Matching of vertebrate cardiac energy demand to energy metabolism

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DRIEDZIC, WILLIAM R., BRUCE D. SIDELL, DAVID STOWE, AND RHODA BRANSCOMBE. *Matching of vertebrate cardiac energy demand to energy metabolism.* Am. J. Physiol. 252 (Regulatory Integrative Comp. Physiol. 21): R930–R937, 1987.—
Concentrations of high-energy phosphates and activities of key enzymes of energy metabolism were assessed in hearts from species with differing levels of cardiac power output. Positive correlations were found between resting power output and the total adenylate pool and between citrate synthase activity and the total adenylate pool. Maximum in vitro activity levels of enzymes from energy metabolism were compared with calculated resting cardiac power output and maximal cardiac power output (as reflected by total oligomycin-insensitive adenosinetriphosphatase activity). Three indexes of carbohydrate metabolism (hexokinase, pyruvate kinase, and L-lactate dehydrogenase) all plateau at relatively low levels of energy demand. In contrast, enzymes required for aerobic fatty acid metabolism, (carnitine palmitoyltransferase and 3-hydroxyacyl-CoA dehydrogenase) and for tricarboxylic acid and electron transport (citrate synthase and cytochrome-*c* oxidase) show consistent increases as ATP demand is elevated. It appears that as capacity for power development by vertebrate hearts, increases across taxa, the elevated demand for ATP is met by expansion of fatty acid based aerobic metabolism and not carbohydrate metabolism.

heart metabolism; heart power; energy metabolism

ALL HEARTS HAVE A CONTINUOUS NEED for ATP to meet the needs of the contractile apparatus; energy necessary to phosphorylate ADP is derived from oxidation of metabolic fuels. The level of ATP is extremely constant within an individual heart over a wide range of ATP demand (23). Even within a single cardiac cycle, rates of ATP breakdown and synthesis are so closely matched that very little oscillation in content is apparent (35). In rat heart, glucose is the primary metabolic fuel at low levels of energy demand, but at increased rates of work glucose breakdown is curtailed, and fatty acid metabolism is enhanced (25). It is not known if these relationships hold true for a wide spectrum of animals. The present study examines the activity levels of key enzymes of energy metabolism and the content of ATP and associated compounds in hearts from a variety of vertebrates from hagfish to birds and mammals. The objective is to assess what features of energy metabolism covary with energy demand.

The primary predictors of cardiac power output and hence energy demand are phylogeny and size. Evolutionary development of the vertebrate heart involved the transition from a single-circuit, low-pressure system found in fish to the complete double-circuit, high-pressure system characteristic of birds and mammals. Coincident with this evolutionary progression in cardiovascular circuitry has been a transition from poikilothermy to homeothermic endothermy. Hearts of endotherms have both an increased capacity for pressure development and an inherently higher frequency. At first approximation, these factors lead to a greater level of energy demand in hearts of higher than of lower vertebrates. Allometric relationships between cardiovascular performance and body size further influence demands for cardiac power. Within a class, small animals have much higher weight-specific cardiac outputs than large animals. Heart mass, though, remains relatively constant with respect to body size. This leads to higher levels of cardiac energy demand per unit heart weight in small relative to large animals. Each of these features was exploited in selecting animals with widely divergent cardiac energy demands. The most salient finding is that, when the full spectrum of cardiac energy demands among vertebrates is considered, the enzymatic machinery for aerobic fatty acid but not carbohydrate metabolism is expanded to meet increased power output across species. This major trend is in marked contrast to the apparently equivalent efficacy of carbohydrate and fatty acid fuels in supporting cardiac work of teleost hearts (29) and emerges only when data from higher taxa are considered.

MATERIALS AND METHODS

Animals. Hagfish (*Myxine glutinosa*), flounder (*Pseudopleuronectes americanus*), ocean pout (*Macrozoarces americanus*), and sea raven (*Hemitripterus americanus*) were obtained from the Huntsman Marine Laboratory, St. Andrews, NB. Dogfish (*Squalus acanthias*) and skate (*Raja erinacea*) were obtained from the Mount Desert Island Biological Laboratory, Mount Desert Island, ME. Trout (*Salvelinus fontinalis*) were obtained from the St. John Fish Culture Station, St. John, NB. Mudpuppies (*Necturus maculosus*), grass frogs (*Rana pipiens*), bullfrogs (*R. catesbeiana*), turtles (*Pseudemys sp.*), *Iguana iguana*, budgerigar, finches, mice, rats, guinea pigs, and rabbits were obtained from commercial suppliers. Pigs

and chickens were obtained from local distributors. Pigeons were trapped alive locally in either Sackville, NB, or Orono, ME; western fence lizards (*Scleroporus undulatus*) were live trapped in Arizona. Approximate animal weights are presented in the text.

