The following is the abstract of the article discussed in the subsequent letter:

**Tobita and Keller BB.** Maturation of end-systolic stress-strain relations in chick embryonic myocardium. *Am J Physiol Heart Circ Physiol* 279: H216–H224, 2000—The embryonic myocardium increases functional performance geometrically during cardiac morphogenesis. We investigated developmental changes in the in vivo end-systolic stress-strain relations of embryonic chick myocardium in stage 17, 21, and 24 white Leghorn chick embryos (n = 10 for each stage). End-systolic stress-strain relations were linear in all developmental stages. End-systolic strain decreased from 0.50 ± 0.02 to 0.31 ± 0.01 (mean ± SE, P < 0.05), while average end-systolic wall stress was similar at 3.29 ± 0.34 to 4.19 ± 0.43 mmHg (P = 0.14) from stage 17 to 24. Normalized end-systolic myocardial stiffness, a load-independent index of ventricular contractility, increased from 2.98 ± 0.19 to 6.03 ± 0.39 mmHg from stage 17 to 24 (P < 0.05). Zero-stress midwall volume increased from 0.024 ± 0.002 to 0.124 ± 0.004 μl from stage 17 to 24 (P < 0.05). These results suggest that the embryonic ventricle increases normalized ventricular “contractility” while maintaining average end-systolic wall stress over a relatively narrow range during cardiovascular morphogenesis.

**Stress-Strain Relations in Embryonic Chick Heart**

*To the Editor: In the July 2000 issue of the American Journal of Physiology-Heart Circulatory Physiology,* Tobita and Keller (6) used experimental data and a theoretical model to compute wall stress in stages 17, 21, and 24 embryonic chick hearts, and they proposed a way to characterize material properties. Because mechanical stress likely affects heart development, this is an important problem. We feel, however, that several errors in their analysis render many of their results and conclusions questionable. Below we list six areas of concern.

First, a main conclusion of this study is that average end-systolic wall stress remains nearly constant during development (see the above abstract). This statement defies the laws of mechanics, because ventricular pressure increases by two orders of magnitude during development, whereas the ratio of radius to thickness changes much less. In fact, models for the mature human heart have shown that the peak wall stress is about 50 kPa = 375 mmHg (1) compared with the peak of about 6 mmHg reported by Tobita and Keller (6) for the embryonic heart. Because mature human and chick hearts have roughly similar (scaled) geometry and pressure, the stresses should be similar. Hence, even if the authors’ estimates are correct and wall stress does not change much between stages 17 and 24, it clearly must increase dramatically at some point during development.

Second, the authors used the equations of Mirsky et al. (2) to compute stress. These equations are valid if the ventricular wall is composed of a linear, isotropic, homogeneous material. Although it is likely that none of these qualifications are true for the embryonic heart, the authors justify using the Mirsky equations by showing that a more realistic, inhomogeneous model (4) predicts a similar value for the peak stress. There is, however, a serious problem with their analysis. Namely, as stated in the paragraph above, Eq. A8 in Ref. 6, they modified the material parameters for the myocardium in the Taber et al. (4) model so that the peak stress given by that model matched the peak stress predicted by the Mirsky model (2). They then claim that this agreement between the two models confirms the validity of the Mirsky model. This is a circular argument. In fact, for the same pressure, equilibrium considerations dictate that the peak stresses in pressurized homogeneous and inhomogeneous tubes cannot be equal.

Third, because Eq. 5 in Ref. 2 was derived using linear stress-strain relations, there should be no surprise that Eq. 5 itself is linear. Below this equation, however, the authors state that “Equation 5 indicates that the stress-strain relationship is linear.” Clearly, this is another circular argument.

Fourth, the end-systolic “stress-strain relations” shown in Fig. 4 of the Tobita and Keller paper apparently were obtained by drawing a straight line through two points. The caption to this figure reads “End-systolic stress-strain relations were linear in all developmental stages.” Once again, the argument appears to be circular. The paper presents no data supporting this contention such as stress-strain loops for variations in preload and afterload.

Fifth, ventricular wall volume was determined by applying NaCl solution to the heart to elicit maximum contraction. The authors then estimated the volume of the remaining mass, assuming that all blood had been forced out of the heart. This method has been used in previous papers, but, to our knowledge, this crucial assumption has never been verified. In fact, it seems likely that some blood would remain trapped in the spaces of the porous wall. (The embryonic heart trabeculates during the studied stages.) Furthermore, the in vivo wall thickness was computed by assuming that the ventricular wall is incompressible. Whereas this may not be a bad assumption at stage 17, it clearly is not valid at stages 21 and 24.

Sixth, the quantity $D_{0, m}$ is identified as a zero-stress diameter, but in this paper, it actually represents the diameter of the unloaded ventricle at peak activation.

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This state is not free of stress. The zero-stress state must be determined by cutting the heart (3).

