHA diet, dormice significantly delayed onset of hibernation by 4.3 ± 2.1 days compared with high-LA diet individuals. Further, hibernation onset correlated positively with WAT-DHA levels and negatively with WAT-LA amounts prior to hibernation. Subsequently, hibernating patterns did not differ between the two dietary groups, despite a significant difference in WAT-LA but not in WAT-DHA proportions. In contrast, SR-PL fatty acid composition and SERCA activity were similar in torpid individuals from the two dietary groups during mid-winter. In line with our previous findings, we found that SERCA activity levels correlated positively with LA and negatively with DHA contents of SR-PL in torpid dormice. Further, the higher SERCA activity was, the lower was minimal T_b reached during torpor. We conclude that (1) high DHA levels prevent hibernators from entering into torpor and (2) fatty acid composition of SR membranes modulates cardiac SERCA activity, hence determining the minimum T_b tolerated by hibernators.

16. WORKING WITH ANIMALS IN RESEARCH: EDUCATION AND TRAINING WITH THE ONLINE PLATFORM LAS INTERACTIVE

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The validity of scientific results and the well-being of animals used in animal studies depend to a great extent on the competence of all personnel involved. Therefore, any person concerned in animal research has to be adequately educated, competent and continuously trained. Online tools are ideally suited to make information easily accessible and available. The implementation of videos, slide shows and interactive tools contribute to the 3R concept (Reduction, Replacement, Refinement of procedures

involving animals), since they illustrate methods and techniques prior to practical training programs or work settings.

LAS interactive is a free online learning platform for working with animals in a research environment (**L**aboratory **A**nimal **S**cience). Learning modules provide information on legislation and ethics in animal experimentation; on general and species specific biology, husbandry and strain assessment as well as experimental techniques; and much more. The platform addresses a variety of vertebrate species, from the most commonly used rodents in the laboratory to e.g. non humane primates and birds. Due to the long research experience in the field of torpor at the University of Marburg, the platform also deals with hibernating and daily heterothermic species such as marmots, bats and hamsters.

The future aim of LAS interactive is (in collaboration with experts from other institutions) to expand the content to more species such as wildlife and farm animals, outlining the different challenges when working with these animals in lab or field. By providing information that links species specific needs with research methods, legislation and the 3Rs, LAS interactive promotes a best practice for working with animals in research. The online availability and the multilingualism of the platform contribute to an up to date education and a harmonization of qualification. Both will support high research standards and in the end facilitate the international exchange of researchers between countries.

17. THE ROLE OF HYDROGEN SULFIDE IN THE HYPOXIA TOLERANCE OF HIBERNATORS

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One of the many physiological mysteries of small-bodied hibernators is their marked tolerance to severe hypoxia, which occurs during rewarming arousal from deep torpor bouts. The goal of this project is to identify mechanisms that support hypoxia tolerance of hibernating ground squirrels in the brain - normally a hypoxia–sensitive tissue. In non–hypoxia tolerant species, including mice, damaging or lethal hypoxia is mediated in the brain by an acute increase in the gasotransmitter hydrogen sulfide (H_2S), which inhibits the electron transport chain. This study will examine the hypothesis that the brain cells of deep hibernators are able to metabolize H_2S, and that this innate ability underlies their survival of severe hypoxic events. We will combine in vivo and in vitro approaches in the brain of 13-lined ground squirrels (*Ictidomys tridecemlineatus*) with a comparative genomics approach, to evaluate selective pressures on the key genes of H_2S production and metabolism within the rodent lineage. Preliminary evidence for negative selection of target H_2S pathway enzymes across the rodent lineage suggests that any differential alteration in H_2S metabolism in 13-lined ground squirrel might occur not by altered amino acid sequences, but rather non-coding, regulatory genomic differences.

