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**13. ABSTRACT** *(Maximum 200 words)*

We have successfully identified genes controlling the physiological characteristics of hibernation in the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*). Identification of these specific genetic markers has allowed us to study the function of their gene products under highly controlled conditions of temperature, light and humidity. To accomplish this goal specific instrumentation was purchased with this DURIP grant. Measurements of low-temperature physiology in hibernating mammals under controlled conditions required an environmental chamber that allows us to maintain a variety of ambient temperatures ranging from -5 to 20°C. The response and adaptation of our ground squirrel species to environmental change has been monitored by precisely measuring activity, core body temperature and heart rate using miniature, battery-free, implantable transmitters that allow uninterrupted lifetime studies. Tissues collected from these animals were quick-frozen in liquid $N_2$ and placed in a -86°C freezer where they are stored until we analyze patterns of gene expression controlling the physiological characteristics of hibernation in mammals.

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REPORT TITLE: Monitoring Low-Temperature Physiology in Hibernating Mammals

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SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

Sincerely,

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Enclosure 3

b. FINAL PROGRESS REPORT

## DURIP: Monitoring Low Temperature Physiology in Hibernating Mammals

Matthew T. Andrews, P. I.

### Statement of the problem studied

This DURIP grant funded the purchase of equipment used on two ARO-sponsored projects: *A new regulatory protein expressed in hibernating mammals* (DAAH04-96-1-0072) and our current grant, *Regulation of genes controlling carbohydrate metabolism in the heart of a hibernating mammal* (DAAD19-01-1-0014). Both of these projects dealt directly with the genetic control of mammalian hibernation using the thirteen-lined ground squirrel as a model organism.

Unlike model genetic systems, where defined strains can be obtained from a stock center, ground squirrels used for the study of hibernation are collected directly from the wild. To inventory and categorize parameters of animal size, sex and location, we purchased a portable laptop computer that can be utilized in the field. Measurements of low-temperature physiology in hibernating mammals under controlled conditions required purchase of environmental chambers to maintain a variety of ambient temperatures ranging from -5 to 20°C. The response and adaptation of our ground squirrel species to environmental change is now being monitored by precisely measuring activity, core body temperature and heart rate using miniature, battery-free, implantable transmitters that allow uninterrupted lifetime studies. Finally, tissues collected from these animals are quick-frozen in liquid $N_2$ and placed in a -86°C freezer where they are stored until we analyze patterns of gene expression controlling the physiological characteristics of hibernation. All of the equipment items described above were purchased with funds from this DURIP grant.

### Summary of the most important results

During winter, limited supplies of food make energy management an important issue for mammals trying to survive cold, harsh conditions where heat must be continuously generated to replace heat lost to the environment. Consequently, the colder the surroundings, the more energy required for an animal to maintain this equilibrium. Some mammals, however, have the ability to reduce their metabolic rate and survive for up to six months without food in an inactive state where body temperatures can approach 0°C. This inactive state is called hibernation.

Mammals prepare for hibernation during late summer and early fall by depositing lipid in the form of triacylglycerols in their main fat storage depot, the white adipose tissue (WAT; 2, 3). Hibernating mammals have a respiratory quotient of 0.7 indicating that stored lipids, not carbohydrates, are their main source of energy during hibernation (reviewed in 4). Although fat stores provide the necessary metabolic fuel, the mechanism of lipid mobilization and utilization during hibernation is not completely understood. In euthermic mammals the enzyme typically responsible for the hydrolysis of triacylglycerols stored in WAT is hormone sensitive lipase (HSL; 5). Therefore it has been presumed that HSL is the main mediator of lipolysis in the hibernator as well (6). In this report we describe evidence that a second lipolytic enzyme, pancreatic triacylglycerol lipase (PTL), is expressed in the WAT of thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) before and during hibernation.

