Regulation of energy consumption in cardiac muscle: analysis of isometric contractions

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Landesberg, Amir, and Samuel Sideman. Regulation of energy consumption in cardiac muscle: analysis of isometric contractions. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H998–H1011, 1999.—The well-known linear relationship between oxygen consumption and force-length area or the force-time integral is analyzed here for isometric contractions. The analysis, which is based on a biochemical model that couples calcium kinetics with cross-bridge cycling, indicates that the change in the number of force-generating cross bridges with the change in the sarcomere length depends on the force generated by the cross bridges. This positive-feedback phenomenon is consistent with our reported cooperativity mechanism, whereby the affinity of the troponin for calcium and, hence, cross-bridge recruitment depends on the number of force-generating cross bridges. Moreover, it is demonstrated that a model that does not include a feedback mechanism cannot describe the dependence of energy consumption on the loading conditions. The cooperativity mechanism, which has been shown to determine the force-length relationship and the related Frank-Starling law, is shown here to provide the basis for the regulation of energy consumption in the cardiac muscle.

calcium control; cooperativity; intracellular; sarcomere

The existence of a linear relationship between oxygen consumption ($\dot{V}_{O_2}$) and the mechanical energy generated by the left ventricle (LV) has been demonstrated by Suga et al. (28, 32). The total mechanical energy generated by the LV is quantified by the pressure-volume area (PVA) in the LV pressure-volume plane (Fig. 1A), where PVA is the sum of the external work (EW) done by the LV and the mechanical potential energy (PE) given by

$$PVA = EW + PE$$

(1)

PE is defined as the area bounded by the end-systolic pressure-volume relationship (ESPVR) curve, the passive end-diastolic pressure-volume relationship, and the end-systolic volume (21, 32).

Suga's experimental data yields (32)

$$\dot{V}_{O_2} = (a \cdot PVA) + b$$

(2)

where PVA, the mechanical energy, corresponds to the energy consumed by the actomyosin ATPase and b represents the basal metabolic energy consumption, i.e., the energy used by the sodium-potassium pumps and by the calcium pumps. The parameters a and b are constants (31).

Using ferret papillary muscle fibers, Hisano and Cooper (16) have shown that the force-length area (FLA), the cardiac fiber analog of the ventricular PVA for the mechanical energy, is also closely correlated with oxygen consumption as

$$\dot{V}_{O_2} = (k_1 \cdot FLA) + k_0$$

(3)

where $k_1$ and $k_0$ are constants. The FLA is defined, similarly to the ventricular PVA, as the sum of EW and PE (Fig. 1B). As was experimentally established at the LV level, the relationship between FLA and $\dot{V}_{O_2}$ is independent of the mode of contraction, either isometric or shortening. Similarly, Mast and Elzinga (26) have shown, using rabbit papillary muscles, that the FLA correlates with the tension-dependent heat in isometric contraction.

Alpert et al. (3), Hisano and Cooper (16), and Wannenburg et al. (35) have demonstrated the existence of a linear relationship between the energy consumption and the force-time integral (FTI), i.e., the integral over the time course of the force, for isometric contractions. Thus

$$\dot{V}_{O_2} = (k_1 \cdot FLA) + k_0 \equiv (C_1 \cdot FTI) + C_0$$

(4)

where $C_1$ and $C_0$ are constants. Both the FLA and the FTI correlate with energy consumption for isometric contractions (16, 26, 35).

The linear relationship between the FLA and $\dot{V}_{O_2}$ at the level of the muscle fiber (Eq. 3) (16, 26) suggests that the linear relationship between PVA and $\dot{V}_{O_2}$ at the LV level (Eq. 2) (28, 32) is not due to some unique characteristics of the LV but is an integrated result of the basic characteristics of the myocytes. The cellular mechanism underlying this tight biochemical-mechanical coupling is still not fully understood.

Here, we analyze the correlation between the energy consumption and the FLA for isometric contraction and relate it to our current model of the biochemical-mechanical coupling by the sarcomere.

Our earlier studies (22–25) describe the intracellular control mechanisms of the contractile filaments and couple the kinetics of calcium binding to troponin with the regulation of cross-bridge cycling by the troponin regulatory proteins. The analyses of skinned (24) and intact muscle function (25) suggest the existence of two intracellular feedback mechanisms: the positive feedback, i.e., the cooperativity mechanism (22, 24), and the negative feedback, i.e., mechanical feedback (25).

The cooperativity mechanism (24), the dominant positive-feedback mechanism in the sarcomere, relates to the dependence of the affinity of troponin for calcium on the number of cross bridges in the strong, force-generating conformation. This mechanism determines the force-length relationship (FLR) in the cardiac muscle (22, 24) and the related Frank-Starling law.
The negative mechanical feedback (25) assures that the rate of cross-bridge weakening, i.e., the rate of transition from the “strong” force-generating conformation to the “weak” non-force-generating conformation, is linearly dependent on the cross-bridge strain rate and, thus, on the filament shortening velocity. The negative mechanical feedback determines the force-velocity relationship (FVR) and the ability of the muscle to generate power. The analytic derivation of the FVR in the cardiac muscle (25) is in agreement with the well-established, experimentally derived Hill’s equation. A detailed description of the dependence of the shortening velocity on the sarcomere length, the free calcium concentration, the time during the contraction, and other parameters is given elsewhere (25). Also, the negative mechanical feedback provides the basis for the linear relationship between energy consumption and the generated mechanical energy (23).

The intracellular control model (21–24), which is based on biochemical and physiological assumptions and includes these two feedback loops, successfully describes a wide variety of experimental observations and well-established phenomena at the cardiac fiber level as well as at the global LV level. These include the FLR (24, 25), the FVR (25), and, at the LV level, the time-varying elastance (TVE), the effect of shortening velocity on the generated force, and the positive effect of ejection on the end-systolic pressure (22).

The study considers isometric contractions and the related mechanical potential energy (32). The paper highlights the role of the cooperativity mechanism in the regulation of energy consumption by the sarcomere. The cooperativity mechanism determines the amount of calcium bound to troponin and, hence, the rate of energy consumption by the actomyosin ATPase. It provides the adaptive mechanism whereby the loading conditions affect energy consumption.

Specifically, this study aims to 1) prove, analytically, that the experimentally observed linear relationship between VO₂ and mechanical energy indicates that cross-bridge recruitment depends on the generated force, which corresponds to the role of the positive “cooperativity” feedback mechanism in the regulation of cross-bridge recruitment; 2) show that a model that does not include a positive cooperativity feedback cannot explain the control of energy consumption; and 3) show that utilizing the cooperativity mechanism enables the derivation and simulation of the experimentally established linear relationship between the energy consumption and FTI for isometric contraction.

**PHYSIOLOGICAL MODEL**

The model utilized here describes the intracellular control mechanisms of contractile filament contraction and couples the kinetics of calcium binding to troponin with the regulation of cross-bridge cycling. The basic assumptions underlying the model are detailed elsewhere (24, 25), and the mathematical description is summarized in the **APPENDIX**. Only the assumptions relevant to the control of the energy conversion are briefly summarized here for coherency and convenience.

**Assumption 1.** The cross bridge cycles between the weak, non-force-generating conformation and the strong, force-generating conformation due to nucleotide binding and release (9). The hydrolysis of ATP occurs as the cross bridge turns from the weak to the strong conformation (7, 9). Thus energy consumption is proportional to the total amount of cross-bridge turnover from the weak to the strong conformation.

