

## Research Article

# Contractile Strength during Variable Heart Duration Is Species and Preload Dependent

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We investigate the effect of beat-to-beat variability on cardiac contractility. Cardiac trabeculae were isolated from the right ventricle of rabbits and beagle dogs and stimulated to isometrically contract, alternating between fixed steady state versus variable interbeat intervals. Trabeculae were stimulated at physiologically relevant frequencies for each species (dog 1 and 4 Hz; rabbit 2 and 4 Hz) intercalating fixed periods with 40% variability. A subset of the trabeculae (at 90% of optimal length) was stretched prior to stimulation between 5 and 13% and stimulated at the same frequencies with a fixed versus 40% variation. Fixed rate response at the same base frequency was measured before and after each variable period and the average force reported. In canine preparations no change in force was observed as a result of the imposed variability in beat-to-beat duration. In the rabbit, we observed a non-significant decrease in force between fixed and variable pacing at both 2 and 4 Hz ( $n = 8$ ) when 40% variability was introduced. When a 5% and 13% stretch was applied, the correlation coefficient sharply increased, indicating a more prominent impact of the prebeat duration on the following cycle with higher preload.

## 1. Introduction

Bowditch [1] described the influence of the interval between beats on force development over 100 years ago. His landmark experiments in frog hearts ascribed prime importance to the effect of the interval between beats on the strength of contraction. Widely recognized as one of the most important relationships in cardiac physiology, the force frequency relationship has ever since been subject of intense investigation in the field of cardiac mechanics. The force-frequency relationship (FFR) describes that an increase in force development is observed as the interval between beats decreases (higher frequency) and has been shown *in vivo* and *in vitro*, occurs in all healthy mammals [2], and this FFR is impaired in patients with heart failure [3]. Typically, these changes in contractile force develop over several beats and are generally derived from steady-state observations at different frequencies [4].

Other intrinsic and seemingly opposite phenomena in mammalian myocardium act on a more immediate non-steady-state beat-to-beat basis. Postrest potentiation des-

cribes the stronger force development seen when stimulation is resumed after a longer than usual interval, that is, a period or rest, between beats [5]. Mechanical restitution describes the dependency of the recovery of contractile force on the interval between contractions, namely, that a shorter interval leads to a weaker beat and a longer interval leads to a stronger beat [6, 7]. Postextrasystolic potentiation is yet another mechanism whereby a shorter interval that precedes a longer interval determines a stronger contraction in the subsequent beat [8].

In our previous work in rat, we were able to show small gains in average contractile force over the fixed rate period given an equivalent number of beats in each period [9]. Here, to translate these findings to species that more closely resemble human cardiac function, we investigated R-R interval modulation (such as what may occur during atrial fibrillation) may have on the inotropic status of the myocardium in rabbits and dogs and seek to verify the impact of this strategy on different species with different calcium handling and EC coupling properties.

