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Strategies of biochemical adaptation for hibernation in a South American marsupial, *Dromiciops gliroides*: 4. Regulation of pyruvate dehydrogenase complex and metabolic fuel selection

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ABSTRACT

Mammalian hibernation is characterized by extensive adjustments to metabolism that typically include suppression of carbohydrate catabolism and a switch to triglycerides as the primary fuel during torpor. A crucial locus of control in this process is the pyruvate dehydrogenase complex that gates carbohydrate entry into the tricarboxylic acid cycle. Within the complex, the E1 enzyme pyruvate dehydrogenase (PDH) is the main regulatory site and is subject to inhibitory phosphorylation at three serine residues (S232, S293, S300). To determine if marsupial hibernators show a comparable focus on PDH to regulate fuel metabolism, the current study explored PDH control by site-specific phosphorylation in the South American marsupial, monito del monte (*Dromiciops gliroides*). Luminex multiplex technology was used to analyze PDH responses in six tissues comparing control and hibernating (4 days continuous torpor) animals. Total PDH content did not change significantly during hibernation in any tissue but phospho-PDH content increased in all. Heart PDH showed increased phosphorylation at all three sites by 8.1-, 10.6- and 2.1-fold for S232, S293 and S300, respectively. Liver also showed elevated p-S300 (2.5-fold) and p-S293 (4.7-fold) content. Phosphorylation of S232 and S293 increased significantly in brain and lung but only S232 phosphorylation increased in kidney and skeletal muscle. The results show that PDH suppression via enzyme phosphorylation during torpor is a conserved mechanism for inhibiting carbohydrate catabolism in both marsupial and eutherian mammals, an action that would also promote the switch to fatty acid oxidation instead.

1. Introduction

Many small mammals (including monotremes, marsupials and eutherians) utilize daily torpor and/or multi-day/week bouts of hibernation to endure periods of food limitation and/or low environmental temperatures (McNab, 1978; Wang and Lee, 1996; Storey and Storey, 2010; Ruf and Geiser, 2015). Best known are the obligate seasonal hibernators that live in climates with long cold winters but more flexible patterns are found in other situations or more generally in species located basally in the phylogeny of mammals (Nespolo et al., 2010). Indeed, monotremes and many small marsupials show considerable heterothermy. Hibernation is typically characterized by a decrease in body temperature (T_b) to near ambient values, strong reductions in physiological parameters (e.g. heartbeat and respiratory rates), and suppression of basal metabolic rate (BMR) (typically to < 10% of euthermic values) (Nespolo et al., 2010; Storey and Storey, 2010; Ruf and Geiser, 2015). Many small hibernating species also employ daily torpor in the non-hibernating seasons to provide energy savings during their daily resting phase. One such species is the monito del monte (*Dromiciops gliroides*), a small South American marsupial native to the Valdivian forests of southern Chile. *D. gliroides* can employ either daily torpor in the warmer months or prolonged hibernation for days or weeks in the

Abbreviations: MRD, metabolic rate depression; PDC, pyruvate dehydrogenase complex; PDP, Pyruvate dehydrogenase phosphatase; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; RQ, respiratory quotient; TCA, tricarboxylic acid

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winter as survival strategies (Greer, 1965; Grant and Temple-Smith, 1987; Bozinovic et al., 2004; Nespolo et al., 2010). However, initiation of hibernation in monito del monte is considered to be opportunistic rather than an engrained circannual or seasonal event as is common in eutherians (Nespolo et al., 2010), and this makes *D. gliroides* a fascinating model for studying the torpid state of facultative hibernators.

