Tissue-specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus*

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Lewis JM, Driedzic WR. Tissue-specific changes in protein synthesis associated with seasonal metabolic depression and recovery in the north temperate labrid, *Tautogolabrus adspersus*. Am J Physiol Regul Integr Comp Physiol 293: R474–R481, 2007. First published March 22, 2007; doi:10.1152/ajpregu.00594.2006.—The tissue-specific changes in protein synthesis were tracked in relation to the seasonal metabolic depression in cunner (*Tautogolabrus adspersus*). In vivo protein synthesis rate and total RNA content were determined in liver, white muscle, brain, heart, and gill during periods of normal activity before metabolic depression, entrance into and during winter dormancy, and during the recovery period. The decrease in water temperature from 8°C to 4°C was accompanied by a 55% depression of protein synthesis in liver, brain, and heart and a 66% depression in gill. Protein synthesis in white muscle fell below detectable levels at this temperature. The depression of protein synthesis is an active process (Q₁₀ = 6–21 between 8°C and 4°C) that occurs in advance of the behavioral and physiological depression at the whole animal level. Protein synthesis was maintained at these depressed levels in white muscle, brain, heart, and gill until water temperature returned to 4°C in the spring. Liver underwent a hyperactivation in the synthesis of proteins at 0°C, which may be linked to antifreeze production. During the recovery period, a hyperactivation of protein synthesis occurred in white muscle, which is suggestive of compensatory growth, as well as in heart and liver, which is considered to be linked to increased activity and feeding. Seasonal changes in total RNA content demonstrate the depression of protein synthesis with decreasing temperature to be closely associated with translational capacity, but the stimulation of protein synthesis during recovery appears to be associated with increased translational efficiency.

low temperature; dormancy; teleost; ribonucleic acid

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ANIMALS UTILIZE METABOLIC depression as a way to survive periods of unfavorable environmental conditions such as low temperature, hypoxia, and desiccation. Behavioral studies suggest the cunner (*Tautogolabrus adspersus*), a north temperate member of the mainly tropical labridae family of teleosts that is indigenous to the North-West Atlantic Ocean, enters a period of inactivity once water temperature decreases to 4°C in the fall of the year. During this time, cunner aggregate in rock crevices, remain motionless, and refrain from feeding until water temperature returns to 4°C in the spring (15). Measurements of physiological parameters during the period of behavioral depression are sparse; however, the metabolic rate of cunner in the winter was found to be ~75% lower than in summer (19). More recently, a study that tracked oxygen consumption rates in cunner over a full year has shown an 83% depression in resting metabolism during winter, with a Q₁₀ value of 9.9 between 5°C and 0°C (5). This elevated Q₁₀ value suggests the winter dormancy in cunner observed by Green and Farwell (15) to be a result of an active depression in metabolism. A similar active depression in metabolic rate was not found in the brown bullhead (*Ictalurus nebulosus*) or the largemouth bass (*Micropterus salmoides*) between 17°C and 3°C, despite the behavioral observations of dormancy at low temperatures (7). The occurrence of metabolic depression in the cunner provides a novel model to study the biochemical mechanisms behind hypothermic metabolic depression in an ectothermic vertebrate, without the confounding factor of low oxygen as in turtles or temperate amphibians.

The syntheses of macromolecules, such as protein and RNA, are major contributors to overall metabolic rate, with protein synthesis accounting for 18–26% and RNA synthesis accounting for ~10% of cellular energy expenditure (16, 20). As such, it is not surprising that these two processes have been demonstrated to be sensitive to ATP supply (2, 35). Several studies have investigated tissue-specific changes in rates of protein synthesis during metabolic depression in various vertebrate classes in response to a plethora of environmental stressors and have demonstrated levels of depression ranging from ~50% to a complete suppression of the synthesis of protein (1, 11, 12, 13, 23, 30, 36, 37). Of those vertebrate classes that have been studied, information on in vivo protein synthesis rates and metabolic depression in teleosts is limited, with only one study (30) investigating the changes in protein synthesis during anoxia-induced metabolic depression in crucian carp. This investigation of the seasonal changes in tissue-specific protein synthesis rates in the cunner is the first study to investigate in vivo protein synthesis in a teleost in relation to hypothermia-induced metabolic depression.

