# The force-frequency relationship in human heart failure: effect of pyruvate and isoproterenol

**Martisiene I*, Gendviliene V, Zablockaite D, Gurskaite H**

Institute of Cardiology, Kaunas University of Medicine, Kaunas, Lithuania

## Abstract

**Purpose:** **The purpose of present study was to investigate the effect of metabolic substrate pyruvate and β-adrenergic agonist isoproterenol and combination of these agents on the force- and relaxation-frequency relationship in human heart failure.**

**Material and methods:** **The experiments were performed on isolated human ventricle strips from patients undergoing cardiac corrective open heart surgery, using conventional method of registration of electromechanical activity. The stimulation frequency of myocardial strips was 0.2, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 Hz.**

**Results:** **In control, i.e. at perfusion of myocardial strips by Tyrode solution and stimulation frequency 1 Hz, the contraction force (F) was 0.94±0.18 mN, half time of relaxation ($t_r$) – 178.8±9.3 ms (n=12). Pyruvate (10 mmol/L) increased F to 176.0±13.4%, $t_r$ – 104.6±3.1% (n=8, p<0.05) vs control. By the action of isoproterenol ($10^{-5}$ mol/L) F increased to 122.1±10.2%, $t_r$ decreased to 58.9±3.1% (n=4, p<0.05) vs control. The relationship of F and $t_r$ from stimulation frequency in the absence of pyruvate and isoproterenol was negative. Pyruvate and isoproterenol didn't alter the shape of force-frequency relationships but F was augmented at all stimulation frequencies. The positive inotropic effect of isoproterenol was potentiated by pyruvate.**

**Conclusions:** **Pyruvate and isoproterenol alone can improve cardiac contractility in wide-range of stimulation frequency. The combination of these inotropic agents results in even more effective increase of contractile performance and therefore may be of therapeutic value in heart failure.**

* CORRESPONDING AUTHOR:
Institute of Cardiology, Kaunas University of Medicine
Sukileliu 17, LT-50009
Kaunas, Lithuania
Fax: 370 37 302872
e-mail: membiof@kmu.lt (Irma Martisiene)

