J Comp Physiol B (1992) 162:23–28

Journal of Comparative Physiology B Biochemical, Systemic, and Environmental Physiology

Mechanisms of glycolytic control during hibernation in the ground squirrel *Spermophilus lateralis*

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Accepted August 19, 1991

Summary. The mechanisms of glycolytic rate control during hibernation in the ground squirrel *Spermophilus lateralis* were investigated in four tissues: heart, liver, kidney, and leg muscle. Overall glycogen phosphorylase activity decreased significantly in liver and kidney to give 50% or 75% of the activity found in the corresponding euthermic organs, respectively. The concentration of fructose-2,6-bisphosphate (F–2,6–P_2) decreased significantly in heart and leg muscle during hibernation to 50% and 80% of euthermic tissue concentrations, respectively, but remained constant in liver and kidney. The overall activity of pyruvate dehydrogenase (PDH) in heart and kidney from hibernators was only 4% of the corresponding euthermic values. Measurements of phosphofructokinase (PFK) and pyruvate kinase (PK) kinetic parameters in euthermic and hibernating animals showed that heart and skeletal muscle had typical rabbit skeletal M-type PFK and M_1-type PK. Liver and kidney PFK were similar to the L-type enzyme from rabbit liver, whereas liver and kidney PK were similar to the M_2 isozyme found primarily in rabbit kidney. The kinetic parameters of PFK and PK from euthermic vs hibernating animals were not statistically different. These data indicate that tissue-specific phosphorylation of glycogen phosphorylase and PDH, as well as changes in the concentration of F–2,6–P_2 may be part of a general mechanism to coordinate glycolytic rate reduction in hibernating *S. lateralis*.

Key words: Hibernation – Control of glycolysis – Phosphofructokinase – Pyruvate kinase – Pyruvate dehydrogenase – *Spermophilus lateralis*

Abbreviations: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenonine triphoshate; EDTA, ethylenediaminetetra-acetic acid; EGTA, ethylene glycol tetra-acetic acid; F–6–P, fructose 6-phosphate; F–1,6–P_2, fructose 1,6-bisphosphate; F–2,6–P_2, fructose-2,6-bisphosphate; K_a, activation coefficient; I_{50}, concentration of inhibitor which reduces control activity by 50%; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PFK, 6-phosphofructo-1-kinase, PK, pyruvate kinase

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Introduction

Many species of mammals hibernate during the winter months to conserve fuel when food supplies are scarce. Hibernation results in substantial energy savings [ca. 90% compared with the cost of remaining euthermic throughout the winter (Wang 1978)]. During hibernation, metabolic rates are reduced to values less than 5% of those of euthermic animals and body temperature is reduced to values between 2 and 15 °C (Deavers and Musacchia 1980; Snapp and Heller 1981; Geiser 1988). The biochemical mechanisms underlying this profound metabolic reduction are relatively poorly understood for mammalian hibernators, although aspects of the comparable processes in lower vertebrate and invertebrate facultative anaerobes are well characterized (Storey 1988a, 1989). Specifically, the mechanisms that regulate glycolytic rate during periods of depressed metabolism are: (1) covalent modification of key glycolytic enzymes (e.g. PFK, PK, and glycogen phosphorylase) to alter kinetic constants and decrease activity in the hypometabolic state; (2) reversible aggregation of glycolytic enzymes into supra-molecular complexes capable of metabolizing glucose at rates greater than that of the free enzymes (Srivastava and Bernhard 1986; Friedrich 1988); and (3) changes in the concentration of enzyme allosteric activators such as F–2,6–P_2. Tissue-specific combinations of all three mechanisms occur in different species to down-regulate glycolytic rate in order to conserve glycogen fuel and reduce the accumulation of toxic end-products. These mechanisms represent part of an overall metabolic depression that is crucial to long-term survival in hypometabolic states.

Recent studies with the meadow jumping mouse *Zapus hudsonius* (Storey 1987a, b) and earlier studies with bats (Behrish 1974; Borgmann and Moon 1976) demonstrated the usefulness of these mechanisms in regulating glycolysis in mammalian hibernators. Changes in PFK and PK in liver, glycogen phosphorylase in brain, and F–2,6–P_2 concentrations in brain, heart, and kidney were consistent with an overall decreased glycolytic rate

in hibernating *Z. hudsonius* (Storey 1987a). Changes in PK and fructose 1,6-bisphosphatase (F–1,6–P_2) from hibernating bat liver were also consistent with an overall decreased glycolytic rate (Borgmann and Moon 1976). These data agree with measurement of glucose utilization in Arctic ground squirrels, which showed a profound glycolytic rate depression during hibernation (Tashima et al. 1970).