In experiments involving enzyme analyses, all animals were either decapitated or stunned by a blow to the head; hearts were excised, rinsed, weighed, and subsequently homogenized. In experiments involving metabolite analyses, ectotherms, with the exception of hagfish, were either decapitated or stunned. Hagfish were anesthetized with buffered tricaine (0.1% in seawater). Endothermic animals were overdosed with Somnotol (M.T.C. Pharmaceuticals, Mississauga, ONT) delivered either intravenously or intraperitoneally. Hearts were rapidly excised and freeze-clamped between aluminum plates cooled in liquid nitrogen.

Metabolite analyses. Frozen tissue was powdered with a stainless steel pulverizer cooled to the temperature of liquid nitrogen. Finely ground tissue was placed in a centrifuge tube containing cold 6% $HClO_4$ and homogenized to a paste with a Tissumizer (Tekmar, Cincinnati, OH). The suspension was centrifuged at 20,000 *g* for 15 min, and the supernatant was neutralized with KOH. $KClO_4$ precipitate was removed by centrifugation, and the supernatant was immediately analyzed for creatine phosphate (CP), ATP, ADP, and AMP by standard enzymatic procedures (4). Data are expressed as moles metabolite per gram wet weight. Energy charge was calculated as ATP + (0.5) ADP/ATP + ADP + AMP (2).

Enzyme analyses. Tissues were excised, rinsed in extraction medium (see below), dried on absorbent paper, weighed, and transferred to cold medium (5–10% wt/vol) consisting of 10 mM tris(hydroxymethyl)aminomethane (Tris) and 1 mM ethylenediaminetetraacetic acid (EDTA) adjusted to pH 7.4. Hearts were homogenized in ground glass homogenizers and subsequently sonicated. Enzyme activities were determined on crude extracts under conditions of substrate saturation and optimal pH. Hexokinase [(HK) EC 2.7.1.1], pyruvate kinase [(PK) EC 2.7.1.40], L-lactate dehydrogenase [(LDH) EC 1.1.1.27], carnitine palmitoyltransferase [(CPT) EC 2.3.1.23], 3-hydroxyacyl-CoA dehydrogenase [(HOAD) EC 1.1.1.36], citrate (Si)-synthase [(CS) EC 4.1.3.7], and cytochrome-*c* oxidase [(Cyt Ox) EC 1.9.3.1] were assayed by well-established procedures (10, 15). Assay medium for adenosinetriphosphatase (ATPase) contained 50 mM Tris, 3.5 mM $MgSO_4$, 0.1 mM $CaCl_2$, 50 mM KCl, 5 mg/100 ml oligomycin, and 5.0 mM ATP, pH 7.2 at 20°C (29) Assay temperatures are indicated in the text. Enzyme activities are expressed as units per gram wet weight, where 1 unit is defined as 1 mole of substrate converted to product per minute.

Resting power calculation. Resting power output was calculated from the product of mean aortic pressure, cardiac output, and gravitational acceleration. Cardiovascular parameters utilized are for resting animals. Body weights for mammals (with the exception of seals) are taken from the present study. Weights for bullfrogs and grassfrogs are from Refs. 33 and 28, respectively. Body weights for turtle and fish have been set to 1.0 or 0.1 kg in the case of hagfish to allow use of the available cardiac output information. Body weights for which the cardiovascular parameters were originally determined for the turtle, amphibians, and fish are similar to the weights for which enzyme activities and metabolite information is here reported.

Heart mass and cardiac output for mammals and birds were calculated from scaling equations, Refs. 16 and 14, respectively. Mean ventral aortic pressure was set to 130 cmH_2O for mammals (27) and calculated for birds from scaling equations (14). Cardiovascular parameters for turtle at 20°C are reported in Ref. 34. A 1.0-kg turtle has a heart mass of 3.0 g. Cardiac output (at 25°C) and aortic pressure for the bullfrog are from Refs. 32 and 31, respectively. A 250-g bullfrog has a heart mass of 1.1 g. Cardiovascular parameters at 20°C for grassfrogs are reported in Ref. 28. A 20-g grassfrog has a heart weight of 0.08 g. Cardiovascular parameters for sea raven, ocean pout (12), and dogfish (19) are reported at 17°C and those for flounder at 10°C (6). The cardiosomatic indexes for these species are 0.86, 0.69, 0.85, and 0.46 g heart/kg body wt, respectively (29). A 100-g hagfish has a heart weight of 0.09 g. Power output was estimated at 10°C from values presented in Refs. 5 and 18.