**Assumption 2.** Calcium binding to the low-affinity troponin sites regulates the actomyosin ATPase activity (8) and the rate of phosphate dissociation from the myosin-ADP-P complex, which is required for the transition of the cross bridges to the strong conformation (9). Thus calcium binding to troponin regulates cross-bridge recruitment and the energy consumption by the sarcomere.

**Assumption 3.** The sarcomere contains three regions of overlap between the thin (actin) and the thick (myosin) filament: a nonoverlap region, a single-overlap region, and a double-overlap region. The double overlap of the actin filaments in the double-overlap region does not interfere with cross-bridge cycling (29). However, the net force generated by the cycling cross bridges in the double-overlap region is zero (29). This assumption is discussed further in Activation level and energy consumption.

The troponin regulatory units are divided into four different physiological states defined by their calcium-binding and cross-bridge conformation, i.e., the states are characterized by the biochemical kinetics of calcium binding and dissociation from troponin and by the cross-bridge cycling between the weak and the strong conformations (24, 25). The different states in the single-overlap region at isometric regime are depicted in Fig. 2. State A_s represents the rest state; the cross bridges are in the weak conformation, and no calcium is bound to the troponin. Calcium binding to troponin leads to state A_0. State A_s denotes a regulatory unit “activated” by calcium binding, but in which the adjacent cross bridges are still in the weak conformation. State A_s represents the level of the mechanical activation, i.e., the number of available cross bridges in the weak conformation that can turn to the strong, force-generating conformation. (The importance of this definition to the calculation of energy consumption is discussed in Activation level and energy consumption.) Cross-bridge cycling leads to state T_s, a state in which calcium is bound to the low-affinity sites and the cross bridges are in the strong conformation.
conformation. Calcium dissociation at state $T_s$ leads to state $U_s$, in which the cross bridges are still in the strong conformation but without bound calcium.

The loose coupling in state $U_s$ suggests that calcium can dissociate from troponin while the adjacent cross bridge is still in the strong conformation and, as shown by Peterson et al. (27), calcium dissociation from tropinin can precede the cross-bridge weakening and force relaxation. The loose-coupling component in Fig. 2 is essential for the description of the time course of force development and the force-calcium relationship. This is discussed in detail elsewhere (24, 25).

The cross bridges at states $T_s$ and $U_s$ generate the same average force, and the rate of cross-bridge weakening is identical in these states. Hence, the linear relationship between the energy consumption and FTI or the FLA is not related to the loose-coupling concept of the biochemical model.

We define $R_s$, $A_s$, $T_s$, and $U_s$ as the densities of the four troponin states existing within the single-overlap region. For example, $R_s = R_s/L_s$, where $L_s$ is the length of the single overlap. The isometric force generated by the sarcomere is proportional to the number of regulatory tropinin units associated with force-generating cross bridges in the single-overlap region, states $T_s$ and $U_s$. With $F_{cb}$ denoting the unitary isometric force developed by each regulatory unit, the isometric force generated by the contractile element per unit filament cross section ($F_{ce}$) is given by

$$F_{ce} = F_{cb} \cdot (T_s + U_s) = \alpha \cdot L_m \cdot F_{cb} \cdot (T_s + U_s)$$

where $L_m$ is the thick filament length that is carrying myosin heads and $\alpha$ is the overlap ratio ($\alpha = L_s/L_m$).

The force generated by the fiber per unit cross section is given by $F = F_{ce} + F_{pe}$, where $F_{pe}$ is the internal load (force/unit cross section) and is simulated by the parallel element (24, 25), which has a passive elastic property.

Activation level and energy consumption. The analysis of cardiac muscle mechanics is commonly based on two fundamental mechanical characteristics: the FLR and FVR (11). These phenomenological relationships are usually related to the activation level (11) and the prevailing mechanical conditions. Clearly, the ability to describe these fundamental characteristics and to simulate cardiac muscle dynamics depends on a reasonable quantitative description of the activation function and the complex intracellular relationship between calcium kinetics and cross-bridge cycling.

Following the method of Ford (11), we have defined the mechanical activation level as the ability of the muscle to generate new force-producing cross bridges (24, 25). On the basis of reported biochemical studies (8, 9), the activation level is defined here as the number of available cross bridges in the weak conformation that can turn to the strong, force-generating conformation, i.e., the activation level is described here by state $A_s$ in Fig. 2.

The transition from state $A_s$ to state $T_s$ describes cross-bridge turnover from the weak to the strong conformation, which requires one ATP hydrolysis and phosphate release (9) for each cross-bridge turnover. Thus the rate of energy consumption, i.e., the rate of ATP hydrolysis by the actomyosin ATPase in the single-overlap region (Eq. 5) is given by the amount of available cross bridges in the weak conformation that can turn to the strong conformation, i.e., by the activation level, state $A_s$ ($A_s = \bar{A}_s \cdot \alpha \cdot L_m$) and by the rate of cross-bridge turnover ($f$) from the weak to the strong conformation, and is given by

$$E_s(t) = E_{cb} \cdot f \cdot \alpha \cdot L_m \cdot \bar{A}_s(t)$$

where $E_{cb}$ denotes the free energy of hydrolysis of a single ATP molecule.

Cross-bridge cycling occurs in the double-overlap region as well as in the single-overlap region (29). With the assumption that the distribution of the myosin heads along the whole thick filament is uniform (24), the rate of energy consumption in the double-overlap region (Eq. 5) is given by

$$E_d(t) = E_{cb} \cdot f \cdot (1 - \alpha) \cdot L_m \cdot \bar{A}_s(t)$$

Combining Eqs. 6 and 7 gives the rate of energy consumption ($E$) and total amount of energy consumed ($E$) by the whole sarcomere as

$$\dot{E}(t) = \dot{E}_s(t) + \dot{E}_d(t) = E_{cb} \cdot f \cdot L_m \cdot \bar{A}_s(t)$$

and

$$E = E_{cb} \cdot f \cdot L_m \cdot \int_0^T \bar{A}_s(t) \cdot dt$$

where $T$ is the time during the twitch.

RESULTS

Analysis of experimental observations. The total amount of energy consumed by the cross bridges in isometric contraction is given by

$$E = N_{cb} \cdot E_{cb}$$

where $N_{cb}$ is the total number of cross bridges that are in the strong conformation during the twitch in the single- and double-overlap regions.

Consider Fig. 3, in which we depict isometric contractions at two different fiber lengths ($L_1$ and $L_2$). The total number of force-generating cross bridges increases as the muscle fiber length increases (by $\Delta L_2$) from $L_2$ to $L_1$.

On the basis of experimental data (16) and Eq. 3, the increase in the energy consumption ($\Delta E$), when going from $L_2$ to $L_1$, is given by

$$\Delta E = \Delta V_{O_2} = k_1 \cdot \Delta FLA$$
where \( F_m(L) \) is the peak isometric force at sarcomere length \( L \).

Combining Eqs. 12 and 13 for a differential length step \( dL \) yields the relationship between the increase in the number of cycling cross bridges \( dN_{cb} \) and the increase in the sarcomere length \( dL \) as

\[
\frac{dN_{cb}}{dL} = \frac{k_1}{E_{cb}} \cdot F_m(L)
\]  

Equation 19 is based on the TVE concepts (Eq. 16) and states that the ratio of energy consumption to peak isometric force is constant. However, returning to Eqs. 11 and 12, which are based on experimental data, and rearranging yields

\[
\frac{dE(L)}{dF_m(L)} = \frac{k_1}{\chi(L)} \cdot F_m(L)
\]
where \( \chi(L) = \frac{dF_m(L)}{dL} \) and is the local elastance at sarcomere length \( L \).