The present study further examines metabolic processes during hibernation by investigating mechanisms of glycolytic control in the golden-mantled ground squirrel *Spermophilus lateralis*. During the hibernation season these squirrels typically alternate between bouts of torpor lasting 5–20 days and short periods of euthermia lasting only 12–24 h. Body temperature drops from a euthermic value of approximately 34 °C to 8–10 °C while hibernating; the Q_{10} value for this change is approximately 2.5 (Snapp and Heller 1981). In the present study, we measured the relative activities of key glycolytic enzymes (PFK, PK, glycogen phosphorylase, and PDH) to determine whether they are regulated during hibernation in a fashion similar to that seen in *Z. hudsonius* and bat liver. Identification of down-regulated enzymes would present evidence for the universality of glycolytic control mechanisms in hibernating mammals.

Materials and methods

Animals and chemicals. All *Spermophilus lateralis* tissues were graciously supplied by Dr. H.C. Heller (Department of Biological Sciences, Stanford University, Stanford, CA., USA) and transported to Carleton University frozen on dry ice. Euthermic animals were held at 23 °C under natural daylength conditions and fed ad libitum. Hibernating ground squirrels were sampled after 3 days of continuous hibernation at 5 °C (core temperature 5–10 °C) with a 12:12 h light:dark cycle. All animals were sacrificed by decapitation and tissues were immediately frozen on dry ice. Leg muscle was of mixed fiber types obtained from the hips and thighs. All chemicals were obtained from Sigma Chemical Co (St. Louis, Mo. USA) or Boehringer Mannheim (Montreal, Quebec, Canada) and were of the highest purity available.

Preparation of samples. Frozen tissues samples were homogenized 1:4 (w:v) with 50 m*M* phosphate buffer (pH 7.0), 30 m*M* β-mercaptoethanol, 5 m*M* EDTA, 5 m*M* EGTA, 100 m*M* NaF for 10–15 s in an Ultra-turrax homogenizer at 80% full speed. For measurement of glycogen phosphorylase activity, samples were allowed to settle for 0.5 h at 0 °C. Samples used for PFK and PK measurements were centrifuged for 20 min at $20000 \times g$. The resulting supernatant was centrifuged (60 s) through a Sephadex G–25 column (equilibrated in 50 m*M* imidazole, pH 7.0, 0.5 m*M* EDTA, 20 m*M* β-mercaptoethanol, and 30 m*M* NaF) to remove low molecular weight species (Helmerhorst and Stokes 1980).

Samples used for measurement of F–2,6–P_2 concentrations were homogenized 1:20 (w:v) in hot (80 °C) NaOH, incubated at 80 °C for 10 min, and stored on ice until assay. Liver homogenates required 10 min centrifugation at $20000 \times g$ to clarify the sample. Samples were stored on ice until assayed.

PDH activity was measured according to Elnageh and Gaitonde (1988). Briefly, samples were homogenized 1:9 (w:v) in 50 m*M* phosphate (pH 7.8), 2 m*M* β-mercaptoethanol, 1 m*M* EDTA, 0.1% Triton X–100. Samples were frozen at −20 °C for at least 1 h, removed, thawed, and assayed for active enzyme. In order to obtain total PDH activity, samples were mixed 1:1 (v:v) with 20 m*M* $MgCl_2$, 2 m*M* $CaCl_2$, and incubated at 30 °C for 2 h.

