THEORETICAL ANALYSIS OF THE RELATIONSHIP BETWEEN REGIONAL AND GLOBAL MEASURES OF MYOCARDIAL CONTRACTILITY

HIDEO KUSUOKA, M.D., Ph.D.

Contractile function of the left ventricle is often measured regionally, particularly in intact animals. In this paper I dispute the validity of the common assumption that regional shortening accurately reflects whole-heart mechanics, even when contractility is homogeneous, by using an ellipsoidal model of the left ventricle to examine the relationship between measures of regional function and whole-heart function. Segment shortening and wall thickening are the most common measures of regional function, while the end-systolic pressure-volume relationship, or $E_{\max}$, is a well-validated index of contractility in the whole heart. Assuming that the arterial end-systolic pressure vs stroke volume relationship is constant, segment shortening shows a monotonically increasing, convex relation to $E_{\max}$. If, in addition, myocardium is considered an incompressible material, then wall thickening also shows a monotonically increasing, convex relation to $E_{\max}$. Thus, segment shortening and wall thickening are quite insensitive when measuring high contractility and overly sensitive when measuring low-contraction. Finally, I consider the non-linear relationship between regional measures of function and global contractility, and thereby resolve apparently contradictory findings regarding the inotropic responsiveness of stunned myocardium.

(*Jpn Circ J* 1993; 57: 904–911)

THE end-systolic pressure-volume relationship (ESPVR) in ejecting mode, or simple isovolumic peak pressure in non-ejecting mode, has been validated as a rigorous index of contractile function in whole hearts.^{1–3} The slope of ESPVR ($E_{\max}$) is a measure of elastance, which is a material property of the myocardium of a ventricle.^{1–3} The counterpart of $E_{\max}$ in regional mechanics is the stiffness constant of myocardium at end-systole.^{4,5} Although an index of regional strain alone, such as segment

shortening or wall thickening, cannot represent the stiffness, changes in segment length or wall thickness of the left ventricle are frequently used as indices of regional function in *in situ* hearts. Furthermore, segment shortening has been criticized due to its significant regional variability^{6,7} as well as dissociation between epicardial and transmural function.⁸ On the other hand, wall thickening has been considered to be a more reliable index of regional function,⁸ although nonuniformity has been observed across the myocardial wall.^{7,9}

This article considers the relation between indices for regional function and whole-heart function, and discusses the theoretical limitations of segment shortening and wall thick-

Key words:

End-systolic pressure-volume relation
Segment shortening
Wall thickening
Theoretical analysis
Stunned myocardium

(Received October 22, 1992; accepted January 21, 1993)

Department of Tracer Kinetics, Biomedical Research Center, Osaka University Medical School, Suita Osaka 565, Japan

Mailing address: Hideo Kusuoka, M.D., Ph.D. Department of Tracer Kinetics, Biomedical Research Center, Osaka University Medical School, 2-2 Yamada Oka, Suita Osaka 565, Japan

Fig.1. The relation between pressure and volume in whole heart (left) and the relation between force and length in regional myocardium (right). A: Lines a and b represent ventricular end-systolic pressure volume relation (ESPVR) and arterial ESPVR, respectively. Curve c represents the diastolic pressure-volume relation of a ventricle. B: Solid curve shows the end-systolic force-length relation.

ening in quantifying changes in contractility.

METHODS

The entire approach was computational. Equations were solved analytically. The details of model assumptions are given with the results.

RESULTS

We will first consider the relation between segment shortening and contractility, and then move on to consider the relation between wall thickening and contractility.

Model assumptions

A thin-layer model is adopted for analyzing the relation between segment shortening and contractility because segment shortening is usually measured in the sub-endocardium. Let us consider the relation between pressure-volume and force-length in a whole heart, and assume that the end-systolic pressure-volume relationship of the heart (ESPVR) is linear! Thus,

$$P = E_{\max} \cdot V + V_0 \quad \text{Eq (1)}$$

where P , $E_{\max}$, V and V_0 are the intraventricular pressure, the slope of ventricular ESPVR, the ventricular volume, and the unstressed volume to generate zero peak

isovolumic pressure, respectively. The other assumptions adopted in this model are follows:

1) The shape of the heart is an ellipsoid!^{10,11} When ejection is allowed during systole, myocardium contracts concentrically to the weight point of the ventricle, retaining its shape.