Hyperactivity during arousal from metabolic depression is commonly observed at the behavioral level as increased feeding and activity; however, research into the biochemical changes during the recovery period is limited. Those few studies that investigated changes in protein synthesis during the recovery period have provided contradictory results. In vitro studies on various tissues from hibernating ground squirrels and hepatocytes from anoxia-tolerant turtles have shown a definite hyperactivation of protein synthesis during recovery (23, 37). In contrast, the only in vivo analysis of protein synthesis, which was also on the anoxia-tolerant red-eared slider turtle, did not show any hyperactivation of protein
synthesis rates after acute anoxia exposure (11). These contradictory results may indicate a problem with comparing in vitro vs. in vivo applications or due to the varying exposure time to the environmental stress.

The objective of the present study was to utilize the cunner as a novel model to investigate changes occurring at the cellular level in relation to the observed metabolic depression that occurs in response to seasonal low temperatures. Distinct time periods over the annual temperature cycle were selected for measurement: periods of normal activity before metabolic depression, entrance into and during winter dormancy, and during the recovery period. It was hypothesized that rates of protein synthesis in the cunner would be actively depressed to levels greater than would be predicted from temperature effects alone, and this change would be supported by changes in the capacity of the tissue to synthesize proteins. During the recovery period, a hyperactivation of in vivo rates of protein synthesis is expected to occur to repay the protein debt accumulated during the lengthy winter dormancy experienced by cunner.

MATERIALS AND METHODS

Animal collection. Cunner were collected via hoop net in Portugal Cove, Newfoundland, Canada, and transported to the Ocean Sciences Centre where they were held in tanks with flow through seawater from an ambient water source. Cunner were fed chopped herring ad libitum one or two times a week while animals were active. During winter dormancy, cunner do not normally feed; however, food was offered one time per week with uneaten food removed the following day. All animals in this study were held and treated in accordance with Canadian Council of Animal Care guidelines and the methodology was approved by the Animal Care Committee of Memorial University of Newfoundland (protocol #0603WD).

Validation of the flood dose methodology. The measurement of protein synthesis was achieved through the application of a flooding dose of radiolabeled phenylalanine based upon the methods first described in Garlick et al. (14) and since used extensively for the measurement of protein synthesis in teleosts (21 and references within). Because protein synthesis rates had not been previously measured in cunner or in a teleost that experiences metabolic depression induced by low temperatures, it was essential that an appropriate incubation time be chosen to ensure the validation criterion were fulfilled. The measurement of protein synthesis has been validated in teleosts over a range of water temperatures using an incorporation time up to 9 h (21, 32). Compared with the pelagic lifestyle of many of these study species, cunner are a sluggish, benthic species and have a substantially lower resting metabolic rate (40% lower than Atlantic cod, based on mass independent oxygen consumption measurements; see Ref. 6). Therefore, a lengthy incubation time was selected to accommodate for the lower metabolic rate of this species and its torpid-like state at low water temperatures.

Seasonal changes in tissue-specific rates of protein synthesis. Seasonal changes in the protein synthesis rates in cunner were tracked from October 2003 through August 2004 at five key points throughout the year relating to various states of activity: 8°C (November) when fish were fully active and feeding, 4°C (December) during the entrance into winter dormancy, 0°C (March) while fish are fully dormant, 4°C (June) when fish begin recovery from winter dormancy, and 8°C (July) once fish had returned to an active state and resumed feeding. Fish were allowed to reach the above experimental temperatures by following the ambient water temperature cycle (see Ref. 24 for example of typical temperature profile for the water source). Once the desired experimental temperature was reached, fish (92–270 g, average 174 ± 5.1 g) were randomly selected, tagged for individual recognition, and placed in a separate experimental tank 1 wk before sampling. Water temperature in this tank was maintained at the experimental temperature by mixing ambient with either heated or chilled seawater. During this time, food was withheld to ensure complete clearance of the gut. Fish were injected intraperitoneally with 1.0 ml/100 g of [2,3-3H]phenylalanine (Amersham International) solution. This injection solution consisted of 135 mM phenylalanine in addition to sufficient [2,3-3H]phenylalanine to ensure a dosage of 100 µCi/ml in a saline solution containing (in mM) 150 NaCl, 5 KCl, 2 CaCl2, 1 MgSO4, 10 NaHCO3, 5 d-glucose, 5 HEPES, and 2 Na2HPO4, pH 7.8 at 20°C. Fish were immediately placed back in the experimental tank postinjection, and based on behavioral observations fish returned to a quiescent state within minutes. After an incubation time of 4, 8, 16, or 24 h, four fish were killed by a blow to the head followed by a severing of the spine. Brain, heart, liver, white muscle, and gill were excised, blotted dry, and frozen in liquid nitrogen. Samples were stored at −70°C until analysis.

