06 Mitochondrial respiration and proton leak in hibernation and daily torpor

James Staples, Alexander Gerson & Jason Brown

Biology, University of Western Ontario, London ON, N6A 5B8, Canada; jfstaple@uwo.ca

Abstract

Potential mechanisms of metabolic suppression were compared between hibernation and daily torpor using isolated liver mitochondria. State-3 respiration was suppressed in both conditions by 30–40%, but only when measured at temperatures above 30°C. This respiratory suppression corresponded primarily to reduced substrate oxidation. Hibernation did not alter proton leak, whereas in daily torpor proton leak increased by the same extent that substrate oxidation decreased, resulting in no change in non-phosphorylating respiration. Increased proton leak in daily torpor may prevent excessive oxidative damage from reactive oxygen species. In ground squirrels fed diets with high or low levels of linoleic acid (18:2*n*-6) for several weeks prior to hibernation, proton leak was significantly reduced in hibernation, but state-3 respiration was not. Reducing proton leak under these circumstances may permit suppression of mitochondrial metabolism in hibernation when diet prevents suppression of substrate oxidation.

Introduction

Active, regulated metabolic suppression may have a different role in mammalian hibernation vs daily torpor (Heldmaier & Elvert 2004), but in both cases coordinated downregulation of cellular processes that generate and consume ATP is required. Key ATP-consuming processes appear to be downregulated (e.g. Diaz *et al.* 2004; MacDonald & Storey 1999) and glycolysis may be suppressed by reversible phosphorylation (Brooks & Storey 1992; Heldmaier *et al.* 1999). Several groups (including ours) have demonstrated that mitochondrial oxidative capacities are reversibly downregulated during hibernation in some tissues, such as liver (Martin *et al.* 1999; Pehowich & Wang 1984; Roberts & Chaffee 1972), but not in others, including skeletal muscle (Barger *et al.* 2003; Muleme *et al.* 2006). Comparable measurements for mammalian daily heterotherms were not available until recently. Comparing mitochondrial metabolism between hibernation and daily torpor may elucidate important regulatory targets for metabolic suppression.

Lovegrove, B.G. & McKechnie, A.E. (eds) *Hypometabolism in animals: hibernation, torpor and cryobiology.* University of KwaZulu-Natal, Pietermaritzburg.

The leak of protons across the inner mitochondrial membrane (LEAK) is thought to contribute up to 20% of mammalian standard metabolic rate (Rolfe *et al.* 1999). Under conditions of low ATP turnover, LEAK normally stimulates mitochondrial respiration (and heat production) without concomitant ATP synthesis, and therefore has been considered a "waste" of energy, and reducing LEAK might conserve energy and prolong survival under energy-challenging conditions, including mammalian hibernation. It has been reported, however, that LEAK in liver mitochondria does not differ between animals in deep hibernation and summer euthermic controls (Barger *et al.* 2003). Again, comparable data for daily heterotherms were lacking until recently. The regulation of inner mitochondrial membrane (IMM) proton permeability is poorly understood, but appears to be related to temperature (Chamberlin 2004) and the unsaturation of IMM phospholipids (Brand *et al.* 2003; Brookes *et al.* 1998). The phospholipid composition of cellular membranes changes among stages of the hibernation season (Cremel *et al.* 1979; Platner *et al.* 1976) and even within phases of a hibernation bout (Pehowich 1994). These changes may affect mitochondrial function within a hibernation/torpor bout as body temperature (T_b) changes.

Augmenting dietary linoleic acid (18:2*n*-6) content increased the proportion of animals entering torpor, and lowered core body temperature (T_b) and metabolic rate in hibernation and daily torpor, permitting greater energy savings (Frank 1992; Geiser 1991; Geiser & Kenagy 1987). Changes in phospholipid composition correlate with suppression of mitochondrial metabolism in other hypometabolic states (Stuart *et al.* 1998). Comparing mitochondrial properties among conditions known to alter whole-animal metabolism in hibernation might also elucidate mechanisms involved in metabolic suppression.

We measured respiration rates and LEAK kinetics in liver mitochondria isolated from torpid and euthermic *Phodopus sungorus* (Dwarf Siberian Hamster), and compared these data with similar measurements from a hibernator, *Spermophilus tridecemlineatus* (Thirteen-lined Ground Squirrel). We also manipulated dietary linoleic acid (18:2*n*-6) and examined the effect on hibernation patterns, mitochondrial respiration and LEAK kinetics in *S. tridecemlineatus*.

