

---

**PHARMACOLOGY**

---

**IMPACT OF ISOPRENALINE AND CAFFEINE ON DEVELOPMENT OF LEFT VENTRICULAR HYPERTROPHY AND RENAL HEMODYNAMIC IN WISTAR KYOTO RATS****ASHFAQ AHMAD<sup>1\*</sup>, MUNAVVAR Z. A. SATTAR<sup>1\*</sup>, HASSAAN A. RATHORE<sup>1</sup>, SAFIA AKHTAR KHAN<sup>1</sup>, MOHAMMED A. LAZHARI<sup>1</sup>, FAYAZ HASHMI<sup>1</sup>, NOR A. ABDULLAH<sup>2</sup> and EDWARD J. JOHNS<sup>3</sup>**<sup>1</sup> School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, 11800, Malaysia<sup>2</sup> Department of Pharmacology, Faculty of Medicine, Universiti of Malaya, Kuala Lumpur, Malaysia<sup>3</sup> Department of Physiology, University College Cork, Cork, Ireland

**Abstract:** Left ventricular hypertrophy (LVH) is a compensatory mechanism in response to an increased work load on the heart. This study investigated the impact of chronic isoprenaline and caffeine (I/C model) administration on cardiac geometry, systemic hemodynamic and physiological data in rats as LVH develops. LVH was induced by administering isoprenaline (5 mg/kg s.c. every 72 h) and caffeine (62 mg/L) in drinking water for 14 days to Wistar Kyoto (WKY) rats. Mean arterial pressure (MAP), systolic blood pressure (SBP), heart weight, LV weight, LV chamber diameter and thickness of myocardium were observed as LVH indicators. MAP was significantly higher ( $142 \pm 13$  vs.  $119 \pm 2$  mmHg, respectively) while heart rate (HR) in LVH was lower ( $314 \pm 9$  vs.  $264 \pm 18$  BPM) compared to control WKY. Heart weight, LV weight and kidney weight were 31%, 38% and 7%, respectively, greater in the LVH group as compared to the control WKY (all  $p < 0.05$ ). The myocardium thickness was 101% greater while LV chamber diameter was 44% smaller in the LVH group as compared to the control WKY ( $p < 0.05$ ). The superoxide dismutase (SOD), glutathione reductase (GSH) and total antioxidant capacity (T-AOC) levels were significantly reduced while malondialdehyde (MDA) level increased in LVH as compared to control WKY (all  $p < 0.05$ ). In conclusion, isoprenaline and caffeine (I/C) induces LVH and cardiac hypertrophy with increases in blood pressure, fluid excretion and reduced renal hemodynamics. Prooxidant mechanism of the body and arterial stiffness are dominant in this disease model. This model of LVH is easily generated and associated with low mortality.

**Keywords:** isoprenaline, caffeine, left ventricular hypertrophy, hemodynamics, renal function

Left ventricular hypertrophy (LVH) is defined as an increase in mass and size of the left ventricle which frequently occurs as a result of an elevated resistance within the circulatory system. This increase in mass of the myocardium results from a chronically raised workload on the heart (1) and is taken as an early sign of cardiomyopathy, which ultimately may lead to symptomatic heart failure. LVH is an independent predictor of cardiovascular morbidity and mortality (1, 2). There are two types of LVH: (i) pressure overload hypertrophy (POH) or, (ii) volume overload hypertrophy (VOH). LVH has been studied using different animal models based on catecholamine administration, for example, isoprenaline, caffeine, torbafylline, as well as a combination of isoprenaline with caffeine (I/C) (3)

and isoprenaline with torbafylline (4) for varying periods up to 7 days. Isoprenaline given as an intraperitoneal injection (i.p.) of 5 mg/kg/day for 7 days has been used as a model for heart failure (5). A further variation has been when isoprenaline was administered for the development of myocardial necrosis in which 2 injections of isoprenaline (s.c.) were used some 72 h apart (6).