Sample preparation and scintillation counting. Samples were homogenized with a Polytron in 9 vol of 6% perchloric acid (PCA) except for liver, which was homogenized in 4 vol of PCA. Homogenized samples were left on ice for 10–15 min; after thorough mixing, a 1-ml aliquot was transferred to a microcentrifuge tube and centrifuged for 5 min at 15,600 g to separate out the protein pellet. Supernatant was removed and frozen at −20°C until analysis for the free pool phenylalanine content and specific radioactivity.

The protein pellet was washed by resuspending the pellet in 1.0 ml of 6% PCA, vortexing, and then centrifuging as described above. The supernatant was discarded afterward. This wash step was repeated until the radioactivity in the discarded supernatant was at background levels to ensure only protein-bound [3H]phenylalanine was being measured in the protein pellet. After sufficient washing, 1.0 ml of 0.3 M NaOH was added to the tube containing the protein pellet. The protein pellet was incubated in a water bath held at 37°C until fully dissolved. The dissolved protein was stored at −20°C until analysis for protein content and protein-bound radioactivity.

Aliquots of the original supernatant from the homogenized tissue and the dissolved protein were added to 10 ml of Ecolume scintillation cocktail and counted on a Packard Tri-carb 2100TR liquid scintillation counter to obtain the [2,3-3H]phenylalanine content of the free phenylalanine and protein-bound phenylalanine pools of the tissues, respectively.

Biochemical assays. Free pool phenylalanine content was measured from the PCA extraction supernatant and phenylalanine standards in 6% PCA by a fluorometric assay following the protocol described in McCaman and Robins (27). Protein content of the tissue was determined from the NaOH solubilized protein pellet by using the Bio-Rad Dκ kit (Bio-Rad Laboratories), based on the Lowry assay, using standards made from BSA.

Determination of total RNA content. A second cohort of cunner of comparable size to the fish used for the analysis of protein synthesis (92–482 g, average 194 ± 170 g, P = 0.252) was tracked over a full temperature cycle (March 2005 to January 2006) to obtain samples for analysis of the total RNA content in all tissues analyzed for protein synthesis. Six cunner were sampled at the same temperatures and corresponding time of the year as described for the protein synthesis experiment. Fish were killed, and tissues were excised and frozen as described above. Total RNA was extracted from the samples using TRIzol reagent (Invitrogen), which is a commercially available modification of the single-step RNA isolation method (4) following the procedure outlined in Treberg et al. (32). Total RNA, expressed as microgram of RNA per gram tissue (wet weight), was determined by subtracting the absorbance at 320 nm from the absorbance at 260 nm.

Statistical analysis. Mean tissue phenylalanine content, specific activity over the incubation time, and RNA data were compared using a one-way ANOVA with Tukey’s post hoc test for multiple comparisons. The incorporation of radioactivity into protein was determined by linear regression. Once data were confirmed to fit the validation criteria; phenylalanine incorporation rates between temperatures for
each tissue were compared using a one-way ANOVA with Tukey’s post hoc test. Data were log-transformed when necessary, and in all cases $P < 0.05$ was considered significant.

RESULTS

Validation of flooding dose methodology. Accurate interpretation of in vivo protein synthesis rates via the flooding dose methodology is contingent upon fulfillment of several validation criteria. The injection dose must fully flood the phenylalanine pool, and the free-pool specific radioactivity must rapidly elevate and remain stable over the incubation period. As well, the incorporation of phenylalanine into tissues must be significant and linear over the time that protein synthesis is being measured. Demonstrating the fulfillment of these criteria is particularly important for this study, since it not only explored the in vivo measurement of protein synthesis in a previously unstudied teleost species but also involved the added complication of successful measurements being obtained during a period of metabolic depression occurring in response to extreme low environmental temperatures.