Statistical analyses. Values are expressed as means ± SE. Data were transformed and fitted by linear least squares to functions that generally have been shown to have physiological relevance. These included linear, exponential, power, and hyperbolic functions. Relationships with the highest F value are shown (30). Equations are presented only for those relationships that are highly significant ($P < 0.005$). As data may fit more than one general function with $P < 0.05$, the ultimate resolution of equations is dependent on greater sample sizes. Regardless, it may be stated with reasonable confidence that relationships reported here that fit linear, exponential, or power functions all represent positive correlations between variables.

RESULTS AND DISCUSSION

Enzyme activity levels. Enzyme activity levels at their respective assay temperatures are shown in Table 1. HK, PK, and LDH are indexes of carbohydrate metabolism. In rat heart, HK has the lowest maximal activity of all the glycolytic enzymes and most closely approximates in vivo rates of glucose utilization (25). PK and LDH are qualitative predictors of in vivo carbon flux. CPT and HOAD are necessary for oxidation of fatty acids. In many muscle systems, the maximal activity of CPT closely matches calculated rates of fatty acid oxidation in vivo (8). CS catalyzes the point of entry of acetyl-CoA into the tricarboxylic acid cycle regardless of the source of carbon. Activities of this enzyme in vitro exceed maximal in vivo flux rates and thus must be viewed only as a qualitative indicator of citric acid cycle activity (7). Cyt Ox is considered to be a measure of the electron transport system. Total oligomycin-insensitive ATPase activity reflects the maximal ATP demand by the tissues (see below). The most striking feature of the data is that, despite large differences in assay temperature, there is

TABLE 1. *Maximum activity levels of enzymes in hearts from selected vertebrates*

	HK	PK	LDH	CPT	HOAD	CS	Cyt Ox	ATPase	Assay Temperature, °C
Mammals									
Weddell seal	2.0[a]	217.5[a]	1,032[a]			28.8[a]			37
Pig	1.27±0.08 (4)	157.6±4.3 (4)	350±15 (4)	0.098±0.02 (4)		20.11±0.71 (4)	38.17±3.02 (4)	2.57±0.38 (4)	25
Rat	1.30±0.24 (6)	69.36±13.50 (6)	485±62 (6)	1.20±0.11 (6)	54.5[b]	70.75±10.03 (6)	97.50±9.6 (6)	18.48±1.54 (6)	25
Birds									
Chicken	2.33±0.11 (5)	58.18±6.75 (5)	343±54.2 (5)	0.292±0.09 (5)		64.64±5.5 (5)	49.62±6.05 (5)	5.46±0.55 (5)	25
Pigeon	1.10±0.15 (4)	74.92±10.44 (4)	353.2±80.9 (4)	0.665±0.09 (4)	39.0[b]	88.78±13.3 (4)	36.50±6.5 (4)	10.70±1.21 (4)	25
Reptiles									
Fence lizard	7.29±0.15 (3)	140.5±4.2 (3)	332±11 (3)	1.00±0.03 (3)		24.5±3.6 (3)	21.98±1.48 (3)	6.23±1.14 (6)	25
Iguana	10.71 (2)	44.7 (2)	257.4 (2)	ND	9.1 (2)	29.0 (2)	13.2 (2)	5.84 (2)	25
Turtle	2.60±0.15 (4)	37.30±3.09 (4)	187.1±5.8 (4)	0.08±0.03 (4)	2.8[c]	15.88±0.46 (4)	7.88±0.52 (4)	1.44±0.22 (4)	15
Amphibians									
Grass frog	3.37±0.16 (5)	51.40±2.26 (5)	198±10 (5)	0.43±0.06 (5)	5.8[c]	17.13±0.82 (5)	21.55±0.88 (5)	3.81±0.57 (5)	15
Mud puppy	1.24±0.24 (3)	14.39±1.69 (3)	89.31±8.1 (3)	0.06±0.03 (3)	1.50 (2)	3.63±0.53 (3)	6.08±0.25 (3)	1.17±0.29 (5)	15
Fishes									
Sea raven	2.52[d]	37.04[d]	154.7[d]	0.11[f]	2.42[d]	12.05[d]	35.8[d]	8.10[f]	10
Ocean pout	2.45[d]	36.34[d]	127.8[d]	0.21[d]	1.79[d]	12.78[d]	29.8[d]	8.30[f]	10
Trout	8.3[g]	37.7[g]	225.2[g]	0.8[g]	13.2[g]	16.4[g]	32.4[g]	16.54±0.74 (7)	15
Dogfish[f]	4.35	66.69	250.5	ND	1.78	21.32	9.49	8.26	15
Hagfish	1.70[e]	35.98[e]	114.4[e]	0.06[e]	1.78[f]	6.92[e]	4.15[e]	5.31[f]	15

