Molecular Medicine 5: 110–116, 1999

Molecular Medicine

The Myocardial Profile of the Cytosolic Isozymes of Creatine Kinase Is Apparently Not Related to Cyanosis in Congenital Heart Disease

Gania Kessler-Icekson,[1] Einat Birk,[2] Hadassa Schlesinger,[1] Yael Barhum,[1] Niv Ad,[3] Menahem Friedman,[3] and Bernardo A. Vidne[3]

[1]Basil and Gerald Felsenstein Medical Research Center, The Rabin Medical Center, Petach Tikva, Israel
[2]Department of Pediatric Cardiology, Schneider Children's Medical Center of Israel, Petach Tikva, Israel
[3]Department of Cardiothoracic Surgery, Beilinson Hospital, The Rabin Medical Center, Petach Tikva, Israel

Abstract

Background: CKMB, the cardiac-specific heterodimer of cytosolic creatine-kinase (CK), is developmentally and physiologically regulated, tissue hypoxia being a proposed regulator. In patients with cyanotic heart disease the myocardium is perfused with partially saturated blood. We questioned whether the myocardium of cyanotic subjects contains higher proportions of CKMB.
Materials and Methods: CK activity, the distribution of cytosolic CK isozymes, activity of lactic dehydrogenase (LDH), and tissue protein content were determined in obstructive tissues removed at corrective surgery of patients with congenital heart defects. Cyanotic (n = 13) and acyanotic (n = 12) subjects were compared.
Results: In cyanotic and acyanotic patients, CK activity was 8.4 ± 0.6 and 7.6 ± 0.6 IU/mg protein and the proportion of CKMB was 21 ± 1.4 and 22 ± 2.0% (mean ± S.E.M), respectively. In the two groups of patients, the activity related to the B subunit corresponded to the steady-state level of the CKBmRNA. The tissue content of protein and the activities of CK and LDH were similar in cyanotic and acyanotic subjects and increased with the age.
Conclusions: The lack of difference in CKMB distribution between the cyanotic and acyanotic patients may either indicate that hypooxygenation is not a regulator of CK isozyme expression, or may be attributed to the already high proportion of this isozyme in hypertrophied, obstructive tissues. Recruitment of additional CKMB, in the cyanotic hearts, may thus not be required.

Introduction

Creatine kinase (CK, EC 2.7.3.2.) catalyzes the transfer of high-energy phosphate between creatine and ATP, maintaining a proper intracellular ratio of ATP to ADP, and playing a key role in the energy metabolism of skeletal and cardiac muscles. Distinct CK isozymes, localized at both the mitochondria and the cytoplasm, act as an intracellular system for the efficient transfer of energy between the site of production and the site of consumption (1,2). The native forms of the cytosolic CK are either homodimers of the M (CKMM, muscle) and B (CKBB, brain) subunits or a heterodimer of M and B (CKMB). The three isozymes differ in their kinetics and substrate

Address correspondence and reprint requests to: Dr. G. Kessler-Icekson, Basil and Gerald Felsenstein Medical Research Center, Beilinson Campus, The Rabin Medical Center, Petach Tikva 49100, Israel. Phone: 972-3-937 6806/7291; Fax: 972-3-921 1478; E-mail: icekson@post.tau.ac.il

affinity, CKMM having lower affinity than CKMB and CKBB (3–6). The heterodimer CKMB is unique for the cardiac muscle and its proportion depends on the expression of the B subunit, which is developmentally and physiologically regulated. A transition from B to M predominance occurs in mammalian heart at a relatively early embryonic stage (7–11). Increased proportions of CKMB and CKBB, as well as the CKBmRNA, have been described in several models of cardiac hypertrophy, ischemia, and failure (12–22). Biopsy samples of left ventricular myocardium from human subjects with left ventricular hypertrophy due to aortic stenosis exhibit lower CK activity and higher CKMB isozyme content, whereas similar specimens from patients suffering of coronary artery disease without hypertrophy have normal CK activity and increased CKMB isozyme ratio, as compared with normal adult hearts containing almost no CKMB isozyme activity (21). The observation that myocardium of patients with left ventricular hypertrophy or coronary artery disease contains higher amounts of CKMB isozyme has led to the suggestion that tissue hypoxia may play a role in stimulating the transition in cytosolic CK isozyme distribution (21).