Equation 20, which is derived from experimental data (16, 26, 35), shows that the relationship between the energy consumption and the peak isometric force is not constant. The relationship will be constant, and Eqs. 20 and 19 will be equivalent, only if the cardiac muscle FLR is exponential, i.e., if

\[
\frac{dF_m(L)}{F_m(L)} = dL
\]  

(21)

However, the cardiac FLR is not exponential (1). Therefore, Eq. 21 is not valid for the general case, and Eqs. 20 and 19 are not equivalent. Consequently, the TVE concepts cannot explain the linear relationship between energy consumption and the FTI for isometric contractions (Eq. 4).

Force-time integral. The isometric force is determined in our four-state model (24, 25) by the number of cross bridges in the strong conformation in the single-overlap region (Eq. 6), i.e., states \( T_s \) and \( U_s \) in Fig. 2. The temporal change in the density of cross bridges in the strong conformation is given by

\[
\frac{d[T_s(t) + U_s(t)]}{dt} = f \cdot \mathcal{A}_s(t) - g \cdot [T_s(t) + U_s(t)] \]  

(22)

Integrating both sides of Eq. 22 over the twitch, i.e., from \( t = 0 \) to \( t = T \), gives

\[
\int_{t=0}^{t=T} f \cdot \mathcal{A}_s(t) \cdot dt - \int_{t=0}^{t=T} g \cdot [T_s(t) + U_s(t)] \cdot dt = 0
\]  

(23)

The left-hand side of Eq. 23 is zero, because both \( T_s \) and \( U_s \) return to their initial values at the end of the twitch. The first term on the right-hand side of Eq. 23 represents the energy consumption by the cross bridges, as defined by Eq. 9. Rewriting Eq. 23 and utilizing Eq. 5 for the whole cross section of the sarcomere gives the FTI as

\[
\text{FTI} = \int_{t=0}^{t=T} f(T_s(t) + U_s(t)) \cdot dt = \alpha \cdot L_m \cdot F_{cb} \]  

(24)

and by utilizing Eq. 9 we obtain

\[
\text{FTI} = \frac{F_{cb}}{E_{cb}} \cdot \frac{1}{g_0} \cdot \alpha \cdot E
\]  

(25)

Equation 25 states that FTI is proportional to the product of \( E \), the energy consumption by the cross bridges, and \( \alpha \), the single-overlap ratio, and is inversely related to the isometric rate of cross-bridge weakening, \( g_0 \). The proportionality coefficients correspond to the unitary force per cross bridge and the free energy per hydrolysis of one ATP.

The linear relationship between FTI and \( E \) provided by this model is in agreement with the experimental studies of Alpert et al. (3), Hisano and Cooper (16), and Wannenburg et al. (35), who have shown that the FTI correlates linearly with the energy consumption for isometric contraction. The effect of \( \alpha \) on the FTI is relatively small for the cardiac muscle compared with the changes in \( E \) (see DISCUSSION).

Examining the effect of the cooperativity mechanism. As emphasized in Activation level and energy consumption, state \( A_s \) represents the activation level, and the product \( A_s \times f \) gives the rate of cross-bridge turnover from the weak to the strong conformation and determines the rate of ATP hydrolysis (see Assumption 1 and Eq. 9). The rate of change of the activation level is given by

\[
\frac{dA_s}{dt} = (k_1 \cdot [Ca] \cdot \mathcal{R}_c) - ([f + k_-] \cdot A_s) + (g_0 \cdot T_s) \]  

(26)

from Eq. A3 in the APPENDIX.

In the absence of a feedback-cooperativity mechanism, the coefficients \( k_1 \), \( k_- \), \( f \), and \( g_0 \) in Eq. 26 are constants. Because the state variables are distributed uniformly along the sarcomere, the right-hand side of Eq. 26 is length independent. Equation 26 thus suggests that the activation level \( (A_s) \) and, hence, the energy expenditure, calculated without a cooperativity mechanism, are independent of the sarcomere length

\[
\int_{0}^{T} A(t) \cdot dt = \text{constant}
\]  

(27)

However, the isometric force and, hence, the mechanical energy depend on the sarcomere length (Eq. 5). Thus the analytic expressions for energy consumption (Eq. 9) and force generation (Eq. 5) imply that without a cooperativity mechanism there is a dissociation between energy consumption and the generated force. The energy expenditure is then constant and independent of the muscle length, whereas the force is length dependent.

Figure 4 presents simulations of isometric contractions of various sarcomere lengths, in which no cooperativity mechanism is assumed to exist and the affinity of troponin for calcium \( (K_{Ca}) \) is assumed to be constant \( (K_{Ca} = 100,000 \, \text{M}^{-1}) \). Figure 4A depicts the time course of force development at various sarcomere lengths. Figure 4B demonstrates that the time course of the activation level, state \( A_s \), is identical for the entire range of sarcomere lengths. As shown in Fig. 4C, the developed peak force and energy consumption are not correlated. The energy expenditure is independent of the muscle length and, consequently, as shown in Fig. 4D, the energy consumed by the cross bridges is independent of the FLA and the FTI. The excess energy is consumed by the double-overlap region. Also note the very moderate slope of the FLR in Fig. 4B, which is
inconsistent with the steep FLR observed in the cardiac muscle (1).

It is noteworthy that the relationships observed in Fig. 4 can be obtained in the skeletal muscle when the skeletal muscle is forced to contract in the ascending limb of the FLR (29). Indeed, the effect of the cooperativity mechanism diminishes at high and constant free calcium concentrations. Moreover, the depicted results of the simulation in Fig. 4 are also in agreement with experimental data obtained with skinned cardiac muscle at constant and full activation (19), where pCa = 4.3.

The effect of incorporating the cooperativity mechanism is seen in Fig. 5, in which cardiac fiber isometric contractions are simulated at various sarcomere lengths. Figure 5A depicts the time course of the force at various sarcomere lengths. Figure 5B shows the corresponding time course of the activation level, state $A_s$. Figure 5C describes peak force and the total energy consumption at the different sarcomere lengths. As shown, accounting for the cooperativity mechanism yields the steep FLR observed in the cardiac muscle (1). Figure 5D demonstrates the tight linkage between the energy consumption and the FLA or the FTI in isometric contractions.

As shown in Fig. 5C, the decrease in the generated force at shorter sarcomere lengths is somewhat steeper than the decrease in energy consumption. The noted increase in the difference between the normalized peak force and the normalized energy consumption, as the sarcomere length is shortened, is due to the increase in the double-overlap region at shorter sarcomere lengths and the consequent increase of the energy consumption by the cycling cross bridges in the double-overlap region. However, because the cooperativity mechanism determines cross-bridge recruitment along the whole sarcomere, in both the single- and double-overlap regions the tight relation between the FTI and the energy consumption is preserved, despite the increased energy consumption at the double-overlap region with the decrease in the sarcomere length.

Fig. 4. Simulated isometric contractions at various sarcomere lengths without incorporation of the cooperativity mechanism. A: time course of active force development. B: time course of activation level (state $A_s$). C: peak force and total energy consumption. D: FLA and FTI versus energy consumption by cross bridges.