A common biochemical signature in hibernation is a change in metabolic fuel use. Animals typically switch from catabolizing carbohydrates and other nutrients acquired during feeding to a primary reliance on lipid oxidation arising from the mobilization of large internal fat reserves that are accumulated in the weeks/months leading up to the hibernation season (Buck and Barnes, 2000; Dark, 2005; Storey and Storey, 2010). Indeed, measured respiratory quotients (RQ) of ~0.7 during hibernation in various species are indicative of the primary use of fatty acid oxidation to generate ATP (Lyman and Chatfield, 1955; Burlington and Klain, 1967; Buck and Barnes, 2000). The switch to lipid catabolism in a starved or torpid state is typically facilitated by strong inhibitory controls on carbohydrate catabolism. Such carbohydrate sparing helps to preserve carbohydrates for tissues that must continue a high dependence on carbohydrates as primary fuel sources (e.g. brain). One approach to assessing changes in fuel metabolism during torpor/hibernation is to evaluate the responses of enzymatic loci that are known to be crucial regulatory sites involved in carbohydrate sparing. A main enzyme in the regulation of this response is pyruvate dehydrogenase (PDH).

The mammalian pyruvate dehydrogenase complex (PDC) catalyzes the irreversible oxidative decarboxylation of pyruvate to produce acetyl-CoA, CO_2, and NADH. Acetyl-CoA can then be oxidized by mitochondria to produce ATP or channeled into various biosynthetic pathways including production of fatty acids in certain tissues. The PDC is one of the largest and best-characterized multi-enzyme complexes and contains three main catalytic subunits: pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), and dihydrolopoamide dehydrogenase (E3). Also present in the complex are one E3 binding protein (E3BP) and two regulatory enzymes, pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP) (Patel and Korotchkina, 2003). A main factor in PDH regulation is reversible protein phosphorylation that responds to changes in the ratios of $NADH/NAD^+$, acetyl-CoA/CoA, and plasma glucose levels as well as to hormones including insulin and glucocorticoids and metabolic conditions such as starvation (Patel and Roche, 1990; Korotchkina and Patel, 2001; Kolobova et al., 2001; Patel and Korotchkina, 2003). Phosphorylation of PDH (E1) at S232, S293 or S300 by one of 4 PDKs inhibits its activity (Harris et al., 2002). Oppositely, PDH is activated by inhibition of PDKs, allowing PDPs to dephosphorylate the enzyme to reactivate it; conditions promoting this include high cellular levels of pyruvate and ADP. As such, PDH is a crucial regulatory site in metabolism that functions as a metabolic switch in the control of carbohydrate versus fatty acid oxidation (Zhou et al., 2001; Patel and Korotchkina, 2006; Rardin et al., 2009). Indeed, adjustments to PDH activity are considered to be an indicator of the status of carbohydrate use as a fuel, and because PDH activity typically correlates negatively with fatty acid oxidation, changes in PDH activity are also useful indicators of the relative state of fuel use (carbohydrate versus lipid) in tissues. During torpor/hibernation, suppression of PDH activity would also reduce acetyl-CoA availability for biosynthetic reactions and thereby contribute to the overall suppression of anabolic functions that is a common characteristic of hypometabolism (Storey and Storey, 2004, 2010).

The current study hypothesized that PDH activity would be strongly suppressed during hibernation in *D. gliroides* tissues. Relative changes in the levels of total PDH and the phosphorylation state at S232, S293, and S300 were assessed in six organs of *D. gliroides*, comparing torpid and euthermic animals. Our results indicated inhibition of PDH in all six organs due to elevated phosphorylation at one, two or all three serine sites during hibernation. Such inhibition would provide a mechanism to achieve carbohydrate sparing and enhance lipid oxidation as a fuel in the tissues of torpid animals.

2. Materials and methods

2.1. Animal protocol

Expanded details of animal studies are available in Wijenayake et al. (2018). Adult *D. gliroides* were collected, handled, acclimated at 20 ± 1°C with 12 h:12 h photoperiod for 2 weeks and then induced to hibernate by reducing ambient temperature in a climate-controlled chamber over 2–3 days to 10 °C. All animals had entered torpor by ~15°C and were sampled after 4 days of continuous hibernation. Torpor was assessed according to physiological criteria described by Franco et al. (2013). Active animals at 20 °C were sampled as controls. Tissue samples were collected, immediately frozen in liquid nitrogen, and then packed in a dry shipper and air freighted to Carleton University (Ottawa, Canada) where they were stored at − 80 °C until use.