Materials and methods

Animals

All aspects of ground squirrel care and monitoring have been described previously (Muleme *et al.* 2006). Animals were fed one of four semi-synthetic isocaloric, isolipemic (9%) diets (formulated by TestDiets) that differed in 18:2*n*-6 content: 16, 22, 35, and 55 mg g^{-1}, respectively. Newly-weaned *P. sungorus* were obtained from a breeding colony (Katherine Wynne-Edwards, Queens University, Kingston, ON) and maintained at 18°C with a photoperiod of 14L:10D for four weeks. Subsequently the temperature and photoperiod were changed to 15°C and 8L:16D and maintained for 3–5 months. T_b was used to monitor torpor (Muleme *et al.* 2006).

Mitochondrial isolation, bioenergetics and phospholipid analysis

Liver mitochondria were isolated and respiration rates measured as previously described (Muleme *et al.* 2006). Proton motive force was estimated by measuring the IMM transmembrane

potential ($\Delta\Psi_m$) using tetraphenylphosphonium (TPP^+) and TPP^+-sensitive electrodes (World Precision Instruments), essentially as described by Barger *et al.* (2003). The kinetics of proton leak were determined by inhibiting succinate oxidation using incremental additions of malonate and measuring the effect on $\Delta\Psi_m$ while simultaneously measuring non-phosphorylating respiration. $\Delta\Psi_m$ was calculated using a modified Nernst equation assuming a TPP^+ binding constant of 0.16 (Marcinkeviciute *et al.* 2000). For *P. sungorus* the kinetics of mitochondrial oxidation and phosphorylation systems were determined as previously described (Barger *et al.* 2003), allowing for elasticity analysis of mitochondrial bioenergetics (Ainscow & Brand 1999).

Phospholipids from ground squirrel mitochondrial samples (stored at -80°C) were extracted, separated from other lipids, transesterified and quantified by gas chromatography as previously described (Maillet & Weber 2007).

Results

Hibernation in *S. tridecemlineatus* significantly increased the unsaturation index (2.27 ± 0.05 vs 1.55 ± 0.20) of mitochondrial phospholipids. Dietary 18:2*n*-6, however, had no significant effect on unsaturation, body mass, hibernation bout duration, T_b or metabolic rate during hibernation (Table 1).

When fed moderate levels of 18:2*n*-6 (22 mg g^{-1}, comparable to commercial rodent foods), state-3 respiration was 41% and 35% lower in *S. tridecemlineatus* hibernation than in summer-active controls when measured at 37°C and 31°C, respectively (Figure 1), but there were no differences when measured at 25°C or 10°C. From 37°C to 10°C, Q_{10} values did not differ between the hibernation and summer-active states (hibernation 2.09, summer 1.94), regardless of diet.

Table 1 . The effect of dietary 18:2*n*-6 on hibernation and IMM composition. Values are mean ± SEM, except for the unsaturation index and *n*-6/*n*-3 in summer active animals fed 22 mg 18:2*n*-6 g^{-1}, as sufficient material from only one sample was available. * indicates significant difference regardless of diet.

Dietary 18:2*n*-6 (mg g^{-1})	**16**	**22**	**35**	**55**
Body Mass (g)	209.96 ± 6.0	224.94 ± 4.74	224.80 ± 11.65	233.73 ± 2.91
T_b (°C)	4.96 ± 0.19	4.79 ± 0.12	6.27 ± 1.28	5.73 ± 1.04
MR (µmol O_2 h^{-1} g^{-1})	2.37 ± 0.53	2.83 ± 1.63	1.77 ± 0.61	1.88 ± 0.64
Bout Duration (d)	7.42 ± 1.02	7.73 ± 0.36	6.59 ± 0.96	7.69 ± 0.83
Unsaturation Index				
Summer	1.67 ± 0.17	2.04	1.45 ± 0.49	1.27 ± 0.48
Hibernating	2.29 ± 0.07	2.17 ± 0.13	2.09 ± 0.09	2.17 ± 0.51
***n*-6/*n*-3**				
Summer	5.67 ± 0.16	2.03	1.47 ± 0.81	2.87 ± 1.50
Hibernating	4.24 ± 0.46	4.68 ± 0.66	5.83 ± 0.39	4.79 ± 0.81