Fig. 5. Simulated isometric contractions at various sarcomere lengths with incorporation of cooperativity mechanism. A: time course of force development. B: time course of activation level (state $A_s$). C: peak force and total energy consumption. D: FLA and FTI versus energy consumption by cross bridges.
DISCUSSION

Validating the cooperativity mechanism. The basic hypothesis underlining the present study is that it is possible to describe the mechanical performance and the regulation of biochemical to mechanical energy conversion in the cardiac muscle on the basis of the intracellular control of calcium kinetics and cross-bridge cycling.

The linear relationship between the energy consumption and the generated mechanical energy represents a fundamental characteristic of the cardiac muscle. Using ferret papillary fibers, Hisano and Cooper (16) have shown a linear relationship between the FLA or FTI and energy consumption in isometric contraction with correlation coefficients of 0.965 ± 0.05 and 0.970 ± 0.06, respectively. The linear relationship between the FLA and the energy consumption is independent of the mode of contraction, whether isometric or shortening (16). Mast and Elzinga (26) have also obtained a linear relationship between the FLA and the energy consumption by the cross bridges for isometric contractions of rabbit papillary muscle. This linear relationship is simulated here for isometric contraction in Fig. 5 by utilizing the cooperativity mechanism.

The cooperativity mechanism provides the feedback loop whereby the afterload affects the kinetics of calcium binding to troponin and the amount of calcium bound to troponin. The cooperativity mechanism regulates the energy consumption by the cross bridges in the strong conformation and is the dominant feedback mechanism that regulates calcium binding to troponin (24, 25). This mechanism is responsible for the “length-dependent calcium sensitivity” (1), i.e., the increase in calcium sensitivity with increasing sarcomere length (24, 25). Lengthening the sarcomere increases the number of available cycling cross bridges in the single-overlap region and, thus, increases the activation level $A_0$. Elevation of the activation level increases the number of cross bridges in the strong conformation and, through the cooperativity feedback, elevates the affinity of troponin for calcium (23). Thus the cooperativity feedback mechanism has two major components: the number of force-generating cross bridges (input) and the affinity of troponin for calcium (output). Consequently, the cooperativity mechanism determines the FLR and provides the basic intracellular mechanism for the Frank-Starling law (22).

The present study suggests that the cooperativity mechanism regulates the energy consumption by the sarcomere and explains the linear correlation between energy consumption and the FLA or the FTI for isometric contractions. Interestingly, the cooperativity mechanism also provides the cellular basis for the phenomenological TVE model (22). In this context, the TVE model can be viewed as a simple presentation of the more complex intracellular control of contraction and, as such, is insufficient to explain the wide spectrum of experimental observations.

Calcium binding to troponin regulates cross-bridge recruitment (24) and determines the amount of the activated actomyosin ATPase. The ATP hydrolysis mediates cross-bridge turnover from the weak to the strong conformation (9), i.e., force development and mechanical work generation. Thus the rate of cross-bridge recruitment is linked to the rate of ATP consumption by actomyosin ATPase (7). The quantitative expressions of these phenomena are given by our four-state control model, which couples the kinetics of calcium binding to troponin with cross-bridge cycling kinetics.

It is instructive at this point to return to the simulation of the four-state control model without a feedback mechanism (Fig. 4). In this case the affinity of troponin for calcium is constant, and a constant amount of calcium will bind to the troponin for the different sarcomere lengths. Consequently, the energy consumption will be length independent, and the experimentally observed linear relationship between energy consumption and the generated mechanical energy cannot be analytically reconstructed (Fig. 4C).

The observed increase of the generated force and energy consumption with the increase in the sarcomere length cannot be explained without the suggested cooperativity loop, unless it is argued that the amount of calcium released from the sarcoplasmic reticulum is length dependent (1). Alternatively, we can argue that the length-dependent calcium sensitivity of the sarcomere is due to the dependence of the affinity of troponin for calcium on the sarcomere length (1). However, these two arguments are inconsistent with reality.

Measurements of energy consumption at various loading conditions suggest that the PVA-independent energy consumption is constant at the same “contractility” (32). Higashiyama et al. (15) have used 2,3-butanedione monoxime to inhibit cross-bridge cycling and to quantify the nonmechanical energy cost at different LV volumes. They have shown that the LV end-diastolic volume does not affect the nonmechanical energy consumption. Therefore, the energy expenditure for calcium sequestration from the cytoplasm, which is the major constituent of the PVA-independent energy consumption, is length independent (15, 28, 32). These studies suggest that the amount of calcium released from the sarcoplasmic reticulum is practically independent of the sarcomere length. This is consistent with Allen and Kentish (1), who have suggested that the main mechanism for the FLR is “length-dependent calcium sensitivity of the sarcomere” and not the length-dependent calcium release from the sarcoplasmic reticulum. Moreover, if the length-dependent calcium release from the sarcoplasmic reticulum regulates the FLR in the cardiac muscle, an increase in the peak free calcium transient with the increase in sarcomere length is expected. However, no significant effect of the sarcomere length on the free calcium transient has been observed experimentally (17).

A length-dependent calcium affinity of the troponin cannot, by itself, explain the linear relationship between the energy consumption and the mechanical energy. If we assume that the amount of calcium bound to
troponin depends on the preload, then the same amount of calcium will be bound to the troponin at the same preload but at different afterloads, whereas the mechanical work and the mechanical energy depend on the loading conditions, including the afterload. Hence, the relationship between the energy consumption and the generated mechanical energy will not be linear. This inconsistency is resolved here by showing that the length-dependent calcium sensitivity of the sarcomere is consistent with, and can be explained by, the cooperativity feedback mechanism (24).

As shown in Fig. 5, the cooperativity mechanism regulates the energy consumption of the sarcomere and, for the isometric contraction, provides the linear relation between energy consumption and the generated mechanical energy, which is quantified by the FLA or the FTI. Moreover, this mechanism is responsible for the ability of the muscle to adapt to the loading conditions, because elevation in the afterload will increase the rate of ATP hydrolysis and afterload reduction will reduce energy expenditure.

The experimental evidence supports the hypothesis that the isometric force, or the number of force-generating cross bridges, and not the length by itself, is the activating parameter. Allen and Kentish (2) have measured the force and the free calcium response to length perturbation in skinned ventricular ferret muscles. They have shown that 1) the magnitude of the changes in the free calcium concentration correlates with that of the changes in the tension rather than with the changes in length; and 2) the time course of the changes in the free calcium concentration correlates with the time course of the tension changes rather than with the time course of the length changes. Rapid stretches of the muscle length (10 ms) resulted in a slow increase in tension (τ = 410 ms) and a slow decrease in the fluorescent light of the calcium dye (τ = 330 ms). These findings are consistent with Gordon and Ridgway (13), who have shown in barnacle muscle that the decrease in the affinity of troponin for calcium and the extra calcium release after quick release relate to the decrease in the force. Kurihara and Komukai (21) have studied the effect of muscle-length perturbation on the free calcium and the generated force in the ferret ventricle muscle. They have shown that the changes in the free calcium concentration, produced by quick release, depend on the magnitude of the fall in the force rather than the length change.

These studies on the effects of length and force on the free calcium transient are supported by the study of Hofmann and Fuchs (17). Using 45Ca2+, they have shown that the amount of calcium bound to the troponin increases with the increase in sarcomere length. However, the addition of vanadate, an ATP analog that interferes with cross-bridge cycling, reduces the amount of bound calcium. Moreover, the amount of calcium bound to troponin was independent of the muscle length in the presence of vanadate.