Until recently, PTL was believed to be expressed exclusively in the pancreatic acinar cells and secreted into the small intestine as a means to digest dietary fat. However, we found that PTL is differentially expressed in the heart of thirteen-lined ground squirrels where it provides low-temperature lipolysis during hibernation (1). PTL is expressed in WAT in the form of a novel chimeric retroviral-PTL mRNA that is distinct from the PTL message found in the heart. The expression of both PTL and HSL in WAT is seen as well as PTL enzymatic activity. In this report we describe the use of recombinant PTL from both humans and ground squirrels to determine the requirements for PTL-mediated lipolysis at temperatures as low as 0°C. We suggest that the unusual expression of PTL in WAT may have arose from a retroviral insertion event, and that PTL plays a significant role in the mobilization of fatty acids from stored triacylglycerols during the near freezing body temperatures associated with hibernation.

An obvious explanation for the expression of PTL in WAT is that PTL retains high activity at low temperatures. We have shown that extracts containing PTL from the heart of hibernating thirteen-lined ground squirrels showed 61% and 34% maximal colipase-dependent activity at 7°C and 0°C, respectively (1). To determine whether this cold lipolysis is unique to ground squirrel PTL, or possibly the result of a hibernation-specific modification of the lipase, we expressed cDNAs for both human and thirteen-lined ground squirrel PTL in the yeast *Pichia pastoris* (7). Expression of the two recombinant PTLs produces identical-length 449 amino acid proteins lacking the 16 amino acid N-terminal signal peptide. Because both lipases are expressed in the same yeast background, we would not anticipate post-translational modifications that are unique to a specific mammalian tissue and/or state of animal activity.

Activity of the two recombinant lipases was assayed by the pH-STAT method using triacylglycerol substrates tributyrin and triolein. As expected, PTL shows higher overall activity against the 4-carbon acyl chain tributyrin than the 18-carbon chain triolein (7). Figure 1 shows that both human and ground squirrel PTL perform remarkably well at low temperatures. At 0°C the ground squirrel enzyme still maintains 48% and 33% of maximal activity (seen at 37°C)

with tributyrin and triolein, respectively. Human PTL showed 42% maximal activity at 0°C using tributyrin and an amazing 55% maximal activity with triolein. The cold-adapted character of PTL is also confirmed by calculating the $Q_{10}$ (fold-reduction in two reaction rates 10°C apart) over a range of body temperatures typically seen in mammals entering hibernation. A $Q_{10}$ of 1.28 with tributyrin is calculated over a range of 37° to 7°C for both ground squirrel and human PTL. Similarly low $Q_{10}$ values of 1.35 for ground squirrel PTL and 1.32 for human PTL are seen over the same temperature range with triolein. We conclude that low-temperature lipolysis is a property of both human and thirteen-lined ground squirrel PTL, and that PTL does not require modifications specific to mammalian cells in order to function in the cold.