Because of limited mitochondrial yield, LEAK kinetics in *S. tridecemlineatus* were measured only at 37°C. In the group fed moderate 18:2*n*-6, LEAK did not differ between hibernation and summer (Figure 2b). For all other diet groups (Figure 2a, c, d) LEAK was significantly reduced in hibernation, i.e. for any value of $\Delta\Psi_m$ the mitochondrial respiration rate was greater in the summer-active condition. Moreover, proton conductance (at 130 mV, data not shown) was significantly greater in the summer for all diets, except the 22 mg g^{-1} 18:2*n*-6 group. Within diet groups, maximal non-phosphorylating respiration rates (similar to state-4; uppermost point in curves from Figure 2) did not differ significantly between summer and hibernation, but maximum membrane potential was significantly higher in hibernation for the 16 and 35 mg 18:2*n*-6 g^{-1} diets, and tended to be higher for the 55 mg 18:2*n*-6 g^{-1} diet. However, in the 22 mg 18:2*n*-6 g^{-1} group, maximal membrane potentials were virtually

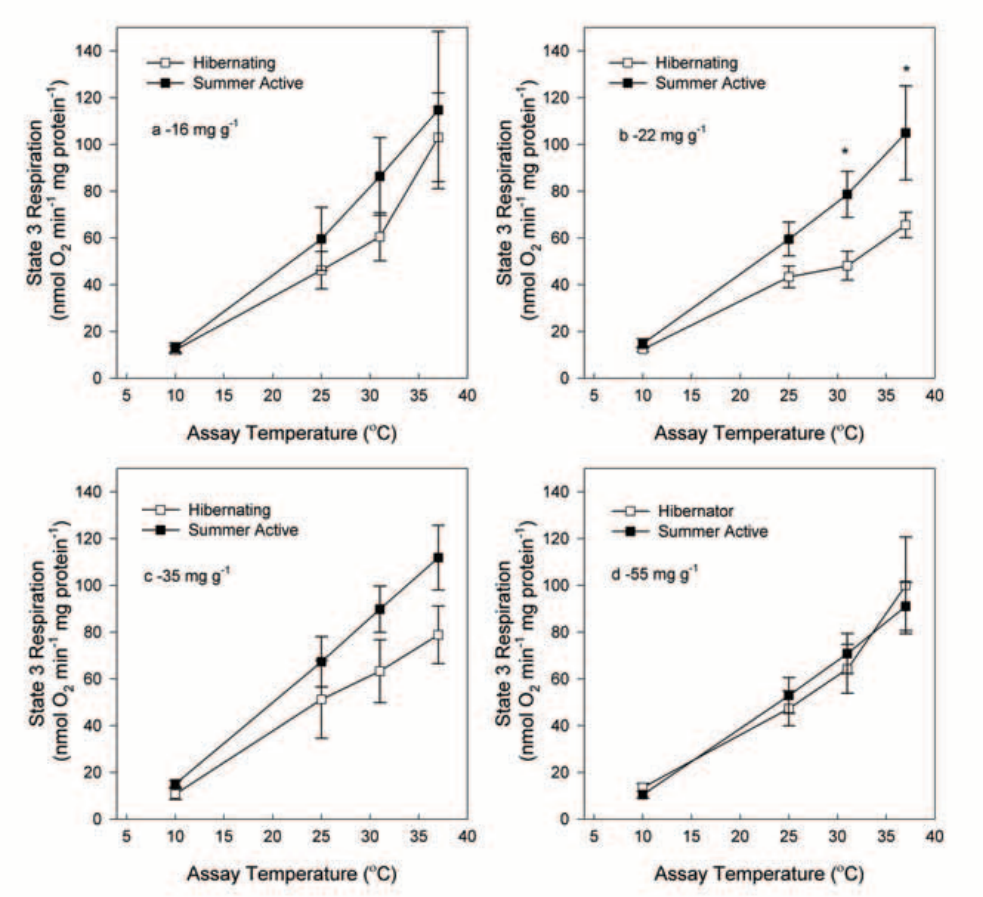

Figure 1. The effect of dietary 18:2*n*-6 and assay temperature on state-3 respiration of liver mitochondria isolated from summer active or hibernating *Spermophilus tridecemlineatus*. Values are means ± SEM. * indicates significant difference.

indistinguishable between the two states (Figure 2b). These patterns were also evident when aspects of LEAK were calculated relative to cytochrome c oxidase activity (COX; an estimate of IMM surface area, data not shown), rather than relative to mitochondrial protein, as reported in Figure 2.

