J Mol Cell Cardiol **21**, 367–373 (1989)

Biochemical Characteristics of Mammalian Myocardia

Sally Blank, Victor Chen,[1] Nancy Hamilton, Tomas A. Salerno[2] and C. David Ianuzzo*

Department of Biology and Physical Education, York University, Toronto, Ontario, Canada
[1]Department of Pathology, Yale University, New Haven, CT, USA, and
[2]Department of Cardiovascular Surgery, University of Toronto, Ontario, Canada

(*Received 26 May 1988, accepted in revised form 22 December 1988*)

S. BLANK, V. CHEN, N. HAMILTON, T. SALERNO AND C. D. IANUZZO. Biochemical Characteristics of Mammalian Myocardia. *Journal of Molecular and Cellular Cardiology* (1989) **21**, 367–373. Selected biochemical parameters of the ventricular myocardium were compared among several orders of adult mammals with established differences in resting heart rate (cattle, 51 beats/min; swine, 68; canine, 107; rabbit, 256; guinea-pig, 273; rat, 355; mouse, 475). It was hypothesized that the biochemical character of mammalian myocardia is associated with the chronic functional demand on the muscle. Therefore, differences observed in the myocardial biochemical potential among the species could reflect differences in resting heart rate. Myocardia from smaller mammals with higher resting heart rate had significantly ($P < 0.05$) higher maximal activities of citrate synthase, 3-hydroxyacyl-CoA dehydrogenase, lactate dehydrogenase (muscle/total), hexokinase and oxidation rates of glucose and palmitate than did larger mammals with lower resting heart rate. Maximal activities of phosphorylase and phosphofructokinase were more uniform across the animals. Correlation coefficients determined among average values of measured biochemical parameters and resting heart rate indicated that resting heart rate was closely associated with: citrate synthase ($r = 0.86$), 3-hydroxyacyl-CoA dehydrogenase ($r = 0.93$), ratio muscle/total lactate dehydrogenase ($r = 0.89$), hexokinase ($r = 0.89$), glucose oxidation ($r = 0.88$), and palmitate oxidation ($r = 0.93$). Significant correlations were observed among all of these parameters with the exception of citrate synthase vs. 3-hydroxyacyl-CoA dehydrogenase, and glucose oxidation vs. muscle/total lactate dehydrogenase. It was concluded that the oxidative capacity of mammalian myocardia was closely associated with resting heart rate, whereas the glycolytic potential of the myocardia was more uniform among the species.

KEY WORDS: Myocardial metabolism; Heart rate; Biochemical correlates: Citrate synthase; Phosphofructokinase; Phosphorylase; Hexokinase; Substrate oxidation; 3-hydroxyacyl-CoA dehydrogenase.

Introduction

The relationship between heart weight, body weight, and resting heart rate in different sized adult mammals is well known. Over a wide range of mammalian orders, body weight is inversely proportional to resting heart rate and directly proportional to heart weight of the animal [*8, 11, 12, 21*]. Comparisons of the energetic requirements of the myocardium from vastly different sized animals (i.e., shrew vs. cattle) have demonstrated that hearts of smaller animals have considerably higher energetic requirements (per unit time and body mass) than do larger animals [*11, 24*]. Myocardial metabolic parameters compared among different sized mammals have also illustrated a commensurate shift toward enhanced oxidative capacity, and higher myosin ATPase and sarcoplasmic reticulum ATPase activities in the hearts of smaller animals [*2, 11, 14, 18*]. Because resting heart rate of mammals is directly proportional to the metabolic rate per unit body weight [*12*] and representative of functional demand imposed on the myocardium, we postulated that selected metabolic and biochemical parameters that may be modulated by chronic myocardial activity would also correlate with interspecies differences in resting heart rate. Therefore, the purpose of this study was two-fold: (1) to determine whether the biochemical character of the heart is

* Please address all correspondence to: C. David Ianuzzo, T115 Steacie Building, York University, 4700 Keele Street, North York, Ontario M3J 1P3, Canada.