All animal protocols were approved by the Ethics of Animal Experiments Committee of the Universidad Austral de Chile and were conducted in accordance with guidelines of the American Society of Mammologists (Sikes et al., 2011) and authorized by the Chilean Agriculture and Livestock Bureau (SAG: Servicio Agricola Ganadero de Chile, permit No. 1054/2014).

2.2. Total protein extraction

Soluble protein extracts from 30 to 50 mg samples of frozen tissues were prepared in lysis buffer as described in Wijenayake et al. (2018) and protein concentrations were determined by the Coomassie Blue dye binding method using the Bio-Rad prepared reagent. All samples were then adjusted to a final soluble protein concentration of 10 μg/μL with lysis buffer and stored at − 80 °C until use.

2.3. Luminex multiplex analysis

A pyruvate dehydrogenase (PDH) complex magnetic bead panel (Millipore, Cat#. PDHMAG-13 K) was used to analyze total PDH protein levels as well as PDH phosphorylation at S232, S293, and S300. Aliquots of tissue protein extracts were appropriately diluted with the assay buffer provided in the kit (Cat#. 43-010) and then 25 μL aliquots containing 13 μg of total protein were added to each sample well. Additional wells were prepared as blanks or with aliquots of manufacturer-provided positive and negative controls that were run to assure functionality and performance of the assay. Unstimulated HepG2 cell lysate (Millipore, Cat#. 47-234) and dichloroacetate (DCA)-treated HepG2 cell lysate (Millipore, 47-232) were used as the positive and negative controls, respectively, prepared according to the manufacturer's instructions.

To conduct the assay, capture antibody-conjugated beads (premixed) were sonicated for 30 s, vortexed, and then 25 μL of aliquots were added into each well. The plate was sealed and covered with aluminum foil to prevent light exposure and then incubated for 2 h on a plate shaker with agitation at room temperature. Using a magnetic plate to retain the beads, the liquid was removed by gentle inversion, followed by washing the plate 3 times with 1 × wash buffer (Millipore, Cat# L-WB) as per manufacturer instructions. An aliquot of detection antibody (50 μL) (Millipore, Cat# PDH-1013) was then added to each well and the plate was sealed, covered with aluminum foil, and allowed to incubate for 1 h at room temperature with agitation. The plate was again washed 3 times before 50 μL aliquots of 1 × streptavidin-phycoerythrin (SAPE) (Millipore, Cat#. L-SAPE5) were added for a 30 min incubation at room temperature with mild agitation and avoiding light. Finally, the plate was washed again and the beads were re-suspended in 100 μL of Sheath Fluid. After 5 min shaking on a plate shaker, the plate was read on a Luminex 200 instrument (Luminex, Austin, TX) using Milliplex Analyst software (Millipore, Billerica, MA). Beads were analyzed using the following instrument parameters: Events: 50 beads; Sample Size: 50 μL; Gate settings: 8000 to 15,000.

2.4. Data and statistics

Data were collected as median florescence intensity (MFI) values and expressed as mean ± SEM for control and hibernating groups. Statistical analysis used the Student's *t*-test with Sigmaplot 11 software (Systat Software Inc., San Jose, CA); $p < 0.05$ was considered a statistically significant difference.

3. Results

PDH protein levels and the relative phosphorylation of PDH at serine residues S232, S293 and S300 were measured in six tissues from *D. gliroidies* comparing control and hibernation (4 days continuous torpor). Total PDH protein levels did not change in any tissue between the two states (Figs. 1–6). However, all tissues showed significant changes in PDH phosphorylation between control and hibernating animals.