Cyanosis is characterized by a slate-blue color of the skin which results from the presence, in the blood, of deoxygenated hemoglobin at concentrations higher than 5 g/dl. In patients with cyanotic congenital heart disease, diminished volume of pulmonary blood flow and a right-to-left shunt increase the relative volumes of deoxygenated blood pumped to the coronary and peripheral circulation. Arterial oxygen saturation is variable, ranging from very low values to almost normal range, depending on the degree of obstruction at the right ventricular outflow tract and the magnitude of the right-to-left shunt. Oxygen carrying capacity in cyanotic patients increases by producing more red blood cells and an eventual elevation of hemoglobin and hematocrit. As a result, blood viscosity is higher and the effective pulmonary and systemic resistance increase, causing more strain on the respective ventricles (23,24).

Tetralogy of Fallot (TOF) is a complex congenital malformation whose clinical spectrum extends from cyanotic hypoxemic newborns to young adults with mild or no cyanosis. TOF consists of four components that may vary in severity: (i) subpulmonary infundibular stenosis causing right ventricular outflow obstruction; valvar and supravalvar pulmonary stenosis may also exist; (ii) ventricular septal defect (VSD) allowing variable mixing between right and left ventricles; (iii) a dextroposed aorta; and (iv) right ventricular hypertrophy, secondary to the increased right ventricular pressure overload. Double-chamber right ventricle (DCRV) is a congenital malformation in which hypertorphied muscle bands divide the right ventricular cavity into a proximal high-pressure chamber and a distal low-pressure chamber. DCRV is commonly associated with a VSD but most patients in this group are not cyanotic.

We have undertaken the present study to determine whether the condition of hypooxygenation, which characterizes the cyanotic group of patients, potentiates increased expression of the CKMB isozyme as compared with the acyanotic group. Right ventricular obstructive tissues removed at corrective surgery of patients with TOF or DCRV were taken for the analysis. Our observations indicate high CKMB content and activity in all patients but no difference in isozyme distribution between the cyanotic and acyanotic patients.

Materials and Methods

Tissue Specimens

Obstructive muscles of the septoparietal bands (Septomarginal trabeculae) and moderator bands were removed from 25 patients undergoing corrective surgery for congenital malformation. The biopsied tissues, which are otherwise discarded, were rapidly frozen in the operation theater and stored at −70°C until processed. Patients having arterial oxygen saturation of 92% or lower, and/or clinical history of cyanosis, were defined as cyanotic. All other patients were considered acyanotic. The clinical details of all specimens were unmasked only when the biochemical and molecular analyses had been completed. In the cyanotic group (n = 13), 12 had TOF and one had DCRV plus severe subpulmonary stenosis. The acyanotic group (n = 12) included five patients with TOF, two with DCRV plus valvar pulmonary stenosis, two with DCRV and VSD, two with DCRV and no VSD, and one with VSD plus subpulmonary stenosis. All patients had systemic systolic right ventricular pressure except one who had 75% of the systolic pressure in the right ventricle. The study was approved by the Institutional Review Board.

Determination of Protein, DNA, and Enzyme Activities

Samples (20–120 mg) were cut from the frozen tissues and homogenized in ice cold 0.1 M phosphate buffer, pH 7.4, 1 mM EDTA, and 1 mM β-mercaptoethanol, using a polytron (Kinematica). The crude homogenate was sonicated (15 sec at 40% duty cycle) and aliquots were removed in duplicates for the determination of DNA, protein, and the activities of CK and LDH. Prior to enzymatic determination the samples were centrifuged in a minifuge for 5 min, 4500 rpm, 4°C. DNA was measured fluorimetrically using calf thymus DNA as a standard (25), and noncollagen protein was measured spectrophotometrically using BSA as a standard (26). CK activity was measured with a commercial kit (Sigma, St. Louis, MO), and CK isozymes were determined electrophoretically using the Helena Laboratories equipment and procedure (Clinin Scan II, agarose plates). Only the MM, MB, and BB isozymes were identified on the plates and scans (27). LDH was determined by the pyruvate/lactate reverse reaction as previously described (28). Calculation of the CKB-related activity was based on the assumption that all the B subunits are assembled in functional CK dimers and that the M and B subunits contribute equally to the activity of CKMB. The CKB-related activity in each specimen equaled the sum of activities of CKBB and CKMB/2, each calculated from the isozyme ratio (%) and the CK activity (IU/DNA) in that tissue.