Values are means ± SE; sample population size in parentheses; activities are U/g wet wt. Additional data: rabbit CS 69(3) [Ref. 1]; guinea pig CS 61(2), HOAD 9.7(2); mouse CS 146(3) [Ref. 1]; budgerigar CS 120.4(2), HOAD 13.3(2); finch CS 146(2), HOAD 27.1(2); flounder CS 21.9 ± 4.7(3), HOAD 13.4(2); assay temperature 25°C. Mean values only are shown; [a] Ref. 22, [b] Ref. 20, [c] Ref. 20 (adjusted to 15°C), [d] Ref. 10, [e] Ref. 15, [f] Ref. 29, [g] Ref. 11. ND, not determined. See MATERIALS AND METHODS for other abbreviations.

considerable overlap in enzyme activity levels between higher and lower vertebrates. For instance, HK activities from fish, assayed at 10–15°C, are as high or higher than activity levels of the enzyme from endothermic organisms assayed at 25°C. The same relationship generally holds true for all other enzymes. If activity levels for enzymes from birds and mammals are doubled to approximate the activity at physiological temperatures, then levels for the non-rate-controlling enzymes are in most cases higher for endotherms than ectotherms. However, the activities of HK and CPT, the enzymes considered to be most predictive of flux rates in vivo, still show a great deal of overlap.

Both maximal ATPase activities and maximal O_2 consumption rates are known for two species only. In situ perfused heart preparations of sea raven consumed O_2 at ~1.3 μmol $O_2 \cdot g^{-1} \cdot min^{-1}$ at 10°C (13). Assuming a P/O ratio of 3, this equates to 7.8 $\mu mol \cdot g^{-1} \cdot min^{-1}$ of ATP turned over. ATPase activity from ventricular tissue measured at 10°C is 8.1 U/g (29), which precisely matches the predicted ATP supply. Maximal O_2 consumption by rat heart is ~5 mmol $O_2 \cdot h^{-1} \cdot g$ dry wt^{-1} at 37°C (32). This corresponds to ~45 μmol $ATP \cdot min^{-1} \cdot g$ wet wt^{-1} turned over at 25°C assuming a rate change of a process with 10°C increase (Q_{10}) of 1.8. The calculated rate of ATP supply is close to the maximal measured in vitro ATPase activity (18 U/g), given the assumptions involved and potential size and strain differences between the two groups of animals.

Metabolite contents. It is extremely difficult to excise and freeze-clamp heart before discharge of high-energy phosphates. However, the relatively high-energy charge of all except bird hearts suggest that metabolite measurements closely reflect in vivo status (Table 2). In heart, the contents of CP and ATP are similar. This is in contrast to the situation in vertebrate skeletal muscle in which CP is usually about threefold greater in content than ATP (3). The sum of the adenylates is more resistant to sampling error due to discharge of high-energy phosphates than any of the individual metabolites. Content of the total adenylate pool is higher in birds and mammals than in lower vertebrates. As opposed to the enzyme activity distribution, there is no overlap between ectotherms and endotherms with respect to this parameter.

Resting power output. Resting cardiac power output per gram heart of small ectothermic animals is as high as that of large endotherms, despite differences in body temperature (Table 3). Maximum power output for any individual organism is probably three- to fivefold higher than resting levels. In addition, cardiac efficiency among individuals possibly differs at maximum levels of power output. These constraints render definition of a precise relationship between resting and maximal power output

TABLE 2. *Levels of creatine phosphate and adenylates in hearts from selected vertebrates*

	n	CP	ATP	ADP	AMP	Adenylates	EC
Mammals							
Rabbit	5	3.58±0.42	3.81±0.09	1.05±0.08	0.35±0.04	5.20±0.07	0.83±0.01
Guinea pig	5	2.00±0.71	3.17±0.28	0.95±0.22	0.41±0.05	4.65±0.28	0.80±0.02
Rat	5	1.59±0.35	3.39±0.23	1.46±0.09	0.62±0.07	5.47±0.22	0.75±0.02
Mouse	7	1.83±0.31	2.78±0.22	1.34±0.06	0.68±0.08	4.80±0.17	0.72±0.02
Birds							
Chicken	9	0.62±0.10	2.55±0.21	1.58±0.20	0.80±0.15	4.93±0.44	0.69±0.03
Pigeons	6	1.74±0.31	3.62±0.24	1.18±0.12	0.42±0.05	5.39±0.39	0.80±0.01
Budgerigar	6	0.84±0.05	2.50±0.16	1.30±0.07	0.67±0.03	4.47±0.19	0.70±0.01
Zebra finch	6	1.16±0.52	2.05±0.30	1.14±0.12	0.64±0.08	3.82±0.22	0.67±0.03
Reptiles							
Turtle	5	2.03±0.35	1.60±0.23	0.50±0.14	0.07±0.01	2.17±0.17	0.85±0.04
Iguana	3	2.77±0.89	2.10±0.55	0.72±0.20	0.34±0.09	3.16±0.74	0.77±0.04
Amphibians							
Bullfrog	8	2.06±0.20	1.72±0.23	0.39±0.04	0.11±0.01	2.21±0.26	0.87±0.01
Mudpuppy	6	2.89±0.47	1.73±0.12	0.22±0.05	0.17±0.04	2.11±0.18	0.87±0.02
Grass frog	6	2.73±0.28	1.80±0.22	0.28±0.02	0.93±0.02	2.18±0.24	0.89±0.01
Fishes							
Sea raven	5	1.69±0.21	1.91±0.12	0.38±0.02	0.10±0.02	2.39±0.10	0.88±0.01
Flounder	7	2.22±0.17	2.16±0.16	0.63±0.10	0.27±0.04	3.06±0.25	0.81±0.02
Trout	5	1.86±0.23	2.50±0.04	0.54±0.04	0.13±0.02	3.18±0.09	0.88±0.01
Skate	4	1.24±0.33	1.66±0.13	0.56±0.10	0.39±0.08	2.61±0.15	0.75±0.04
Hagfish	6	1.51±0.29	0.47±0.14	0.34±0.09	0.08±0.02	0.89±0.24	0.72±0.24