The left ventricular hypertrophy and altered cardiac output will impact on systemic hemodynamic and the function of other organs, including the kidney. The functional capacity of renal adrenergic receptors in the kidney has been studied using the I/C model where treatment lasted for 7 days, which probably induced a degree of cardiac hypertrophy, and combined with diabetes in Sprague-Dawley

---

\* Corresponding authors: e-mail: munavvar@usm.my; e-mail: raza\_chohan487@hotmail.com; mobile: 006016-4872009

(SD) rats (7). This study demonstrated that  $\alpha_{1A}$  and  $\alpha_{1D}$  subtypes mediate adrenergically induced vasoconstriction responses in this combined state of heart failure and diabetes. In another I/C model study, caffeine was administered as 62 mg/L while isoprenaline was injected (s.c.) at the dose of 5 mg/kg for 14 days in anesthetized Wistar Kyoto rats (3). The investigators reported that although cardiac function was not impaired in LVH, there was decreased high pressure baroreceptor control of sympathetic outflow. These investigators went on to show that in the same I/C model as well as one in which heart hypertrophy was induced by giving thyroxin (1 mg/kg, s.c.) for 1 week (8) there was a defective baroreceptor regulation of renal sympathetic nerve activity. These reports found no significant difference in baseline value of blood pressure, heart rate or RSNA in vehicle control, isoprenaline/caffeine-treated or thyroxin-treated groups of anesthetized animals. However, following an acute saline volume expansion in these models, the renal sympatho-inhibition was prevented. This model was presumed to be one of a transition from LVH to heart failure (HF).

Metabolic changes are likely to occur during the induction of LVH and an increased plasma level of angiotensin II has been observed in catecholamine induced LVH (9). In the I/C model, both isoprenaline and caffeine act as cardiac stimulants. Caffeine is an adenosine receptor antagonist and increases heart rate along with activation of noradrenergic neurons (10). This increased adrenergic activity will affect the renal hemodynamic and excretory functional capacity of kidney.

Left ventricular remodelling is a multifactorial process with the involvement of inflammatory cytokines and production of reactive oxygen species (11), oxidative stress and inflammatory reactions in injured myocardium (12). It is there important to know the balance between prooxidant and antioxidant. Plasma levels of oxidative stress parameters (SOD), malondialdehyde and nitric oxide (NO) levels should be altered in LVH. To the best of our knowledge, these parameters of oxidative stress have not been studied yet in this isoprenaline and caffeine model of LVH and cardiac hypertrophy.

Based on previous investigations, the current study used a modification of the original isoprenaline and caffeine regime to induce LVH by extending isoprenaline therapy to achieve consistent cardiac dysfunction. This study also aimed to investigate the impact of LVH on circulatory status, cardiac geometry, and systemic hemodynamic and oxidative stress parameters. This was extended to

examine how blood perfusion through kidney was altered in LVH and to determine whether endothelial status had been altered as reflected in arterial stiffness.

## MATERIALS AND METHODS

### Animals

Fourteen male Wistar Kyoto rats (WKY) weighing  $200 \pm 20$  g, were brought from the Animal Facility of Universiti Sains Malaysia (USM) and were acclimatized in the transit room for 5 days before starting any treatment. Animals were given free access to water and food. Animals were randomly divided into control and LVH groups. The control group and LVH groups were given the same food for 14 days while in the LVH group on day 1, the animals began treatment with isoprenaline (5 mg/kg s.c., 72 h apart) and caffeine (62 mg/L) in the drinking water until day 14 as stated by (3) when they were taken for acute study. According to previous study (3), isoprenaline s.c. 4 injections were administered after 72 h apart but present study used 5 injections of isoprenaline 72 h apart on day 1, 4, 7, 10 and 13 to prolong the effect of drug before acute experiment. Caffeine dose was continued as stated (3). Experimental procedures were approved by the Animal Ethical Committee of USM approval no. 2012/(76) (364).

### Measurements of electrocardiogram (ECG) data

Animals were fastened overnight and then anesthetized with injection of pentobarbital sodium (Nembutal; CEVA, Lebourne, France) at a dose of 60 mg/kg, i.p. A 3 lead surface ECG recordings of all the animals were done with gold plated needles electrodes (ADInstruments, Sydney, Australia) as previously reported (13, 14).