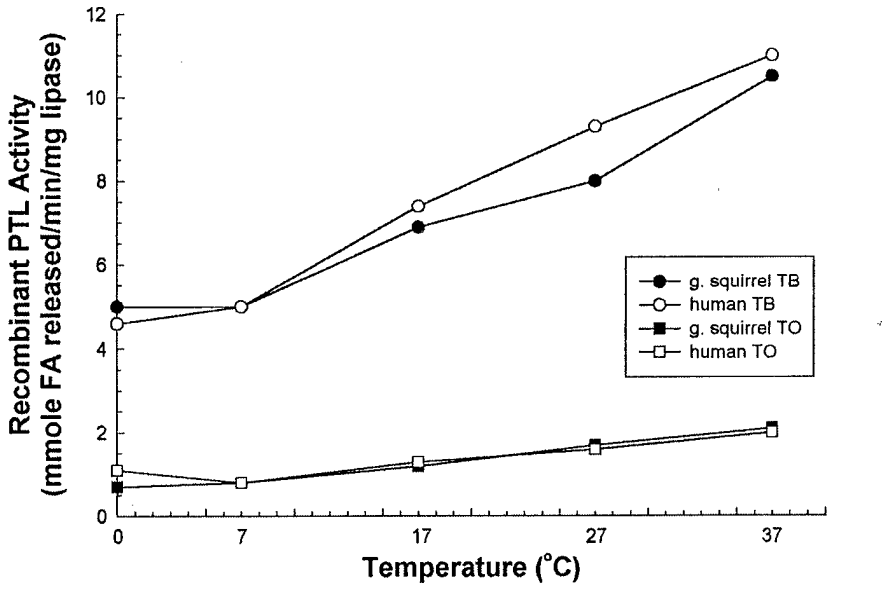


**Figure 1:** Lipolytic activity of human and thirteen-lined ground squirrel recombinant PTLs. cDNAs encoding human and ground squirrel PTLs were expressed in *Pichia pastoris* according to Yang and Lowe (7). Each purified recombinant PTL was assayed using the pH-STAT method with two triacylglycerol substrates, 4-carbon tributyrin (TB) and 18-carbon triolein (TO). Assays were performed in the presence of a five-fold molar excess of colipase and 4 mM taurodeoxycholate at 0, 7, 17, 27 and 37°C. The data represented are averages of two independent trials.

**Perspectives.** A growing number of advances in both genomic and bioinformatic technologies have recently allowed systematic investigations of previously intractable biological systems. Understudied species showing unique physiological properties are now on the brink of being explored at a level of

detail only previously realized with certain model organisms. Mammalian hibernation is one example of a complex physiological process that is now poised for such an investigation. As illustrated in this report, researchers have begun to characterize the genes responsible for the physiological characteristics of hibernation with the intent of escalating this effort through the use of high-throughput genomic and proteomic strategies. The potentially wide functional temperature range of proteins expressed during hibernation is especially attractive due to various practical uses and versatility of handling. Another possible application derived from the strategies of mammalian hibernation includes the achievement of stasis states for purposes of enhanced organ preservation. Identifying the gene products that specify the physiological extremes of the hibernating phenotype has the potential of greatly increasing our understanding of responses to human stresses such as hypoxia, hypothermia and starvation.

**List of all publications and technical reports**

**Published articles:**

Squire, T.L. and Andrews, M.T. (2000) Genetic control of carbon utilization during hibernation: mechanistic considerations, in *"Life in the Cold"* (G. Heldmaier, S. Klaus, M. Klingenspor, Eds.) pp. 325-337, Springer-Verlag, Berlin.

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Bauer, V.W., Squire, T.L., Lowe, M.E., and Andrews, M.T. (2001) Expression of a chimeric retroviral-lipase mRNA confers enhanced lipolysis in a hibernating mammal. *Am. J. Physiol.*, in press.

**Abstracts:**

Squire, T.L., Bauer, V.W., and Andrews, M.T. (1999) Role of hibernation-specific gene activity in controlling low-temperature metabolism. Hibernation and Adaptations to the Cold, May 20-22, 1999, Estes Park, Colorado.

Bauer, V.W., Squire, T.L., Lowe, M.E., and Andrews, M.T. (1999) Novel expression of pancreatic lipase during hibernation in the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. Hibernation and Adaptations to the Cold, May 20-22, 1999, Estes Park, Colorado.

Buck, M.J., Squire, T.L., and Andrews, M. T. (1999) Differential expression of genes encoding mitochondrial proteins in cardiac and skeletal muscle of the hibernator, *Spermophilus tridecemlineatus*. Hibernation and Adaptations to the Cold, May 20-22, 1999, Estes Park, Colorado.

Squire, T.L., Bauer, V.W., Buck, M.J., and Andrews, M.T. (1999) Genetic control of low-temperature metabolism in a hibernating mammal. Fifth International Congress of Comparative Physiology and Biochemistry, August 23-28, 1999, Calgary, Alberta, Canada.

Buck, M.J. and Andrews, M.T. (2000) Coordinated regulation of PDK-4 in cardiac, skeletal muscle, and adipose tissue during hibernation in the thirteen-lined ground squirrel. Eleventh International Hibernation Symposium, Jungholz, Austria, August 13-18, 2000.

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**UNDERGRADUATE RESEARCH PROJECTS DIRECTED**

Michael Fielder, 1998-99
Luis Miguel Gonzalez, 1999-2000

**Report of inventions**

none

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