Values are means ± SE in μmol/g wet wt. CP, creatine phosphate; EC, energy charge.

TABLE 3. *Resting cardiac power output in selected vertebrates*

	Size, kg	Pressure, cmH_2O	Cardiac Output, ml/min	Heart Mass, g	Power, mW/g
Mammals					
Seal	425	130	19,563	1,844	2.25
Pig	75	130	5,326	325	3.47
Rabbit	2.66	130	435	11.5	7.99
Guinea pig	0.65	130	150	2.8	11.38
Rat	0.23	130	70	1.0	14.67
Mouse	0.03	130	14	0.1	24.95
Birds					
Chicken	1.08	166	307	9.4	8.85
Pigeon	0.31	173	131	3.0	12.25
Budgerigar	0.04	181	32	0.5	20.67
Zebra finch	0.01	188	14	0.1	28.66
Reptiles					
Turtle	1.0	36	55	3.0	1.07
Amphibians					
Bullfrog	0.25	22	21	1.0	0.69
Grass frog	0.02	33	2	0.08	1.27
Fishes					
Sea raven	1.0	40	15	0.86	1.13
Ocean pout	1.0	47	18	0.69	2.00
Flounder	1.0	35	23	0.46	2.85
Dogfish	1.0	28	12	0.85	0.64
Hagfish	0.1	5	0.5	0.09	0.05

See text for genus and species.

impossible at the present time. Regardless, it seems acceptable to assume that major increases in cardiac power output among individuals must be associated with increases in ATP demand.

Matching of enzyme activity levels to resting power output. To assess the relationship between metabolic capacity to supply ATP and ATP demand, enzyme activities were converted (on the basis of a Q_{10} of 1.8) to the temperatures at which power output was determined. Mammalian and bird enzyme activity levels were corrected to 37 and 40°C, respectively. Activity levels of enzymes of carbohydrate metabolism reached their maximum at very low levels of energy demand (Fig. 1). On the other hand, two markers of fatty acid metabolism (CPT and HOAD) are expanded as resting power output increases. The exponential relationship between CPT activity and resting power development is highly significant. Values for CPT and HOAD from dogfish have not been included in this analysis because elasmobranch heart apparently has very little capacity to oxidize fatty acids for reasons independent of energy demand (9, 36). The relationship between Cyt Ox activity and resting power output best fits a power function; however, a linear relationship is also significant. There is a highly significant linear correlation between CS activity and power output. Collectively, the data indicate that increased resting power output is underwritten by expansion of aerobically based fatty acid metabolism. ATPase activity increases exponentially with respect to resting power output. This implies that the ratio between maximal cardiac work and resting cardiac work is not a constant; the scope for cardiac power output is expanded as resting power output increases.

Potential relationships between enzyme content and resting power output were explored by adjusting enzyme activities to a common temperature of 25°C and regressing against resting power output at body temperatures. In all cases, except for CS activity, plots yielded a random distribution of points. There is a linear increase in CS activity and content with respect to resting power output (Fig. 2). In mammals, total mitochondrial volume is proportional to resting cardiac power (17). Therefore, CS activity, measured at a common temperature, appears proportional to mitochondrial volume in heart from a broad spectrum of vertebrate animals.