In the liver, relative phosphorylation of PDH at S293 and S300 increased by 4.72 ± 0.83 fold and 2.48 ± 0.14 fold, respectively (both $p < 0.05$), during hibernation whereas p-PDH (S232) content did not change compared to controls (Fig. 1). In the heart, relative phosphorylation rose significantly at all three serine sites; increases were 8.15 ± 2.0 fold for p-PDH (S232), 10.6 ± 2.66 fold for p-PDH (S293), and 2.09 ± 0.34 fold for p-PDH (S300) (all $p < 0.05$) (Fig. 2). In brain, significant changes were noted in p-PDH (S232) and p-PDH (S293) during hibernation with fold increases of 5.74 ± 1.35 fold and 3.57 ± 0.70 fold ($p < 0.05$), respectively (Fig. 3). These same two residues showed increased phosphorylation in lungs with values in hibernation being 1.43 ± 0.21 fold higher for S232 and 1.50 ± 0.14 fold higher for S293 as compared with controls (both $p < 0.05$) (Fig. 4). In kidney p-PDH (S232) content increased significantly by 2.55 ± 0.67 fold ($p < 0.05$) and in skeletal muscle the same residue showed a 3.69 ± 1.04 fold increase ($p < 0.05$) during hibernation as compared to the euthermic control (Figs. 5, 6). Phosphorylation of the other two residues was unaffected in these latter two tissues.

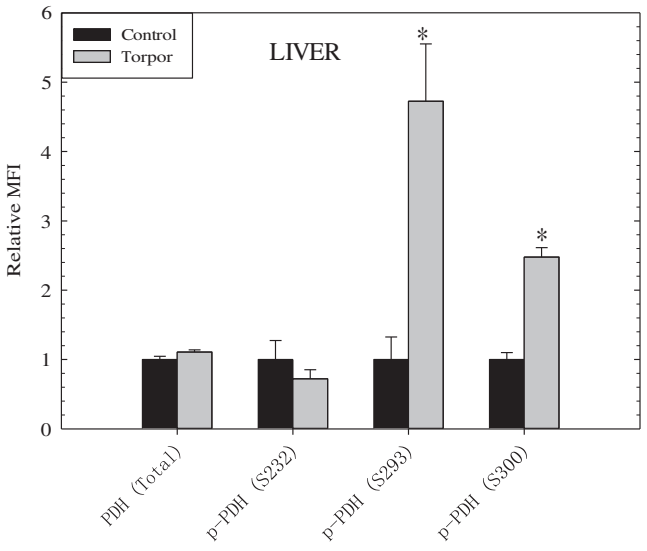

Fig. 1. Changes in total PDH protein and PDH phosphorylation at S232, S293 and S300 in *D. gliroides* liver during hibernation (four days of continuous torpor). Data were quantified as median fluorescence intensity (MFI) for each biological replicate and are shown as mean ± SEM, $n = 4$ independent samples from different animals. For each parameter, values for the hibernating condition were expressed relative to the mean control value that was standardized to 1.0. Data were analyzed using a two-tailed Student's *t*-test. * - Significantly different from the corresponding control value, $p < 0.05$.

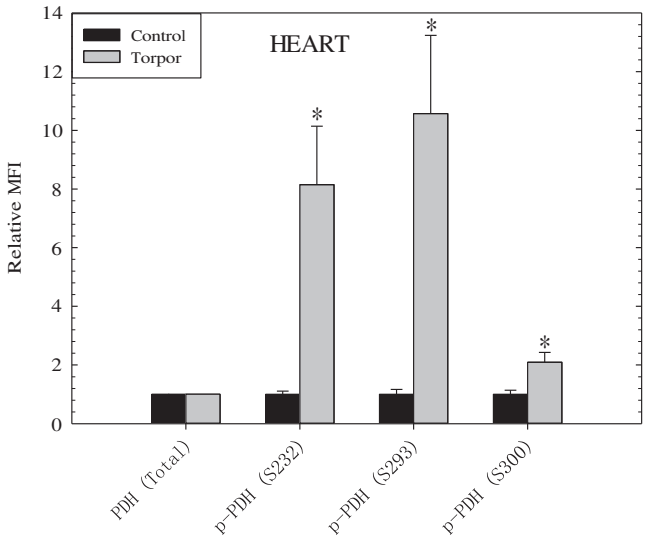

Fig. 2. Changes in total PDH protein and PDH phosphorylation at S232, S293 and S300 in *D. gliroides* heart during hibernation. Other information as in Fig. 1.