In *P. sungorus*, state-3 respiration was 30% and 22% lower in mitochondria isolated during torpor compared to those isolated during normothermia when measured at 37°C and 30°C, respectively (Figure 3). Elasticity analysis showed that this suppression was the result of decreased substrate oxidation in torpor, with no change in the phosphorylation system (Figure 5a). At 23°C or 15°C state-3 respiration did not differ between normothermia and torpor. Maximal non-phosphorylating respiration did not differ between torpor and normothermia at either assay temperature (Figure 4, uppermost points). Between 37°C and 15°C, the Q_{10} values for

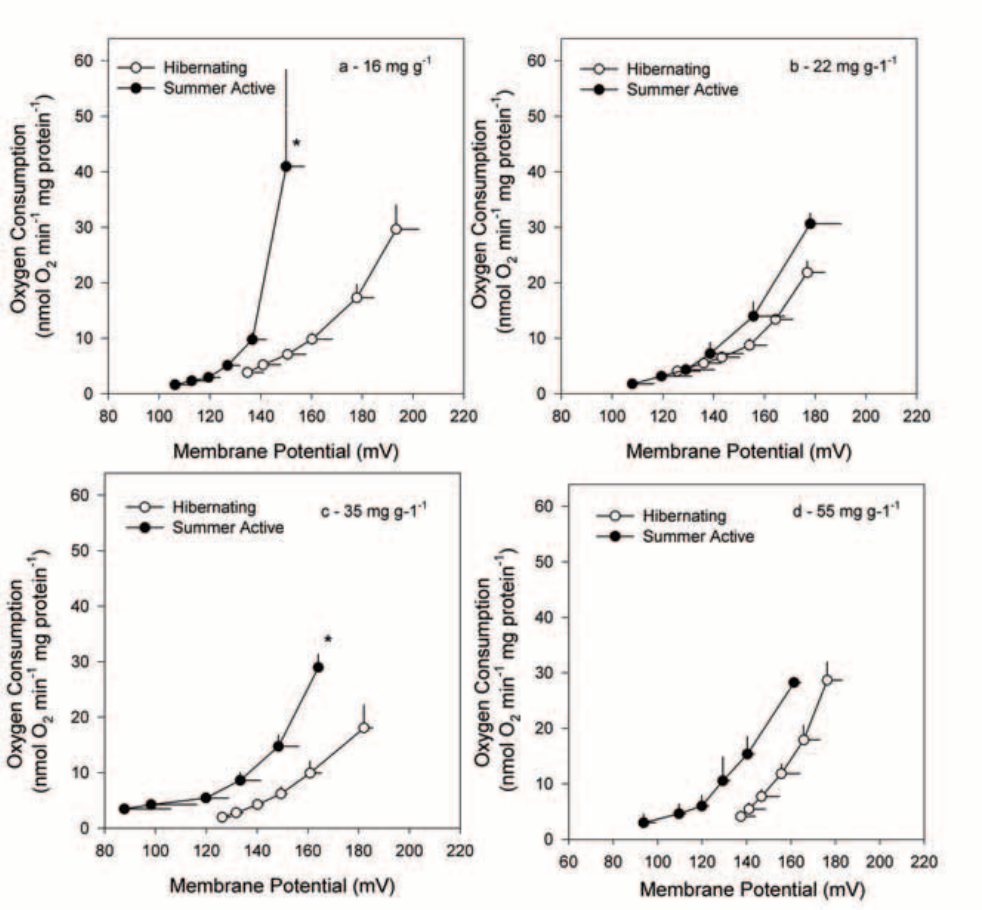

Figure 2. The effect of dietary 18:2*n*-6 on liver mitochondrial proton leak kinetics (at 37°C) in summer active and hibernating *Spermophilus tridecemlineatus*. Values are means ± SEM. * indicates significant difference in maximum membrane potential.

state-3 respiration were 2.26 and 2.24 for normothermia and torpor, respectively. For non-phosphorylating respiration, Q_{10} for normothermia and torpor were 1.94 and 2.12, respectively.

Kinetic curves suggest that the proton permeability of the IMM *increased* in torpor, especially when measured at 15°C (Figure 4). Elasticity analysis allowed us to calculate partial integrated response coefficients (which represent the relative change in respiration that would result from a change in the kinetics of a given oxidative-phosphorylation component if only that component underwent a kinetic change), and confirms the increased LEAK in torpor, at both 37°C and 15°C (Figure 5b).