RNA Analysis

Samples (100–300 mg) from the same frozen biopsies as above were extracted by the lithium chloride–urea/phenol extraction method (29). Total cellular RNA of the individual specimens was size fractionated on 1% agarose-formaldehyde gels (15–20 μg per lane), transferred to nitrocellulose membranes (Hybond-C Extra, Amersham, Little Chalfont, Buckinghamshire, U.K.), heat or UV immobilized, and subjected to hybridization with ^{32}P-labeled oligonucleotide probes using T4 polynucleotide kinase and [γ-^{32}P]-ATP. A 46-mer oligonucleotide corresponding to nucleotides 3779–4024 at the nontranslated 3′ end of the human CKBmRNA was used to detect the CKBmRNA (30). Variations between lanes were corrected by normalization to ribosomal RNA measured by hybridization with a 45-mer oligonucleotide specific for the human 28S ribosomal RNA (Oncogene Science, Uniondale, NY). Radiolabeled nucleotides were purchased from Amersham or Rotem Industries (Beer-Sheva, Israel). Hybridizations were performed overnight at 42°C in 6× SSC, 50 mM phosphate buffer, pH 6.5, 10× Denhardt's solution, 1% SDS, 50% formamide, and 200 μg/ml salmon sperm DNA. The membranes were rinsed as follows: four times briefly at room temperature (RT), 30 min at 65°C, 5 min at RT, then briefly with 2× SSC at RT. The membranes were exposed to Kodak film type X-OMAT-AR and the intensity of labeled bands was quantified by soft laser densitometry (Biomed Instruments, Fullerton, CA). The mRNA scores are expressed in arbitrary units (AU) summarizing results obtained from at least two repetitions for each RNA specimen.

Statistical Analysis

The data for all variables were normally distributed. The two-tail *t*-test and the nonparametric Mann-Whitney test were used to compare cyanotic and acyanotic patients. Analysis of covariance was further performed to correct for variations dependent on the age of the patients. Probability values of <0.05 were considered statistically significant.

Results

Twenty-five patients were included in this study; 13 were defined as cyanotic and 12 were grouped as acyanotic (Table 1). The cyanotic patients had lower arterial oxygen saturation and higher hemoglobin content, and were admitted for surgery at an average younger age than the acyanotic subjects. Since all our specimens were removed from obstructive tissues, we assessed in each of them the protein-to-DNA ratio as a measure for protein accumulation and hypertrophy. As shown in Figure 1, a marked increase in tissue protein content occurred with age in both cyanotic and acyanotic patients, and was significantly correlated with age. The mean protein-to-DNA ratio of acyanotics was higher (not statistically significant), apparently because they were operated on at older ages (Fig. 1, Table 2). The two groups of patients, hypooxygenated and normoxygenated, exhibited the same rate of right ventricular growth and hypertrophy.

The activity of CK per protein and the profile of the cytosolic CK isozymes did not change with age and was similar in the cyanotic and acyanotic

Table 1. Details of cyanotic and acyanotic patients

	Patients (*n*)	Mean ± S.E.M	Range
Cyanotic			
Age (years)	13	3.5 ± 0.88	0.3–8.7
Hemoglobin (g/dl)	12	16.0 ± 0.86*	10.8–20.1
O_2 (% saturation)	12	82.5 ± 2.40*	66.0–92.0
Acyanotic			
Age (years)	12	8.2 ± 2.21	0.9–24
Hemoglobin (g/dl)	9	12.4 ± 0.42	10.3–14.3
O_2 (% saturation)	9	97.7 ± 0.17	97.0–98.0

*$p < 0.05$ compared with acyanotic patients.

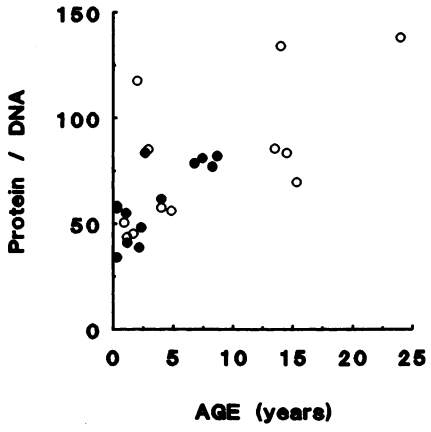

Fig. 1. Correlation of age with protein content. Closed circles, cyanotic; open circles, acyanotic; linear regression analysis: $r = 0.71$; $p = 0.00007$.

subjects (Table 2). The observed augmentation of cytosolic CK and each of its isozymes thus corresponded to the increase in tissue protein (Fig. 2). Similar to cytosolic CK, LDH activity per protein displayed neither variation according to age nor any difference between cyanotic and acyanotic subjects (Table 2), and its amount in the tissue increased as protein accumulated (not shown).