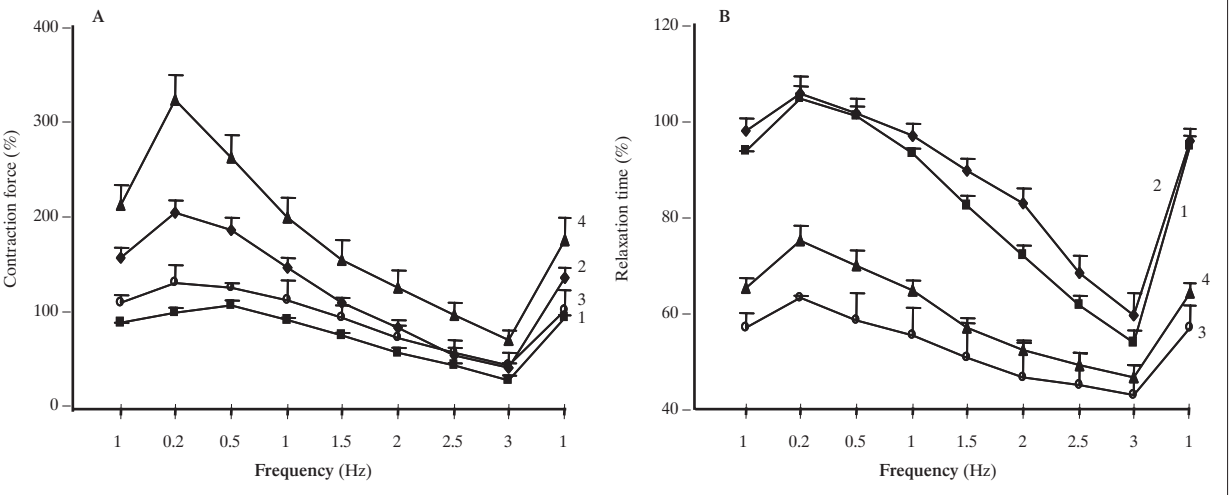
Received 25.05.2005 Accepted 02.06.2005

**Key words:** failing human myocardium; force-frequency relationship; pyruvate; isoproterenol.

## Introduction

The main feature of failing human myocardium is a decline of contraction force caused by disorders of cellular systems regulating intracellular $Ca^{2+}$ concentration, such as sarcolemmal L-type $Ca^{2+}$ channel, $Ca^{2+}$-ATPase, $Na^{+}$-$Ca^{2+}$ exchanger, sarcoplasmic reticulum (SR) $Ca^{2+}$-ATPase (SERCA2) and phospholamban [1]. An informative indicator and convenient methodical tool for evaluating the defects of behavior of these systems and severity of contractile dysfunction, cardiac reserve capacity and effectiveness of therapeutic agents is the force-frequency relationship (FFR). Normal human heart exhibits a positive FFR, i.e. increasing of pacing frequency augments the contraction force of myocardium, while negative FFR is the characteristic of failing myocardium [1,2]. It has been reported that β-adrenergic stimulation of failing human myocardium increases the contractility and partly reverses the negative FFR [2]. However, the increased energy demand (ATP) that is observable during β-adrenergic stimulation limits the efficiency of this inotropic intervention in failing myocardium. It has been recently shown that pyruvate, a natural aliphatic monocarboxylate and central metabolic intermediate in mammalian cells, increases phosphorylation potential, improves contractility and potentiates β-adrenergic inotropism in failing human heart [3,4]. However, the influence of pyruvate and combination of pyruvate with β-adrenergic agonists on the force-frequency relationship in human heart failure is not determined. The purpose of present study was to investigate the effect of pyruvate and isoproterenol and combination of these agents on the force- and relaxation-frequency relationship in failing human heart.

*Figure 1.* **Influence of stimulation frequency on the contraction force (A) and relaxation time (B) in failing human myocardium: without pyruvate and isoproterenol – curve 1, (n=12); in the presence of pyruvate (10 mmol/L) – curve 2, (n=8), or isoproterenol ($10^{-5}$ mol/L) – curve 3 (n=4), or pyruvate and isoproterenol – curve 4 (n=8) in Tyrode solution. Changes in contraction force and relaxation time are given in % change from the basal value at 1 Hz**




## Material and methods

The experiments were performed on strips of human ventricle myocardium from patients undergoing mitral or aortic valve correction surgery in Department of Cardiosurgery of Kaunas University Hospital. Isolated myocardium strips were placed in an experimental chamber and superfused with oxygenated (100% $O_2$) Tyrode solution (in mmol/L): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.9, glucose 5, Hepes 10, pH 7.4 at 36±0.5°C. The stimulation frequencies was 0.2, 0.5, 1, 1.5, 2, 2.5 and 3 Hz, the duration of pulses – 2-5 ms, amplitude – twice the diastolic threshold. Isometric contraction was recorded using a linear force-displacement transducer (Harvard Apparatus, U.S.A.). The action of pyruvate (10 mmol/L) and isoproterenol ($10^{-5}$ mol/L) on the contraction force (F) and half time of relaxation ($t_r$) was investigated. Changes of parameters were expressed in percentage in respect to control (Tyrode solution, 1 Hz). All values were presented as means ±SEM. The significance of data was assessed using Student's t-test and the results were considered significant at $p<0.05$.

## Results

An average of contraction force of ventricular strips from human failing heart was 0.94±0.18 mN, half time of relaxation – 178.9±9.3 ms (n=12). After addition of pyruvate (10 mmo/L) F increased to 176.0±13.4%, $t_r$ – 104.6±3.1% (n=8, $p<0.05$) vs control. Isoproterenol ($10^{-5}$ mol/L) increased F to 122.1±10.2% and diminished $t_r$ to 58.9±3.1% (n=4, $p<0.05$) vs control. The combination of pyruvate and isoproterenol resulted in an increase of contraction force to 236.9±2.5% (n=8, $p<0.05$), which was higher than the addition of the individual effects of these agents. The half time of relaxation under the action of these agents decreased to 67.7±2.4% (n=4, $p<0.05$) vs control.