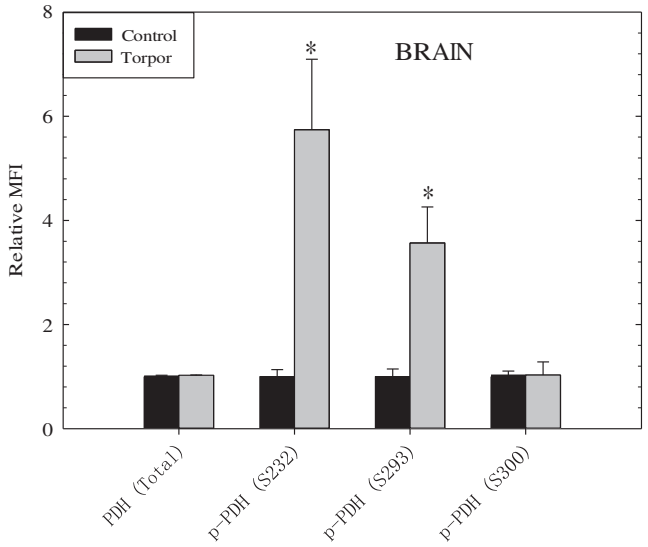

Fig. 3. Changes in total PDH protein and PDH phosphorylation at S232, S293 and S300 in *D. gliroides* brain during hibernation. Other information as in Fig. 1.

4. Discussion

Physiological adaptations along with metabolic rate depression and the activation of cytoprotective pathways are some of the well-known characteristics of hibernation in eutherian mammals (Storey, 1989; Storey and Storey, 2004, 2010). However, the molecular machinery behind metabolic fuel selection to achieve a primary reliance on β-oxidation of fats (Kelley et al., 1993; Dark, 2005) during hibernation still warrants further study. Early on, Tashima et al. (1970) showed that hibernating mammals produce very little CO_2 from glucose catabolism and suggested that pyruvate was being blocked from entering the TCA cycle. Zimny and Tyrone (1957) also reported that plasma lactate concentration was reduced in the torpid state. Both were indications of reduced glucose breakdown. Since then, evidence of a metabolic shift from carbohydrate to lipid dependence during torpor has been reported in multiple eutherian species.

PDH is the rate-limiting enzyme that regulates the catabolism of pyruvate arising from carbohydrate breakdown (Patel and Korotchkina, 2006). PDH is sensitive to a number of allosteric regulators and as well

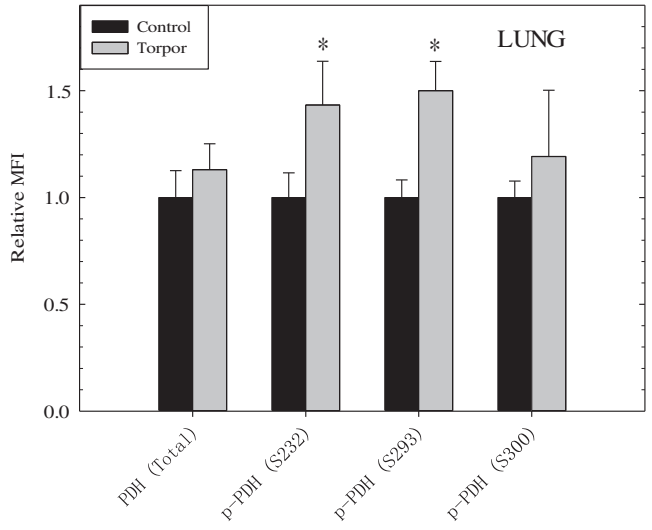

Fig. 4. Changes in total PDH protein and PDH phosphorylation at S232, S293 and S300 in *D. gliroides* lung during hibernation. Other information as in Fig. 1.