Phosphofructokinase and pyruvate kinase activity determinations. All assays were performed in 50 m*M* imidazole (pH 7.0), 50 m*M* KCl, 5 m*M* $MgCl_2$, 0.15 m*M* NADH, and excess coupling enzymes at 21 ± 0.5 °C in a total volume of 1 ml. Assays were monitored by following NADH oxidation at 340 nm in a single beam spectrophotometer. For PFK assays, the K_m for fructose 6-phosphate (F–6–P), the activation coefficient (K_a), and the concentration of inhibitor reducing activity by 50% (I_{50}) were determined at 0.5 m*M* ATP. The F–6–P concentrations used for measuring I_{50} values were: 10 m*M* (liver and kidney), 1.5 m*M* (heart), or 0.6 m*M* (leg muscle). F–6–P concentrations used to determine the K_a values for phosphate, AMP, and F–2,6–P_2 were: 1 m*M* (liver), 0.3 m*M* (heart), 3 m*M* (kidney), or 0.2 m*M* (leg muscle). All PK assays were performed at 1 m*M* ADP except for the measurement of the ADP K_m values. The I_{50} values for L-alanine were determined at either 0.5 m*M* phosphoenolpyruvate (PEP) (kidney), 0.05 m*M* PEP (heart), 0.1 m*M* PEP (liver), or 0.02 m*M* PEP (leg muscle). Activation coefficients for F–1,6–P_2 were determined at 0.1 m*M* PEP (liver), 0.005 m*M* PEP (heart), 0.025 m*M* PEP (liver), or 0.02 m*M* PEP (leg muscle). Assays were initiated by the addition of either F–6–P or PEP. ATP and ADP were added as 1:1 mixtures with $MgCl_2$. All kinetic constants were determined by non-linear least-squares regression to either the Hill equation (K_m, K_a) or to a linear inhibition equation (I_{50}). Values were compared using a one-tailed Student's *t*-test. Values were considered significant at $P < 0.05$.

Other assays. Glycogen phosphorylase activity was assayed in 50 m*M* potassium phosphate buffer, pH 7.0, 2 mg glycogen · ml^{-1}, 0.4 m*M* NADP, 10 μ*M* glucose 1,6-bisphosphate, 0.25 m*M* EDTA, 10 m*M* $MgCl_2$, and excess dialyzed phosphoglucomutase and glucose 6-phosphate dehydrogenase with (for total activity) or without (for *a* form alone) the addition of 1.6 m*M* AMP. The tissue concentration of F–2,6–P_2 was assayed as previously described (van Schaftingen 1984). PDH activities were measured using *p*-iodonitrotetrazolium violet and lipoamide dehydrogenase as previously described (Elnageh and Gaitonde 1988).

Results

Phosphofructokinase kinetic constants

Table 1 presents PFK kinetic parameters measured at pH 7.0 in heart, liver, kidney, and leg muscle from euthermic *S. lateralis*. All enzymes showed allosteric kinetics with respect to F–6–P; the Hill coefficients were significantly different from 1.0 as determined by Student's *t*-test. The liver and kidney PFK isozymes were similar to those from mammalian liver (Dunaway 1983; Storey 1987a), and were typical of L-type isozymes. L-type PFK isozymes are sensitive to citrate inhibition, have low sensitivity to activation by AMP and phosphate or to inhibition by ATP, and have relatively high K_m values for F–6–P. Both liver and kidney isozymes did not show any inhibition at higher ATP concentrations (up to a value of 20 m*M*). PFK from *S. lateralis* heart and leg muscle were typical of rabbit M-type PFK. Rabbit M-type PFK is characterized by a relatively high sensitivity to inhibition by citrate, a high sensitivity to activation by AMP and phoshate, and a high affinity for F–6–P as compared with the L-type enzyme. Thus, heart and leg muscle PFK had a 10-fold lower I_{50} for citrate, K_a for AMP, and K_m for F–6–P than did the liver or kidney isozymes. PFK

Table 1. Phosphofructokinase kinetic parameters determined in heart, liver, kidney, and leg muscle of euthermic *S. lateralis*

Parameter	Heart	Liver	Kidney	Leg muscle
$S_{0.5}$ F–6–P (m*M*)	0.64±0.06	5.6± 1.0	10.2 ± 0.4	0.52 ±0.04
Hill coefficient	1.8 ±0.1	1.6± 0.2	1.5 ± 0.3	6.8 ±1.6
K_m ATP (μ*M*)	20 ±3	39 ± 5	10 ± 2	45 ±6
I_{50} ATP (m*M*)	1.3 ±0.2	NE	NE	1.1 ±0.2
I_{50} citrate (m*M*)	0.09±0.02	3.0± 0.7	0.48± 0.08	0.020±0.005
K_a P_i (m*M*)	0.64±0.14	1.2± 0.4	2.2 ± 0.3	2.2 ±0.3
K_a AMP (μ*M*)	25 ±4	262 ±80	236 ±41	32 ±8
K_a F–2,6–P_2 (n*M*)	6.7 ±1.2	40 ± 9	250 ±60	39 ±4
V_{max} (IU · g ww^{-1})	8.9 ±2.8	2.7± 0.5	1.3 ± 0.3	21.1 ±3.9