## 2. Methods

Male NZW rabbits (2-3 months) were anesthetized with pentobarbital (at 60 mg/kg intraperitoneal). In dogs (weighing  $19.0 \pm 0.4$  kg, range, 13.6–24.1 kg, 2-3 years old), an intravenous catheter was placed in the cephalic vein and a surgical plane of anesthesia was induced by the bolus injection (over 1-2 min) of sodium pentobarbital (50 mg/kg, Nembutal, Abbott, North Chicago, ILL). Under deep anesthesia, the hearts were rapidly removed and perfused retrogradely through the aorta with Krebs Henseleit solution (120 mM NaCl, 5 mM KCl, 2 mM  $MgSO_4$ , 1 mM  $NaH_2PO_4$ , 20 mM  $NaHCO_3$ , 0.25 mM  $Ca^{2+}$ , and 10 mM glucose) in equilibrium with 95% $O_2$ /5% $CO_2$ . 2,3-Butanedione monoxime (BDM) was added to the dissection solution to stop the heart from beating and to prevent damage during dissection [10]. This investigation conforms with the *Guide for the Care and Use of Laboratory Animals published by the US national Institutes of Health (NIH Publication Ns 83-23, revised 1996)*. Suitable thin, uniform, nonbranched trabeculae along the free wall of the right ventricle were dissected carefully and without touching the muscle mounted in the bath as previously described for rabbit [11] and dog [12]. Average width was  $\sim 120$ – $140 \mu m$  in order to prevent hypoxic cores of the muscles [13]. The dissected specimen contained small cubes of ventricular tissue attached to each end to facilitate mounting the muscle onto the experimental setup. The cube of ventricular tissue at one end of the trabecula was connected to a hook, and the cube at the other end rested in a basket-shaped extension of the force transducer. The muscle was bathed in a continuous flow of oxygenated K-H solution (without BDM), and with 1.75 mM  $Ca^{2+}$ , at 37°C. The dimensions (length, width, and thickness) of the muscle were measured under 30x magnification. Initially, the muscle was stimulated at 1 Hz at a temperature of 37°C and stretched until small increases in length resulted in about equal increases in resting tension and active developed tension. This fixed optimal preload is equivalent to that near the end diastolic volume in the *in vivo* situation (sarcomere length of  $\sim 2.2 \mu m$ ) [14]. Trabeculae were stimulated at physiologic frequencies for each species (1 and 4 Hz in dog,  $n = 9$ , and 2 and 4 Hz in rabbit,  $n = 8$ ) with or without a 40% variation imposed on interbeat duration. In order to define the load dependence of contractility during heart rate variability (HRV), a subset of rabbit trabeculae was subsequently subjected to a variable preload protocol by utilizing a high-speed high-accuracy servomotor as a displacement device ( $n = 5$ ). One end of the trabeculae was attached to a length displacement device (i.e., a high-speed, high-accuracy servomotor). Once peak tension is reached and forces start declining (150 ms after initiation of the twitch), the servomotor reduces the muscle length by 10% (to 90% of optimal) within 2 ms, after which it is immediately stretched at the velocity of one muscle length per second until the next beat is triggered. At that point, the muscle length is kept constant. This range of lengths was chosen to be close to the *in vivo* sarcomere length range the heart undergoes during a contraction [14]. The protocol is then repeated (Figure 1). By actively stretching the muscle at a rate of 1 muscle length

per second, a certain percent stretch of muscle length will result, which is identical to the percentage of muscle that is released. Thus, average preload is identical both during the fixed preload and variable preload protocol.

In all protocols, total number of beats in each fixed versus variable period was the same. A fixed-rate steady-state response at the same base frequency was measured before and after each variable period, and average force was reported.

*Data Analysis.* Data were obtained and analyzed through custom written software (LABVIEW). Correlation coefficients and slopes were obtained for prebeat duration and force development. Kaleidagraph statistical package was utilized to perform Students *t*-test and repeated measures ANOVA where appropriate. All data are reported as means  $\pm$  SEM.

## 3. Results

First, we compared the average active force development between protocols with base frequency with a random 40% variation in the interval between beats compared. In dog myocardium (Figure 2), there was no significant change on average force development at both 1 Hz (cycle time of 1000 ms) and 4 Hz (cycle time of 250 ms).

Next, we compared these protocols in the rabbit. Since during *in vivo* beats, a prolonged beat-interval increases filling time, we mimicked this increase an increase in preload by stretching the muscle during the interbeat period until stimulated. In Figure 3, we depict the observed nonsignificant decrease in strength when imposing a 40% variable protocol until a stretch (increased pre load) is applied. Albeit not significant, there is a reversal of the negative tendency at 5% stretch for both baseline frequencies 2 and 4 Hz. Only at the more extreme 13% stretch at 4 Hz (the high end of the frequency range for rabbit) do we again observe a nonsignificant trend towards a negative effect.

In Figure 4, we show the correlation between prebeat duration and average force development in the dog at both 1 and 4 Hz during a 40% variable pacing protocol. Note the static relationship at the different frequencies. There is no difference with the increase in frequency.

In Figure 5, we show that as the preload stretch increases, while the baseline frequency increases too, a much higher correlation between beatduration and developed force. At 4 Hz, the increase in correlation becomes statistically significant. The longer the prebeat duration, the higher the contractile force observed, and the shorter the interval, the weaker the following beat.