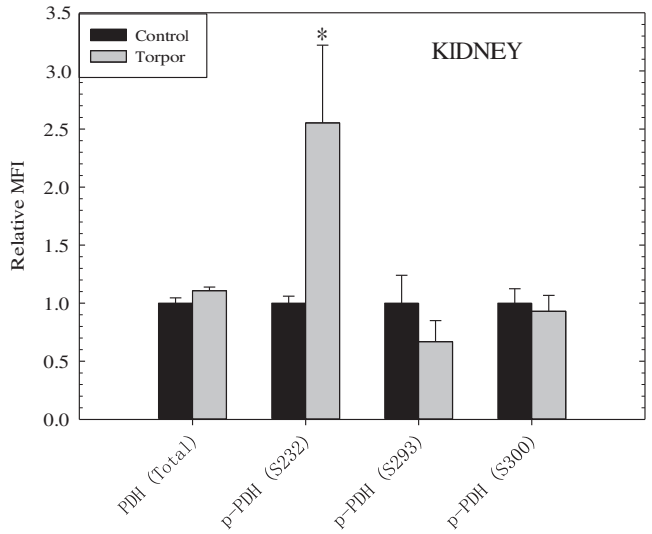

Fig. 5. Changes in total PDH protein and PDH phosphorylation at S232, S293 and S300 in *D. gliroides* kidney during hibernation. Other information as in Fig. 1.

as to strong inhibitory control via phosphorylation at S232, S293, and/or S300 residues by PDH kinases. High ratios of acetyl-CoA/CoA, NADH/NAD^+, or ATP/ADP facilitate PDH phosphorylation and inhibition (Patel and Roche, 1990; Rardin et al., 2009). Overall, the current data showing hibernation-responsive tissue-specific phosphorylation of PDH in *D. gliroides* is indicative of a reduction in PDH enzymatic activity during torpor in all six tissues analyzed. This concurs with previous reports of PDH inhibition in eutherian hibernators (e.g. ground squirrels, bats, jumping mice) (Brooks and Storey, 1992; Storey, 1997; Buck et al., 2002; Wijenayake et al., 2017), as well as during daily torpor in species including gray mouse lemurs and hamsters (Heldmaier et al., 1999; Tessier et al., 2015). Thus, the evidence suggests that carbohydrate sparing is a feature of torpor/hibernation in marsupial species, as it is in eutherians.

Liver of *D. gliroides* showed a 4.7-fold increase in phosphorylation at S293 and a 2.5-fold increase in S300 phosphorylation in response to torpor (Fig. 1). As the major metabolic center of the body and the largest site of glycogen storage, liver regulates the glucose supply to most organs, as well as catalyzing a host of other functions that are vital for hibernation survival, including ketogenesis, gluconeogenesis, and

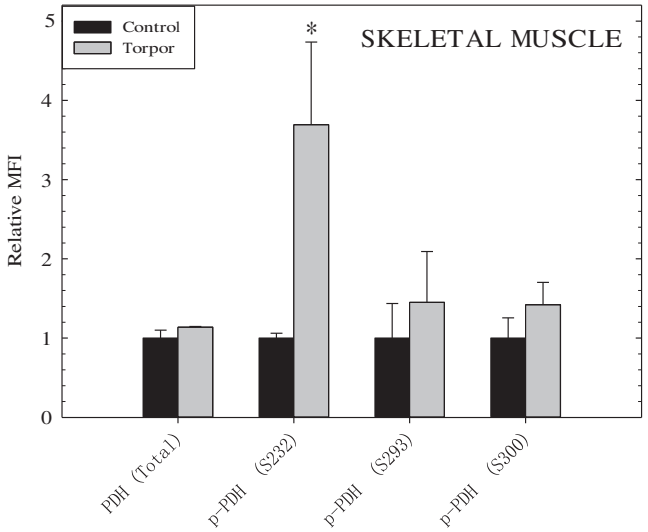

Fig. 6. Changes in total PDH protein and PDH phosphorylation at S232, S293 and S300 in *D. gliroides* skeletal muscle during hibernation. Other information as in Fig. 1.