*Fig. 1* shows the influence of stimulation rate on the contraction force (*A*) and half time of relaxation (*B*) of ventricular strips without (curve 1) and with pyruvate (10 mmol/L) (curve 2) or isoproterenol ($10^{-5}$ mol/L) (curve 3), or combination of these both agents (curve 4) in Tyrode solution. In the absence of pyruvate or isoproterenol the contraction force and relaxation time slightly increased at low stimulation frequency (0.2-0.5 Hz) and continuously declined at higher stimulation rate (curve 1 in *Fig. 1A* and *B*, respectively). Pyruvate as well as isoproterenol didn't alter the shape of FFR, however, contraction force was higher at all stimulation frequencies, as compared to untreated muscles (*Fig. 1A*, curves 2 and 3, respectively). The combination of these inotropic agents resulted in a more significant increase of contraction force at investigated range of stimulation rate as compared to their individual effects (*Fig. 1A*, curve 4). The decrease of relaxation time at high stimulation rate was lesser under the action of pyruvate and higher under the action of isoproterenol as compared to untreated muscles (*Fig. 1B*, curve 2 and 3 respecively). The less significant acceleration of relaxation was observed by combination of pyruvate and isoproterenol as compared to isoproterenol action alone (*Fig. 1B*, curve 4).

## Discussion

The present study demonstrates that an increase of stimulation frequency reduced the contraction force and relaxation time in failing human myocardium. The main cause of negative force-frequency relationship may be the alterations in the intracellular $Ca^{2+}$-handling caused by reduced activity of the SERCA2 in failing myocardium [2]. The metabolic substrate pyruvate and β-adrenoceptor agonist isoproterenol didn't alter the shape of FFR, however, they improved contractile function in wide-range of stimulation frequency in failing human myocardium. The main mechanisms by which pyruvate increases

contractility include the stimulation of SERCA2 activity and an increase of SR $Ca^{2+}$- uptake due to an increase in phosphorylation potential and free energy available from ATP hydrolysis [4,5]. The positive effect of β-adrenoceptor agonist isoproterenol can be explained by an increase in intracellular cAMP level, phosphorylation of L-type $Ca^{2+}$ channel and phospholamban what removed the inhibition of SERCA2, increased $Ca^{2+}$ load in SR and contractility in failing myocardium [1,2]. Our results show that the combination of these inotropic agents resulted in a more effective increase of contractile performance in wide-range of stimulation frequency. This effect may be interpreted as a potentiation of β-adrenergic inotropism by metabolic substrate pyruvate due to improved cardiac energetic state in failing human myocardium. We conclude that combination of pyruvate and β-adrenergic agents may be of therapeutic value in heart failure.

## References

1. Endoh M. Force-frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance. Europpean Journal of Pharmacology, 2004; 500: 73-86.

2. Bavendiek U, Brixius K, Münch G, Zobel C, Müller-Ehmsen J, Schwinger RHG. Effect of inotropic interventions on the force-frequency relation in the human heart. Basic Research in Cardiology, 1998; 93: 76-85.

3. Hermann H-P, Zeitz O, Lehnart SE, Keweloh B, Datz N, Hasenfuss G, Janssen PML. Potentiation of beta-adrenergic inotropic response by pyruvate in failing human myocardium. Cardiovascular Research, 2002; 53: 116-23.

4. Hasenfuss G, Maier LS, Hermann H-P, Lüers C, Hünlich M, Zeitz O, Janssen PML, Pieske B. Influence of pyruvate on contractile performance and $Ca^{2+}$ cycling in isolated failing human myocardium. Circulation, 2002; 105: 194-9.

5. Mallet RT, Sun J. Mitochondrial metabolism of pyruvate is required for its enhancement of cardiac function and energetics. Cardiovascular Research, 1999; 42: 149-61.