Discussion

The pattern of state-3 respiration is very similar in hibernation (with moderate dietary PUFA) and daily torpor – significant suppression when measured at high temperatures, but not below about 30°C. This suggests that regulated suppression may be more important during the early stages of entrance into a hibernation/torpor bout when T_b is relatively high. Below these temperatures, passive thermal effects appear to be more important in reducing respiration. In *P. sungorus* between normothermia at 37°C and torpor at 15°C, the Q_{10} of state-3 respiration rate is 2.64. On its own, this value might be interpreted to mean that passive thermal effects alone are responsible for the observed changes in respiration. Our data, however, clearly

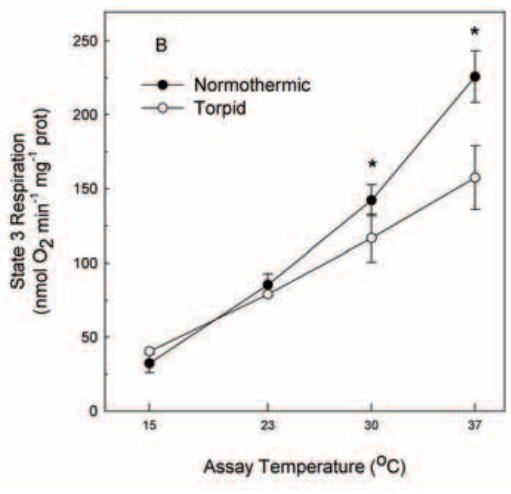

Figure 3. The effect of temperature on liver state-3 mitochondrial respiration in normothermic and torpid *Phodopus sungorus*. Values are means ± SEM. * indicates significant difference.

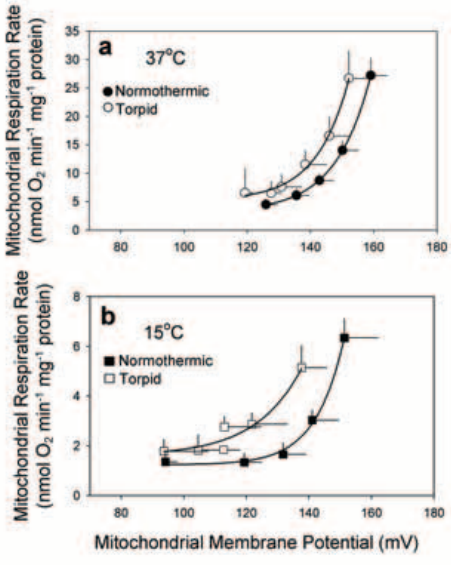

Figure 4. Mitochondrial proton leak kinetics from normothermic and torpid *Phodopus sungorus*. Values are means ± SEM.

demonstrate a regulated, "active" suppression. If Q_{10} values cannot discern active metabolic suppression in our relatively simple, tightly-controlled experimental system, one should heed warnings (e.g. Geiser 2004) when using Q_{10} to analyse whole-animal data in mammalian heterotherms where T_b and metabolic have complex interactions with several physiological and behavioural systems.

At first glance it appears that respiration is reduced in hibernation and daily torpor by similar mechanisms. Our data (Figure 5) suggest that the transition from normothermia to torpor at 37°C involves a suppression of substrate oxidation (i.e. Krebs cycle, electron transport, substrate transport), but not the phosphorylation system (i.e. ATPases, ATP synthase, adenine nucleotide transporter (ANT)). We have demonstrated significant downregulation of electron transport chain flux between complexes II and IV in hibernation (Muleme *et al.* 2006) and daily torpor (Brown *et al.* 2007). In hibernators suppression of state-3 respiration is readily reversible during arousal, implying that regulatory mechanisms respond very quickly. In *P. sungorus*, however, the maximal activities of succinate dehydrogenase (SDH) and COX do not change between normothermia and torpor, and succinate-cytochrome c reductase activity actually increases (J.C.L. Brown unpubl. data), implying that other mechanisms regulate the