detoxification of xenobiotics. A strong reduction in liver PDH activity and increased reliance on fatty acid oxidation is part of the glucose sparing effect, in which liver glycogen stores are reserved to supply tissues that have a continuing high reliance on glucose, such as brain and red blood cells (Storey, 1997; Storey and Storey, 2010; Wijenayake et al., 2017). Although glucose sparing is clearly indicated by our results for PDH regulation during hibernation in *D. gliroides*, other lines of evidence that would support this are still unresolved. For example, RQ values are normally about 1.0 for carbohydrate oxidation and 0.7 for lipid oxidation but available RQ data for *D. gliroidies* is inconsistent and influenced by food availability and ambient temperature (Nespolo et al., 2010). Furthermore, control of liver PDH for the purpose of glucose sparing probably also needs to be integrated with another function in monito del monte liver since liver appears to be the site of nonshivering thermogenesis in marsupial species (Villarin et al., 2003). Evidence consistent with this function was provided in the previous papers in this series on *D. gliroides*: changes in cell signaling during hibernation indicated possible cell growth responses in liver (Wijenayake et al., 2018, this issue; Luu et al., 2018a, this issue) and increased MDM2 levels suggested stimulation of fatty acid oxidation in liver (Luu et al., 2018b, this issue). Although much remains to be explored about marsupial thermogenesis, PDH inhibition to restrict carbohydrate consumption would be crucial during lipid-based thermogenesis in liver. Furthermore, PDH inhibition is also consistent with an overall suppression of glycolysis during torpor as seen in studies of liver enzyme responses in the meadow jumping mouse, *Zapus hudsonius*, where three main regulatory enzymes of carbohydrate catabolism (phosphofructokinase, pyruvate kinase, PDH were all suppressed in parallel (Storey, 1987a, 1987b).

In the heart, PDH was phosphorylated at all three serine residues with 8-, 10- and 2-fold increases at S232, S293 and S300, respectively, indicating strong suppression of enzyme activity (Fig. 2). Similar to the liver, this indicates reduced dependence on carbohydrates as a fuel source during hibernation and a probable switch to a main dependence on fatty acid oxidation. Indeed, upregulation of triglyceride lipase in heart is a prominent event of hibernation in ground squirrels (Andrews et al., 1998; Dark, 2005). Strong suppression of PDH in heart has also been reported in small eutherian hibernators: e.g. the percentage of PDH in the active, non-phosphorylated form fell from 15% in euthermia to 1% in hibernation in heart of meadow jumping mice (*Zapus hudsonius*) (Storey, 1989) and from 5.3% to 1.5% in bat heart (*Myotis lucifugus*) (Storey, 2012). Moreover, cardiac PDH activity was strongly reduced via phosphorylation in 13-lined ground squirrels during

hibernation (Wijenayake et al., 2017). In addition to carbohydrate sparing, suppression of PDH activity in heart probably also reflects the dramatic reduction in heart rate (ie. less energy expenditure needed) that occurs during torpor; in eutherian hibernators this can be a decrease to < 5% of the euthermic value (Storey, 1989).

D. gliroides brain also showed large increases in PDH phosphorylation at S293 (3.6-fold) and S232 (5.7-fold) during hibernation, indicating a strong suppression of PDH activity (Fig. 3). Phosphorylation-mediated inhibition of PDH also occurred in brain of torpid bats (*M. lucifugus*) where the percentage of PDH in the active (non-phosphorylated) form dropped from 63% in euthermia to just 9% during torpor (Storey, 2012). Again, PDH suppression may reflect both an overall decrease in metabolic demand at the reduced body temperature of the torpid state and a restructuring of fuel use. Although brain is typically the most glucose-dependent vertebrate organ, the brain of hibernating eutherians can also catabolize ketone bodies (acetoacetate, β-hydroxybutyrate) that are produced as byproducts of fatty acid oxidation in liver and delivered via the blood (Dark, 2005). Indeed, plasma ketone concentrations are known to rise substantially during hibernation. Furthermore, heart and skeletal muscle preparations from hibernating ground squirrels showed reduced uptake of glucose when incubated in the presence of physiological concentrations of ketones (Krilowicz, 1985). Overall then, it appears that glucose sparing, mediated by PDH inhibition as well as adaptations that promote oxidation of fatty acid and ketone fuels, is a general strategy of organ metabolism in the hibernating state for both marsupial and eutherian hibernators.