2) The relation between pressure and force (F) is governed by Laplace's law!¹² With the above assumptions, the following end-systolic force-length relationship can be derived from Eq. 1.

$$F = E_{\max} \cdot P(L^4 - L \cdot L_0^3)/q \quad \text{Eq (2)}$$

where

F : force generated by a unit of myocardium,

L : length of a segment in the epicardium at given volume V ,

L_0 : length of a segment in the epicardium at volume V_0 ,

p : factor in the relation between a volume, V , and a segment length, i.e., $V = pL^3$ and $V_0 = pL_0^3$,

q : factor in Laplace's law for the relation between pressure and force, i.e., $P = qF/L$.

As is shown in Fig. 1B, Eq. 2 yields a curvilinear relationship between F and L . Such a relationship between force and length at end-systole has been found experimentally in papillary muscles,¹³ cardiac trabeculae,¹⁴ and

Fig.2. The relationship between segment shortening and contractility. Contractility is expressed by $E_{\max}$. $E_{\max}$ in control is assigned a value of 7.6 mmHg/ml per 100 g wt (arrow) where segment shortening is 0.25. The slope of arterial ESPVR is set to 5.55 mmHg/ml per 100 g wt. V_0 is held at zero.

in isolated cardiac myocytes.¹⁵

Relationship between $E_{\max}$ and segment shortening

In a whole heart, pressure and volume at end-systole are determined from where the ventricular ESPVR line crosses the arterial end-systolic pressure vs stroke volume relationship (Fig 1A¹⁶). When the heart contracts from end-diastolic volume V_1 to end-systolic volume V_2 , the ejection fraction (EF) is given as

$$EF = (V_1 - V_2)/V_1 = E_{\max} \cdot (1 - V_0/V_1)/(E_{\max} + E_a) \quad \text{Eq (3)}$$

where E_a is the slope of the arterial ESPVR line. If we define the segment shortening(s) as

$$s = (L_1 - L_2)/L_1,$$

where L_1 and L_2 denote the segment lengths corresponding to V_1 and V_2 , respectively (Fig. 1B), then Eq. 3 can be rewritten as

$$E_{\max} (1 - (L_0/L_1)^3)/(E_{\max} + E_a) = s^3 - 3s^2 + 3s$$

or,

$$s = 1 - ((E_a + E_{\max} \cdot (L_0/L_1)^3)/(E_{\max} + E_a))^{1/3} \quad \text{Eq (4)}$$

It is not surprising that the relation between

$E_{\max}$ and s is nonlinear. Nevertheless, it should be noted that this relations is strictly monotonically increasing and convex because $ds/dE_{\max} > 0$ and $d^2s/dE_{\max}^2 < 0$ at $E_{\max} > 0$. These results indicate that the increment of segment shortening is smaller as contractility progressively increases. In other words, segment shortening is always less sensitive to increases in contractility and more sensitive to decreases in contractility. Note also that these characteristics of the relation between segment shortening and $E_{\max}$ are independent of V_0 . When afterload is held constant with $V_0 = 0$, the relationship between $E_{\max}$ and s is as illustrated in Fig. 2. In Fig. 2, $E_{\max}$ is assigned a value of 7.6 mmHg/ml per 100 g wt^{3,17} when segment shortening(s) is 0.25¹⁸. In this case, the slope of the arterial ESPVR (E_a) is 5.55 mmHg/ml per 100 g wt, according to Eq. 4.

Relation between $E_{\max}$ and wall thickening

Let us assume that myocardium is incompressible and that the model is a thick-walled spheroid with homogeneous wall thickness. Other assumptions are the same as above. The following equations can then be derived:

$$V_i = \alpha R_i^3 \quad \text{Eq (5)}$$

Fig.3. The relationship between wall thickening and contractility. Contractility is expressed by $E_{\max}$. $E_{\max}$ in control is assigned a value of 7.6 mmHg/ml per 100 g wt (arrow) where segment shortening is 0.25. End-diastolic volume and muscle volume are set to 25 ml and 70 ml, respectively. A slope of arterial ESPVR is 5.55 mmHg/ml per 100 g wt. V_0 is held at zero.

$$V_o = \alpha R_o^3 = V_i + m \quad \text{Eq (6)}$$

where

V_i : volume of an inner spheroid, i.e., that of the ventricle,

V_o : volume of an outer spheroid, i.e., total volume of myocardium,

R_i : inner short radius,

R_o : outer short radius,

m : muscle volume,

α : factor in the relation between volume, V , and radius, R , i.e., $V = \alpha R^3$.