Values are means±SEM for $n=8$ separate determinations. NE no effect up to an ATP concentration of 20 m*M*. P_i phosphate, $S_{0.5}$ concentration of F–6–P giving half maximal activity

Table 2. Pyruvate kinase kinetic parameters determined in heart, liver, kidney, and leg muscle of euthermic *S. lateralis*

Parameter	Heart	Liver	Kidney	Leg muscle
K_m PEP (μ*M*)	20 ± 2	948 ±111	577 ±32	38 ± 3
Hill coefficient	1.2 ± 0.2	1.3 ± 0.1	1.4 ± 0.1	1.0 ± 0.1
K_m ADP (m*M*)	0.31± 0.03	0.16± 0.02	0.15± 0.02	0.22± 0.06
I_{50} L-alanine (μ*M*)	NE	NE	44 ± 9	NE
K_a F–1,6–P_2 (m*M*)	0.43± 0.05	NE	NE	0.46± 0.07
V_{max} (IU · g ww^{-1})	98.9 ±11.2	13.0 ± 1.4	20.7 ± 1.3	255 ±31

Values are means±SEM for $n=8$ separate determinations. NE no effect up to a concentration of 20 m*M*

kinetic constants from tissues of hibernating *S. lateralis* were similarly fully characterized but revealed no significant differences with respect to the enzymes from euthermic animals.

Pyruvate kinase kinetic constants

Table 2 presents data on K_m values and Hill coefficients for PEP, K_m values for ADP, I_{50} values for L-alanine, and K_a values for F–1,6–P_2 determined at pH 7.0 from euthermic animals. Comparison of the kinetic parameters from *S. lateralis* with rabbit liver and kidney PK parameters (Seubert and Schoner 1971) indicated that *S. lateralis* liver and kidney PK had M_2-type isozymes. Thus, liver and kidney PK showed sigmoidal kinetics with respect to PEP as indicated by Hill coefficients significantly greater than 1.0 ($P>0.05$; Table 2). These enzymes also had a relatively lower affinity for PEP as shown by a 100- to 200-fold K_m lower compared with the heart and leg muscle enzymes. The data of Table 2 further show that PK from both liver and kidney were not activated by increasing concentration of F–1,6–P_2, and that liver PK was not inhibited by up to 50 m*M* L-alanine under assay conditions.

Both heart and leg muscle PK kinetic constants were similar to M_1-type PK (Hall and Cottam 1979). The K_m for PEP was approximately 10-fold lower than the values determined in liver and kidney, and velocity vs substrate the curve was square hyperbolic as shown by Hill coefficients not significantly different from 1.0 (Table 2). Table 2 also demonstrates that heart and leg muscle PK were not inhibited by L-alanine; absence of L-alanine inhibition is also consistent with M_1-type enzyme kinetic parameters (Hall and Cottam 1979; Engstrom et al. 1987). However, contrary to other M_1-type isozymes, both heart and leg muscle enzymes were activated by F–1,6–P_2, although this activation was only 2- to 3-fold under our conditions.

A comparison of PK kinetic parameters from euthermic and hibernating *S. lateralis* revealed no significant differences between these two groups.