18. COMPARISON OF BAT MITOCHONDRIAL PROTEOMES BETWEEN TORPID AND ACTIVE STATES

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Hibernation is an adaptive strategy of some mammals to survive cold winter. Fat is used instead of glucose as the main energy supply during mammalian hibernation. Mitochondria have a dominant function in producing energy for the cells. Our previous findings suggested that mitochondria play a critical role in hibernation of *Myotis recketti* bats. To learn the molecular mechanism in regulation of mitochondrial function and mammalian hibernation, we isolated liver mitochondrial proteins from torpid and active states of these bats and compared the mitochondrial proteomes between the two states. We found that that 18% (45 proteins) of identified proteins with significant changes were associated with fatty acid metabolism, citric acid cycle, and respiratory chain. The expression levels of proteins 14% (36

proteins) involved in amino acid metabolism, mitochondrial translation, and protein quality control systems were also significantly altered in torpid bats. Approximately 15% (37 proteins) of differentially expressed proteins were associated with mitochondrial apoptosis and morphology. Results of *gene ontology* and *ingenuity pathway analysis* imply that activation of eukaryotic initiation factor 2 signaling pathway is a pivotal control of cell survival and death in hibernation of bats. Our study provides novel insights in liver protection during bat hibernation.

19. HIGHER EXPRESSION OF WARM-SENSITIVE TRANSIENT RECEPTOR POTENTIAL VANILLOID SUPERFAMILY MEMBERS IN THE BRAIN OF TORPID BATS

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Some small mammals (e.g., bats and squirrels) use hibernation to survive the harsh environment. During torpor, the body temperature of these hibernators can be reduced to an extremely low level that is lethal for euthermic animals. The molecular mechanisms involved in control of thermal sensation in hibernating mammals are not fully understood. Temperature-sensitive transient receptor potential channels, referred to as thermo-TRPs, are critical factors for thermal sensation. Since torpid bats maintain their ability in response to elevated environmental temperature, we hypothesize that warm-sensitive TRPs are highly expressed in bats during torpor. We compared gene expressions of ten thermo-TRPs, including TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM4, TRPM5, TRPM8, TRPC5, and TRPA1 in the brain between torpid and active bats using quantitative RT-PCR. Results showed that the mRNA level of warm-sensitive TRPV1, TRPV2, TRPV3, and TRPV4 and cold sensitive TRPA1 was significantly higher in torpid bats. In contrast, cold sensitive TRPM8 and TRPC5 and the thermo-sensitive TRPM2, TRPM4, and TRPM5 had lower expression in torpid bats. The data imply that warm-sensitive vanilloid receptors have indispensable roles in bats during torpor. The regulatory mechanism for expression of these thermo-TRPs remains to be investigated.

20. WATER-FAT MRI SUGGESTS AN ENDOGENOUS RHYTHM OF BROWN ADIPOSE TISSUE PROLIFERATION IN A HIBERNATOR

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Hibernating mammals, such as the 13-lined ground squirrel (*Ictidomys tridecemlineatus*), spend most the winter torpid with body temperatures near 5°C. Individual bouts of torpor last for several days and are interrupted spontaneously by arousal periods during which body temperature increases to approximately 37°C in just a few hours. During these arousals brown adipose tissue (BAT) is the primary source of heat production. In non-food-caching hibernators, including ground squirrels, body mass and white adipose tissue proliferation are controlled by an endogenous rhythm, allowing animals to store food energy to fuel the hibernation season. The seasonal rhythms of BAT quantity are less well studied. In non-hibernating eutherians, BAT proliferation requires extensive acclimation to cold environmental temperatures. In hibernators, however, BAT genes related to thermogenesis are upregulated in autumn, even when animals are housed at warm temperatures. We predicted that the total volume of BAT would also increase as the hibernation season approached, even in the absence of cold acclimation. We used water-fat MRI to measure total BAT volume in ground squirrels, housed at 23^{o}C, at intervals of approximately 10 days from late spring until hibernation began in late autumn. During that period BAT volumes increased significantly from 0.59% to 4.44% of total body volume. Moreover the proportion of the MRI signal related specifically to lipid (the fat fraction) in BAT decreased over that period, suggesting a relative increase in BAT water content. The decrease in fat fraction may be due to proliferation of mitochondria and/or vasculature within BAT. Our data demonstrate that BAT quantity

and, probably, mass-specific thermogenic capacity, increase in hibernators without cold acclimation. This raises the possibility that BAT function and amount are also controlled by an endogenous rhythm.