The wall thickening (w) is defined as

$$w = (R_{o2} - R_{i2}) / (R_{o1} - R_{i1}) - 1 \quad \text{Eq (7)}$$

where, the subscripts 1 and 2 indicate end-diastole and end-systole, respectively. Eq. 3 can be rewritten as

$$\frac{E_{\max} \cdot (1 - V_0/V_{i1})}{(E_{\max} + E_a)} = (V_{i1} - V_{i2})/V_{i1}, \quad \text{Eq (3')}$$

and following relation can be derived from Eqs. 3', 5, 6 and 7:

$$w = \left[\frac{\{E_a/(E_{\max} + E_a) + E_{\max} \cdot V_0/(E_{\max} + E_a)/V_{i1} + m/V_{i1}\}^{1/3} - \{E_a/(E_{\max} + E_a) + E_{\max} \cdot V_0/(E_{\max} + E_a)/V_{i1}\}^{1/3}}{(1 + m/V_{i1})^{1/3} - 1} \right] \quad \text{Eq (8)}$$

It is not surprising that the relation between $E_{\max}$ and w is also nonlinear. Nevertheless, it should be noted that this relation is also strictly monotonically increasing and convex, because $dw/dE_{\max} > 0$ and $d^2w/dE_{\max}^2 < 0$ at $E_{\max} > 0$ (see Appendix). Thus, these results indicate that wall thickening and segment shortening have the same theoretical limitations when used as measures of contractility. Apparently, this relationship depends upon both V_{i1} (enddiastolic volume; EDV) and m (muscle volume), but is independent of V_0 . When afterload, EDV and muscle volume are held constant with $V_0 = 0$, the relationship between $E_{\max}$ and w is as illustrated in Fig. 3. EDV (V_{i1}) and muscle volume (m) are set to 25 ml and 70 ml, respectively,¹⁹ while other parameters are the same as in Fig. 2.

Ca_o responsiveness of contractility

Because measurements of regional function are so commonly utilized, the implications of this work will be widespread. Nevertheless, it is instructive to illustrate the likely magnitude of the correction using an actual example. For this purpose I have chosen to examine an apparent discrepancy

Fig. 4. Responsiveness to extracellular calcium in normal and stunned myocardium. A: The observed relation between $E_{\max}$ and $[Ca]_o$ in isolated, perfused hearts (reproduced from Kusuoka et al²⁰). $E_{\max}$ is normalized by that of normal hearts at $[Ca]_o = 2$ mM. B: The calculated relation between segment shortening and $[Ca]_o$ derived from panel A. C: The ratio of $E_{\max}$ or segment shortening in stunned myocardium to that in control obtained in isolated hearts. D: The calculated relation between $E_{\max}$ and infused Ca derived from panel E. $E_{\max}$ is normalized by that in the hearts before ischemia without Ca infusion indicated by "C". E: The observed relation between segment shortening and infused Ca in *in situ* hearts (reproduced from Ito et al¹⁸). F: The ratio of $E_{\max}$ or segment shortening in stunned myocardium to that in control obtained in *in situ* hearts. In panels A, B, D and E, open symbols connected by a solid line, and filled symbols connected by a dotted line, show the relation before and after ischemia, respectively. M: Maximal Ca^{2+} -activated state. C: Control, i.e., before calcium infusion.

in the literature regarding the responsiveness of stunned myocardium to extracellular calcium.

Ca_o responsiveness is determined from measurements of contractility as a function of extracellular Ca concentration ($[Ca]_o$)²⁰. Recently, Ca_o responsiveness was pointed out to differ in *in situ* hearts and in isolated, perfused hearts under similar experimental conditions²¹. In this discussion I consider the possibility that the use of different indicators for contractile functions, (i.e., segment shortening vs. isovolumic pressure) may cause an apparent discrepancy even if there

is no difference in the underlying biology.

Fig. 4A shows an example of the effect of Ca_o on $E_{\max}$ measured during isovolumic contraction in isolated, perfused hearts (modified from the data in Kusuoka et al²⁰). When hearts are reperfused after a brief period of ischemia, contractility is depressed for a long while without any irreversible morphological changes. Such a prolonged depressed state has been referred to as "stunned" myocardium^{21,22}. In Fig. 4A, $E_{\max}$ at different $[Ca]_o$'s for both normal and stunned hearts is normalized with respect to its value in the normal hearts at $[Ca]_o = 2$ mM.