0022-2828/89/040367 + 08 $03.00/0

associated with differences in chronic functional demand (as represented by resting heart rate of the animal), and (2) to define the extent to which selected biochemical capacities may exist in mammalian myocardia.

Materials and Methods

Biochemical parameters of ventricular myocardia were compared among several different mammalian orders (C.D.-1 mouse, $n = 3$ to 4; Sprague Dawley rat, $n = 4$ to 5; Hartley guinea-pig, $n = 4$; New Zealand white rabbit, $n = 6$; mongrel dog, $n = 6$ to 10; Yorkshire swine, $n = 5$ to 9; Hereford cattle, $n = 5$ to 6). With the exception of cattle, hearts were removed following anesthesia of the animals with sodium pentobarbital (25 to 60 mg/kg, i.p.). Cattle hearts were removed following exsanguination of the animals. Myocardial tissue was either quick frozen in liquid nitrogen and stored at $-70°C$ or weighed and subsequently processed for enzymatic analyses. Citrate synthase and phosphofructokinase activities were spectrophotometrically determined according to Srere [*23*] and Shonk and Boxer [*22*], respectively. Total phosphorylase activity was measured as described elsewhere [*17*]. Hexokinase and 3-hydroxyacyl-CoA dehydrogenase activities were spectrophotometrically or fluorometrically analyzed [*2, 16*]. Lactate dehydrogenase activity was determined [*13*] and reported as the ratio of muscle isozyme activity:muscle plus heart (total) lactate dehydrogenase activity. Total protein was determined according to Lowry *et al.* [*15*]. Substrate utilization was measured by the production of radioactively labelled $^{14}CO_2$ from [U-^{14}C]-glucose or [-^{14}C]-palmitate in the incubation media using a modification of the procedure of Beatty *et al.* [*3, 4*] and Baldwin *et al.* [*1*] as described previously [*6*].

One-way analysis of variance and Duncan's Multiple Range Test were used to determine statistical differences between biochemical parameters. The degree of association between two average values was determined by the Pearson product moment correlation coefficient. Linear regression analysis was used for estimation of the equation of the line of best fit for related variables. Statistical significance was determined at alpha ≤ 0.05.

Results

Body weight, heart weight and the heart weight/body weight ratio of the mammals are shown in Table 1. Body weight was significantly correlated with heart weight ($r = 0.99$, $P < 0.001$). Ranges in body weight and heart weight exceeded four orders of magnitude between the smallest (mouse) and the largest (cattle) mammal. The ratio of heart weight/body weight varied among the animals. Smaller mammals had significantly lower heart weight/body weight ratios than larger mammals.

Ventricular myocardia from smaller mammals (mouse and rat) had significantly higher maximal citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activities and substrate oxidation rates than did larger mammals (Table 1). Maximal citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activities were approximately three-fold higher in the myocardia from smaller vs. larger mammals. There was approximately a 10-fold difference observed in glucose oxidation and palmitate oxidation rates among these animals.

Maximal activities of the enzymes associated with glycogenolysis and glycolysis (phosphorylase and phosphofructokinase) were generally more uniform among the mammalian hearts (Table 1). Maximal hexokinase activities of the ventricular myocardium were significantly higher in smaller vs. larger mammals. The ratio of the maximal activities of the lactate dehydrogenase isoforms of the myocardium significantly decreased from the smaller to the larger mammals (Table 1). The ratio of muscle:total lactate dehydrogenase activity was 37% higher in mouse than cattle myocardia.