21. UNDERSTANDING THE MOLECULAR MECHANISM OF PAIN SENSATION IN *S. TRIDECEMLINEATUS*

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Thirteen-lined ground squirrels (*I. tridecemlineatus*) can tolerate cold better than mice or rats, providing a new perspective for studying general principles of cold sensitivity and cold tolerance at the level of the somatosensory system. Nav1.9 (Scn11a) is a voltage-gated ion channel from the somatosensory neurons of dorsal and trigeminal ganglia. Studies in mice and rats show that the channel contributes to neuronal excitability linked to noxious cold (≤ 10°C). Here, we put this hypothesis to a test by comparing Nav1.9 (Scn11a) function in neurons from dorsal root ganglia (DRG) of mice and active or hibernating squirrels. We cloned Scn11a from squirrel DRG and characterized its neuronal distribution and functional properties by RNA in situ hybridization and patch-clamp electrophysiology. We found that the abundance and size distribution of Scn11a-expressing neurons did not vary between mice and active or hibernating squirrels, suggesting that the pattern of Scn11a expression is conserved among the neuronal subpopulations in rodents. Consistently, electrophysiological analysis of dissociated DRG neurons from active and hibernating squirrels revealed the presence of a voltage-gated sodium current with kinetic characteristics similar to mouse Nav1.9. Lowering the temperature of the recording solution from 20°C to 10°C led to a decrease in the Nav1.9 peak current density by 25% and 35% in, respectively, mouse and squirrel neurons, supporting the idea that Nav1.9 activity is modulated by temperature. However, in comparison to mice, squirrel neurons exhibited only ~50% of the initial peak current density even at 20°C, suggesting a decrease in the basal activity of the channel. We did not detect differences in Nav1.9 activity in neurons from active and hibernating animals. We hypothesize that the low basal Nav1.9 activity leads to a suppressed excitability of neuronal cold receptors in squirrel DRG, providing an explanation to the remarkable cold tolerance exhibited by squirrels in both active and hibernating state.

22. INCREASED HOMEOTHERMY FOLLOWING PARTURITION IN COMMON TENRECS

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Endothermy may be defined as an increase in metabolism, which leads to increased body temperatures (T_b). Basal protoendothermic mammals like monotremes, marsupials, and afrotherians have lower and more variable T_bs. An appealing theory for the evolution of increased endothermy/homeothermy predicts increased endothermy results in greater resource allocation to young (parental care model). We monitored T_b in common tenrecs *(Tenrec ecaudatus)* during late pregnancy and following birth. During pregnancy, T_b was highly variable. Animals housed at room temperature (~24 °C) experienced T_bs that varied between 25 and 34 °C during pregnancy. Remarkably, pregnant animals are able to enter torpor. Indeed, an animal that was pregnant for ~ three weeks was able to go torpid when housed at 12 °C and maintained T_b at ~13 °C for ~22 h. Following parturition, female tenrecs maintain less variable and warmer T_bs. While these data support the parental care model, a surprising outcome was that increased homeothermy is restricted to the post-natal period and not during pregnancy.

23. A DIRECT TEST OF THE AEROBIC CAPACITY MODEL FOR THE EVOLUTION OF ENDOTHERMY

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A definition of endothermy may be an increase in metabolism which leads to increased body temperatures (T_b). Basal protoendothermic mammals like afrotherians, marsupials, and monotremes maintain lower and more variable T_bs. Some hypothesize that the evolution of increased endothermy/homeothermy was to allow increased exercise performance. The hypothesis centered on an increased ability to run longer or faster when T_b was higher. Common tenrecs *(Tenrec ecaudatus)* have extremely variable T_bs. Animals housed at 12 °C are able to run or swim when T_b = ~14 °C. We swam tenrecs while monitoring oxygen consumption at T_a/T_b at 16, 20, 24, 28, and 32 °C. We calculated $VO_{2\ max}$ at the different temperatures. $VO_{2\ max}$ was decreased at lower T_bs. Endurance (time at 80% of $VO_{2\ max}$) did not change across T_b. We suspect that selection for speed and $VO_{2\ max}$ may have contributed to the ability to perform endothermy. However, not all indices of performance are affected equally by temperature.