Fig. 4B shows the calculated relation between segment shortening and $[Ca]_o$, in which the relation in Fig. 4A has been transformed with Eq. 4 (shown in Fig. 2). Fig. 4B is based on the assumption that the heart contracts against the same afterload used in Fig. 2. A relationship similar to that shown in Fig. 4B has been obtained in normal and regionally stunned *in situ* hearts by selective infusion of calcium into a coronary artery (Fig. 4E, reproduced from Ito et al¹⁸).

Fig. 4D illustrates the calculated effect of $[Ca]_o$ on E_{max} in *in situ* hearts derived from the observed relation between $[Ca]_o$ and segment shortening in both normal and stunned myocardium (Fig. 4E). In this calculation, the heart is assumed to have the same uniform contractile properties as were measured regionally. The afterload is assumed to be unchanged before and after regional ischemia, which is consistent with the data of Iro et al¹⁸. Again, the calculated Ca_o responsiveness of E_{max} in *in situ* hearts shows change similar to that observed in isolated, perfused hearts (Fig. 4A). These results indicate that Ca_o responsiveness of either E_{max} or segment shortening is similar in isolated and *in situ* preparations.

Fig. 4C and 4F show the ratio of E_{max} or segment shortening in stunned myocardium to its corresponding control value obtained from isolated hearts and *in situ* hearts, respectively. Note that the changes in E_{max} induced by different conditions are much more sensitive than the corresponding changes in segment shortening. For example, E_{max} under standard conditions decreased to 63% in isolated hearts ($[Ca]_o = 2$ mM; panel C) and to 34% in *in situ* hearts (before calcium infusion; panel F) due to stunning, whereas segment shortening decreased only 75% and 47%, respectively. During activation of myocardium by higher $[Ca]_o$, the percent changes in E_{max} are always greater than those in segment shortening in both preparations. Thus, segment shortening underestimates the deteriorated function in stunned myocardium, as compared to that obtained using E_{max} .

DISCUSSION

The end-systolic pressure-volume relationship (ESPVR) and its slope, E_{max} , are rigor-

ously validated measures of contractility in myocardium over a wide range of physiological conditions.¹⁻³ However, E_{max} can be measured only in whole-heart preparations, and therefore can not be used directly to evaluate regional function. Recently, other indices, such as the stress-strain relationship, have been proposed to evaluate regional function, and may be comparable to ESPVR^{4,5} but such methods are still not widely used. In contrast, segment shortening and wall thickening have gained broad acceptance as indices of regional function because they can be easily applied in experiments and because they measure parameters that are intuitively reasonable. Nevertheless, neither segment shortening nor wall thickening is a true index of myocardial contractility because they are both only indices of regional strain and apparently depend on afterload and preload of the ventricle. Furthermore, several practical problems have been pointed out in both when used as measures of regional function^{8,9}.

To translate data expressed by segment shortening or wall thickening to that expressed by E_{max} , several assumptions are required. In the present analysis, an ellipsoidal model of the heart and simple laws characterizing myocardial mechanics were used to derive the relationship between regional indices in *in situ* hearts and E_{max} in isolated hearts. These results indicate that both segment shortening and wall thickening are less sensitive to increases in contractility, and more sensitive to decreases in contractility. Thus, under conditions of higher contractility achieved by increasing $[Ca]_o$, the difference between regional indices at two different contractile states becomes small, even though the true difference in contractility may be large. This fundamental non-linearity may cause the apparent saturation in segment shortening with high $[Ca]_o$, as observed in Fig. 4D.

Recently, Bolli²¹ pointed out in a review of stunned myocardium that the sensitivity to extracellular calcium observed in *in situ* hearts¹⁸ differs from that measured in isolated hearts²⁰. Differences between species (canine vs. ferret) and the methods used for stunning (regional vs. global ischemia) may have produced this discrepancy. However, two additional factors may help to explain

the discrepancy. First, the different measures of contractile function used in the two studies may have produced different results. As indicated above, Ca_o sensitivity, as expressed by segment shortening, exhibits a smaller shift with stunning than the change in E_{max} , due to the non-linear relationship between segment shortening and E_{max} . Second, the definition of Ca_o sensitivity may have had an effect. Kusuoka et al²⁰ normalized contractility by its true maximal value, whereas Ito et al¹⁸ normalized the increment of segment shortening induced by calcium infusion by its maximal response under each experimental condition. The latter method tends to minimize the difference between normal and stunned myocardium. It is clear from Fig. 4D that Ca_o sensitivity is depressed in stunned myocardium when the data are plotted using the former definition. Thus, the changes in Ca_o sensitivity in *in situ* hearts induced by stunning are consistent with those observed in isolated whole-heart preparations.