Comparison of the enzymatic activities and substrate oxidation rates expressed in ratio form (Table 2). illustrate relatively constant proportions of 3-hydroxyacyl-CoA dehydrogenase/citrate synthase, phosphorylase/ phosphofructokinase, muscle/total lactate dehydrogenase/phosphofructokinase, muscle/total phosphorylase, and hexokinase/citrate synthase ratios among mammalian myocardia. General trends of increasing values of myocardial phosphofructokinase/citrate synthase, phosphorylase/hexokinase, hexokinase/

TABLE 1. Average values for mammalian myocardial parameters

	BW (kg)	HW (g)	HW/BW (g/kg × 10^3)	PHOS	PFK	HK	M-LDH/ M + H LDH	CS	HADH	GLU	PAL	RHR* (beats/min)
Mouse	0.04^{a}	0.1^{a}	3.3^{a}	21.7^{d}	42.0^{ab}	6.70^{c}	0.63^{c}	180.6^{d}	42.5^{d}	219.1^{e}	49.1^{d}	475
Rat	0.33^{a}	0.8^{a}	2.4^{ab}	20.3^{cd}	49.3^{b}	4.40^{b}	0.62^{bc}	142.6^{c}	35.4^{c}	117.0^{d}	33.6^{c}	355
Guinea-pig	0.50^{a}	1.3^{a}	2.7^{a}	12.2^{a}	37.8^{ab}	3.78^{b}	0.58^{bc}	108.2^{b}	21.4^{b}	60.2^{c}	18.3^{b}	273
Rabbit	4.33^{a}	6.7^{a}	1.5^{a}	14.2^{ab}	37.9^{ab}	2.23^{a}	0.49^{ab}	67.2^{a}	31.7^{c}	45.1^{bc}	11.8^{ab}	256
Canine	23.22^{ab}	169.2^{a}	8.5^{d}	22.7^{d}	47.2^{ab}	1.70^{a}	0.49^{ab}	96.9^{b}	10.9^{a}	—	—	107
Swine	33.44^{b}	197.2^{a}	5.4^{c}	17.6^{bc}	30.6^{a}	2.21^{a}	0.49^{ab}	65.9^{a}	17.4^{ab}	35.5^{ab}	7.4^{a}	68
Cattle	472.67^{c}	2583.3^{b}	5.5^{c}	14.3^{a}	31.3^{a}	2.40^{a}	0.40^{a}	73.0^{a}	13.4^{a}	26.0^{a}	4.6^{a}	51

* Abbreviations: body weight (BW); heart weight (HW); phosphorylase (PHOS); hexokinase (HK); muscle: muscle plus heart lactate dehydrogenase ratio (M-LDH/M + H LDH); citrate synthase (CS); 3-hydroxyacyl-CoA dehydrogenase (HADH); glucose oxidation (GLU); palmitate oxidation (PAL); resting heart rate (RHR).

Activities for phosphorylase, phosphofructokinase, hexokinase, citrate synthase, and 3-hydroxyacyl-CoA dehydrogenase are expressed as μmol/g/min.

Glucose oxidation and palmitate oxidation rates are expressed as nmol/g/min.

Mean values within a given parameter that have the same letter superscript are not significantly different.

Resting heart rates were determined from literature values (see Results).

TABLE 2. Ratios of average values

	HADH / CS	GLU[a] / CS	PAL[a] / CS	PFK / CS	PHOS / PFK	HK / PFK	(M − LDH/ M + H LDH) / PFK	PHOS / HK	(M − LDH/ M + H LDH) / PHOS	HK / GLU	HK / CS	HK/ M − LDH / M + H + LDH	GLU[a] / HADH	PAL[a] / HADH
Mouse	0.24	1.21	0.27	0.23	0.52	0.16	0.02	3.3	0.03	30.6	0.04	10.6	5.16	1.16
Rat	0.25	0.82	0.24	0.35	0.41	0.09	0.01	4.6	0.03	37.6	0.03	7.1	3.33	0.85
Guinea-pig	0.20	0.56	0.17	0.35	0.32	0.10	0.02	3.2	0.05	62.8	0.03	6.5	2.81	0.85
Rabbit	0.47	0.67	0.18	0.56	0.37	0.06	0.01	6.4	0.03	49.4	0.03	4.6	1.42	0.37
Canine	0.11	—	—	0.48	0.48	0.04	0.01	13.4	0.02	—	0.02	3.5	—	—
Swine	0.26	0.53	0.11	0.46	0.58	0.07	0.02	8.0	0.03	62.3	0.03	4.5	2.04	0.42
Cattle	0.18	0.36	0.06	0.43	0.46	0.08	0.01	6.0	0.03	92.3	0.03	6.0	1.94	0.34

Abbreviations as Table 1.