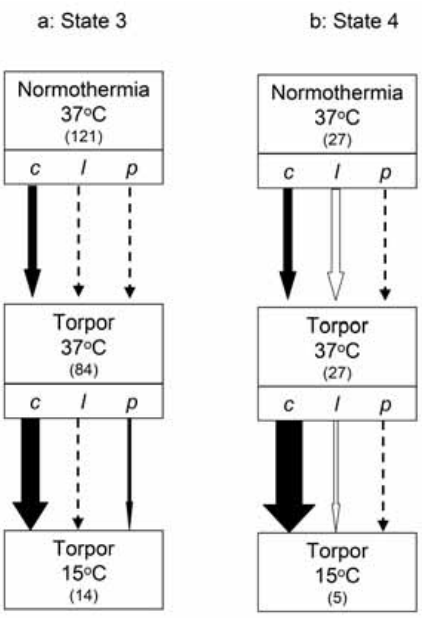

Figure 5. Elasticity analysis of liver mitochondrial metabolism during daily torpor in *Phodopus sungorus*. Each box represents a combination of metabolic state and temperature, and the number is the respiration rate (nmol O_2 min^{-1} mg^{-1} protein). Arrows represent the partial integrated response coefficients (the product of integrated elasticity coefficients and flux control coefficients; see Brown *et al.* (2007) for details) for each component of oxidative phosphorylation. Letters indicate the oxidative phosphorylation component that the arrow represents (by convention, *c*, substrate oxidation; *l*, LEAK; *p*, phosphorylation), and the size of the arrow indicates the relative magnitude of the partial integrated response coefficient. Filled and open arrows represent negative and positive partial integrated response coefficients, respectively, whereas dashed arrows represent zero values. Substrate oxidation accounted for the decrease in state-3 respiration in the transition from normothermia to torpor at 37°C (a). In torpor, the change in temperature from 37°C to 15°C caused decreases in both substrate oxidation and phosphorylation. For non-phosphorylating respiration in the presence of oligomycin (similar to state 4; b) the transition from normothermia to torpor at 37°C decreases substrate oxidation with a corresponding increase in LEAK, resulting in no change in respiration rate. Once in torpor, the drop in temperature from 37°C to 15°C decreased substrate oxidation with a slight increase in LEAK.

decreased substrate oxidation. In hibernation, however, SDH and succinate-cytochrome c reductase are significantly downregulated (Gehnrich & Aprille 1988), suggesting that specific sites of oxidative suppression may differ between hibernation and daily torpor.

Dietary 18:2*n*-6 did not significantly alter the unsaturation of mitochondrial phospholipid fatty acyl components, but hibernation did. An increase in phospholipid unsaturation is assumed to increase membrane fluidity, permitting continued function at low T_b. Kraffe *et al.* (2007), however, propose that saturation changes may alter the activity of specific membrane associated enzymes with minimal effects on fluidity. The membrane phospholipid environment may influence the function of SDH, COX, and ANT, all of which could alter respiration, LEAK, or both (Hulbert 2003; Paradies *et al.* 1993). Increased mitochondrial phospholipid unsaturation correlates with decreased LEAK in hypothyroid rats (Pehowich 1999) and among other mammalian species (Porter *et al.* 1996). In our study, hibernation is associated with increased unsaturation and decreased LEAK in all of the diet groups for which we have sufficient data. Given that cellular membranes appear to be remodelled during arousal from hibernation (Pehowich 1994), it would be interesting to measure LEAK and IMM composition during this phase where whole-animal metabolism and liver mitochondrial respiration are massively activated.

In contrast to hibernation, IMM proton permeability increased in daily torpor, despite decreased substrate oxidation. In fact, LEAK kinetics from normothermia at 37°C overlap significantly with the curve from torpor at 15°C (Figure 6). It appears that the IMM proton conductivity is being defended despite dramatic drops in substrate oxidation and temperature. This "homeoconductivity" may protect hamsters from excessive production of reactive oxygen species (ROS) (Brookes 2005) in the relatively shallow (compared with hibernation at 5°C) hypometabolic state of daily torpor. We are currently comparing rates of liver mitochondrial ROS production between normothermia and torpor. The experiments presented in this paper could not identify potential mechanisms that increased LEAK and/or reduced oxidation in daily torpor, though ANT content does not change (Brown *et al.* 2007). Experiments currently in

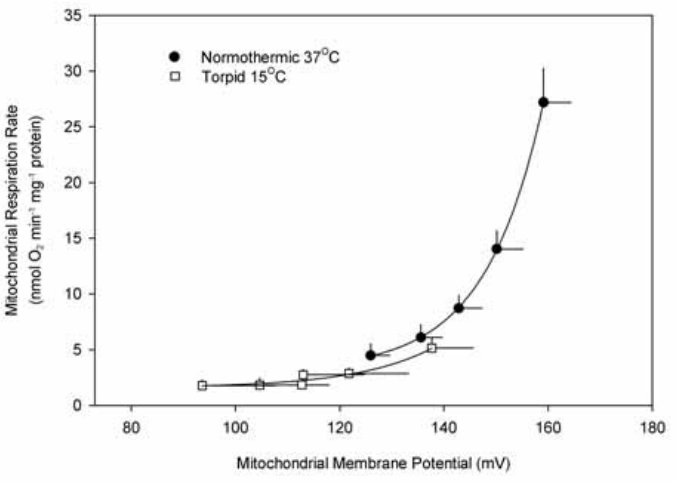

Figure 6.