## 4. Discussion

In our previous work, we had suggested and shown that an algorithm that imposed a beat-to-beat variability can lead to an average slight increase in force in rat myocardium [9]. In subsequent studies, we showed that in the rabbit, up to three preceding beats contribute to the amplitude of a given beat but that the average amplitude is not significantly

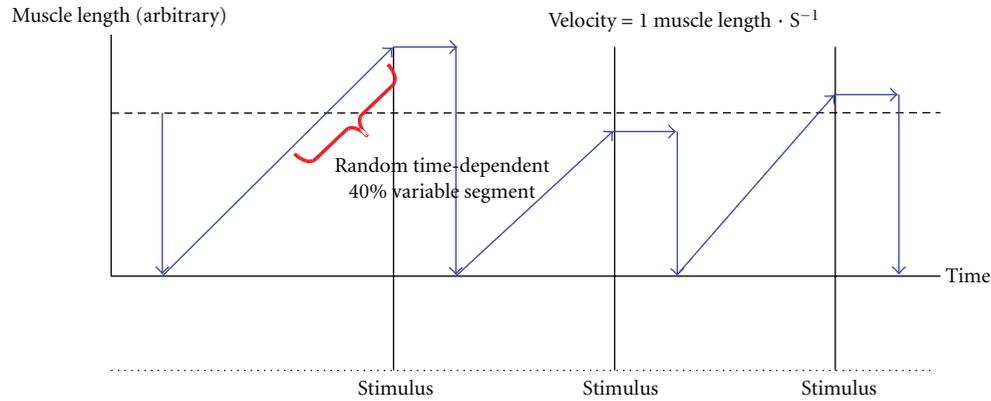


FIGURE 1: Following a stimulus, muscle length ( $y$ -axis) is kept constant till well after peak active force development has been reached. At 150 ms after the stimulation, muscle length is quickly reduced to 90% of optimal length and thereafter restretched at 1 muscle length per second. The duration of this stretch varied resulting in successive beats with different preloads, where the preload is increased linearly with the interbeat duration.