[a] Value × 10^{-3}.

TABLE 3. Correlation coefficients for mammalian myocardial parameters

	$\frac{M - LDH}{M + H\ LDH}$	HK	CS	HADH	GLU	PAL
RHR	0.89[a]	0.89[a]	0.86[a]	0.93[a]	0.88[a]	0.93[a]
LDHr	—	0.81[a]	0.85[a]	0.76[a]		
HK	0.81[a]	—	0.93[a]	0.81[a]		
CS	0.85[a]	0.93[a]	—	0.72		
HADH	0.76[a]	0.81[a]	0.72	—		
GLU	0.78	0.97[a]	0.96[a]	0.86[a]		
PAL	0.87[a]	0.97[a]	0.98[a]	0.89[a]		

Abbreviations as for Table 1.
[a] Significant at least at $P < 0.05$.

glucose oxidation ratios and decreasing values of hexokinase/phosphofructokinase, hexokinase/lactate dehydrogenase ratio, glucose oxidation/citrate synthase, palmitate/citrate synthase, glucose oxidation/3-hydroxyacyl-CoA dehydrogenase, palmitate/3-hydroxyacyl-CoA dehydrogenase ratios were observed from the smaller to the larger animals.

Established values for average resting heart rate of the mammals were taken from other sources [5, 7]. Correlation coefficients calculated among the average values for the biochemical parameters and established values of resting heart rate are listed in Table 3. Resting heart rate was significantly correlated with the lactate dehydrogenase ratio, the maximal activities of hexokinase, citrate synthase, 3-hydroxyacyl-CoA dehydrogenase and with the glucose oxidation and palmitate oxidation rates of the myocardium. Significant correlations were also obtained among all of these parameters with the exception of correlations between citrate synthase and 3-hydroxyacyl-CoA dehydrogenase, and between glucose oxidation and lactate dehydrogenase. Linear regression plots of the measured biochemical parameters versus resting heart rate (Figs 1 and 2) illustrate the association of the selected maximal enzyme activities and oxidation rates with resting heart rate.

Discussion

Enzyme markers for substrate oxidative metabolism (citrate synthase and 3-hydroxyacyl-CoA dehydrogenase) were significantly higher in smaller vs. larger

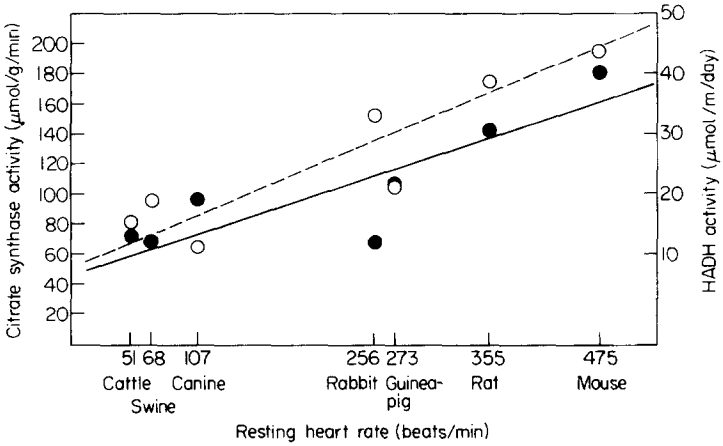

FIGURE 1. Activities of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase as correlates to resting heart rate. Linear regression plots for average enzyme activities vs. resting heart rate are represented as a continuous line for citrate synthase (●) and a discontinuous line for 3-hydroxyacyl-CoA dehydrogenase (○) activity. $y_{CS} = 0.234x + 51.95$; $y_{HADH} = 0.070x + 8.78$.