### Acute experiment

The experimental procedure and protocols for the acute studies were based on those previously published studies (15). Overnight fasted rats were anesthetized using pentobarbital (Nembutal; CEVA, Lebourne, France) at a dose of 60 mg/kg, i.p. A tracheal tube PP 240 (propylene tubing 240; Portex, UK) was inserted to facilitate ventilation. The left jugular vein was cannulated with PP-50 to allow maintenance doses of anesthetic to be given during the course of surgery. The right carotid artery was cannulated with PP-50 and connected to a pressure transducer (P23 ID Gould; Statham Instruments, Nottingham, UK), linked to a computerized data acquisition system (Power Lab; AD

Instrumentation, Sydney, Australia) to monitor blood pressure, heart rate, cyclic duration and mean arterial pressure (MAP). The cyclic duration is taken as the time interval between one cardiac cycle and second cardiac cycle. The left kidney was exposed *via* an abdominal midline incision and a laser Doppler probe (OxyFlow, ADInstruments, Australia Model No. ML191) was placed on the cortical surface of the kidney to measure cortical blood perfusion, expressed as blood perfusion units. The left iliac artery was cannulated with PP-50 tubing to allow recording of iliac blood pressure. The iliac cannula was connected to a pressure transducer which was attached to a Powerlab (ADInstruments) and *via* a side arm was attached to an infusion pump programmed to deliver saline (0.9% NaCl) at 6 mL/h. After stabilization for about 60 min, pulse wave velocity was measured by exposing the aorta and measuring the distance from the point of insertion of the carotid artery cannula to the insertion point of the iliac artery cannula (16, 17). The left ureter was cannulated with PP-10 tubing for urine collection. At the end of acute experiment, animals were euthanized by means of an overdose of pentobarbital. The weight of the heart, left ventricle and kidney were taken after careful isolation and removal from experimental animals. After weighing, left ventricle tissues were put in 10% formalin for histopathology study. The thick-

ness of the myocardium and LV chamber internal diameter was estimated as described previously (18). Thickness was measured with a Vernier caliper just below the level of the mitral valve. These values were used to generate heart index, kidney index and LV index as follows:

$$\text{Heart index} = \text{Heart weight} / \text{body weight} \times 100$$

$$\text{Left ventricle index} = \text{Left ventricle weight} / \text{body weight} \times 100$$

$$\text{Kidney index} = \text{Kidney weight} / \text{body weight} \times 100$$

The study protocol is shown in Figure 1.

### Measurement of creatinine and electrolytes in plasma and urine samples

Both groups had fluid intake and urine output measured using metabolic cages before and after treatment (day 0 and day 14). Water intake was measured by subtracting the remaining water in the bottle after 24 h from the initial total volume. Blood (1 mL) was drawn from the lateral tail vein, centrifuged at 10,000 rpm for 10 min using a centrifuge (Gallenkamp, UK) and the supernatant layer was taken as plasma. Sodium and potassium in the plasma was estimated using a flame photometer (Jenway Ltd. Felsted, Essex, UK) while creatinine in plasma and urine was estimated using a colorimetric method (19) and the samples were read using a 96 well microplate reader. Creatinine was measured from

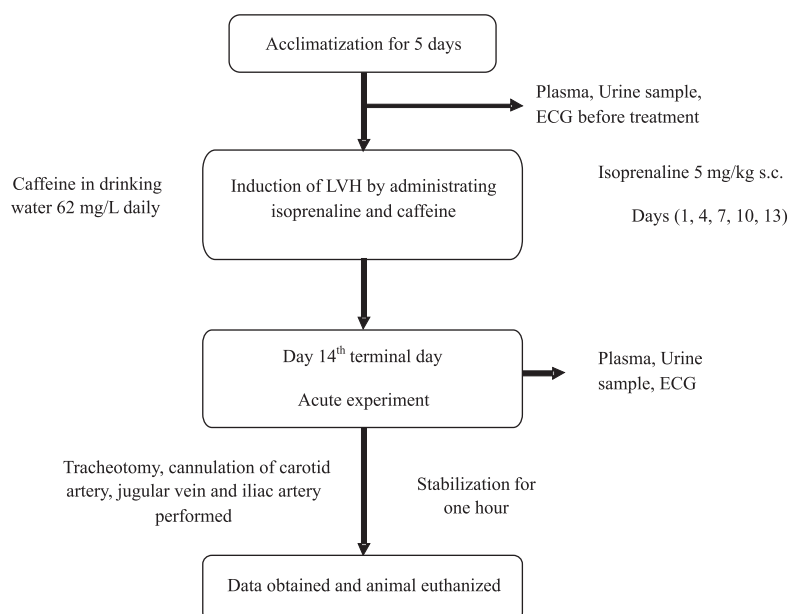


Figure 1. Study protocol conducted for validation and evaluation of left ventricular hypertrophy and hemodynamic parameters

plasma and urine to assess creatinine clearance (mL/min/kg b.w.) and fractional excretions of sodium and potassium (%).