24. HIBERNATION INDUCES A PRO-INFLAMMATORY SHIFT IN WHITE ADIPOSE TISSUE OF GROUND SQUIRRELS

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Every year from late summer through fall, hibernators go through a period of hyperphagia and rapid weight gain in order to increase white adipose tissue (WAT) stores needed to survive the winter. This large increase in adipose mass is similar to that seen in obesity. A major part of obesity-driven disease in humans is associated with a more pro-inflammatory phenotype and increases production of pro-inflammatory cytokines in WAT. Because hibernating ground squirrels enter an obese state in preparation for winter, we hypothesized that the immune populations in WAT of an early season hibernator would mirror that in other obese mammals and generally have a pro-inflammatory phenotype. We used flow cytometry was used to determine the relative numbers of macrophages (CD11b/c+) and T cells (CD3+) in WAT in the summer, hibernating and spring seasons. We also quantified the production of a pro-inflammatory cytokine, tumor necrosis factor (TNF)-α, and a marker of anti-inflammatory regulatory T cells, FoxP3, in WAT using qRT-PCR. We found that the percentage of macrophages decreased from summer to hibernation and increased again in the spring. Although not significant, CD3+ T cells showed a similar trend, but without the increase in spring. TNF-α levels were significantly higher in hibernation compared to other seasons. Although FoxP3 showed no significant change, there was a trend for a decrease during hibernation. Together, these data suggest that the immune phenotype of WAT shifts to a more pro-inflammatory state during hibernation.

25. IDLE REDUCTION BY TORPOR: TORPOR EXPRESSION WITHOUT ENERGY CONSTRAINTS IN THE MUSK SHREW *SUNCUS MURINUS*

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In addition to importance for energy conservation, torpor may play an important role in the colonization of new habitats. The musk shrew (*Suncus murinus*, Soricidae) is originally native to south Asia and part of southeast Asia, but has expanded its distribution to include Africa, west Asia and islands of southeast and east Asia largely thanks to introduction by humans. This small insectivore is capable of daily torpor, and we predicted that this ability might aid adjustment to new habitats, where ambient temperatures (T_a) could affect both energy expenditure and the availability of insect prey. We examined the influence of T_a on expression of daily torpor in fifteen shrews allocated to three groups

and maintained at 30°C, 26°C and 20°C for two weeks at a time in a different order for each group. All groups were exposed to long photoperiods (L: D = 16h: 8h) and fed *ad libitum*. Nesting materials were provided for one week at each experiment T_a. Body temperature was logged continuously during the experiment. Our results showed that all shrews expressed daily torpor irrespective of T_a. Incidence of torpor was not affected by T_a, sex, or availability of nest materials. The lower thermoneutral limit for this species is ca. 28°C, so individuals maintained at 30°C could not be experiencing any thermal challenge. The results suggest that the shrew expresses daily torpor spontaneously even without energy constraints. Moreover, multiple torpor bouts were frequently observed under constant environmental conditions. The expression of daily torpor in this species may not be governed exclusively by a circadian clock but also by other mechanisms. Routine and multiple torpor bouts in shrews may serve to reduce energy requirements more effectively than in other endotherms of similar body size. These characteristics may have contributed to the species' colonization success.

26. REDUCED NEUTROPHIL FUNCTIONALITY DURING EARLY AROUSAL IN HIBERNATING SYRIAN HAMSTER

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Hibernation induces a reversible immunosuppression by affecting the innate as well as the adaptive immune system. In all hibernating mammals studied so far, the number of circulating neutrophils decreases after entrance into torpor, which we demonstrated to be due to storage. Whereas numbers of circulating neutrophils rapidly restore upon arousal, it is unknown whether they are functional. Here, we investigated the functionality of neutrophils from Syrian hamsters (*Mesocricetus auratus)* upon arousal.