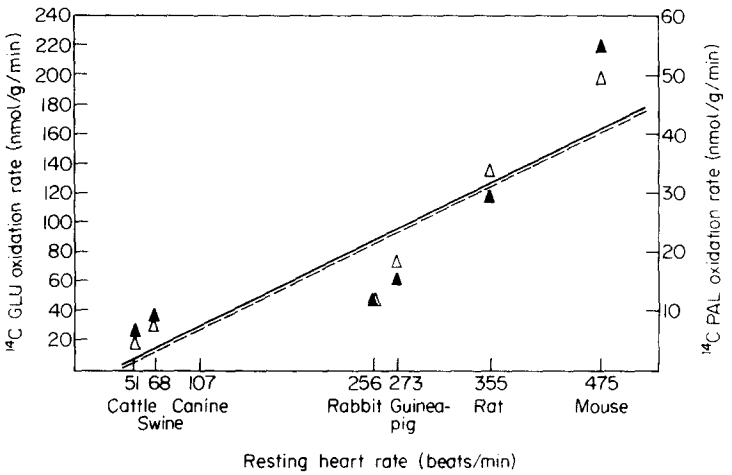

FIGURE 2. ^{14}C-glucose and ^{14}C-palmitate oxidation rates as correlates to resting heart rate. Linear regression plots are shown for average ^{14}C-glucose oxidation (▲—▲) and ^{14}C-palmitate (△- - -△) oxidation rates vs. resting heart rates. $y_{GLU} = 0.396x - 13.78$; $y_{PAL} = 0.098x - 3.43$.

mammals as were oxidation rates for radioactively labelled ^{14}C-glucose and ^{14}C-palmitate. The positive correlations between each of these parameters and resting heart rate represent an enhanced aerobic capacity of the ventricular myocardium that is associated with increased chronic activity of the heart. Supportive of this observation are the high correlations among myocardial citrate synthase activity and substrate oxidation rates shown in Table 3.

The glycolytic potential of the heart was more uniform among the mammals studied (Fig. 3). A narrow range of maximal activities was observed for phosphorylase, hexokinase and phosphofructokinase. However, hexokinase activity and the lactate dehydrogenase ratio were significantly correlated to resting heart rate. These findings suggest that the expression of an increased proportion of the muscle lactate dehydrogenase isoform and its concomitant high affinity for substrate with increasing pyruvate concentration [*10*] could enhance the potential for carbon flux through the glycolytic pathway in the hearts of smaller mammals. This potential was demonstrated by the approximately ten-fold difference in the *in vitro* glucose oxidation rates between mouse and cattle myocardia (Table 1). Furthermore, the increasing ratio of phosphorylase/hexokinase activity from smaller to larger animals suggest that glucose phosphorylation catalyzed by hexokinase could also play a role in supplementing the potential for increased carbon flux through the myocardial glycolytic pathway in mammals with resting heart rates exceeding 300 beats/min.

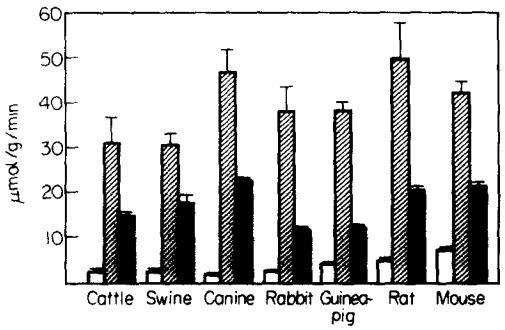

FIGURE 3. Phosphorylase, hexokinase, and phosphofructokinase activities in mammalian myocardia. The histogram illustrates the relatively constant proportions of the glycolytic enzyme activities of phosphorylase (■), hexokinase (□), and phosphofructokinase (▩) among mammalian myocardia. Values represent mean + S.E.M.