Homeoconductivity of *Phodopus sungorus* liver mitochondria in the transition from normothermia to daily torpor. The proton leak kinetic curve from active hamsters measured at 37°C (filled circles) overlaps with that measured at 15°C from torpid animals (open squares). Values are means ± SEM.

progress will compare mitochondrial phospholipid headgroups and saturation between normothermia and torpor, and other mechanisms, including mitochondrial K_{ATP} channels, known to regulate LEAK in energy-challenging systems, will also be explored (Buck & Pamenter 2006).

References

AINSCOW, E.K. & BRAND, M.D. 1999. The responses of rat hepatocytes to glucagon and adrenaline. Application of quantified elasticity analysis. *European Journal of Biochemistry* 265:1043–1055.

BARGER, J., BRAND, M.D., BARNES, B.M. & BOYER, B.B. 2003. Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels. *American Journal of Physiology* 284:R1306–R1313.

BRAND, M.D., TURNER, N., OCLOO, A., ELSE, P.L. & HULBERT, A.J. 2003. Proton conductance and fatty acyl composition of liver mitochondria correlates with body mass in birds. *Biochemical Journal* 376:741–748.

BROOKES, P.S. 2005. Mitochondrial H^+ leak and ROS generation: an odd couple. *Free Radical Biology and Medicine* 38:12–23.

BROOKES, S.J., BUCKINGHAM, J.A., TENREIRO, A.M., HULBERT, A.J. & BRAND, M.D. 1998. The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlation with standard metabolic rate and phospholipid fatty acid composition. *Comparative Biochemistry and Physiology* 119B:325–334.

BROOKS, S.P.J. & STOREY, K.B. 1992. Mechanisms of glycolytic control during hibernation in the ground squirrel *Spermophilus lateralis*. *Journal of Comparative Physiology B* 162:23–28.

BROWN, J.C.L., GERSON, A.R. & STAPLES, J.F. 2007. Mitochondrial metabolism during daily torpor in the dwarf Siberian hamster: the role of active regulated changes and passive thermal effects. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* 293:R1833–R1845.

BUCK, L.T. & PAMENTER, M.E. 2006. Adaptive responses of vertebrate neurons to anoxia – matching supply to demand. *Respiratory Physiology and Neurobiology* 154:226–240.

CHAMBERLIN, M. 2004. Top-down control analysis of the effect of temperature on ectotherm oxidative phosphorylation. *American Journal of Physiology* 287:R794–R800.

CREMEL, G., REBEL, G., CANGUILHEM, B., RENDON, A. & WAKSMAN, A. 1979. Seasonal variation of the composition of membrane lipids in liver mitochondria of the hibernator *Cricetus cricetus*. Relation to intramitochondrial innermembanal protein movement. *Comparative Biochemistry and Physiology* 63A:159–167.

DIAZ, M.B., LANGE, M., HELDMAIER, G. & KLINGENSPOR, M. 2004. Depression of transcription and translation during daily torpor in the Djungarian hamster (*Phodopus sungorus*). *Journal of Comparative Physiology B* 174:495–502.

FRANK, C.L. 1992. The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophlilus lateralis*). *Physiological Zoology* 65:906–920.

GEHNRICH, S.C. & APRILLE, J.R. 1988. Hepatic gluconeogenesis and mitochondrial function during hibernation. *Comparative Biochemistry and Physiology* 91B:11–16.

GEISER, F. 1991. The effect of unsaturated and saturated dietary lipids on the pattern of daily torpor and the fatty acid composition of tissues and membranes of the deer mouse (*Peromyscus maniculatus*). *Journal of Comparative Physiology B* 161:590–597.

GEISER, F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology* 66:239–273.

GEISER, F. & KENAGY, G.J. 1987. Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. *American Journal of Physiology* 252:R897–R901.