The contribution of CKMB to CK activity was relatively high in all patients, ~22%,

Table 2. Protein content and enzyme activities in cyanotic and acyanotic patients

	Patients (*n*)	Mean ± S.E.M	Range
Cyanotic			
Protein/DNA	13	61.2 ± 4.9	34.1–83.4
CK (IU/mg)	13	8.4 ± 0.6	6.1–13.5
CKMM (%)	13	78 ± 1.6	72–87
CKMB (%)	13	21 ± 1.4	13–27
CKBB (%)	13	1 ± 0.2	0–2.3
LDH (IU/mg)	13	1.60 ± 0.07	1.18–2.14
Acyanotic			
Protein/DNA	12	80.5 ± 9.7	43.7–138
CK (IU/mg)	12	7.6 ± 0.6	3.9–10.9
CKMM (%)	12	77.0 ± 2.3	60–86
CKMB (%)	12	22.0 ± 2.0	14–38
CKBB (%)	12	1.0 ± 0.3	0–2.6
LDH (IU/mg)	12	1.48 ± 0.10	0.85–2.09

IU/mg, enzyme activity expressed as international units (IU) per mg protein; %, percent of total CK activity.

whereas the proportion of CKBB activity was found to be negligible (Table 2). No correlation was observed between the distribution of CK isozymes and the degree of tissue hypertrophy (protein/DNA). The total activity of CKMB in the tissue increased, however, parallel to the increase in tissue protein and was the same in cyanotic and acyanotic patients (Fig. 2). The amount of CKMB and CKBB isozymes depends on the availability of the B subunit. As shown in Figure 3, the estimated amount of B-subunit, expressed as CKB-related activity, correlated with the abundance of CKBmRNA, indicating regulation at the level of transcription. Notably, no correlation was found between the proportion of CKMB and the arterial oxygen saturation or the hemoglobin content in either group of subjects as well as in the combined population.

Discussion

We were interested in finding out whether the activity of cytosolic CK and the relative amount of CKMB isozyme are altered in the myocardium of cyanotic patients as compared with similar tissues of acyanotic subjects. Obstructive muscle

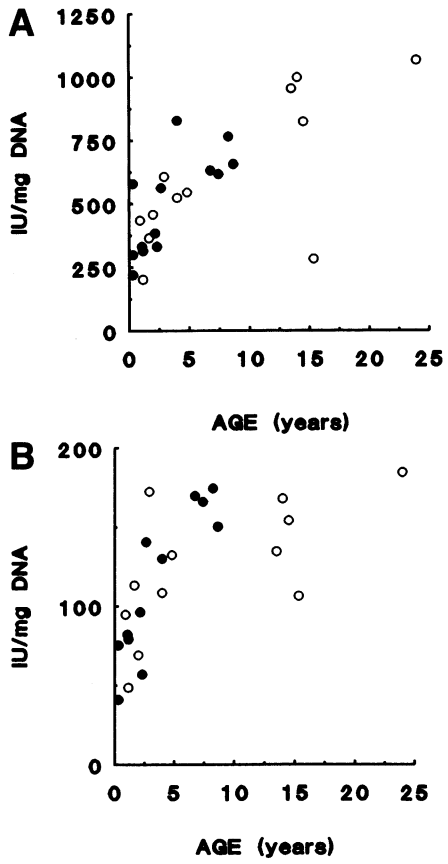

Fig. 2. Correlation of age with tissue CK (A), and CKMB (B) activities. Closed circles, cyanotic; open circles, acyanotic. Linear regression analysis: (A) $r = 0.72$; $p = 0.00005$; (B) $r = 0.66$; $p = 0.0004$.