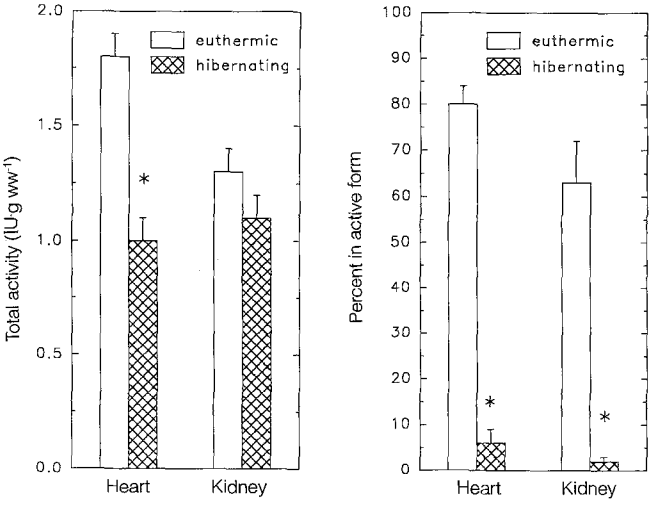

Fig. 1. Total PDH activity in units per gram of wet weight *(left)* and percentage of enzyme in the active *a* form *(right)* in euthermic and hibernating *S. lateralis* heart and kidney. Values are means± SEM ($n=4$ separate determinations). No activity was detectable in liver or skeletal muscle preparations. * Significant difference ($P<0.05$)

Table 3. Glycogen phosphorylase activity in euthermic and hibernating *S. lateralis*

Tissue	Glycogen phosphorylase	
	total activity (IU · g ww^{-1})	% *a*
Heart:		
Euthermic	4.9±0.8	20±1
Hibernating	5.5±0.3	21±2
Liver:		
Euthermic	4.3±0.3	70±1
Hibernating	2.9±0.6*	53±4*
Kidney:		
Euthermic	6.8±0.3	13±4
Hibernating	3.3±0.8*	20±3
Leg muscle:		
Euthermic	44.1±3.9	9±3
Hibernating	46.4±1.6	13±3

Values are means±SEM for $n=4$ separate determinations
%*a*, percentage of total enzyme present in the active (*a*) form
* Significant difference ($P<0.05$)

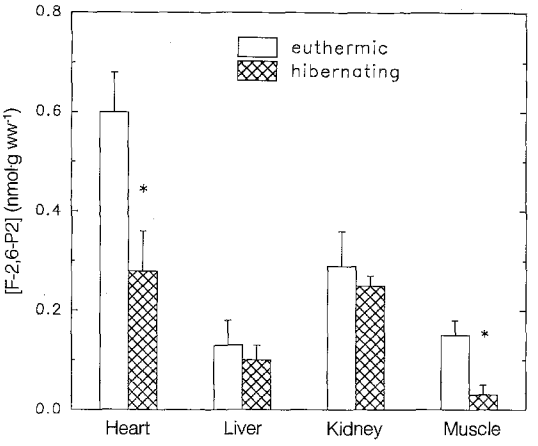

Fig. 2. Fructose 2,6-bisphosphate concentrations (nanomoles per gram of wet weight) in euthermic and hibernating *S. lateralis*. Values are means±SEM for $n=4$ separate determinations. * Significant difference ($P<0.05$)

Pyruvate dehydrogenase

Measurement of PDH activity was possible only in heart muscle and kidney tissue of *S. lateralis* using the procedure of Elnageh and Gaitonde (1988). This was due to the low concentration of mitochondria in liver and leg muscle; in these latter tissues, PDH activity did not exceed the background rate. The data of Fig. 1 show both total PDH activity (obtained by dephosphorylating the enzyme using the endogenous phosphatase) and the percentage of active PDH in tissues of euthermic and hibernating animals (% in the active form, Fig. 1). These data show that hibernating heart and kidney tissue had a significantly lower percentage of PDH in the active *a* form when euthermic and hibernating animals were compared. Heart tissue also showed a reduction in total measurable PDH activity from 1.8 to 1.0 IU · g wet weight^{-1}. These reductions resulted in PDH activities in hibernating tissue which were only 3% (kidney) or 4% (heart) of euthermic activities.

Glycogen phosphorylase activity and F–2,6–P_2 concentrations

The data in Table 3 show measurements of glycogen phosphorylase activity and Fig. 2 presents F–2,6–P_2 concentrations in tissues from euthermic and hibernating animals. Significant decreases in total glycogen phosphorylase activity occurred in liver and kidney tissue during hibernation and the percentage of glycogen phosphorylase in the active *a* form decreased in liver but increased in kidney during hibernation. These values show that glycogen phosphorylase activity was 51% and 75% of the euthermic activity in liver and kidney, respectively (Table 3). The tissue levels of F–2,6–P_2 did not change in liver or kidney but decreased significantly in heart and leg of hibernating animals: F–2,6–P_2 concentrations decreased to 50% of euthermic heart values and to 20% of euthermic leg muscle values (Fig. 2).