HELDMAIER, G. & ELVERT, R. 2004. How to enter torpor: thermodynamic and physiological mechanisms of metabolic depression. Pp. 185–198 in Barnes, B.M. & Carey, H.V. (eds.) *Life in the cold*. University of Alaska Fairbanks, Fairbanks.

HELDMAIER, G., KLINGENSPOR, M., WERNEYER, M., LAMPI, G.J., BROOKS, S.P.J. & STOREY, K.B. 1999. Metabolic adjustments during daily torpor in the Djungarian hamster. *American Journal of Physiology* 276:E896–E906.

HULBERT, A.J. 2003. Life, death and membrane bilayers. *Journal of Experimental Biology* 206:2303–2311.

KRAFFE, E., MARTY, Y. & GUDERLEY, H. 2007. Changes in mitochondrial oxidative capacities during thermal acclimation of rainbow trout *Oncorhynchus mykiss*: roles of membrane proteins, phospholipids and their fatty acid compositions. *Journal of Experimental Biology* 210:149–65.

MACDONALD, J.A. & STOREY, K.B.1999. Regulation of ground squirrel Na^+K^+-ATPase activity by reversible phosphorylation during hibernation. *Biochemical and Biophysical Research Communications* 245:424–429.

MAILLET, D. & WEBER, J.M. 2007. Relationship between n-3 PUFA content and energy metabolism in the flight muscles of a migrating shorebird: evidence for natural doping. *Journal of Experimental Biology* 210:413–20.

MARCINKEVICIUTE, A., MILDAZIENE, V., CRUMM, S., DENIM, O., HOEK, J.B. & KHOLODENKO, B. 2000. Kinetics and control of oxidative phosphorylation in rat liver mitochondria after chronic ethanol feeding. *Biochemical Journal* 349:519–526.

MARTIN, S.L., MANIERO, G.D., CAREY, C. & HAND, S.C. 1999. Reversible depression of oxygen consumption in isolated liver mitochondria during hibernation. *Physiological and Biochemical Zoology* 72:255–264.

MULEME, H.M., WALPOLE, A.C. & STAPLES, J.F. 2006. Mitochondrial metabolism in hibernation: metabolic suppression, temperature effects, and substrate preferences. *Physiological and Biochemical Zoology* 79:474–83.

PARADIES, G., RUGGIERO, F.M., DINOI, P., PETROSILLO, G. & QUAGLIARIELLO, E. 1993. Decreased cytochrome oxidase activity and changes in phospholipids in heart mitochondria from hypothyroid rats. *Archives of Biochemistry and Biophysics* 307:91–95.

PEHOWICH, D.J. & WANG, L.C.H. 1984. Seasonal changes in mitochondrial succinate dehydrogenase activity in a hibernator, *Spermophilus richardsonii*. *Journal of Comparative Physiology* 154:495–501.

PEHOWICH, D.J. 1994. Modification of skeletal muscle sarcoplasmic reticulum fatty acyl composition during arousal from hibernation. *Comparative Biochemistry and Physiology* 109B:571–578.

PEHOWICH, D.J. 1999. Thyroid hormone status and membrane n-3 fatty acid content influence mitochondrial proton leak. *Biochimica et Biophysica Acta* 1411:192–200.

PLATNER, W.S., STEFFEN, D.G., TEMPEL, G. & MUSACCHIA, X.J. 1976. Mitochondrial membrane liver fatty acids of liver and hear of the euthermic and hibernating ground squirrel (*Citellus tridecemlineatus*). *Comparative Biochemistry and Physiology* 53A:279–283.

PORTER, R.K., HULBERT, A.J. & BRAND, M.D. 1996. Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition. *American Journal of Physiology* 271:R1550–1560.

ROBERTS, J.C. & CHAFFEE, R.R. 1972. Suppression of mitochondrial respiration in hibernation and its reversal in arousal. Pp. 101–107 in Smith, R.E., Shields, J.C., Hannon, P.P. & Horwitz, B.A. (eds), *Proceedings of the International Symposium on Environmental Physiology: Bioenergetics and Temperature Regulation*. FASEB, Bethesda, MD.

ROLFE, D.F.S., NEWMAN, J.M.B., BUCKINGHAM, J.A., CLARK, M.G. & BRAND, M.D. 1999. Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver and to SMR. *American Journal of Physiology* 276:C692–699.

STUART, J.A., GILLIS, T.E. & BALLANTYNE, J.S. 1998. Compositional correlates of metabolic depression in the mitochondrial membranes of estivating snails. *American Journal of Physiology* 275:R1977–R1982.