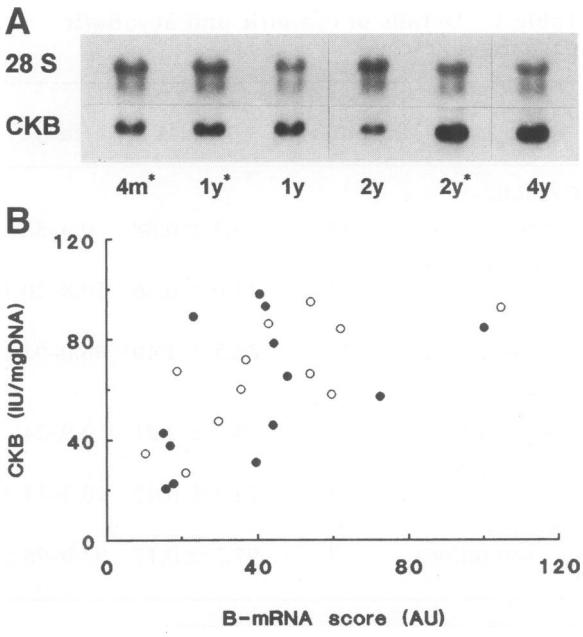

Fig. 3. (A) Representative Northern blots of six specimens probed for CKBmRNA and 28S rRNA. Asterisk denotes a cyanotic patient; m, months old; y, years old. (B) Correlation between CKB-related activity and the abundance of CKBmRNA. Closed circles, cyanotic; open circles, acyanotic. Linear regression analysis: $r = 0.56$; $p = 0.003$.

bands removed from the right ventricle of patients with congenital malformations were explored and no differences in protein accumulation, CK activity, or distribution of CK isozymes were observed between the cyanotic and acyanotic subjects.

Since the tissues analyzed were hypertrophied secondary to the obstructed right ventricular outflow tract, and as most of the patients were still at an age of normal cardiac growth, it is likely that the observed increase in tissue protein was regulated by both developmental and pathophysiological factors. It is clear, however, that the state of cyanosis did not alter the rate of protein accumulation.

Age-matched and equally preserved specimens of normal right ventricle were inaccessible for obvious reasons. We therefore evaluated the results of our enzymatic measurements by comparison with data available from other reports on the human heart. The most detailed study of CK distribution in the diseased human heart thus far reported that in normal, ischemic, and hypertrophic left ventricles, the activities of CK were 12, 11, and 8 IU/mg protein, respectively (21). The mean values of CK activity in our study were comparable to those of activities measured in hearts suffering from aortic stenosis, with or without coronary artery disease. The LDH activity per protein, obtained by us, resembled activities documented in either normal or hypertrophied adult myocardium, supporting the notion that myocardial LDH activity does not alter with age or with hypertrophy (21). Furthermore, the proportions of CKMB in either cyanotic or acyanotic patients were similar to the ratios of CKMB in hearts suffering from aortic stenosis alone or in combination with coronary artery disease. This finding was 20 times higher than the 1% ratio reported by the same authors in the normal human heart. Overall, our findings in the obstructed right ventricle greatly resemble observations made in the obstructed left ventricle (21). Earlier publications claimed that in adult human hearts the proportions of CKMB vary between 4

and 27%, and in infant hearts between 0 and 4% (31,32). The marked discrepancies between studies may result not only from differences in the age and pathophysiological condition of the sampled hearts (which were not always specified) but also from variations in the myocardial sites of sampling, the rapidity and method of tissue preservation, and the procedure of isozyme determination. Our study was restricted to freshly removed and quickly frozen specimens in which we found a 13–38% range of CKMB ratio with an average of 22%. This high ratio of CKMB, apparently maintained through the abundance of CKBmRNA, characterized cyanotic and acyanotic patients. The fact that all our specimens represented hypertrophied muscle explains, at least in part, the high proportion of CKMB. As CKMB is considered beneficial to the myocardium at states of reduced oxygenation, it is reasonable to suggest that an already high proportion of CKMB is sufficient to support the cyanotic hypooxygenated myocardium and recruitment of additional CKMB may therefore not be required. It may also be that higher proportions of CKMB cannot be attained in the postnatal myocardium, as no higher values have been reported in the literature (12–21,31,32).

Alternatively, the lack of difference in the CKMB ratio between cyanotic and acyanotic patients may suggest that arterial oxygen saturation is not an independent regulator of CK-isozyme distribution, or that the reduction in oxygen saturation was not low enough to stimulate it. As was recently reported, simulation of cyanosis in vitro by chronic hypooxygenation of human pediatric cardiocytes impaired mitochondrial aerobic metabolism, and reduced the capacity of ATP production (33). CK activity and isozyme distribution were not measured in that study; however, the model system employed may provide a tool for testing these variables under reduced oxygen pressure, independent of increased workload demands, or hypertrophy. Such measurements are beyond the scope of this clinical study.