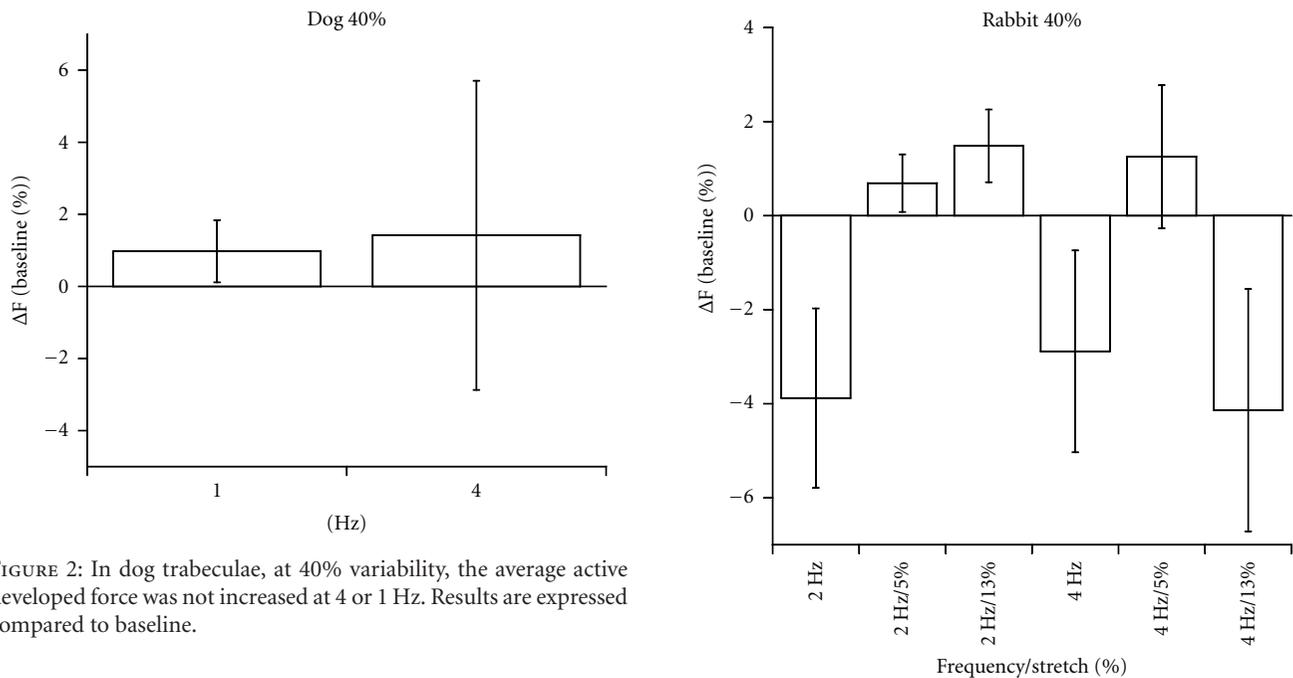


FIGURE 2: In dog trabeculae, at 40% variability, the average active developed force was not increased at 4 or 1 Hz. Results are expressed compared to baseline.

FIGURE 3: At a variability of 40% in the rabbit at both 2 and 4 Hz, there was no statistically significant difference between variable and base protocols. The addition of a 5% and/or 13% stretch just prior to contraction did not seem to improve or impair the average force development.

affected by a pseudovisible heart rate compared to the average of that rate applied as steady state [15, 16]. Here, we tested the efficacy of this initial randomized beat duration strategy in the rabbit and dog. The current data suggest that neither canine or rabbit myocardium seem to benefit (i.e., increase inotropy) from a variable rate with no negative or positive effect of variability on force development or kinetics noticed. The explanation for this species differences is more than likely found in the distinct manner in which EC coupling and calcium regulation are handled in each animal. Of particular interest is the increased contribution of the L-type calcium channels to the overall intracellular calcium pool during each contraction:  $\sim 70\%$  in rabbit and dog compared to less only 2–5% in rat [17, 18]. In addition, it is known that also myofilament isoform composition differs between small and larger mammals, most notably in the

myosin heavy chain (MHC) isoform [19]. However, since the force of contraction highly depends on time passed since the last contraction, dynamic calcium handling and distribution of calcium fluxes that are very much time-dependent are thus more likely contributors, versus fixed differences in isoforms of MHC, or other differences in myofilament protein expression, although these cannot be excluded to contribute to the species differences at this point.

When next we investigated the effect of a variable length (load) under a variable pacing protocol on the rabbit heart,