Hibernating Syrian hamsters were euthanized as summer euthermic (SE, Ta=20 ^{0}C, L:D 14:10) or as hibernators in early arousal (90 min after arousal (AE)). Neutrophils and plasma was collected by Ficoll-Paque isolation from EDTA anticoagulated blood. Neutrophils were incubated for 2 hours with *E. coli* coupled to pHRhodo and fluorescence was measured to quantify phagocytosis. Incubation occurred in the presence of plasma from either SE or AE animals. After incubation, neutrophils were flash frozen and production of free radicals was measured by MDA-assay.

Neutrophils were fully functional in SE animals with respect to phagocytosis of *E. coli* and subsequent free radical production. On the other hand, neutrophils from AE animals were unable to phagocytize *E. coli* and did not produce free radicals. Adding plasma from the AE phase to SE neutrophils reduced their phagocytic capacity, but not subsequent free radical production. Together these results suggest that plasma from AE animals lacks a factor, possibly complement, for optimal binding of neutrophils to *E. coli* particles. These data warrant further investigation of neutrophil functionality in early arousal and the plasma factors involved.

27. PRELIMINARY CHARACTERIZATIONS OF AMMONIUM ACETATE INFUSIONS ON HIBERNATING ARCTIC GROUND SQUIRRELS: TEMPERATURE AND OXYGEN CONSUMPTION MONITORING AND METABOLIC PROFILING

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Our research addresses the question of how the metabolic state of a hibernating Arctic Ground Squirrel (AGS) influences central nervous system control (CNS) of hibernation and induces arousal. NMDA receptor antagonists are known to arouse squirrels out of hibernation and work somewhere in the periphery of the CNS or circumventricular organs. We hypothesize that NMDAR antagonists are mimicking an endogenous decline in available glutamatergic pools directly caused by the metabolic impact of prolonged hibernation. We tested one hypothesis that the natural accumulation in glutamine

(which develops in late torpor) influences glutamatergic signaling pools, potentially arousing the AGS. To do this we shifted the nitrogen balance early in the torpor bout to mimic late stage torpor glutamine levels with an ammonium acetate infusion via indwelling venous catheter. Experiments were started on the third day of a torpor bout with 12 hour infusions of ammonium acetate at 6 mg/kg/hr. Oxygen consumption and carbon dioxide production were measured for 36 hours after the infusion started. Plasma was sampled at baseline, 8, 12, 30 and 54 hours after the start of the experiment via arterial indwelling catheter. Temperature was monitored throughout the winter. Based on response of the animals, preliminary results suggest this intervention was not sufficient to arouse the animals. Complete data analysis to assess the impact of the ammonium acetate infusions on nitrogen balance is in progress.

28. COMPLEMENT COMPONENT C3 TRANSCRIPTION, TRANSLATION AND AND FUNCTION IN THIRTEEN-LINED GROUND SQUIRRELS DURING TORPOR

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Thirteen-lined ground squirrels enter a state of torpor during the winter months, characterized by weight loss, decreased metabolism, and decreased immune function. During torpor the squirrels' white blood cells almost completely disappear from circulation, and are able to quickly return to normal levels within 2 hours of arousal. Bucking the trend of immune suppression during torpor, it has been recently discovered that the transcription of numerous proteins in the complement system are upregulated in the bone barrow during torpor. The complement system is part of the innate immune system and aids in killing infectious bacteria and viruses. Complement component C3 has the most significant increase during torpor. To determine if increased complement C3 transcripts are translated into protein during torpor, a chemiluminescent western blot is being developed to measure and compare complement component C3 levels in the plasma of non-hibernating, hibernating and interbout arousal animals. For this Western blot, a goat anti-mouse C3 polyclonal antibody has been established as reacting specifically with ground squirrel C3, detecting the 125 kDa C3a subunit. Plasma samples from at least eight squirrels at each stage will be compared to determine if C3 protein levels increase during torpor. Quantitative PCR and immunohistochemistry assays are also being used to investigate complement C3 transcription and translation during torpor in tissues other than the bone marrow, for example liver and adipose. Results so far show that unlike in the bone marrow, complement C3 transcription occurs but does not increase during torpor in liver and adipose. Preliminary *E. coli* killing assay results indicate that torpid ground squirrel serum complement is unable to lyse bacterial cells at 37 degrees Celsius. The *E. coli* killing assays using torpid ground squirrel serum will also be performed at four degrees Celsius, a physiologically relevant temperature for torpid animals.