Kentish et al. (20) have observed a steep force-free calcium relationship in the intact and the skinned rat trabeculae. At a constant sarcomere length of 2.15 µm, Hill's coefficient for the force-free calcium relationship was 4.5. This large Hill coefficient cannot be explained by a cooperativity between the calcium regulatory binding sites, and, because the sarcomere length was constant, the only remaining variable is the number of force-generating cross bridges. Our analysis of this data (24) and other data from skinned cardiac muscle supports this hypothesis.

Wang and Fuchs (34) have studied the effect of sarcomere length on the sensitivity of the myofilaments to calcium and tested the hypothesis that the calcium sensitivity is not a function of the length per se but of the spacing between the actin and myosin filaments. They measured force generation and calcium binding at different sarcomere lengths and calcium concentrations in skinned bovine cardiac fibers while the fibers were exposed to varying concentrations of Dextran T-500. Osmotic compression by 5% Dextran T-500 at a sarcomere length of 1.7 µm produced a reduction in the fiber width equivalent to sarcomere stretching from 1.7 to 2.3 µm. This osmotic compression also produced an increase in calcium sensitivity of ~0.25 pCa units so that the normalized force-pCa relation at a sarcomere length of 1.7 µm with the given osmotic compression was almost identical to the normalized force-pCa relation at a sarcomere length of 2.3 µm. Fuchs and Wang (12) were able to determine calcium sensitivity and the calcium binding as a function of sarcomere length under the condition of (almost) constant interfilament spacing. Both calcium binding and calcium sensitivity were found to correlate more closely with changes in filament spacing than with changes in sarcomere length. These studies (12, 13) strengthen the importance of the actin-myosin filament interactions in modulation of the FLR.

The suggested effect of the sarcomere length on interfilament spacing cannot explain the entire complex force-length-calcium relationship observed in the cardiac fiber. A fundamental characteristic of the cardiac fiber is a steep force-pCa relationship with a large Hill coefficient at constant sarcomere length. Consequently, at constant length (and hence, presumably, constant filament spacing), some other important mechanism, such as the suggested cooperativity mechanism, must dictate the force-pCa relationship. Indeed, the cooperativity mechanism provides the steep force-pCa relation and explains the significant sensitivity of the generated force to the number of available cross bridges, i.e., to the filament length and interfilament spacing. Consequently, we suggest that the cooperativity mechanism is the dominant feedback loop that determines the FLR in the cardiac muscle, whereas the sarcomere length, or the filament spacing, only dictates the initial conditions, i.e., the number of available cross bridges for force generation.

All the above-mentioned studies suggest that the cooperativity mechanism requires a close interaction between the troponin regulatory complex and the cross bridges. Babu et al. (4) have suggested that troponin C alone has an intrinsic property that enables it to sense the muscle length and to modulate the sensitivity of the
sarcomere for calcium. They have substituted the native cardiac troponin C with a skeletal troponin C and observed a decline of the steepness of the FLR and a smaller length-induced shift of the force-pCa relationship, i.e., a significant decline of the muscle sensitivity to length changes. However, the studies mentioned above suggest that the changes in the affinity of troponin for calcium are determined by the force, or the number of force-generating cross bridges, and not by the length. Consequently, the troponin complexes bind calcium and determine the activity of the actomyosin ATPase and cross-bridge recruitment. They also interact with the cross bridges, and the affinity for calcium is modulated by the number of cross bridges in the strong conformation.

The cooperativity mechanism seems to depend on the number of force-generating cross bridges in the single overlap rather than on the force itself. Our analysis (24) of the FLR in skinned fibers, in which the force is proportional to the number of force-generating cross bridges, cannot determine whether the input for the cooperativity mechanism is the number of force-generating cross bridges or the resulting force. Quick-release experiments (2, 21) have shown that changes in the free calcium concentration have a slow time constant that correlates with the changes in the isometric force. A rapid length change of >1% of the muscle length causes cross-bridge detachment. However, when quick releases are imposed at various amplitudes (5, 10, and 15% of the muscle length), the changes in the free calcium correlate with the changes in the isometric force and no additional component that relates to cross-bridge detachment is detected. This suggests that the quick detachment of the cross bridges does not affect the affinity for calcium. Consequently, the affinity of troponin for calcium is regulated by the number of cross bridges in the strong conformation and not by the force, which is determined by the number of attached cross bridges.

Double-overlap and energy consumption. Kentish and Stienen (19) have shown that there is little change in the ATPase activity over the sarcomere length range of 2.0–2.4 µm, whereas there is a significant fall in the ATPase activity at sarcomere lengths <1.8 µm. Similar to the observation of Stephenson et al. (29), the force decreases linearly as the sarcomere length is reduced below 2.2 µm. The maximal difference between the normalized force and the normalized ATP consumption, at full activation (pCa = 4.3), was found at a sarcomere length of 1.8 µm, and the difference was 18.9 ± 2.8%. Kentish and Stienen (19) measured the sarcomere length only during rest and could not measure the sarcomere length in the active muscle because the laser-diffraction pattern was too diffuse. This diffused laser pattern suggests that the sarcomeres were not aligned uniformly along the fiber during contraction and that there was a significant inhomogeneity in the sarcomere length along the fiber. Sarcomere shortening at the center of the fiber may reach 10% (~0.2 µm) when the fiber length is held constant (isometric) (1). Hence, the sarcomere lengths in their study, which is based on measurements of the resting length, are overestimated, and the significant fall in the ATPase activity may be at a sarcomere length <1.8 µm.

Kentish and Stienen (19) have suggested that the decrease in the ATPase activity results from the effect of the double overlap and that the double overlap of the thin filament blocks some actin binding sites due to steric effects. However, their hypothesis cannot explain the deviation of the force from the ATPase activity at sarcomere lengths >1.85 µm. At sarcomere lengths of 1.85–2.2 µm, which are greater than the slack length for the rat trabeculae, there are no significant restoring forces. If the double overlap reduces the ATPase activity due to steric effects, the generated force should be parallel to the ATPase activity at the sarcomere length range of 1.85–2.2 µm. The fall in the generated force relative to the ATPase activity and the increase in the difference between these two parameters with a decrease in the sarcomere length to 1.8 µm suggest that the double overlap has different effects on the force and on the ATPase activity. Moreover, if the double overlap blocks the actin binding sites, one would expect a 20% decrease in the ATPase activity at a sarcomere length of 1.8 µm, assuming that, for the rat, the actin length is 1.05 µm, the myosin length is 1.5 µm, and the bar zone and the Z widths are ~0.1 µm. However, the measured decrease in the ATPase activity was only ~10% and, as mentioned above, this may be overestimated due to the inability to measure the sarcomere length during the contraction.

Elzinga et al. (10) have measured the heat production and the force as a function of the sarcomere length in two types of the frog skeletal muscle, the sartorius and the extensor digitorum longus (EDL). The energy consumption of the sartorius muscle, which is a slow-twitch muscle, remains near its maximal value as the sarcomere length decreases below 2.2 µm, whereas the force steadily declines. In contrast, the energy consumption of the EDL muscle decreases in parallel with the decline of the force as the sarcomere length decreases below 2.2 µm.