Acknowledgments

I thank Peter H Backx, Daniel Burkhoff and Eduardo Marban for their helpful comments.

REFERENCES

1. SUGA H, SAGAWA K: Instantaneous pressure-volume relationships and their ratio in the excised supported canine left ventricle. *Circ Res* 1974; **35**: 117–126
2. SAGAWA K: The end-systolic pressure-volume relation of the ventricle: definition, modifications and clinical use. *Circulation* 1981; **63**: 1223
3. SAGAWA K, MAUGHAN L, SUGA H, SUNAGAWA K: Cardiac Contraction and the Pressure-Volume Relationship. New York, Oxford University Press, 1988
4. NAKANO K, SUGAWARA M, TAMIYA K, SATOMI G, KOYANAGI H: A new approach to defining regional work of the ventricle and evaluating regional cardiac function: Mean wall stress-natural logarithm of reciprocal of wall thickening relationship. *Heart Vessels* 1986; **2**: 74–80
5. NAKANO K, SUGAWARA M, ISHIHARA K, KANAZAWA S, CORIN WJ, DENSLOW S, BIEDERMAN RWW, CARABELLO BA: Myocardial stiffness derived from end-systolic wall stress and logarithm of reciprocal of wall thickness. Contractility index independent of ventricular size. *Circulation* 1990; **82**: 1352–1361
6. ARTS T, VEENSTRA PC, RENEMAN RS: Epicardial deformation and left ventricular wall mechanics during ejection in the dog. *Am J Physiol* 1982; **243**: H379–H390
7. SABBAH HN, MARZILLI M, STEIN PD: The relative role of subendocardium and subepicardium in left ventricular mechanics. *Am J Physiol* 1981; **240**: H920–H926
8. GALLAGHER KP, STIRLING MC, CHOY M, SZPUNAR CA, GERREN RA, BOTHAM MJ, LEMMER JH: Dissociation between epicardial and transmural function during acute myocardial ischemia. *Circulation* 1985; **71**: 1279–1291
9. GALLAGHER KP, OSAKADA G, MATSUZAKI M, MILLER M, KEMPER WS, ROSS JR J: Nonuniformity of inner and outer systolic wall thickening in conscious dogs. *Am J Physiol* 1985; **249**: H241–H248
10. MIRSKY I: Left ventricular stress in the intact human heart. *Biophys J* 1969; **9**: 189–208
11. SANDLER H, DODGE HT: Left ventricular tension and stress in man. *Circ Res* 1963; **13**: 91–104
12. YIN FCP: Ventricular wall stress. *Circ Res* 1981; **49**: 829–842
13. HISANO R, COOPER IV G: Correlation of force-length area with oxygen consumption in ferret papillary muscle. *Circ Res* 1987; **61**: 318–328
14. TER KEURS HEDJ, RIJNSBURGER WH, VAN HEUNINGEN R, NAGELSMIT MJ: Tension development and sarcomere length in rat cardiac trabeculae. *Circ Res* 1980; **46**: 703–714
15. PARIKH SS, ZOU SZ, TUNG L: Contraction and relaxation of isolated cardiac myocytes of the frog under varying mechanical loads. *Circ Res* 1993; **72**: 297–311
16. SUNAGAWA K, MAUGHAN WL, BURKHOF D, SAGAWA K: Left ventricular interaction with arterial load studied in isolated canine ventricle. *Am J Physiol* 1983; **245**: H773–H780
17. FUTAKI S, NOZAWA T, YASUMURA Y, TANAKA N, SUGA H: A new cardiogenic agent, OPC-8212, elevates the myocardial oxygen consumption versus pressure-volume area (PVA) relation in a similar manner to catecholamines and canine hearts. *Heart Vessels* 1988; **4**: 153–161
18. ITO BR, TATE H, KOBAYASHI M, SCHAPER W: Reversibly injured, postischemic canine myocardium retains normal contractile reserve. *Circ Res* 1987; **61**: 834–846
19. GOTO Y, IGARASHI Y, YAMADA O, HIRAMORI K, SUGA H: Hyperkinesis without Frank-Starling mechanism in a nonischemic region of acutely ischemic excised canine heart. *Circulation* 1988; **77**: 468–477
20. KUSUOKA H, PORTERFIELD JK, WEISMAN HF, WEISFELD ML, MARBAN E: Pathophysiology and pathogenesis of stunned myocardium. *J Clin Invest* 1987; **79**: 950–961
21. BOLLI R: Mechanism of myocardial “stunning”. *Circulation* 1990; **82**: 723–738
22. BRAUNWALD E, KLONER RA: The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation* 1982; **60**: 1146–1149