As predicted, a much longer incubation time than is the norm for this procedure was necessary to fulfill the validation criteria in the cunner. The injection dosage, which contained 1.35 nmol/mg body mass of unlabeled phenylalanine, should theoretically increase the free-pool phenylalanine content to the same extent. Baseline levels of phenylalanine obtained from noninjected cunner were $0.12 \pm 0.02$, $0.14 \pm 0.04$, $0.09 \pm 0.01$, $0.07 \pm 0.01$, and $0.11 \pm 0.02$ nmol/mg fresh tissue for liver, white muscle, brain, heart, and gill, respectively. The mean postinjection phenylalanine concentration over all time points and all temperatures was $1.38 \pm 0.01$, $1.14 \pm 0.01$, $1.00 \pm 0.01$, $1.04 \pm 0.01$, and $0.9 \pm 0.01$ nmol/mg for liver, white muscle, brain, heart, and gill, respectively, indicating the free phenylalanine pool to be increased by 8.1- to 14.8-fold by the injection dosage, depending on the tissue.

The specific activities of the free pool were found to be elevated and stable over the time protein synthesis was measured, since there was no significant difference in the specific activity of the free phenylalanine pool between sample times within each tissue. Because similar levels of specific activity were obtained for all experimental temperatures, only data from 8°C (November) and 0°C (March) are presented in Fig. 1 to demonstrate the successful fulfillment of the second validation criteria.

The incorporation of phenylalanine into tissues was expressed as nanomole of phenylalanine incorporated per milligram protein. Figure 2 shows regression plots for data obtained at 8°C (November) and 0°C (March) for all tissues as a representation of the range of phenylalanine incorporation in cunner. Liver, brain, heart, and gill tissues exhibited significant and linear incorporation rates of labeled phenylalanine based on the regression equations calculated over the 24-h time period for all five temperature treatments and had intercepts that were not significantly different from the origin. Significant and linear incorporation of phenylalanine was only evident in white muscle at 8°C (November), 4°C (June), and 8°C (July). Protein-bound radioactivity in white muscle from temperature treatments during the winter [4°C (December) and 0°C (March)] was barely above background radioactivity levels, and there was no linear increase in incorporation of phenylalanine into the tissue. Therefore, rates of protein synthesis could not be calculated for white muscle at 4°C (December) and 0°C (March), since the final validation criteria were not fulfilled at these temperatures. The significant linearity of phenylalanine incorporation into the various tissues from injection time onward while cunner were dormant indicates that the disturbance of fish during injection was not substantial enough to rouse the fish and cause a return to increased or “normal” metabolism.

Seasonal changes in tissue-specific rates of protein synthesis. Protein synthesis rates obtained in cunner display the same tissue-specific hierarchy as other species with rates in gill > liver > heart > brain > white muscle across all experimental
temperatures. At 8°C in November, protein synthesis rates for liver, white muscle, brain, heart, and gill were 0.20 ± 0.02, 0.01 ± 0.001, 0.09 ± 0.006, 0.12 ± 0.01, and 0.36 ± 0.05 nmol phenylalanine·g protein⁻¹·h⁻¹, respectively. Significant decreases in protein synthesis occurred in all five tissues as the water temperatures decreased to 4°C (Fig. 3). Rates in white muscle fell below detectable levels at this temperature and did not increase until water temperatures returned to 4°C in June. A decrease of ~55% between 8°C and 4°C was seen in liver, brain, and heart, with the greatest depression occurring in gill tissue, with rates of protein synthesis being depressed by 66%

The only tissue that exhibited a further decrease in rates of protein synthesis as water temperatures dropped to 0°C was the brain. Phenylalanine incorporation dropped to 0.02 ± 0.003 nmol phenylalanine·g protein⁻¹·h⁻¹, a significant decrease from rates at 8°C (78% depression), but not from 4°C. Incorporation rates in the liver underwent an unexpected significant increase to 0.22 ± 0.050 nmol phenylalanine·g protein⁻¹·h⁻¹, bringing protein synthesis to levels comparable to those obtained at 8°C. As water temperature warmed to 4°C in June, liver, heart, and gill tissue maintained protein synthesis at

Fig. 2. Postinjection time course for the incorporation of radiolabeled phenylalanine into protein in cunner at 8°C (November; ○) and 0°C (March; ◯) for liver (A), white muscle (B), brain (C), heart (D), and gill (E) tissues. Values are means ± SE; n = 4 fish at each sample time.