Discussion

This study shows that changes in the degree of enzyme covalent modification and in the concentration of allosteric enzyme activators are an important part of an overall glycolytic rate depression strategy in *S. lateralis* during hibernation. For example, the data in Table 3 illustrate the importance of glycogen phosphorylase covalent modification and changes in F–2,6–P_2 concentration in regulating glycolysis during hibernation in mammals. That these changes represent important control mechanisms in these larger hibernating mammals is best illustrated when the data for *S. lateralis* are compared with those recorded in the relatively smaller hibernator *Z. hudsonius* (Storey 1987a, b). The observed changes in overall glycogen phosphorylase activity and F–2,6–P_2 concentrations were similar for both species, with decreases in overall liver and kidney glycogen phosphorylase activity and in heart and skeletal muscle F–2,6–P_2 concentrations. Decreased glycogen phosphorylase activity is consistent with a switch to fatty acid oxidation during hibernation; decreased liver glycogen phosphorylase will limit glucose export to the blood. The importance of fatty acid oxidation during hibernation is demonstrated by measurements of respiratory quotients that indicate a switch to a primary dependence on lipid oxidation during hibernation supplemented by gluconeogenesis from amino acids (Lyman et al. 1982; Hochachka and Guppy 1987). Furthermore, during hibernation, glycogen phosphorylase activity is down-regulated to spare carbohydrate reserves as opposed to starvation where glycogen is rapidly depleted (Hochachka and Somero 1984).

In general, high F–2,6–P_2 concentrations promote carbohydrate utilization for biosynthetic processes (Hue 1983) and lower F–2,6–P_2 concentrations prevail under conditions of starvation (Hue 1982), anoxia (Storey 1988b), and hibernation (Storey 1987b) where carbohydrate must be preserved. The tissue-specific responses of *S. lateralis* during hibernation illustrate the importance of this allosteric activator in regulating PFK activity in heart and leg muscle. In the case of leg muscle, the concentration decreased from a level five times the K_a value in euthermic animals to one-half the K_a value in hibernating animals, suggesting that F–2,6–P_2 levels may regulate glycolytic rates by directly influencing PFK activity in these tissues.

PDH activity was also dramatically decreased in heart and kidney of hibernating *S. lateralis*, falling to values only 3% (kidney) or 4% (heart) of those in euthermic animals. This change was almost identical to that seen in the small hibernator *Z. hudsonius*, where PDH fell from 15% active (heart) or 29% active (kidney) to approximately 1% in both tissues [total activities were 9 and 5 IU · g wet weight^{-1}, respectively (Storey 1989)]. The PDH complex is responsible for the production of acetyl-CoA in the mitochondrion and represents the major route of pyruvate entry into the tricarboxylic acid cycle. PDH is reversibly phosphorylated to give an inactive enzyme with dephosphorylation occurring via a soluble phosphatase (Denton et al. 1987; Reed and Yeaman 1987). Therefore, PDH activity can be controlled by effectors which regulate kinase and phosphatase activities. For example, PDH kinase activity is activated by acetyl-CoA and inhibited by ADP and pyruvate (Reed and Yeamen 1987). Since PDH activity in heart and kidney dramatically decreased during hibernation, it is likely that this enzyme locus is involved in promoting a metabolism largely based on lipid oxidation during hibernation. Therefore, it is possible that decreases in pyruvate concentration brought about by regulation of PFK and glycogen phosphorylase activity, as well as increases in acetyl-CoA due to increased fatty acid breakdown, combine to stimulate the membrane-bound PDH kinase leading to an inactive enzyme. This would help to arrest glycolysis by stopping pyruvate utilization in mitochondria in heart and kidney tissues. Although we propose that changes in glycolytic flux and fatty acid oxidation may combine to down-regulate PDH, the exact time-course of the changes in PDH, F–2,6–P_2, and glycogen phosphorylase, as well as the switch to fatty acid oxidation, cannot be ascertained from the present study; this is because we compared tissues from active animals fed ad libitum with tissues from 3-day hibernating animals. Consequently, it is possible that changes in F–2,6–P_2, glycogen phosphorylase, and PDH occurred during a fasting phase which precedes hibernation and not during entry into hibernation. The exact nature of the time-course remains to be verified.