In summary, we feel strongly that many of the statements made in this paper are not supported by the data and could potentially mislead future investigators. Moreover, many of the same concerns apply to at least one other paper that has been published previously in the American Journal of Physiology-Heart Circulatory Physiology (5). We hope that the authors can address our concerns satisfactorily.

REFERENCES


Larry A. Taber
Dept. of Biomedical Engineering, Washington University, St. Louis, MO 63130
E-mail: lat@biomed.wustl.edu

Renato Perruchio
Dept. of Mechanical Engineering, University of Rochester, Rochester, NY 14627

REPLY

To the Editor: We would like to respond in detail to the letter by Drs. Taber and Perruchio regarding two of our recent papers (13, 14) and their assertion that “several errors in their analysis render many of their results and conclusions questionable.” This work was focused on the developing embryonic chick heart and our analysis of ventricular mechanics combining in vivo experimental data with simplified numerical models to determine pressure-volume and stress-strain relations during cardiac development. It is important to note that from 1989 to 1994 we actively collaborated with Drs. Taber and Perruchio in the application of biomechanical approaches to the developing myocardium (10–12).

The chick embryo is an excellent model to investigate cardiac function and mechanics during cardiac morphogenesis and has both important similarities and distinctions from the mature heart. The early embryonic heart (stage 16) is smooth walled with thin myocardial and endocardial layers and a thick layer of cardiac jelly. The embryonic ventricle then rapidly transforms into a nonaxisymmetric, variably trabeculated chamber with distinct future right ventricular and left ventricular geometry (stages 17 to 36) and regionally anisotropic contraction patterns (15).

Regarding the specific comments by Drs. Taber and Perruchio:

1) “a main conclusion... is that average end-systolic wall stress remains nearly constant during development...” Taber and Perruchio suggest that the laws of mechanics require that end-systolic wall stress must increase during early cardiac morphogenesis due to the difference between a peak wall stresses of 6 mmHg for the embryonic heart and 375 mmHg for the mature ventricle. The estimated embryonic end-diastolic ventricular volume at stages 17 to 24 is approximately 0.1–0.4 μl, whereas that of the mature human left ventricle is 70–100 ml (approximately 2 × 105 times larger). Peak systolic pressure of the embryonic ventricle at these stages is 1.5–2.2 mmHg versus 100 mmHg for mature chicken (3) and 100–150 mmHg for the mature human heart (approximately 50 to 100 times higher). For an initial approximation, we can calculate peak stress by the simplified equation, \( \sigma = \frac{P}{D/h} \), where \( P \), \( D \), and \( h \) are, respectively, intraventricular pressure, ventricular midwall dimension, and wall thickness. If we assume that ventricular geometry (\( D/h \)) is similar for the embryonic and mature left ventricle, wall stress should be 2–8 mmHg in the embryonic ventricle and 300–500 mmHg in the mature left ventricle, consistent with the calculations in our papers. However, the important point is that over the stages of our investigation that represent the critical period of early cardiac morphogenesis, peak stress remains “relatively constant” versus the peak stress of the adult heart, in contrast to the Taber’s theoretical model (6, 9) adapted from the Rodriguez’s stress-dependent tissue growth theory (8). We certainly agree that peak stress must increase over time, but the majority of this increase likely occurs coincident with the geometric increase in arterial pressure that occurs following the completion of cardiac morphogenesis (3, 16).

2) “the authors use the equations of Mirsky et al. (2) to compute stress... of a linear, isotropic, homogeneous material. none of these qualifications are true for the embryonic heart...” Taber and Perruchio suggest that our application of Mirsky’s model for stress-strain relations in the mature myocardium is not valid for the embryonic heart because we “modified material parameters from an earlier study (10) to allow our data to fit Mirsky’s model.” First, it is important to note that to reach a numerical solution for the calculation of pressure-volume and stress-strain data for the stage 16 embryonic heart, Taber et al. (10) chose arbitrary values for the material properties of the embryonic myocardium due to the absence of supporting in vivo or in vitro data to reach a numerical solution for his laminated shell model. It was due to this specific limitation of Taber’s method that we chose to use the Mirsky’s model (7) because it does not depend on arbitrary

...
material properties to reach a solution for the embryonic heart. Therefore, we acquired in vivo pressure-volume data during changes in ventricular loading conditions and during sequential development stages and then we applied MirsKEY's approach to the embryonic heart to evaluate the relative changes in end-systolic stress-strain relations during these conditions. Thus we selected Mirsky's method because it requires the smallest set of assumptions and is based on in vivo data.