Fig. 3. Rates of protein synthesis (nmol phenylalanine·mg protein⁻¹·h⁻¹) in cunner at the various experimental temperatures in liver (A), white muscle (B), brain (C), heart (D), and gill (E) tissue. Values represent means ± SE; n = 16 fish as linearity was achieved over full incorporation time. Significant differences indicated by differing letters (P < 0.05).
similar rates as measured at 0°C. Brain and white muscle both increased protein synthesis significantly at this temperature, with rates in brain returning to rates similar to those recorded at 4°C in December and white muscle increasing to levels comparable to fully active fish at 8°C in November. Accompanying the return of water temperature to 8°C in July, there were significant increases in rates of protein synthesis in all tissues, except for white muscle in which the increase in average rate was nonsignificant. Brain and gill protein synthesis returned to similar rates as recorded when water temperature was 8°C in the fall of the year. The significant increase of protein synthesis rates to 0.33 ± 0.046 and 0.21 ± 0.028 nmol phenylalanine·g protein⁻¹·h⁻¹ for liver and heart tissue, respectively, indicates a hyperactivation of protein synthesis to 165% for liver and 175% for heart compared with measurements taken at the equivalent temperature in the fall (8°C in November).

Seasonal changes in total RNA content. Total RNA content in fish sampled at 8°C (November) was the highest in liver tissue, followed by gill, brain, and heart, with white muscle exhibiting the lowest levels (6,406 ± 627, 3,214 ± 347, 1,458 ± 488, 1,459 ± 148, 153.9 ± 8.2 μg/g tissue, respectively; Fig. 4). RNA content in liver, brain, heart, and gill was found to decrease with water temperature, whereas levels of RNA in white muscle remained relatively low and similar despite changes in temperature. The decrease in water temperature to 0°C was accompanied by a significant reduction in total RNA content in liver, brain, heart, and gill tissue relative to 8°C sample (decrease of 74, 88, 66, and 60%, respectively). However, as water temperatures increased in the spring, the changes in total RNA content did not mirror the pattern observed during temperature decrease for all tissues. Liver RNA content was significantly increased as water temperatures reached 4°C, bringing total RNA content up to levels comparable to those obtained at 8°C in November. However, RNA levels underwent a significant decrease to 1,102 ± 255 μg/g tissue as water temperatures further increased to 8°C in July. White muscle again maintained constant levels of RNA until water temperatures reached 8°C, when total RNA was significantly increased to 286.5 ± 50.8 μg/g tissue. Brain and heart RNA levels increased significantly along with the spring water temperature increase to 4°C; brain RNA was maintained at these levels despite the further increase in temperature to 8°C, whereas levels in heart returned to 0°C levels. There were no significant changes in total RNA content in gill tissue after the initial decrease that coincided with the entrance into winter dormancy.

Comparison of protein synthesis and RNA content. Although the rates of protein synthesis and content of RNA were obtained from different cohorts of fish, comparison of these values reveals qualitative patterns. Significant correlations were not obtained when comparing mean phenylalanine incorporation rates and mean total RNA content for any of the tissues when all experimental temperatures were included. However, a qualitative analysis of the regression plots demonstrated the values at 8°C in July, during recovery, to be a consistent outlier in brain, heart, and gill tissue (Fig. 5). The removal of the 8°C July data point from the regression produced a significant correlation between protein synthesis and total RNA content, with temperature changes for gill tissue, but not for heart and brain, although a definite trend was observed (Fig. 5).

DISCUSSION

Entrance into metabolic depression. Earlier evidence of metabolic depression in response to low temperature in cunner has been based on behavioral observations (15) and oxygen consumption rates (5, 19). The present study shows a significant depression in the protein synthesis, providing evidence at the cellular level to complement the observations at the whole animal level. Upon the transition from 8°C to 4°C, the incor-
the cellular mechanism an active contributor to metabolic suppression or simply a passive consequence of decreasing temperature. Q10 values are often calculated to determine if the changes observed in a metabolic process are because of direct temperature effects (Q10 value of 2–3) or active alteration of metabolism (Q10 > 3). The incorporation of protein into body tissues is a temperature-dependent process (Q10 values = 2–3), with the rate of protein synthesis being directly related to changes in environmental temperature (17, 18, 25, 34). However, high Q10 values for protein synthesis have been discovered in the toadfish, Opsanus tau (Q10 = 6–7; see Refs. 26 and 28), and the North Sea eelpout, Zoarces viviparous (Q10 = 7–10; see Ref. 31). These high Q10 values were calculated at the lower end of the organism’s temperature profile and often accompanied visual observations of sluggish lifestyle in the eel pout or periods of complete inactivity in the toadfish. The high Q10 values in these two temperate species suggest an active depression in whole animal metabolic rate; however, this has yet to be experimentally determined for these species.