Matching of metabolic enzymes and maximal ATPase

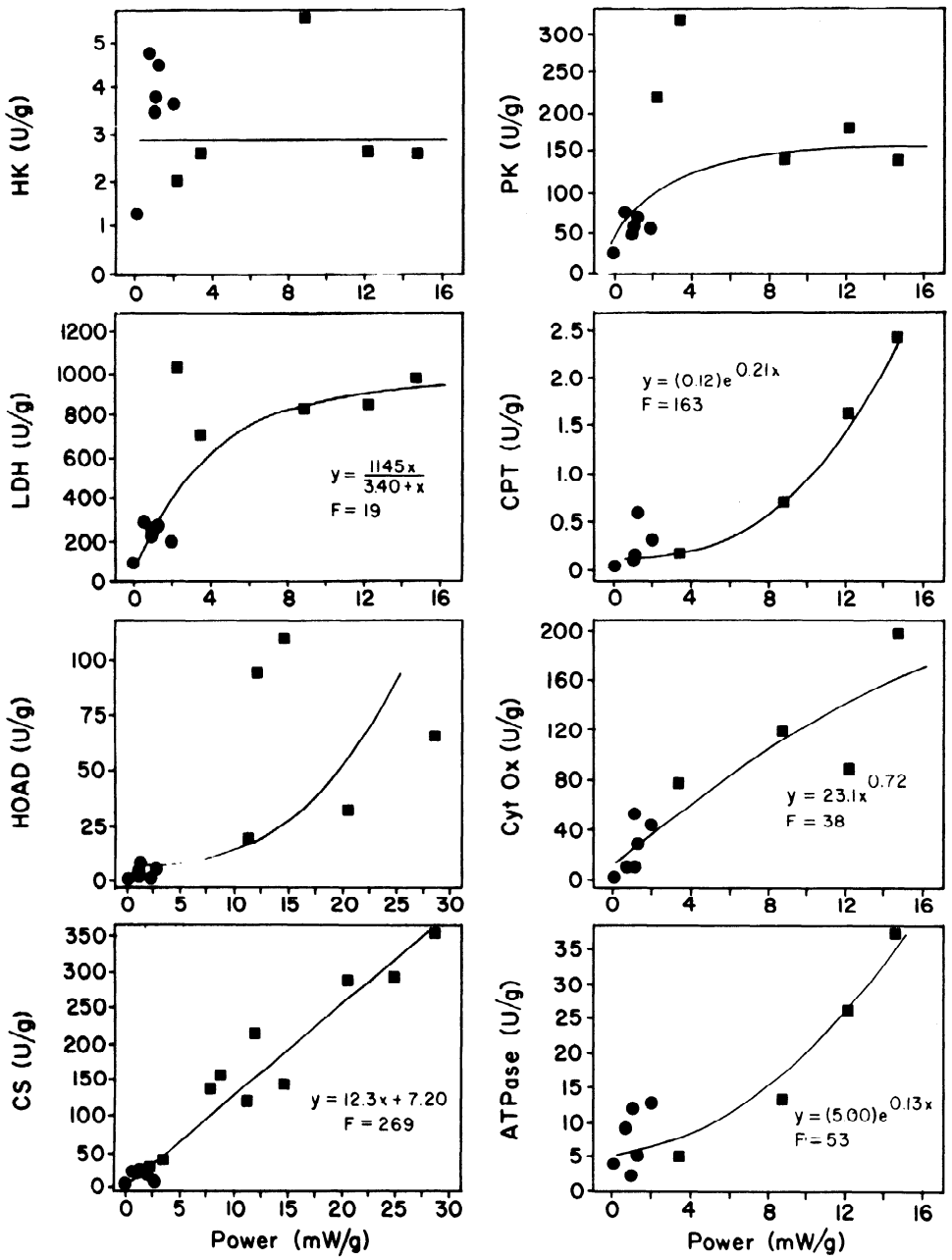

FIG. 1. Enzyme activity (U/g) vs. power (mW/g). Enzyme activities have been adjusted to same temperature as power output on basis of Q_{10} of 1.8. Data taken from Tables 1 and 3. Birds and mammals, *filled squares*; reptiles, amphibians, and fishes, *filled circles*.

activities. Maximal activity levels of metabolic enzymes were regressed against maximal ATPase activities to determine which components of metabolism are expanded in concert with maximal ATP demand. All enzyme activities were entered at their respective assay temperature; thus the analysis avoids any assumptions of Q_{10} values. The same general pattern in the organization of energy metabolism is revealed when metabolic enzyme activities were compared with maximal ATPase activities and compared with resting cardiac output. HK, the key indicator of aerobic glucose utilization, does not increase in association with increased levels of ATPase nor is there a significant relationship between PK and ATPase activities. LDH, the third marker of carbohydrate metabolism, increases in a hyperbolic fashion with respect to ATPase activity but plateaus at moderate levels of energy demand (Fig. 3). The lack of a significant increase in PK activities suggests that the LDH correlation may relate to increased rate of exogenous lactate utilization (i.e., lactate oxidase activity) and not to anaerobic metabolism (pyruvate reductase activity). CPT and HOAD increase linearly and Cyt Ox increases exponentially with respect to ATPase activity. These data collectively indicate that increased maximal ATP demand is matched with expansion of aerobic metabolism, fueled primarily by fatty acids and possibly lactate. Glycolytic potential does not elevate to meet increased energetic demands. The lack of a significant correlation between CS activity and maximal ATPase activity suggests that this component of metabolism is matched to resting and not maximal cardiac power output.