29. EARLY VITAMIN A DEFICIENCY INDUCES CHANGES IN EXPRESSION OF OBESITY-RELATED GENES IN WHITE ADIPOSE TISSUE

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Hibernation is an evolutionary response to periods of reduced energy availability and increased energy demand. Most hibernators, such as the 13-lined ground squirrel (*Ictidomys tridecemlineatus),* rely on energy stores in adipose tissue for the duration of the winter season, making pre-hibernation fattening invaluable. Retinoic acid, the biologically active derivative of vitamin A, plays pivotal roles in the accumulation of adipose tissue via retinoid receptor mediation. A vitamin A-deficient diet was fed to gravid female ground squirrels in order to produce vitamin A deficient squirrels after weaning and subsequently determine the effect of vitamin A deficiency on pre-hibernating adipose accumulation. Using RT-qPCR, expression of the retinoid receptors RXR-β and RAR-α and the adipokines leptin and resistin was examined in intra-abdominal adipose tissue to determine if these adipose accumulation

pathways were interrupted. RAR-α showed no significant difference, while RXR-β expression was significantly different when both season and treatment were included. Deficient squirrels had higher levels of RXR-β in the summer but lower in the pre-hibernating, hibernating, and spring seasons suggesting a disruption of normal RXR-β expression trends. Leptin varied only by season and was not affected by the deficiency. Resistin varied in season as well as treatment suggesting a disruption of required insulin resistance. This novel study suggests that vitamin A deficiency alters adipose accumulation needed for hibernation potentially via effects on retinoic acid signaling.

30. ABSOLUTE DIFFERENCE SUM APPROACH FOR ESTIMATING MOVEMENT ARTIFACTS IN THERMAL IMAGING VIDEOS

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Thermal imaging is generally used to capture surface temperature estimates of objects for inference of heat transfer, especially for applications related to metabolic heat production and inference of peripheral blood flow. Time lapsed thermal imaging videos also offer the possibility of detecting movement or activity patterns without challenges associated with glare, shadows, or changes in lighting common with visual digital imaging. We have developed a frame subtraction algorithm for extracting the pixel-by-pixel relative change in the raw thermal image signal from a fixed focus video file. In combination with simultaneous measurements of metabolic rates via indirect calorimetry, we are able to assign activity scores to thermal imaging data, comparing four species of Darwin's finches of different body sizes during sleep and wake and at a range of different temperatures imposing different metabolic demands. In principle, this would allow for activity data to be standardised to energetic measurements or for quantitative estimates of activity to be used to assist in scoring for estimates of basal metabolism.

31. DNA DAMAGE DURING HIBERNATION IN THE SYRIAN HAMSTER

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Hibernation provokes oxidative stress in specific tissues, such as e.g. intestinal mucosa of ground squirrel, despite upregulation of antioxidant defense mechanisms. Excess production of reactive oxygen species induces DNA damage and inhibits DNA repair, which ultimately results in genome instability. Here, we quantify DNA damage during hibernation in Syrian hamsters (*Mesocricetus auratus*).

Splenocytes and hepatocytes were isolated from male and female hamsters during different phases of hibernation, being torpor late (TL, at least 3 days in torpor, n=6), arousal early (AE, 1.5 h after initiation of arousal, n=6) and arousal late (AL, 8 h after initiation of arousal, n=6). Neutral and alkaline Comet Assay was performed to quantify and classify DNA breaks in > 70 single cells per animal. DNA damage was expressed as the percentage of DNA in the Comet's tail (% DNATail). Further, tissue was examined for markers of DNA damage and repair using immunofluorescence.