Q10 values calculated from the protein synthesis rates in cunner between 8°C and 4°C in the fall of the year are also higher than expected from temperature effect alone, with values of 6.7, 8.0, 9.2, and 21.0 for brain, liver, heart, and gill, respectively. Interestingly, this active suppression of protein synthesis occurs as a precursor to the 69% depression in resting metabolism that occurs between 5°C and 0°C (Q10 = 10.5), the same temperatures over which the behavioral changes are noticeable (5, 15). The theory of active metabolic depression is further strengthened when cunners are faced with an acute temperature challenge (1°C/h, between 5°C and 0°C). During the initial stages of the acute challenge, cunner are capable of thermal compensation, but after 1 h at 0°C a rapid depression of metabolic rate occurs with a peak depression of 64% (Q10 = 8) at 2–3 h at 0°C (5). These results suggest the depression of protein synthesis that occurs between 8°C and 4°C to be an intrinsic response, occurring in anticipation of the extended period of extreme low water temperatures experienced by the cunner during the winter months and before the decrease in whole animal metabolic rate is evident at the physiological level.

Metabolic depression. The only tissue to exhibit further depression in synthesis as water temperatures decreased from 4°C to 0°C was brain, bringing levels of protein synthesis to ~25% of rates obtained in fully active fish (Q10 = 4). The negligible decrease of protein synthesis in heart and gill despite the additional decrease in environmental temperature further supports active control of protein synthesis in relation to seasonal metabolic depression. The level of depression in brain tissue falls in between the complete suppression of brain protein synthesis in anoxic turtles and hibernating ground squirrels (11, 12), which exist in a comatose-like state during metabolic depression, and the maintenance of protein synthesis in the brain of crucian carp, which remain relatively active during anoxia (30). Cunner appear to be in a quiet state during winter dormancy, but fish will become active for a short period of time if disturbed (15), which may explain the maintenance of phenylalanine incorporation at low levels in the brain as representative of neuronal tissue activity during dormancy.

The anomaly of the increase in rates of protein synthesis in liver tissue to predormancy levels is perplexing, since the activation of protein synthesis in this tissue, which is the major

Fig. 5. Comparison of protein synthesis rates (nmol phenylalanine·mg protein−1·h−1) and total RNA content (µg RNA/g fresh tissue wt) in brain, heart, and gill tissue at 8°C in November (■), 4°C in December (●), 0°C in March (△), 4°C in June (□), and 8°C in July (○). Regression equations calculated mean value of protein synthesis (n = 1) for all temperatures except 8°C in July (○); brain: y = 4.22 × 10−5x + 0.02, r² = 0.85, P = 0.078; heart: y = 6.02 × 10−5x + 0.01, r² = 0.41, P = 0.36; gill: y = 9.52 × 10−5x − 0.01, r² = 0.98, P = 0.01.
site of protein synthesis, is an energetically expensive action during a time when energy conservation is essential. Cunner remain in shallow inshore waters where temperatures frequently decline close to the freezing point of seawater, and contact with ice crystals without the protection of an antifreeze mechanism would be fatal to the cunner when they are in this supercooled state. Antifreeze proteins have been found in the skin of cunners and also circulating in the blood during the winter, but the production site of these proteins has not been established (8, 33). Because circulating antifreeze proteins in the blood are commonly expressed in the liver (9), the substantial increase in liver protein synthesis during a time of energy conservation may be linked to the production of antifreeze proteins during exposure to extreme low temperatures to enable survival.

The process of protein synthesis does not stand alone in cellular metabolism; it is closely linked with the degradation of proteins in a continuous cycle known as protein turnover. Protein degradation can account for up to 22% of ATP turnover (22), demonstrating it to be an energetically expensive process (falling just behind protein synthesis and active ion exchange; see Ref. 35). This creates a paradoxical situation in animals that undergo extended periods of metabolic depression. These animals must deal with the need to reduce protein degradation to conserve ATP consumption while avoiding the accumulation of damaged proteins to sustain tissue functionality. A solution to this problem has been described in anoxic turtle hepatocytes. Despite the extensive depression in ATP-dependent proteolysis (93%) during anoxic exposure, total protein degradation was only reduced by 36–41%. Thus the remaining protein degradation is the result of ATP-independent sources (22). Further investigation is needed to determine whether or not T. adsperus employs a similar approach with respect to protein degradation when faced with low temperature-induced metabolic depression.