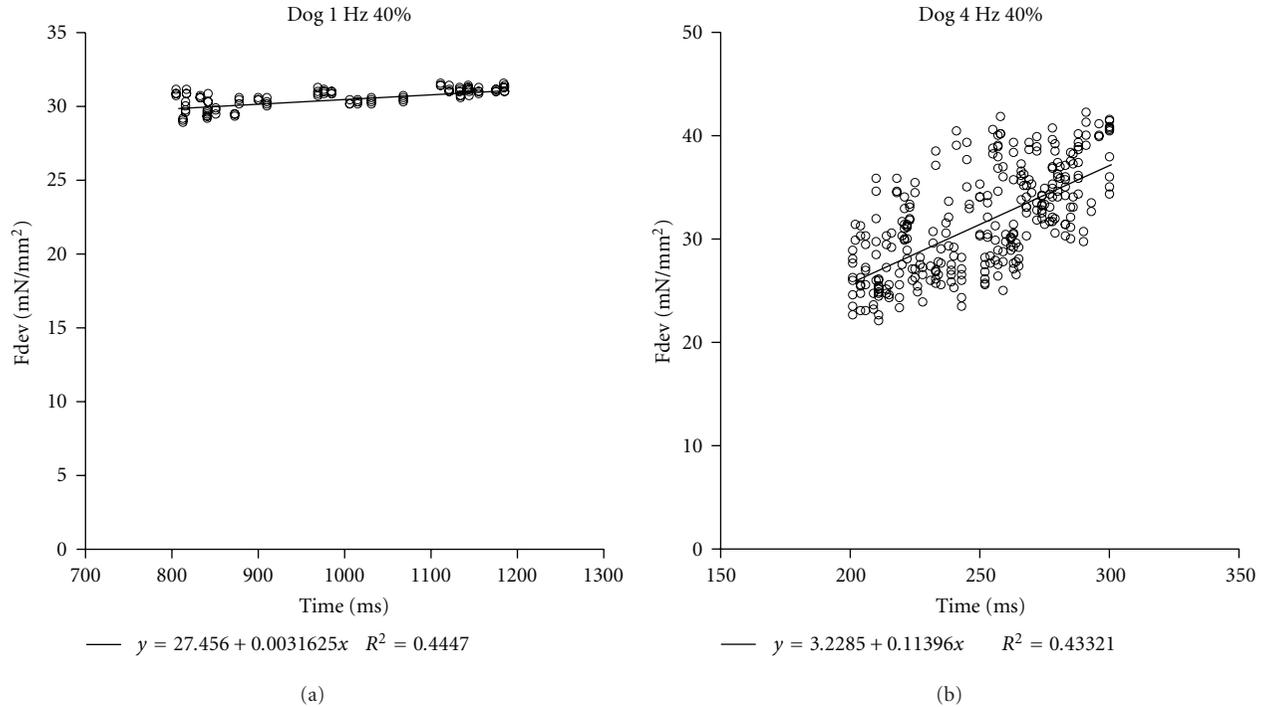


FIGURE 4: The correlation between prebeat duration and average force development in the dog is demonstrated for both 1 and 4 Hz. There is no difference with the increase in frequency.

we noticed a discrete increase on average force of the variable over the fixed rate base frequency that was constant at both 2 and 4 Hz for the 5% stretch group. At 13% stretch, a similar effect was noticed at the 2 Hz frequency group, whereas, at 4 Hz, there was a nonsignificant weakening effect. This latter finding may have resulted as these higher frequencies from the additional stretch may have caused some damage to the myofilament structure by a too rapid stretch.

Of greater importance was the significantly tighter correlation  $R^2$  observed between the prebeat duration and the developed force once a small variable stretch was applied. This correlation was much tighter as the frequency and stretch increased ( $P < 0.001$  at 4 Hz). This suggests that while the interval between beats is important, there is considerable effect that is load dependent affecting the subsequent beat. While several studies have shown an independent and strong effect of the interbeat duration alone and with contribution of the Frank Starling mechanism put into question [7, 20], there are others with findings similar to ours during *in vivo* investigations [21, 22]. At the heart of the problem lie two seemingly opposite phenomena: on one hand a sustained decrease in interbeat duration (thus an increase in frequency) leads to increased contractility, whereas instantaneous decreases in interbeat duration lead to a weaker immediate first beat [23]. We here now show that this interplay appears to be nonlinear in the time domain and that, by manipulating the frequency and variability a host of factors interact dynamically in a non-steady-state of equilibrium that changes every instant. The mechanism of the stretch-dependent “tightening” of the correlation is likely to be found in the interaction between load and calcium handling

and could include stretch-dependent calcium channels and ion homeostasis [24] on one hand, an interaction of the higher calcium sensitivity at greater sarcomere length [25], or a greater contribution of predominantly passive elements such as collagen or titin [26]. Potentially, at larger sarcomere length, the increase in the absolute myofilament calcium buffer capacity is nonlinear with stretch and promotes retention of calcium between two subsequent beats. However, more detailed experiments would be required to test such hypothesis.

On a beat-to-beat basis with instantaneous switching intervals away from steady state, the phenomena of mechanical restitution and postrest potentiation and postextrasystolic potentiation likely play an important role in determining the immediate contractile state of the heart [27]. We speculate that the direction and the magnitude of force modulation possibly relate to the different relative contributions of the sodium calcium exchanger (NCX) versus the sarcoplasmic reticulum calcium ATPase (SERCA) to the contractile process in each species. This paper uniquely highlights the influence of heart rate variability on a beat-to-beat basis and the different effects in diverse species while attempting to account for real life *in vivo* events such as preload stretching of the fibers.