It is possible to estimate the ATP yield from the aerobic oxidation of glucose and fatty acids. Maximal flux through HK could yield at least 54 μmol ATP$\cdot$min$^{-1}\cdot$g^{-1} in all species. Even if in vivo the enzyme functions at only 20% of the maximum in vitro rate as

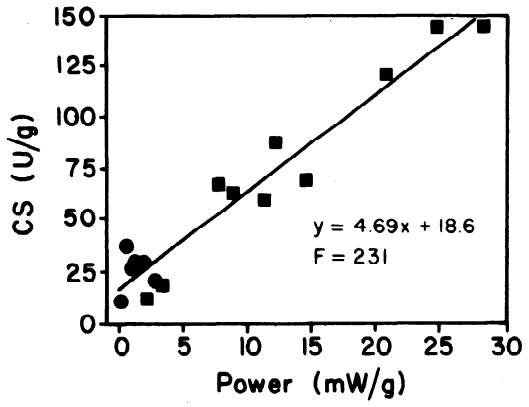

FIG. 2. Citrate synthase activity (U/g) vs. power (mW/g). Enzyme activities have been adjusted to common temperature of 25°C on the basis of Q_{10} of 1.8. Birds and mammals, *filled squares*; reptiles, amphibians, and fishes, *filled circles*.

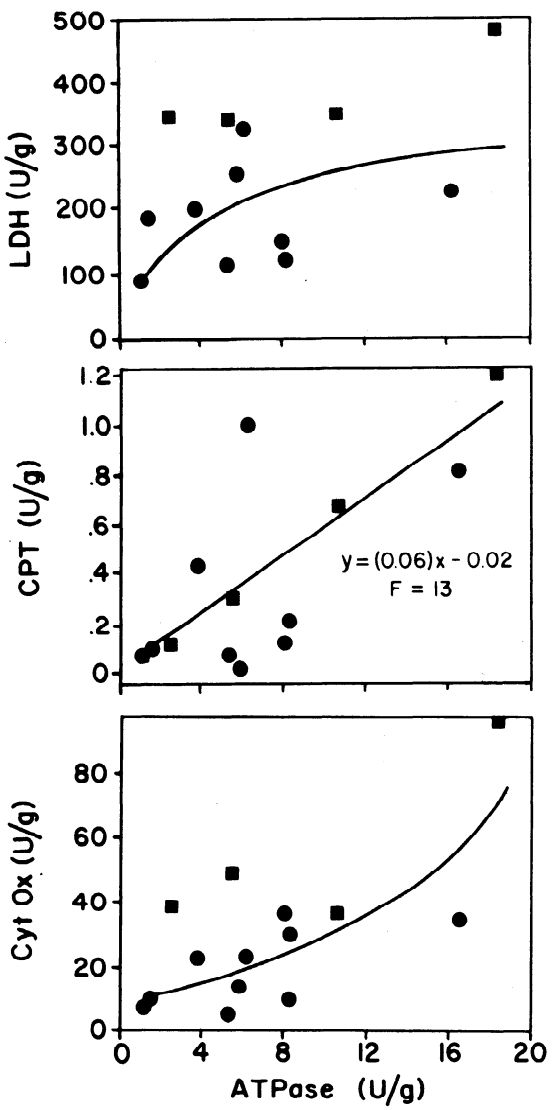

FIG. 3. Activity of metabolic enzymes (U/g) vs. adenosinetriphosphatase (ATPase) activity (U/g). Data taken from Table 1. Birds and mammals, *filled squares*; reptiles, amphibians, and fishes, *filled circles*.

shown for rat heart (25), ATP could be supplied by glucose catabolism alone at all but the highest rates of ATP demand. At the lowest levels of maximal ATPase activity, flux through CPT could yield ATP (based on 65 mol ATP/mol palmitate) at rates similar to that of demand. As demand is increased, capacity to supply ATP through fatty acid oxidation is sharply elevated. These conclusions, based solely on enzyme activities, are consistent with the observation that maximal performance and O_2 consumption by isolated rat hearts is higher if palmitate is available as a metabolic fuel than if glucose is the only exogenous substrate (24, 32).