At euthanization, body temperatures amounted 7±^{0}C, 36.5 ±^{0}C and 36.5 ±^{0}C for TL, AE and AL (n=6), respectively. Median %DNATail of alkaline comets in splenocytes amounted 63.1% in TL, which rapidly decreased to 22.7% and 15.3% in AE and AL, respectively. Median %DNATail of neutral comets significantly decreased from 53.3% in TL to 10.5% in AL, indicating DNA damage to be mainly double strand breaks. Similar pattern was found in liver. Expression of p53bp1, a transducer of the DNA damage checkpoint, was increased in TL, while expression of PARP1, involved in recovery from DNA damage, was highly expressed in AL.

This is the first study demonstrating a substantial amount of genomic DNA damage in the torpor phase of natural hibernation, which seems largely resolved during early arousal. These data imply that the ability to repair DNA damage during arousal may be critical to avoid genomic instability upon

repetitive torpor-arousal cycles. Further, these data fuel the theory that arousals serve to repair organ damage contracted during torpor, ultimately promoting survival of hibernators.

32. ENERGETICS OF BATS WITH WHITE-NOSE SYNDROME: WHY THE EXTRA AROUSALS?

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White-Nose Syndrome (WNS) is a large-scale epizootic killing cave-dwelling bats in North America during hibernation. While the psychrophilic fungus, *Pseudogymnoascus destructans*, is the causal agent of WNS, the proximal cause of death seems to be early depletion of fat reserves. This suggests an elevated torpid metabolic rate (MR_T), and/or more arousals during hibernation, both of which have been demonstrated. The reason for the increased number of arousals is unknown, although a few hypotheses have been proposed. We collected data on MR_T of little brown bats (*Myotis lucifucus*) from hibernacula with and without evidence of WNS. Using these and available data on torpor duration, we show that the higher torpor metabolism could, by itself, cause the more frequent arousals. We estimate that just the higher MR_T of WNS-affected bats would require an additional 0.7 g of fat over the winter, and the increase in arousal frequency could require an additional 2+ g of fat; taken together, it is clear that these little animals can't meet the energy expenditure demands of WNS in addition to the normal energetic requirements of hibernation.

33. ELUCIDATING THE CASPASE CASCADE DURING HIBERNATION

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During hibernation, ground squirrels experience numerous cycles of profound metabolic depression where body temperature approaches ambient (to below 0° C) and oxygen consumption may be as low as 1% of active rates. Numerous conditions known to cause widespread apoptosis in other systems are inherent during hibernation (e.g. hypothermia, bradycardia, and ischemia/reperfusion injury). Central to inflammatory response and apoptosis are a family of cysteine-aspartate proteases known as the caspases. Using a systems-level approach, we found that during hibernation, golden-mantled ground squirrels (*Spermophilus lateralis*) experience incomplete activation of the caspase cascade. Importantly, winter squirrels experience a 2-fold activation of caspase 3. While such activation might be expected to result in 20,000 times more caspase 3 proteolytic activity, proteolysis assays suggest limited activity during hibernation. Similar regulation is found in the activities of other caspase, suggesting a global event. We found no evidence for ICAD activation, PARP cleavage, or DNA nicking, downstream events consistent with significant caspase 3 activation. Therefore, despite *seeming* activation of caspases during hibernation, there was no evidence of apoptosis. These data highlight the need for systems-level approaches in understanding complex physiological states like hibernation.

34. VITAMIN A DEFICIENCY AFFECTS LIVER RETINOIDS AND LECITHIN: RETINOL ACYLTRANSFERASE EXPRESSION IN THE SMALL INTESTINE OF A HIBERNATOR