The energy consumption in both the cardiac muscle and the slow-twitch muscle remains constant for sarcomere lengths between 1.8 and 2.2 µm, whereas the force decreases (11, 19). Contradicting observations have been reported for the fast-twitch muscle (10, 29). A similarity between the cardiac muscle and the slow skeletal muscle is also observed in their force-calcium relationship. Thus the slow skeletal muscles and the cardiac muscle have similar length-dependent sensitivity of the filaments for calcium, whereas the fast skeletal muscle differs in its the length-dependent sensitivity. Consistently, the troponin C of the slow skeletal muscle is similar to the cardiac troponin C, and both have only one regulatory binding site for calcium, whereas the fast skeletal muscle has two sites (4). The difference in the dependence of energy consumption on the sarcomere length between the fast skeletal muscle and the cardiac muscle may be due to the difference in the mode of cross-bridge recruitment (4) or due to basic differences in the effect of the double overlap on the...
energy consumption. However, this difference may also
relate to the difference in the activation mode; Stephe
son et al. (29) have used skinned fiber and very high
calcium concentrations ([Ca] = 0.03 mM), whereas
Elzinga et al. (10) have used intact tetanized muscle
and normal extracellular calcium concentrations. Con
sequently, for the cardiac muscle at the sarcomere
lengths in the range of 1.8–2.3 μm simulated in this
study, we assume (see Assumption 3) that the double
overlap has different effects on the force and the
ATPase activity. At full activation, the ATPase activity
remains constant while the force declines with decreasing
the sarcomere length.

Equation 24 predicts that the FTI depends on the
product of the single-overlap ratio $\alpha$ and the energy
consumption of the cross bridges. Because the cycling
cross bridges in the double-overlap region also consume
energy, Eq. 24 predicts that the discrepancy between
the normalized FTI and the normalized energy con
sumption will increase at short sarcomere lengths.
However, because the cooperativity mechanism deter
mines cross-bridge recruitment along the entire sarco-
mere, including the single-overlap and double-overlap
regions, the tight relationship between the FTI and the
energy consumption is preserved. Indeed, the effect of $\alpha$
is hardly of note in Fig. 5C. Note that the dynamic
range of sarcomere lengths is narrow; a 10% shortening
of the sarcomere length from $L_{\text{max}}$, the length at which
maximal peak force is obtained, results in a 30–50%
reduction in the generated force (1). Thus the energy
consumption is determined mainly by the cooperativity
mechanism that modulates the FLR of the cardiac
muscle. Obviously, Eq. 24 should be further tested
experimentally.

The derived relationship between the energy con
sumption and the FTI (Eq. 24) is further substantiated
by experiments in the fully activated skeletal and
cardiac muscles, in which the cooperativity mechanism
is negligible. Furthermore, this relationship represents
the ability of the model to describe both the cardiac and
the skeletal muscle mechanics and energetics. The study
of Stephenson et al. (29) with the skeletal muscle
shows that energy consumption in the ascending limb
of the FLR of the skeletal muscle is constant and length
independent, whereas the force decreases in proportion
to the decrease in the single-overlap length. Indeed,
the total amount of the cycling cross bridges in the ascend
ning limb of the FLR at full activation is constant, and
thus the energy consumption is constant and indepen
dent of muscle length. However, the force is produced
only in the single-overlap region (29), and thus the
generated force is proportional to $\alpha$. Similar results
were obtained by Kentish and Stienen (19) with skinned
cardiac muscle at steady-state full activation. Only a
small change in ATPase consumption was observed in
the fully activated skinned cardiac muscle at sarcomere
lengths between 2.0 and 2.4 μm, whereas the force fell
almost linearly as the sarcomere lengths was decreased.
Thus there is no linear relationship in the skinned
cardiac fibers between the energy consumption and the
peak force. This characteristic of the skinned
cardiac cell resembles the simulation presented in Fig.
4, because the cooperativity mechanism plays an insig
ificant role at steady-state full activation ($p_{\text{Ca}} = 4.3$).

TVE and pseudo-potential energy. Suga and Sagawa
(28, 31) relate their experimentally observed linear
relationship between the energy consumption and the
mechanical energy (Eq. 2) to their phenomenological
TVE model. Accordingly, the mechanical energy is
determined by the ESPVR, whereas the ESPVR is
determined by $E_{\text{max}}$, the maximal value $E(t)$ can reach,
and the slope of the ESPVR. Thus the linear relation
ship between the energy consumption and the mechani
cal energy is presented as a consequence of the elas
tance concept and substantiates the stated importance
of the ESPVR as an index for LV contractility (28, 29).
However, the linear relationship between the mecha
nical energy and oxygen consumption of the isolated
cardiac muscle fiber (16, 26) implies that the linear
relationship between PVA and VO$_2$ stems from the basic
characteristic of the myocytes and is not due to some
unique characteristic of the LV.

The TVE is a relatively simple phenomenological
model that provides a useful, convenient guide in the
analysis of the LV function and is quite accurate in the
range of low ejection fractions (28), yet it cannot explain
a significant number of physiological observations. For
example, the TVE model cannot explain the close
relationship between the FTI and the energy consump
tion (Eq. 4) for isometric contractions at various sarco
mere lengths. Note that the FLA relates to the peak force,
whereas the FTI relates to the entire twitch. Moreover,
the TVE does not correctly describe the regulation of
power generation. According to the TVE model, the LV
contractility is determined by $E_{\text{max}}$; however, $E_{\text{max}}$
cannot distinguish between two different hearts that gener
ate the same pressure-volume loops on the pressure
volume plane at two different heart rates. Obviously,
these hearts differ in their ability to generate power,
yet, according to the $E_{\text{max}}$ concept, they exhibit the
same “contractility.” Consequently, the TVE model
cannot explain the positive effect of ejection on pressure
generation (18). Clearly, any definition of contractility
should include an index of the LV ability to generate
power.

The TVE model predicts (Eq. 19) that the ratio of the
change in the energy consumption to the change in the
peak isometric force is constant, whereas experimental
data (Eq. 20) suggest that this relation depends on the
peak isometric force, $F_{\text{p}}(L)$. The ratio is only constant if
the cardiac muscle FLR is exponential (Eq. 21), but the
cardiac muscle FLR is not exponential (1).

The inability of the elastance model to explain the
FLR stems from the oversimplified nature of the TVE
model and its inherent assumptions that 1) the elas
tance $e(t)$ is only a function of time and is independent
of the loading conditions, and 2) the isovolumic pres
sure is a product of two independent functions, time
t and volume.

According to the TVE model, all the mechanical
energy in an isometric contraction is stored as potential
energy (28). Thus the energy consumption at isometric
contraction is determined by the maximal elastance and the preload. This approach leads to two conclusions. 1) The energy consumption is independent of the work during the relaxation period, because it is defined by the peak force and does not include the relaxation period. Energy conversion from biochemical to mechanical energy occurs only before the time of peak force, and mechanical perturbations after the peak force have no effect on the energy expenditure. 2) The entire potential energy can be converted to external work, i.e., the potential energy is energetically equivalent to external work.

Suga and co-workers (14, 30, 33, 36) have intensively studied the mechanical work equivalent of the potential energy and the effect of shortening after the peak of contraction on the energy consumption. They suggest that the oxygen consumption is determined only by the energy generated during systole and is independent of the external mechanical work during diastole. However, the LV ejection velocity in their experiment was <200 ml/s. Hata et al. (14) have shown that the external mechanical work during the relaxation period does not significantly affect the myocardial oxygen consumption. Moreover, they have shown that 93% of the potential energy, defined on the basis of PVA, can be converted into external work. However, the maximal ejection velocity in their study was 118 ml/s, or 7 end-diastolic volumes (EDV) per second. With the assumption of a spherical model of the heart for simplicity, this ejection velocity corresponds to a sarcomere velocity of 2.3 μm/s. Additional evidence that they have used a very slow ejection velocity is seen in their pressure recordings: there is hardly any effect of the ejection on the pressure during the relaxation (Fig. 5, Ref. 14), and the pressure during the ejection period is almost identical to that at the isovolumic contraction.