APPENDIX

Proof for $dw/da > 0$ and $d^2w/da^2 < 0$ at $a > 0$. From Eq. 8,

$$\frac{dw}{dE_{\max}} = E_a \cdot \left[\frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}} \right]^{-2/3} - \left\{ \frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}} + \frac{m}{V_{il}} \right\}^{-2/3} \cdot \frac{(1 - V_0/V_{il})/3}{(E_{\max} + E_a)^2} \cdot \frac{1}{(1 + m/V_{il})^{1/3} - 1} \quad \text{..... (A1)}$$

Since $V_{il} > V_0$ and

$\frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}} + \frac{m}{V_{il}} > \frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}}$, $\frac{dw}{dE_{\max}} > 0$. From Eq. A1,

$$\frac{d^2w}{dE_{\max}^2} = 2 \cdot E_a \cdot (1 - V_0/V_{il}) \cdot \left\{ 3 \cdot \frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}} + \frac{m}{V_{il}} \right\}^{-2/3} - \left\{ \frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}} + \frac{m}{V_{il}} \right\}^{-2/3} \cdot \left\{ 3 - \frac{E_a \cdot (V_{il} - V_0)}{(E_{\max} \cdot V_0 + E_a \cdot V_{il})} \right\} - \left\{ \frac{E_a}{(E_{\max} + E_a)} + \frac{E_{\max} \cdot V_0}{(E_{\max} + E_a) \cdot V_{il}} + \frac{m}{V_{il}} \right\}^{-5/3} \cdot \frac{E_a \cdot (1 - V_0/V_{il})}{(E_{\max} + E_a) \cdot 9} \cdot \frac{1}{(E_{\max} + E_a)^2} \cdot \frac{1}{(1 + m/V_{il})^{1/3} - 1} \quad \text{..... (A2)}$$

Let the function $f(x, y)$ be defined as

$$f(x, y) = 3 \cdot \left\{ \frac{E}{(x + E)} + \frac{x \cdot V_0}{(x + E) \cdot V_1} + y \right\}^{-2/3} - \left\{ \frac{E}{(x + E)} + \frac{x \cdot V_0}{(x + E) \cdot V_1} \right\}^{-2/3} \cdot \left\{ 3 - \frac{E \cdot (V_1 - V_0)}{(x \cdot V_0 + E \cdot V_1)} \right\} - \left\{ \frac{E}{(x + E)} + \frac{x \cdot V_0}{(x + E) \cdot V_1} + y \right\}^{-5/3} \cdot \frac{E \cdot (1 - V_0/V_1)}{(x + E)} \quad \text{..... (A3)}$$

$$\text{Then, } f(x=0, y) = (1 + y)^{-5/3} \cdot (2 + V_0/V_1 + 3y) - 2 - V_0/V_1$$

$$\text{Since } \frac{df(x=0, y)}{dy} = -(1 + y)^{-8/3} \cdot (2y + 1/3 + 5V_0/3V_1) < 0 \text{ at } y \geq 0,$$

and $f(x=0, y=0) = 0$, then, $f(x=0, y) < 0$ at $y > 0$.

When the equation $f(x, y)$ is set to 0, the following equation is derived;

$$4yz^7 + (16y^4 + 24ry)z^6(53y^3 + 93ry^2 + 21r^2y)z^5 + (40y^4 + 120ry^3 + 60r^2y^2 + 5r^3y)z^4 + (8y^5 + 60ry^4 + 60r^2y^3 + 10r^3y^2)z^3 + (12ry^5 + 30r^2y^4 + 10r^3y^3)z^2 + (6r^2y^5 + 5r^3y^4)z + r^3y^5 = 0 \quad \text{..... (A4)}$$

where $z = x + (1 - x) \cdot V_0/V_1$ and $r = V_0/V_1$.

The equation A4 apparently has no solution at $z > 0$ and $y > 0$. Considering that $f(x, y)$ has no singular point at $x > 0$ ($\because y > 0$, $E > 0$, and $E \cdot V_1 + x \cdot V_0 > 0$) and $f(x=0, y) < 0$, it is concluded that $f(x, y) < 0$ at $x > 0$ and $y > 0$. Thus, $d^2w/da^2 < 0$.