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Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) hibernate seasonally during which they maintain a torpid, fasted state. As such, nutrient availability can be a major issue during the winter. Vitamin A (retinol) is fat-soluble and absorbed by enterocytes of the small intestine and stored primarily in the liver. The enzyme lecithin:retinol acyltransferase (LRAT) converts retinol to retinyl esters for absorption and storage. We developed a model of early vitamin A deficiency in ground squirrels by feeding gravid females a vitamin A-deficient diet and continuing the pups on that diet until 2 weeks after

weaning. HPLC analysis of liver samples from these animals showed that, compared to controls, squirrels deficient in vitamin A early in life did not store equal amounts of retinyl esters in their livers despite returning to a vitamin A-rich diet at approximately 8 weeks of age. We hypothesized that low LRAT expression in the liver and small intestine of these deficient squirrels led to this difference. We isolated proteins from liver and jejunum (small intestine) and used immunoblotting to quantify LRAT protein expression. Liver LRAT was significantly increased between summer and hibernators but there was no effect of diet on expression. LRAT in jejunum was not effected by season or by diet. Within hibernators, however, higher expression was seen in the jejunum of the previously deficient animals. These results suggest that early vitamin A deficiency does not effect LRAT expression across activity states and that lower retinyl ester storage observed in deficient squirrels was not solely mediated by LRAT expression. Future studies will examine other aspects of retinoid biology that could explain the discrepancy in liver stores.

Late Breaking Abstracts for Poster Presentations

35. ADENOSINE 1AR TARGETED TEMPERATURE MANAGEMENT AND RESULTANT PHYSIOLOGICAL EFFECTS OF A PHARMACOLOGICAL INDUCED HYPOMETABOLIC STATE

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Hype and hope has been generated by promises of inducing suspended animation or a hypometabolic state. Biased reporting of positive results underestimates complications that ultimately limit translation. In this study we apply the Adenosine 1A receptor (A1AR) agonist N6-Cycolhexyladenosine (CHA) in a manner similar to prior work in this lab found to induce a hibernation-like state in rats for therapeutic hypothermia. We report worse case scenarios and steps taken to avoid or manage these scenarios.

Sprague-Dawley rats are administered CHA via IV infusion to inhibit thermogenesis. Core body and brain temperatures, oxygen consumption, heart rate, rhythm and blood pressure are monitored throughout the experiment. Core body temperature is maintained between 30-34°C for 24-48hrs by adjusting the dose of CHA and/or adjusting the surface temperature of the cage via a thermoelectric cooling device. Side effects of bradycardia and hypotension are managed with administration of 8-SPT.

Preliminary results indicate a pronounced individual variation in response to the CHA. Low doses of CHA resulted in bradycardia but had little to no effect on the inhibition of thermogenesis. Administration at a starting does of 0.1mg/kg while doubling the concentration every 4 hours resulted in the development of tolerance to the drug's effect on thermogenesis but not bradycardia. Brain temperature follows core body temperature closely during the cooling and rewarming phases. Oxygen consumption requirements decreased after CHA administration.

CHA is a potent inhibitor of thermogenesis and metabolism at doses greater than 0.4mg/kg. Variations in response and tolerance necessities close monitoring of core body temperature and administration of a loading dose. Changes in core body temperature during targeted temperature management treatment closely resemble changes in brain temperature.

36. HYPOMETABOLISM DURING TORPOR IN MICE IS DOMINATED BY REDUCTION IN THE SENSITIVITY OF THE THERMOREGULATORY SYSTEM

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Some mammals enter a state of active hypometabolism, either torpor or hibernation, when reducing metabolism would benefit survival. Hibernators demonstrate active regulation of body temperature (T_B) by changing both the sensitivity of the thermoregulatory system and the set-point temperature. However,

these properties during torpor remain poorly understood. Here, using *Mus musculus*, we investigated the thermoregulatory mechanism of torpor. We developed a torpor-detection algorithm based on Bayesian inference to evaluate the metabolism of individual mice. Inducing torpor in various ambient temperatures (T_As), we found that the open-loop gain of the thermoregulatory feedback system (H), which is the sensitivity of thermogenesis proportional to the temperature gradient between T_B and T_R, decreased 88% during torpor while the reference temperature (T_R), which is the set-point of T_B only decreased 3.79 °C. These results indicated that thermoregulation during torpor is dominated by the reduction of the sensitivity of thermogenesis but not by lowering T_R.

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