All other organs assessed in *D. gliroides* also showed increased PDH phosphorylation at one or more serine residue, again indicative of PDH suppression in the torpid state. Lung showed elevated phosphorylation of S232 and S293 (Fig. 4) whereas kidney and skeletal muscle both showed increased S232 phosphorylation (Figs. 5, 6). Like the other tissues PDH inhibition in lung, kidney, and skeletal muscle during hibernation could be important to both global metabolic rate depression and a switch to a largely lipid-based metabolism. Comparable responses for kidney have been reported for eutherian hibernators; kidney PDH activity dropped by 97% in hibernating *S. lateralis* (Brooks and Storey, 1992) whereas kidney PDH activity in *Z. hudsonius* fell from 29% active in euthermia to 1% active in hibernating animals (Storey, 1989).

Finally, a few comments on the differences in phosphosite responses among the organs should also be made. Clearly, there were tissue-specific differences in the effects of hibernation on the relative phosphorylation of the three PDH phosphosites but this does not allow us to draw quantitative conclusions about the strength or importance of PDH suppression in the different tissues. PDH is inhibited by phosphorylation at any of the three sites (S232, S293, S300) (Patel and Korotchkina, 2006). Furthermore, the four isozymes of PDK (that catalyze the phosphorylation) have (a) different tissue distributions, (b) can preferentially phosphorylate different phosphosites, and (c) are differentially responsive to various activators and inhibitors (Patel and Korotchkina, 2006). The two PDH phosphatase isozymes (that reverse PDK effects) also show different tissue distributions and responses to allosteric modifiers (Patel and Korotchkina, 2006). This provides enormous flexibility for the regulation of PDH in response to multiple input signals, but also means that phosphorylation control over PDH in response to hibernation signal(s) may be mediated by different phosphosites in different organs. Overall, all tissues examined in this study exhibited hibernation-responsive inhibition of PDH via increased phosphorylation of the enzyme. Coupled with the data from multiple eutherian species, this indicates that PDH is a major conserved site for the regulation of carbohydrate fuel use during mammalian torpor/hibernation.

5. Conclusions

The four papers in this series on the microbiotheriid marsupial, *D. gliroides*, have examined the responses to hibernation by a range of biochemical markers – molecular targets representing stress-responsive signal transduction pathways (Wijenayake et al., 2018), regulators of protein synthesis (Luu et al., 2018a), chaperones that provide cytoprotection and cell cycle markers (Luu et al., 2018b) and the control of carbohydrate fuel use (this paper). Overall, these provide a broad overview of key molecular characteristics of hibernation in this relic marsupial line and allow us to compare and contrast features of marsupial versus eutherian hibernation. In general, our analysis indicated strong conservation of molecular mechanisms of hibernation between *D. gliroides* and data from many previous studies of eutherian hibernators. These included upregulation of heat shock protein chaperones, indicators of cell cycle suppression, strong inhibitory control over carbohydrate catabolism at the PDH locus as well as and the involvement of various signaling pathways in hibernation control. This is perhaps not surprising since these mechanisms are also common features of environmental-stress induced hypometabolism in both lower vertebrates and invertebrates (Storey and Storey, 2004, 2010). However, some novel responses were identified, primarily localized to liver. These included enhanced MAPK signaling (Wijenayake et al., 2018) and activation of Akt and key targets of the Akt pathway (Luu et al., 2018a) in *D. gliroides* liver that we propose could be linked with elevating oxidative capacity during torpor to support a thermogeneic role for marsupial liver. Furthermore, our studies identified MDM2 as a previously unappreciated potential participant in hibernation control. MDM2 has been linked with cytoprotective actions and cell cycle control and, of particular interest to hibernation, also acts as a sensor that stimulates fatty acid oxidation in response to nutrient depletion (such as during fasting or dietary restriction). Hence, a combination of MDM2 upregulation and PDH suppression in *D. gliroides* tissues is a strong indicator of the importance of the metabolic switch to a primary reliance on fatty acid oxidation to fuel torpor. The involvement of MDM2 in eutherian torpor/hibernation certainly deserves further exploration.

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