The importance of the ejection velocity in the energy conversion from potential energy to external work, during relaxation, was addressed by Suga (30). He has shown that there is an optimal, relatively slow (50–100 ml/s) ejection velocity at which maximal energy is converted from potential energy to external work. At higher ejection velocities (~250 ml/s), only one-third of the calculated potential energy can be converted into external work. Using higher ejection rates, Yasumura et al. (36) have experimentally found substantial changes in the energy consumption, which is inconsistent with the elastance model. Linear relationships exist (36) between oxygen consumption during isovolumic contraction (VO2,i) and the PVA, as well as between oxygen consumption during fast releases (VO2,q) and the PVA

\[
\dot{V}_{O2,i} = I + S_i \cdot \text{PVA}
\]

\[
\dot{V}_{O2,q} = I + S_q \cdot \text{PVA}
\]

However, whereas the intercepts (I) of these two linear relationships are identical, the slope during isovolumic contraction (S_i) is steeper than that in the case of quick release (S_q).

Suga et al. (32) suggest that the intercept (I) describes the basal metabolic energy consumption and the energy requirement for calcium handling. The energy consumption by the cross bridges during the isovolumic contraction and the quick releases is given by \( S_i \times \text{PVA} \) and \( S_q \times \text{PVA} \), respectively. Hence, the change in the energy consumption of the cross bridges due to the fast release, relative to the energy consumed during isovolumic contraction (\( \Delta E_v \)), can be calculated from \( S_i \) and \( S_q \) as

\[
\Delta E_v = \frac{S_i - S_q}{S_i} \tag{29}
\]

For ejection velocities >14.1 EDV/s (\( n = 6 \); see Table 1 in Ref. 36), the oxygen consumption due to cross-bridge cycling falls by 32.3 ± 3.8% relative to cross-bridge energy consumption during the entirely isovolumic contractions. Similar results were obtained at the isolated cardiac fiber level; Hisano and Cooper (16) studied the effect of fast release imposed just after end systole on the energy consumption in ferret papillary muscles. They found a 35% reduction in the oxygen consumption in the released contractions, resulting in a shortening velocity of 5 Lmax/s relative to the full isometric contractions.

The potential energy calculated from the PVA equals the energy consumption for isovolumic contraction. However, the observed effects of shortening during the relaxation on energy consumption (16, 36) are inconsistent with the elastance model assumption that the external work during relaxation does not affect energy consumption. Therefore, the calculated potential energy does not accurately describe the energy consumption for contraction with shortening after end systole.

In the present model, cross-bridge recruitment requires energy consumption, and one molecule of ATP is hydrolyzed for each cross-bridge turnover from the weak to the strong conformation. Therefore, the FTI represents the total number of cross-bridge turnovers from the weak to the strong conformation during the twitch and the related energy consumption. This energy is denoted here as “pseudo-potential energy” (Epp) and, utilizing Eq. 24, is given by

\[
E_{pp} = \frac{E_{cb}}{F_{cb}} \cdot \alpha \cdot \text{FTI} \tag{30}
\]

The reported studies (16, 30, 36) that relate to the effect of shortening during relaxation and to the “potential energy” part of the FLA highlight the following differences between the TVE model and the present model and help clarify the meaning of Epp. 1) The potential energy in the TVE model represents mechanical energy that is generated by the cross bridges during systole. This energy is stored in the elastic elements of the LV walls and can be converted to external work. The pseudo-potential energy in the present model, which is quantified by the FTI, represents the free energy from ATP hydrolysis that is stored in the cross bridges that are in the strong conformation. Hence, Epp...
is not the classic mechanical potential energy but a pseudo-potential energy that can only be partially converted to external work. Consequently, \( E_{pp} \) is proportional to the number of cross bridges in the strong conformation and to the FTI. \( E_{pp} \) is identical to Suga's potential energy \((15, 25)\) only under entirely isovolumic conditions. 2) \( E_{pp} \) is defined by the FTI. This is quite obvious for the isovolumic contractions, in which, as shown above, both the FTI and the FLA are proportional to the energy consumption. However, this definition is also valid for the shortening beats, in which the energy consumption is the sum of three components: external work, \( E_{pp} \), and energy dissipation as heat due to the viscoelastic property of the cross bridges \((28)\). 3) According to the TVE model, the external work during relaxation is obtained from the passive elements in the ventricular wall \((30)\). In contrast, the present model suggests that the external work that can be obtained during the relaxation is produced mainly by the cross bridges. The rate of force redevelopment after quick releases during the relaxation is determined by cross-bridge dynamics \((27)\). Hence, the work generated during the relaxation is also produced mainly from the cross bridges and not from other passive elements. 4) The rates of energy conversion from \( E_{pp} \) to heat and external work are determined by the rate constants \( g_0 \) and \( g_V \), respectively \((23)\), where \( g_0 \) is the rate of cross-bridge weakening at isometric contraction, \( g_v \) describes the effect of the filament shortening velocity on the rate of cross-bridge weakening \((25)\), and \( V \) is the sarcomere shortening velocity. There is always some energy dissipation as heat, and \( E_{pp} \) cannot be converted entirely into external work.

As presented here, the cooperativity feedback mechanism quantifies the bound calcium and thus the energy consumption. Another mechanism, the negative mechanical feedback, controls the FVR, the power generation \((25)\), and the efficiency of the biochemical to mechanical energy conversion \((23)\). The interplay between these two feedback loops and the related control of energy consumption and power generation, as well as the general case of the regulation of conversion from biochemical to mechanical energy, are presently under study.

In conclusion, the biochemically based intracellular control \((23, 25)\) model, which describes the basic mechanical properties of the cardiac muscle, i.e., the FLR and the FVR, is extended here to describe the control of energy conversion in the cardiac muscle for isometric contractions. The observed linear correlation between energy consumption and the generated mechanical energy is conveniently and convincingly explained by the cooperativity mechanism. The analysis shows that 1) the experimentally observed linear relationship between oxygen consumption and the generated mechanical work indicates the dependence of cross-bridge recruitment on the generated force, consistent with the established cooperativity mechanism; 2) the control of the energy consumption by the sarcomere cannot be theoretically explained without including a cooperativity mechanism in the four-state model; and 3) the cooperativity mechanism enables the derivation and simulation of the experimentally established linear relationship between energy consumption and mechanical work.

The present study suggests a new approach to the interpretation of physiological observations and describes cardiac muscle function based on a better understanding of the cellular function. Further research in this direction is obviously needed, and the complementary study of the negative mechanical control loop that determines the FVR and the ability of the muscle to generate power is presently under way.

**APPENDIX**

Data Simulation for Isometric Contraction

The equations needed for the simulation of isometric contraction were developed and described elsewhere \((22, 24, 25)\) and are summarized here for clarity and simplicity.