#### Measurement of oxidative stress parameters

Oxidative stress parameters like superoxide dismutase (SOD), malondialdehyde (MDA), glutathione reductase (GSH), nitric oxide (NO) and total antioxidant capacity were measured on day 14<sup>th</sup> from the plasma by using kits.

#### Statistical analysis and presentation of data

The data (the mean  $\pm$  SEM) were analyzed using one way ANOVA along with the *post hoc* Bonferroni test and independent Student's *t* test using Graph Pad Prism (Graph Pad Software, Inc. CA, USA) with significance taken at  $p < 0.05$ .

## RESULTS

#### Effect on systemic hemodynamic

The MAP, HR, PP and cyclic duration was observed principally to validate the model and use them as markers of LVH. MAP, PP and cyclic duration were significantly higher (all  $p < 0.05$ ) in the LVH WKY group as compared to the control WKY (Table 1) while heart rate in the LVH WKY group

was lower than in the control WKY group ( $p < 0.05$ ) (Table 1).

#### Heart weight, left ventricle weight, kidney weight respective indices, myocardial thickness and LV chamber diameter

The physical appearance and weights of vital organs are primary indicators of LV and cardiac hypertrophy. Both heart and LV weights were significantly higher (both  $p < 0.05$ ) in the LVH WKY group (Table 2) as compared to control WKY. The myocardium was thicker in the LVH WKY group compared to the control group while the internal diameter of left ventricles of the LVH WKY group was significantly less than that of the control group (Table 2, Fig. 2).

#### The electrocardiogram (ECG) measurement

The LVH was investigated by studying QRS complex, R-R intervals and R-amplitude in control WKY and LVH WKY rats by using ECG. The ECG morphology showed obvious P, Q, R and S waves in both groups. In this study QRS complex, R-R intervals and R-amplitudes were significantly higher in LVH WKY group as compared to control WKY (all  $p < 0.05$ ) as shown in Table 1.

Table 1. Electrocardiogram of control WKY and LVH WKY done on day 14<sup>th</sup> during acute experiments. (n = 5-6). \* denotes significant difference  $p < 0.05$  compared to control group.

ECG	Control WKY	LVH WKY
R-R (ms)	175.7 $\pm$ 3.9	211.7 $\pm$ 6.*
QRS (ms)	18.6 $\pm$ 0.6	22.2 $\pm$ 0.9*
QT (ms)	72.5 $\pm$ 3.5	88.1 $\pm$ 1.2*
QTc (ms)	200.3 $\pm$ 3.1	210.0 $\pm$ 1.5*
R-Amp (mV)	0.60 $\pm$ 0.01	0.76 $\pm$ 0.03*

ms: milliseconds, mV: millivolts

Table 2. SBP, DBP, MAP, PP, and HR, cyclic duration, time to peak, of control WKY vs. LVH WKY groups (n = 5-6). \* denotes significant difference  $p < 0.05$  compared to control group.

Groups	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Mean arterial pressure (mmHg)	Pulse pressure (mmHg)	Heart rate (BPM)	Cyclic duration (S)
Control	132 $\pm$ 10	102 $\pm$ 4	119 $\pm$ 2	30 $\pm$ 2	314 $\pm$ 9	0.190 $\pm$ 0.0
LVH	159 $\pm$ 13*	125 $\pm$ 6	142 $\pm$ 13*	40 $\pm$ 7*	264 $\pm$ 18**	0.231 $\pm$ 0.03*

Abbreviations: LVH = Left Ventricular Hypertrophy; wt. = weight; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean Arterial Pressure; PP: Pulse pressure; HR: Heart rate.