Determinants of adenylate pool content. The independent relationships between resting power output, CS activity (content), ATPase activity (content), and the total adenylate pool were assessed. There are exponential increases in power output and CS activity vs. the sum of the adenylates (Fig. 4), but no clear relationship exists between ATPase activity and this parameter. Increases in resting ATP demand are therefore associated with increased total cellular adenylate concentration; a main contributor to the increase in total adenylates appears to be expansion of mitochondral volume based on CS measurements. There is no clear relationship between the sum of the adenylate pool and maximum ATP demand as reflected by ATPase activities.

Experimentally induced ischemia leads to a decrease in the sum of the adenylates in rat heart. Recovery of pressure development following an ischemic period is related to the restablishment of adenylate concentrations (21). It now appears that in a wide spectrum of animals an increase in the adenylate pool is associated with increased rates of ATP supply in resting hearts.

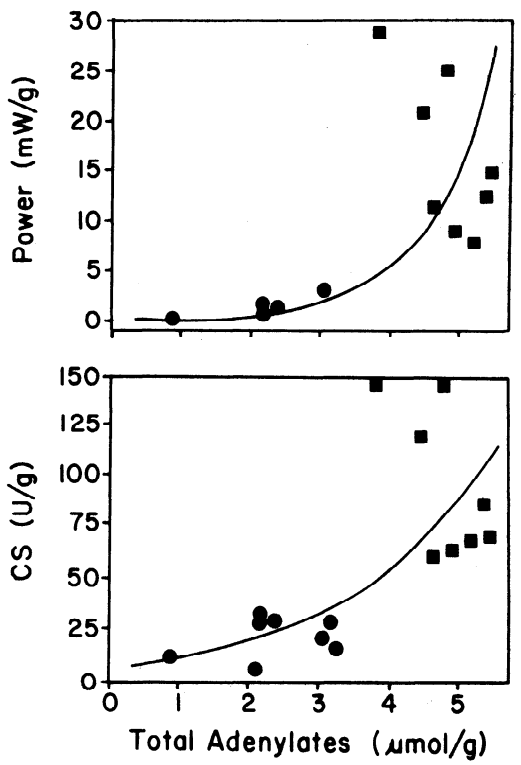

FIG. 4. Power (mW/g) and citrate synthase (CS) activity (U/g) vs. total adenylate pool content (μmol/g). Values are taken from Tables 1, 2, and 3. CS activities have been adjusted to common temperature of 25°C on basis of Q_{10} of 1.8. Birds and mammals, *filled squares*; reptiles, amphibians and fishes, *filled circles*.

General Conclusions

An examination limited to only the activities of key enzymes from hearts of different vertebrate classes reveals no clear pattern in the organization of energy metabolism. However, when these data are referenced to either resting power output per gram heart or maximal ATP demand, general relationships come into focus. Increased ATP demand is associated with an expansion of aerobically based fatty acid metabolism. Expansion of fatty acid catabolic potential is related to cardiac power output and not to phylogenic position. Thus 1 g of heart from an ectotherm operating at 10°C may have a rate of fatty acid metabolism equivalent to that of a large endotherm such as a seal or pig. Glucose catabolism apparently is not increased in concert with increased levels of energy demand. It is possible that machinery for maximal carbohydrate flux is already in place having evolved with the demands of anaerobic metabolism prevalent in the hearts of lower vertebrates (15). Once the lowest range of vertebrate cardiac power development is exceeded, further increases in cardiac work capacity are underwritten almost exclusively by selective expansion of capacity for fatty acid catabolism.

CS measurements at a constant temperature are predictive of, and directly proportional to, resting cardiac power output. Scaling relationships imply that CS activity per content is proportional to total mitochondrial volume. CS activity per content is positively related to total adenylate pool content, further suggesting that mitochondria are prime contributors to the size of the adenylate pool in heart.

Maximal ATP demand, as reflected by ATPase activities, increases exponentially with respect to resting power output. This implies that, across the vertebrate taxa, the scope for cardiac performance is increased exponentially as resting power output increases.

In summary, the most important findings of this study are that *1*) carbohydrate metabolism plateaus at relatively low levels of cardiac power output, *2*) aerobically based fatty acid metabolism is expanded with respect to ATP demand, *3*) CS activity is a predictor of resting cardiac power, and *4*) maximal ATPase activity increases exponentially with respect to resting cardiac power, suggesting that the scope for cardiac performance increases in a similar fashion with respect to resting power development.

We thank the Directors and staff of the Huntsman Marine Laboratory and the Mount Desert Island Biological Laboratory for providing some of the specimens. Western fence lizards were a gift from Dr. J. Hazel. J. M. Stewart assisted with the enzyme activity determinations on iguana hearts.

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada, the New Brunswick Heart Foundation, The Canadian Donner Foundation, the US National Science Foundation (PCM 81-04112), and the American Heart Association, Maine Affiliate.

Received 30 June 1986; accepted in final form 8 December 1986.

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