We conclude that, in larger mammals, no changes in contractile force can be obtained by introducing variation in the beat-to-beat duration, and we confirm the evidence that prebeat duration has an important effect on developed strength [15]. The addition of a prebeat stretch effectively tightens this association that also becomes more significant as the frequency increases. Short-term manipulation of the beat-to-beat

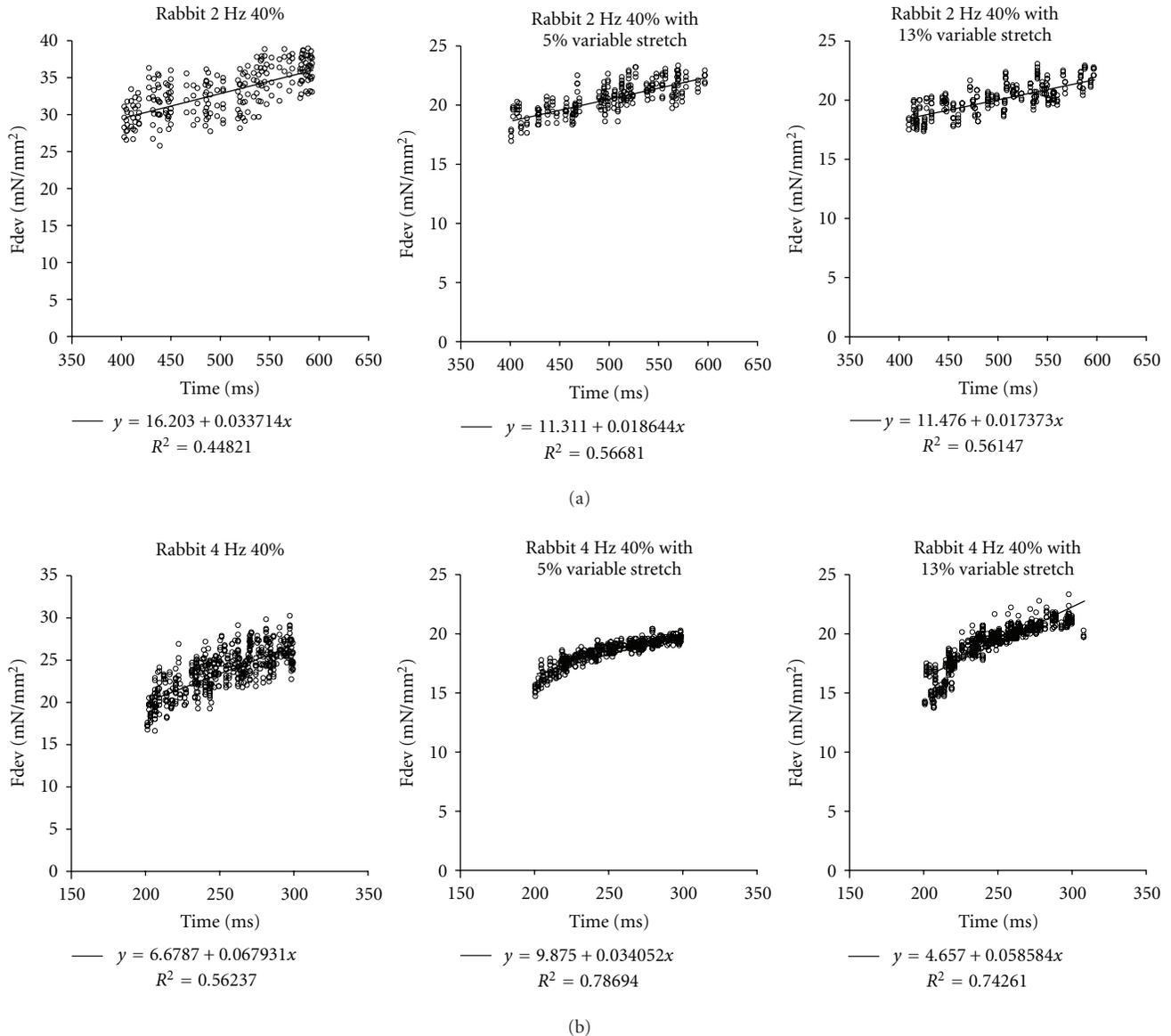


FIGURE 5: Top half displays the interaction between prebeat duration and force development at 2 Hz. The correlation coefficients are increasingly tighter as the preload stretch is increasingly applied. On the bottom half, we depict the same phenomena at the higher frequency (4 Hz). The correlation here is more evident and significantly different statistically ( $P < 0.01$ ) from the initial nonstretch correlation value of 0.56.

interval thus can have variable effects that are species dependent. Its underlying mechanisms require further *in vivo* experiments as well as potential investigation of the long-term effects on protein expression and posttranslational modifications that are known to occur as a result of length [28] of frequency changes [11, 29].

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