The relationships among the measured biochemical parameters expressed in ratio form (Table 2) show generally constant proportions of activities for the measured enzymes of: glycogenolysis/glycolysis, glycogenolysis/lactate dehydrogenase ratio, glucose phosphorylation/tricarboxylic acid cycle, and the tricarboxylic acid cycle/beta oxidation. Although the ratios were somewhat variable, the following ratios increased, from smaller to larger mammals, for the measured enzymes

of: glycolysis/tricarboxylic acid cycle, tricarboxylic acid cycle/substrate oxidation, beta oxidation/substrate oxidation rates, glucose phosphorylation/glucose oxidation, glycogenolysis/glucose phosphorylation, and lactate dehydrogenase ratio/glucose phosphorylation. These data are consistent with the constant and variable proportions of enzyme activities in various striated muscle as measured by others [*2*, *9*, *19*, *20*]. Furthermore, these ratios illustrate that the constant and variable proportions of enzyme activities are not only conserved among skeletal muscle with differing metabolic potentials by also across ventricular myocardia from mammals whose resting heart rate vary by at least one order of magnitude.

According to Bass *et al.* [*2*], heart muscle is characterized as having low potentials for glycogenolysis, glycolysis, and lactate production but high capacities for glucose phosphorylation and high enzymatic activities of the tricarboxylic acid cycle and fatty acid oxidation relative to fast-twitch glycolytic fibers of skeletal muscle. Results from the present study indicate that a continuum exists for the aerobic potential of the mammalian myocardium with smaller animals having at least a ten-fold higher capacity for substrate oxidation rates than larger animals. Similar to skeletal muscle, the relative consistency in the enzyme activities of glycogenolysis and glycolysis of the mammalian myocardium implies that the capacities for these metabolic pathways are in excess to the energetic requirement of the tissue [*10*] and are not as sensitive to chronic functional demand as is the aerobic potential of the heart. Thus, the generally increasing ratios for the enzyme activities of the glycolytic/oxidative metabolism in smaller vs. larger animals can be explained by the uniformly high glycolytic potential of the mammalian myocardia and the different oxidative capacity of the heart in these animals.

Recently, we have reported that selected biochemical characteristics of the mammalian diaphragm muscle are closely associated with the resting breathing frequency of the animal [*6*]. Biochemical correlates to functional demand were observed in the citrate synthase activity and substrate oxidation rates of the diaphragm muscle. The glycolytic potential of the muscle was not associated with breathing frequency of the animal. The results of the present investigation provide further evidence that chronic functional demand is correlated to selected biochemical parameters of mammalian striated muscle.

In summary, the oxidative capacity of mammalian myocardia was closely associated with the resting heart rate of the animal. Maximal hexokinase activity and the proportion of muscle-lactate dehydrogenase isoformic activity were also associated with the resting heart rate of the animal. However, the glycolytic potential of the ventricular myocardia was uniform among mammals. Across the species studied the ten-fold range in the resting heart rate of smaller vs. larger animals is associated with a commensurate enhancement of maximal enzyme activities of glucose phosphorylation, the tricarboxylic acid cycle, and fatty acid oxidation. The continuum observed in the aerobic metabolic potential of mammalian myocardia is also exemplified by substrate oxidation rates that progressively increase with mammalian resting heart rate such that smaller mammals with higher resting heart rate have approximately ten-fold greater capacity for substrate oxidation rates than do larger mammals with lower resting heart rate. Thus, these data appear to support the assumption that functional demand is associated with the biochemical character of mammalian myocardia.

Acknowledgements

The authors thank Holly Williams for her technical assistance and Valerie Baddon for the preparation of the manuscript. Cattle hearts were donated by Beef Terminal of Toronto. This work was supported in part by Canada NSERC Grant-0404.

References

1 Baldwin, K. M., Hooker, A. M., Herrick, R. E., Schrader, L. F. Respiratory capacity and glycogen depletion in thyroid-deficient muscle. J Appl Physiol **49**, 102–106 (1980).

2 Bass, A., Brdiczka, D., Eyer, P., Hofer, S., Pette D. Metabolic differentiation of distinct muscle types at the level of enzymatic organization. Eur J Biochem **10**, 198–206 (1969).