Corrective surgery of the congenitally malformed heart is almost the only source of juvenile myocardial specimens; however, studies of such tissues are relatively few. Alterations in β-adrenoceptor density were observed in the hearts of patients suffering from cyanotic congenital heart disease (34). Also, an abnormality in the temporal expression of myosin light-chain isotypes was reported in the right infundibulum of TOF patients (35), whereas no correlation was found between the expression of myosin light- and heavy-chain isotypes in the right atrial appendage of various heart defects (36). Our study has demonstrated similarity in CK activity and isozyme distribution between myocardial tissues of cyanotic and acyanotic patients with congenital heart defects and right ventricular hypertrophy. Additional investigative efforts are still required for molecular characterization of the human malformed heart.

Acknowledgments

This work was supported by a grant from the Chief Scientist's Office, Israel Ministry of Health, and by the Shlezak Foundation, Tel-Aviv University.

References

1. Bessman SP (1985) The creatine kinase phosphate energy shuttle. *Ann. Rev. Biochem.* **54:** 831–862.
2. Kammermeier H (1987) Why do cells need phosphocreatine and a phosphocreatine shuttle. *J. Mol. Cell Cardiol.* **19:** 115–118.
3. Eppenberger HM, Dawson DM, Kaplan NO (1967) The comparative enzymology of creatine kinases. I. Isolation and characterization from chicks and rabbit tissues. *J. Biol. Chem.* **242:** 204–209.
4. Dawson DM, Eppenberger HM, Kaplan NO (1967) The comparative enzymology of creatine kinases. II. Physical and chemical properties. *J. Biol. Chem.* **242:** 210–217.
5. Wong PCP, Smith AP (1976) Biochemical differences between the MB and MM isozymes of CK. *Clin. Chim. Acta* **68:** 147–158.
6. Szasz G, Gruber W (1978) CK in serum. 4. Differences in substrate affinity among the isozymes. *Clin. Chem.* **24:** 245–249.
7. Hall N, Deluca M (1975) Developmental changes in creatine phosphokinase isoenzymes in neonatal mouse hearts. *Biochem. Biophys. Res. Commun.* **66:** 988–994.
8. Hassebaink HDJ, Labruyere WT, Moorman AFM, Lamers WH (1990) Creatine kinase isozyme expression in prenatal rat heart. *Anat. Embryol.* **182:** 195–203.
9. Cepica S, Jorgensen PF (1977) Ontogenetic development of creatine phoshphokinase in skeletal muscles and hearts from pigs. *Acta Vet. Scand.* **18:** 143–151.
10. Trask RV, Billadello JJ (1990) Tissue specific distribution and developmental regulation of M and B creatine kinase mRNAs. *Biochim. Biophys. Acta* **1049:** 182–188.
11. Wessels A, Vermeulen JLM, Viragh SZ, et al. (1990) Spatial distribution of tissue-specific anti-