3) “... because Eq. 5 was derived using linear stress-strain relations...”. Taber and Perruchio are concerned that our data do not support a linear end-systolic stress-strain relationship for the embryonic heart. In Refs. 13 and 14, we described the method to determine the end-systolic unloaded midwall diameters \( D_{0,m} \) according to Mirsky’s method (7). First, we assumed end-systolic wall stress difference-logarithmic \( D_{0,m} \) relations are linear. We then set \( D_{0,m} \) at a certain point. End-systolic stress-logarithmic midwall dimension points were then fit by a linear-regression analysis. A new \( D_{0,m} \) was then obtained by extrapolation to zero stress. This iterative procedure was continued until the value for \( D_{0,m} \) converged. After we obtained the \( D_{0,m} \), we calculated the end-systolic wall strain. In both studies, we tested whether the end-systolic stress-strain relations were linear and confirmed there are no significant departures from linearity (Fig. 3 and Table 4 in Ref. 13 and Table 2 in Ref. 14). We then estimated myocardial stiffness using Eq. 6 in Ref. 13 and Eq. 5 in Ref. 14. There is no circular argument in this method.

4) “[s]tress-strain relations... were obtained by drawing a straight line through two points... no data supporting this contention... such as stress-strain loops for variations in preload or afterload.” In brief, we obtained simultaneous ventricular pressure-dimension data of at least five to seven cardiac cycles during the acute preload or afterload alteration without altering other hemodynamic parameters and also obtained data during changes in contractility produced by verapamil or \( Ca^{2+} \) administration (13). As stated above, all experiments were completed using protocols to alter ventricular loading characteristics.

5) “[v]entricular wall volume was determined by applying NaCl solution to the heart to elicit maximum contraction... this method has never been verified... furthermore, in vivo wall thickness was computed by assuming that the wall is incompressible... this is not valid at stages 21 and 24... ”. Our use of NaCl in the calculation of ventricular wall volume is based on the experimental observation that the topical application of 2 M NaCl induces a tetanic contraction of the embryonic ventricle that ejects blood from the trabecular wall and ventricular lumen (4). By tracing the epicardial border of the embryonic heart during the cardiac cycle and then following tetanic contraction, we can calculate total ventricular volume and wall volume to calculate the difference, representing the volume of blood within the embryonic heart. We chose this method to be superior to estimating ventricular cavity volume from an optically detected pseudoendocardial border. Whereas a small volume of intratrabecular blood may remain in the heart following NaCl contracture, in our experience the estimated values for ventricular wall volume are very reproducible for each embryonic stage. One additional piece of data that supports our estimate of wall volume is the finding that Clark et al. (2) showed that ventricular dry weight increases approximately 1.4 times between stage 21 and stage 24. Our calculations of ventricular muscle volume for comparable stages also increase approximately 1.4 times, similar to Clark’s data. To our knowledge, there are no better, readily available methods to estimate ventricular volume in ovo from sequential embryonic ventricles. Finally, we agree with Taber and Perruchio that the dimensions of the ventricular wall change dynamically during the cardiac cycle, including the compression of interventricular spaces. We recognize that the NaCl administration is imperfect in representing true muscle volume due to porosity. However, our analysis does not depend on assumptions related to regional porosity or trabecular compression because we assume that the myocardial mass is incompressible during the cardiac cycle despite the ejection of blood from intertrabecular spaces.

6) “... \( D_{0,m} \) is identified as a zero-stress diameter, but in this paper, it actually represents the diameter of the unloaded ventricle at peak activation. Zero stress state must be determined by cutting the heart.” We have made an error in using the term “zero stress” rather than “unloaded” and plan to address this issue in the future. However, this does not invalidate our application of Mirsky’s method to the embryonic heart.

7) “[s]tate that the dimensions of the ventricular wall... during the cardiac cycle... includes contribution of endocardium and myocardium were chosen to aid the numerical solution. Next, Taber applied an active pseudostrain energy function to a single set of end-systolic pressure-volume loops obtained during volume.
infusion. From our experience, during volume infusion to obtain a wide range of ventricular preload (Fig. 3 in Ref. 10), hemodynamic parameters, such as heart rate and arterial resistance, are obviously changed almost simultaneously (13). Finally, Taber chose an active shift ratio (stretch ratio of passive zero-stress state referenced to absolute zero-stress state) based on data from the mature heart (1) to determine the end-systolic point in the Fig. 3 in Ref. 10. This active shift ratio greatly influences the relationship between material constants and calculated end-systolic wall stresses. In fact, we could only reproduce Taber’s published results using a single unique numeric solution for a single pressure-volume loop. Thus, we share Taber and Perruchio’s concern regarding unsubstantiated models and potential impact on the reader with limited background on the developing myocardium but differ as to our opinion regarding which models have been validated for the embryo by in vivo data and which remain “unsubstantiated.” Obviously, further in vivo and in vitro experimental data will provide additional insights in this important area.

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Kimimasa Tobita  
and Bradley B. Keller  
Dept. of Pediatrics,  
University of Kentucky,  
Lexington, KY 40536