In contrast to *Z. hudsonius*, measurement of PFK and PK kinetic constants in *S. lateralis* tisses showed no differences when euthermic and hibernating animals were compared. PFK from *S. lateralis* has typical mammalian-type enzyme analogous to the characteristics of rabbit skeletal muscle M-type enzyme (Dunaway 1983). The M isozyme has a high affinity for F–6–P and ATP, is strongly activated by AMP, and is strongly inhibited by citrate and ATP. These properties support high flux during peak exercise in skeletal muscle when ATP and citrate concentrations are lower and AMP concentrations increase. In tissues with high rates of fatty acid oxidation, such as heart muscle, M-type PFK is relatively inactive owing to the presence of higher ATP and citrate levels. This isozyme is particularly well suited to the metabolic conditions of hibernation where decreases in F–2,6–P_2 and the increased citrate that accompanies a switch to fatty acid oxidation serve to depress PFK activity. Liver and kidney PFK showed characteristics typical of rabbit liver L-type isozymes. L-type PFK has a reduced affinity for F–6–P in the absence of allosteric activators, and a reduced (or absent) sensitivity to ATP inhibition. These properties serve to link enzyme activity to allosteric activator concentrations such as F–2,6–P_2 and AMP to permit carbohydrate use for synthetic purpose, and to turn off activity during gluconeogenesis when F–6–P concentrations are low. In hibernating liver and kidney, lower glycogen phosphorylase activity (Table 3) probably regulates overall flux through PFK by decreasing F–6–P concentrations to values well below the K_m reported in Table 1.

The data in Table 2 showed that *S. lateralis* PK was typical of other mammalian PK enzymes. However, in *S. lateralis* muscle tissues, the M_1-type PK (Hall and Cottam 1979) was unusual in showing activation by F–1,6–P_2 at low PEP concentrations. This effect was rather weak (a 2- to 3-fold activation) and occurred at F–1,6–P_2 concentrations which were much higher than physiological values (Srivastava and Bernhard 1986). This property could serve to link PK control to PFK activity via changes in F–1,6–P_2 concentration in both heart and skeletal muscle tissues. Muscle type PK isozymes are not usually highly regulated but may exhibit inhibition at higher ATP concentrations. These properties are indicative of an enzyme which does not play a large role in regulating glycolytic flux. Liver and kidney PK had a lower overall affinity for PEP similar to other mammalian M_2-type isozymes. Under our assay conditions, liver PK was not inhibited by L-alanine, whereas the kidney isozyme was very sensitive to L-alanine concentrations at low PEP concentrations. Similar to the rabbit M_2-type PK, neither *S. lateralis* liver nor kidney PK were sensitive to addition of F–1,6–P_2. Liver and kidney isozymes are usually inhibited during periods of gluconeogenesis from amino acids because of their sensitivity to L-alanine. These isozymes may also be inhibited during periods of amino acid oxidation when L-alanine concentrations are high.

Finally, in a recent paper, Geiser (1988) observed that overall metabolic rate depression may be regulated by different mechanisms, depending on the size of the hibernating animal and on its body temperature during torpor. This was revealed by a comparison of Q_{10} values during hibernation which decreased from approximately 4.5 to 2.5 as body size increased and as the difference between euthermic and hibernating body temperature

decreased. Since biological processes typically have Q_{10} values between 2 and 3, Q_{10} values above 3 (observed in smaller animals and in animals with higher hibernating body temperatures) imply that these animals actively depress metabolic processes. This paper suggests that this relationship does not apply to mechanisms of glycolytic control in hibernating mammals. The similar nature of the control mechanisms (decreased PDH activity, decreased F-2,6-P_2 levels) in both the small hibernator *Z. hudsonius* (Q_{10} 3–4) and in the relatively large hibernator *S. lateralis* (Q_{10} 2.5) suggests that for these animals glycolytic rate is actively depressed during hibernation and is not a consequence of a change in overall body temperature.

Acknowledgements. The authors thank Drs. C. Heller and T. Kilduff of Stanford University and their co-workers for the gracious supply of ground squirrel tissues, information on the animals and hibernation phenomena, criticism of the manuscript, and continued interest in these studies. This study was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada. We thank J.M. Storey for her editorial criticisms.

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