The sarcomere isometric contraction is described by five state variables: the free calcium concentration \(([Ca])\), the calcium bound to the troponin high-affinity sites \((BTh)\), and the three regulatory troponin unit states \((\bar{A}_u, \bar{T}_u, \bar{U}_u)\). Other variables used in the calculation are derived from these variables. The troponin regulatory state is given by

\[
\bar{A}_u = [\text{Tro}] - \bar{A}_0 - \bar{T}_0 - \bar{U}_0
\]

where \([\text{Tro}]\) is the troponin concentration. The calcium bound to the troponin low-affinity sites \((BTI)\) is given by

\[
BTI = \bar{A}_u + \bar{T}_u
\]

Thus the isometric contraction of the system is described by a set of five ordinary differential equations

\[
\frac{d[Ca]}{dt} = I_1 + I_2 - I_3 - I_4 - \frac{dBTI}{dt} \frac{dBTI}{dt}
\]

\[
\frac{dBTI}{dt} = (2[\text{Tro}] - BTh) \cdot [Ca] \cdot k_{h} - BTh \cdot k_{-h}
\]

\[
\frac{d\bar{A}_u}{dt} = k_{l} \cdot [Ca] \cdot \bar{R}_u - (f + k_{-e}) \cdot \bar{A}_u + (g_0 \cdot \bar{T}_u)
\]

\[
\frac{d\bar{T}_u}{dt} = f \cdot \bar{A}_u - (g_0 + k_{-l}) \cdot \bar{T}_u + k_{l} \cdot [Ca] \cdot \bar{U}_u
\]

\[
\frac{d\bar{U}_u}{dt} = k_{l} \cdot \bar{T}_u - (g_0 + (k_{l} \cdot [Ca])) \cdot \bar{U}_u
\]

The rate coefficients \(k_l\) and \(k_{-l}\) represent the rate constants of calcium binding to, and calcium dissociation from, the low-affinity sites of troponin, respectively. Note that the rate coefficient is not constant because the cooperativity mechanism dictates the dependence of this coefficient on the state variables \((18, 20)\). \(f\) and \(g_0\) represent the cross-bridge turnover rate kinetics; \(g_0\) is the rate of cross-bridge weakening in the isometric regime.

The first differential equation in Eq. A3 describes the free calcium transient, which is determined by the calcium inward current through the sarcolemma \((I_s)\), the calcium release rate from the sarcoplasmic reticulum \((I_u)\), the efflux through the sarclemma \((I_b)\), the calcium uptake rate by the SR \((I_u)\), the calcium binding rate to the troponin high-affinity sites (dBTh/
dt), and the calcium binding rate to the low-affinity troponin regulatory sites (dBTl/dt). The simple phenomenological model used here for the description of the sarcoplasmic reticulum and the sarcoplasmic reticulum currents was described and discussed elsewhere (25). Calcium binding to the troponin high-affinity sites is described by the second differential equation in Eq. A3, where k_i and k_i are the association and dissociation rate constants, respectively. The last three differential equations in Eq. A3 describe the distribution of the troponin regulatory unit between the four states. Calcium binding rate to the low-affinity sites is given by

\[
\frac{dBTI}{dt} = (\left[\text{Tro}\right] - BTI) \cdot \left[Ca\right]k_l - (BTI \cdot k_i) \tag{A4}
\]

Note, however, that BTI is defined by Eq. A2; hence, no additional differential equation is needed and Eq. A4 is just embedded into Eq. A3.

The suggested cooperativity mechanism implies that the affinity of the regulatory site for calcium (K_{[Ca]}) is determined by the number of force-generating cross bridges, which is defined by the number of troponin units in the strong conformation (T_s + U_s) in the single-overlap region. K_{[Ca]} is the ratio k_i/k_i, is a monotonic function of T_s + U_s as based on our previous studies (22, 24, 25) and is described by a simple polynomial function (note 3 in Ref. 25)

\[
K_{[Ca]} = \frac{k_i}{K_i} = K_0 + [K_1 \cdot (T_s + U_s)] + [K_2 \cdot (T_s + U_s)^2] \tag{A5}
\]

The calcium association rate k_i is assumed to be constant, whereas the dissociation rate k_i is given by

\[
k_i = \frac{k_i}{K_{[Ca]}} \tag{A6}
\]

The effect of the cooperativity, as simulated in Fig. 5, is obtained by solving Eqs. A1-A6. The system of the ordinary differential equations described by Eq. A3 was integrated by using the fourth-order Runge-Kutta method. The calculations were made by using the commercially available software MATLAB (MATLAB subroutine: ode45.m) on an IBM-compatible personal computer. The steps of the integration were determined by the desired accuracy, and the chosen tolerance was 10^-8.

The energy consumption by the sarcomere is calculated from the number of cross bridges that are turned over from the weak to the strong conformation, with the assumption that one ATP hydrolysis is required of each cross-bridge cycle. The rate of cross-bridge turnover from the weak to the strong conformation is given by A_s \times f. Hence, the amount of ATP hydrolysis by the sarcomere at the various sarcomere lengths is equal to the area under the time-course plot of the activation level (state A_s in Fig. 2). The energy consumption is the product of this area and the free energy of ATP hydrolysis (Eq. 9).

For the simulation of the isometric contraction without a cooperativity mechanism, shown in Fig. 4, Eqs. A1-A4 were solved by taking the association and dissociation rates of calcium binding to the regulatory sites as constants: k_i = 4.0 \times 10^6 \mu M and k_i = 80 s^-1. The affinity of the troponin regulatory sites for calcium was K_{[Ca]} = 0.5 \times 10^9 \mu M^-1. Note that K_{[Ca]} depends on the experimental condition; whereas the K_{[Ca]} for native troponin is \approx 2 \times 10^9 M^-1, it is smaller during rigor (1.2 \times 10^9 M^-1) and is further reduced by the addition of ATP to 0.25 \times 10^6 M^-1. Moreover, K_{[Ca]} should also be relatively small at end diastole. No active force is detectable at end diastole, when the free calcium concentration is <120 nM; hence, <1% of the troponin regulatory units (BTI/[Tro] \approx 0.01) are at the strong conformation at end diastole. The relationship between the calcium bound to troponin and the total concentration of troponin at steady-state end diastole is given by

\[
\text{BTI} \frac{[\text{Tro}]}{K_{[Ca]} \cdot [\text{Ca}]} \leq 0.01 \tag{A7}
\]

To obtain this value, for [Ca] = 120 nM, K_{[Ca]} should be \approx 8.3 \times 10^9 M^-1. An intermediate value was consequently chosen for the simulation of isometric contraction without cooperativity (Fig. 4).

The above-mentioned variations in the measured (6) and expected values of the troponin affinity with the variation in actin-myosin interaction support the hypothesis that the affinity changes during the twitch and that these variations relate to the cross-bridge conformation.

The values of the coefficients used for the simulation are given in Table 1 of Ref. 25. The simple phenomenological model used to simulate calcium transients was described elsewhere (25). Figures 4 and 5 represent the time courses of the generated force and the activation level (state A_s) at various sarcomere lengths calculated without and with the cooperativity feedback loop. Obviously, Fig. 5 is consistent with the available data. Three other phenomena appear to be due to the cooperativity mechanism: 1) the steep FLR, experimentally observed in cardiac muscle (1) (10% decrease in the sarcomere length reduces the force by 30%); 2) the increase in the peak free calcium transient with the decrease in the sarcomere length, in agreement with experimental observation (5); and 3) the increase in the time to peak force with the increase in the sarcomere length (5).

Finally, the cooperativity mechanism plays an important role during relaxation, too, where it works in the opposite direction, i.e., a decrease in the generated force reduces the affinity of troponin for calcium and facilitates the rate of relaxation. Note the prolonged relaxation obtained in the simulation of isometric contraction without the cooperativity mechanism (Fig. 4) relative to the shorter relaxation obtained when the cooperativity mechanism is considered (Fig. 5).

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