Recovery from metabolic depression. During periods of hypometabolism, levels of protein synthesis and degradation often become mismatched (synthesis < degradation), creating a negative protein balance (22). In this situation, there will inevitably be some accumulation of denatured or damaged proteins, and unless animals can reduce energy demands and enter a maintenance mode (synthesis = degradation) there will also be some loss in protein content. Upon recovery, this relationship is reversed in anoxic turtles, since levels of protein synthesis are hyperactivated (160%) while protein degradation remains below control rates (22). This positive protein balance is most likely necessary to allow for the replacement of damaged proteins that are accumulated during dormancy. In tissues such as white muscle, this increase in synthesis and retention of protein (accompanied by decreased degradation) is manifested at the whole animal level as increased growth (21).

During the initial stages of postdormancy recovery in cunner (4°C, June samples), the synthesis of protein began to increase in a temperature-dependent fashion for brain and heart tissue, whereas rates in gill and liver were maintained at similar levels as measured at the previous temperature. A significant increase in protein synthesis in white muscle occurs during early recovery, bringing rates of phenylalanine incorporation from undetectable levels to rates comparable to those obtained in fully active fish at 8°C in November. As the accretion of proteins in white muscle is representative of increased tissue mass, this hyperactivation of protein synthesis is most likely accompanied by a decrease in degradation and responsible for the stimulation of growth after an extended period of winter dormancy. A comparative study on two geographically different populations of cunner shows that the Newfoundland population experiences a longer period of dormancy than more southerly populations, but the annual growth rates of the two populations remain the same (3). Therefore, the hyperactivation of protein synthesis in white muscle during the recovery period is allowing for compensatory growth to occur to make up for a shorter growth season in cunner inhabiting colder waters. Although metabolism is not decreased to the same extent in Atlantic cod as in cunner, cod also exhibits compensatory protein synthesis after exposure to cold water temperatures (32). Hyperactivation of protein synthesis was also evident in liver (165%) and heart (175%) once cunner returned to a fully active state (8°C, July). A similar level of hyperactivation of protein synthesis was documented in in vitro studies on ground squirrels during arousal from hibernation (37) and turtles during recovery from extended exposure to anoxia (23). As in these previous studies, the mechanism behind the substantial increase in rates of protein synthesis in the cunner remains elusive but is most likely linked to the increase in overall metabolic rate, higher food consumption, and activity level.

Protein synthesis and RNA content. The close relationship between rates of protein synthesis and the translational capacity of a tissue (RNA content) is well established (21). Specifically, translational capacity undergoes significant reductions that parallel the depression of protein synthesis caused by decreasing temperature (11, 29) and during metabolic depression (1, 11, 30). The qualitative comparison of protein synthesis rates with total RNA content in this experiment suggests this to also true in cunner for brain, heart, and gill tissue until cunner begin recovery from winter dormancy. In teleosts, the stimulation of protein synthesis and compensatory growth has been linked to increases in translational efficiency instead of an increase in translational capacity (21, 32). The absence of a significant correlation between protein synthesis rates and total RNA content in liver and white muscle throughout the experiment as well as the increase and hyperactivation observed in brain heart and gill during recovery suggest these changes in protein synthesis are because of variations in the efficiency of the tissue to synthesize protein.

In conclusion, the current study is the first to investigate the relationship between temperature and protein synthesis during both metabolic depression and recovery in a fish species that experiences winter dormancy. The depression of protein synthesis in cunner appears to be an intrinsic response, since it is an active process that occurs in advance of observations of metabolic depression at the whole animal. An unexpected hyperactivation of protein synthesis occurs in the liver while the animals are in a fully dormant state that may be linked to the production of antifreeze proteins to ensure the survival in the ice-laden environment. During recovery, hyperactivation of protein synthesis occurs in white muscle, heart, and liver, presumably to allow for compensatory growth and to repay the protein debt that is accumulated during the extended period of winter dormancy. The biochemical mechanism responsible for the stimulation of protein synthesis remains unclear, although qualitative comparisons of protein synthesis rates and total
RNA content suggest an increase in translational efficiency to be a potential mechanism.

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