3 Beatty C. H., Peterson, R. D., Basinger, G. M., Bocek, R. M. Major metabolic pathways for carbohydrate metabolism of voluntary skeletal muscle. Am J Physiol **210**, 404–410 (1966).

4 Beatty C. H., Young, M. K., Bocek, R. M. Respiration and metabolism by homogenates of various types of muscle. Am J Physiol **223**, 1232–1236 (1972).

5 Biology Data Book. In *Biological Handbooks*. P. L. Altman and D. S. Dittmer (Eds), Vol. III, 2nd ed, pp. 1668–1692. Bethseda, MD: Federation of the American Society of Experimental Biology (1974).

6 Blank, S., Chen, V., Ianuzzo, C. D. Biochemical characteristics of mammalian diaphragms. Resp Physiol **74**, 115–126 (1988).

7 Canadian Council on Animal Care. *Guide to the Care and Use of Experimental Animals*, Vol. I, pp. 83. Ottawa, Ont., (1980).

8 Clark, A. J. *Comparative Physiology of the Heart*. New York: Macmillan (1927).

9 Crabtree, B., Newsholme, E. A. The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase and the glycerol 3-phosphate dehydrogenase in muscles from vertebrates and invertebrates. Biochem J **126**, 49–58 (1972).

10 Hochachka, P. W., Somero, G. N. *Biochemical Adaptation*. Princeton: Princeton University Press (1984).

11 Hoppeler, H., Lindstedt, S. L., Claassen, H., Taylor, C., Mathieu, O., Weibel, E. W. Scaling mitochondrial volume in heart to body mass. Resp Physiol **55**, 131–137 (1984).

12 Holt, J. P., Rhode, E. A., Kines, H. Ventricular volumes and body weight. Am J Physiol **215**, 704–715 (1968).

13 Ianuzzo, C. D., Chen, V. Metabolic character of hypertrophied rat muscle. J Appl Phys **46**, 738–742 (1979).

14 Ianuzzo, D., Blank, S., Hamilton, N., O'Brien, P., Chen, V., Brotherton, S., Salerno, T. The relationship of myocardial chronotropism to the biochemical capacities of mammalian hearts. In: *Biochemistry of Exercise VII*. Champaign, IL: Human Kinetics Publishers. (In press).

15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. Protein measurement with the Folin phenol reagent. J Biol Chem **193**, 265–275 (1951).

16 Lowry, O. H., Passonneau, J. V. *A Flexible System of Enzymatic Analysis*. New York: Academic Press (1972).

17 Noble, E. G., Ianuzzo, C. D. Influence of training on skeletal muscle enzymatic adaptations in normal and diabetic rats. Am J Physiol **249**, E360–E365 (1985).

18 O'Brien, P. J., Ling, E., Williams, H. M., Brotherton, S., Salerno, T. A., Lumsden, J. H., Ianuzzo, C. D. Sarcoplasmic reticulum ATPase and metabolic loads. Can J Cardiol **4**, 243–250 (1988).

19 Pette, D., Luh, W., Bucher, T. A constant-proportion group in the enzyme activity pattern of the Embden-Meyerhof chain. Biochem Biophys Res Commun **7**, 419–424 (1962).

20 Pette, D., Klingenberg, M., Bucher, T. Comparable and specific proportions in the mitochondrial enzyme activity pattern. Biochem Biophys Res Commun **7**, 425–429 (1962).

21 Rihl, J. Die Frequenz des Herzschlages. *Handbuch der Normalen und Pathologischen Physiologie*, Vol. VII, pp. 449–457. Berlin: Springer (1926).

22 Shonk, C. E., Boxer, G. E. Enzyme patterns in human tissue. I. Method for determination of glycolytic enzymes. Cancer Res **24**, 709–721 (1964).

23 Srere, P. A. Citrate synthase. Meth Enzymol **13**, 3–8 (1969).

24 Taylor, C. R. Structural and functional limits to oxidative metabolism: insights from scaling. Ann Rev Physiol **49**, 135–46 (1987).