gens in the developing human heart and skeletal muscle. I. An immunohistochemical analysis of creatine kinase isoenzyme expression patterns. *Anat. Rec.* **228:** 163–176.
12. Vatner DE, Ingwall JS (1984) Effects of moderate pressure overload cardiac hypertrophy on the distribution of creatine kinase isoenzymes. *Proc. Soc. Exp. Biol. Med.* **175:** 5–9.
13. Younes A, Schneider JM, Bercovici J, Swynghedauw B (1985) Redistribution of creatine kinase isoenzymes in chronically overloaded myocardium. *Cardiovasc. Res.* **19:** 15–19.
14. Pauletto P, Nascimben L, Piccolo D, et al. (1989) Ventricular myosin and creatine kinase isoenzymes in hypertensive rats treated with captopril. *Hypertension* **14:** 556–565.
15. Smith SH, Kramer MF, Reis I, Bishop SP, Ingwall JS (1990) Regional changes in creatine kinase and myocyte size in hypertensive and nonhypertensive cardiac hypertrophy. *Circ. Res.* **67:** 1334–1344.
16. Fontanet HL, Trask RV, Haas RC, Strauss AW, Abendschein DR, Billadello RJ (1991) Regulation of expression of M, B, and mitochondrial creatine kinase mRNAs in the left ventricle after pressure overload in rats. *Circ. Res.* **68:** 1007–1012.
17. Sharkey SW, Murakami MM, Smith SA, Apple PS (1991) Canine myocardial creatine kinase isoenzymes after chronic coronary occlusion. *Circulation* **84:** 333–340.
18. Williams RE, Kass DA, Kawagoe Y, et al. (1994) Endomyocardial gene expression during development of pacing tachycardia-induced heart failure in the dog. *Circ. Res.* **75:** 615–623.
19. Schultz D, Su X, Bishop SP, Billadello J, Dell'Italia LJ (1997) Selective induction of the creatine kinase-B gene in chronic volume overload hypertrophy is not affected by ACE-inhibitor therapy. *J. Mol. Cell. Cardiol.* **29:** 2665–2673.
20. Neubaur S, Frank M, Hu K, et al. (1998) Changes of creatine kinase gene expression in rat heart post-myocardial infarction. *J. Mol. Cell Cardiol.* **30:** 803–810.
21. Ingwall JS, Kramer MF, Fifer MA, et al. (1985) The creatine kinase system in normal and diseased human myocardium. *N. Engl. J. Med.* **313:** 1050–1054.
22. Van der Laarse A, Hollar L, Kok SW, et al. (1992) Myocardial creatine kinase-MB concentration in normal and explanted human hearts and released from hearts of patients with acute myocardial infarction. *Clin. Physiol. Biochem.* **9:** 11–17.
23. Driscoll DJ (1990) Evaluation of the cyanotic newborn. *Pediatr. Clin. North Am.* **37:** 1–23.
24. Pinsky WW, Arciniegas E (1990) Tetralogy of Fallot. *Pediatr. Clin. North Am.* **37:** 179–192.
25. Labarca C, Paigen K (1980) A simple, rapid and sensitive DNA assay procedure. *Anal. Biochem.* **102:** 244–252.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall AJ (1951) Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **193:** 265–275.
27. Kessler-Icekson G, Sperling O, Rotem C, Wasserman L (1984) Cardiomyocytes cultured in serum-free medium, growth and creatine kinase activity. *Exp. Cell Res.* **155:** 113–120.
28. Fux A, Sidi Y, Kessler-Icekson G, Wasserman L, Novogrodsky A, Nordenberg J (1991) Dimethylthiourea inhibition of B16 melanoma growth and induction of phenotypic alterations; relationship to ATP levels. *Br. J. Cancer* **63:** 489–494.
29. Auffray C, Nageotte R, Chambraund B, Rougeon F (1980) Mouse immunoglobulin genes: a bacterial plasmid containing the entire coding sequences for a pre-gamma 2a heavy chain. *Nucl. Acids Res.* **8:** 1231–1241.
30. Mariman ECM, Schepens JTG, Wieringa B (1989) Complete nucleotide sequence of the human creatine kinase B gene. *Nucl. Acids Res.* **15:** 6385.
31. Jockers-Wretou E, Pfeleiderer G (1975) Quantitation of creatine kinase isoenzymes in human tissues and sera by an immunological method. *Clin. Chim. Acta* **58:** 223–232.
32. Marmor A, Margolis T, Alpan G, et al. (1980) Regional distribution of the MB isoenzyme of creatine-kinase in the human heart. *Arch. Pathol. Lab. Med.* **104:** 425–427.
33. Merante F, Mickle DAG, Weisel RD, et al. (1998) Myocardial aerobic metabolism is impaired in a cell culture model of cyanotic heart disease. *Am. J. Physiol.* **275:** H1673–H1681.
34. Kozlik R, Krammer HH, Wicht H, Krian A, Ostermeyer J, Reinhardt D (1991) Myocardial beta-adrenoceptor density and the distribution of beta 1- and beta 2-adrenoceptor subpopulations in children with congenital heart disease. *Eur. J. Pediatr.* **150:** 388–394.
35. Auckland LM, Lambert SJ, Cummins P (1986) Cardiac myosin light and heavy chain isotypes in tetralogy of Fallot. *Cardiovasc. Res.* **20:** 828–836.
36. Shi Q, Danilczyk U, Wang J, et al. (1991) Expression of ventricular myosin subunits in the atria of children with congenital heart malformations. *Circ. Res.* **69:** 1601–1607.