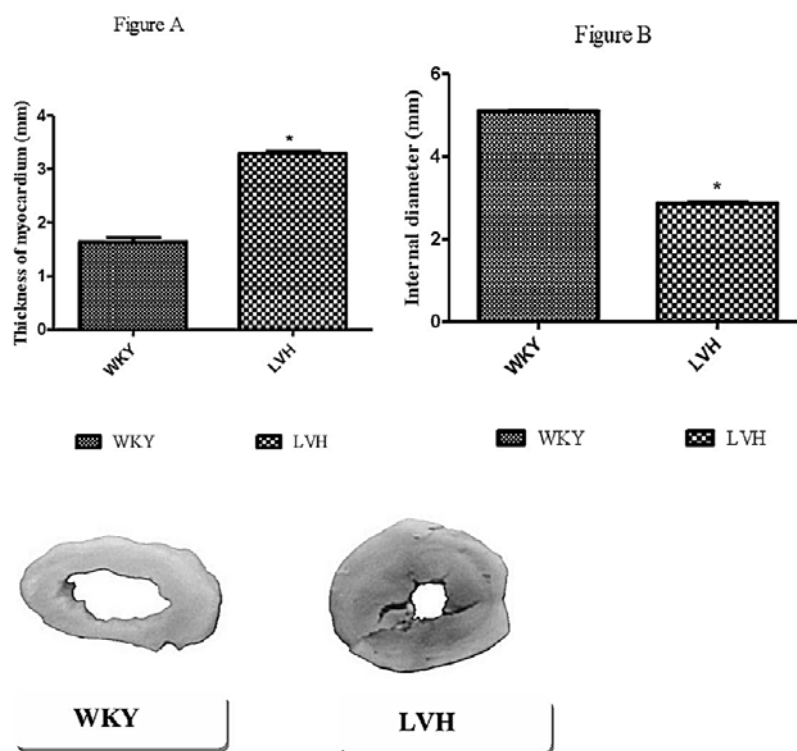


Figure 2. Thickness of myocardium, internal diameter of LV chamber of control WKY, LVH; \* denotes significant difference  $p < 0.05$  from the control group.

Table 3. Heart, left ventricle, kidney indices, myocardial thickness and diameter of LV at day 14<sup>th</sup> of LVH as compared to the control group. (n = 5-6). \* represents mean value significantly different in comparison between control WKY and LVH WKY groups ( $p < 0.05$ ).

Physical parameters	WKY	LVH
Body wt. (g)	298 ± 12	273 ± 8*
Heart wt. (g)	0.79 ± 0.02	1.04 ± 0.05*
LV wt. (g)	0.47 ± 0.02	0.64 ± 0.02*
Kidney wt. (g)	0.87 ± 0.02	0.93 ± 0.05
Heart index	0.26 ± 0.00	0.39 ± 0.01*
LV index	0.46 ± 0.03	0.68 ± 0.06*
Kidney index	0.30 ± 0.02	0.33 ± 0.02*
Myocardial thickness (mm)	1.64 ± 0.18	3.29 ± 0.10*
Diameter of LV (mm)	5.09 ± 0.04	2.85 ± 0.10*

Abbreviations: LVH = Left Ventricular Hypertrophy, wt. = weight

#### Effect on water intake and urine output in control and LVH group

Weight gain in LVH WKY group was significantly less compared to the control WKY group (Table 4a). Water intake and urine output in the two groups were not significantly different as shown in Table 4a.

#### Changes in physiological data in control and LVH groups

Plasma Na concentration of the control group was higher than that of LVH group ( $p < 0.05$ ) while the concentration of Na in urine was significantly lower in the control group as compared to the LVH group (Table 4b). Plasma K<sup>+</sup> was lower in LVH

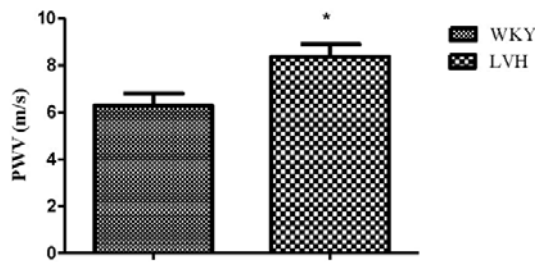
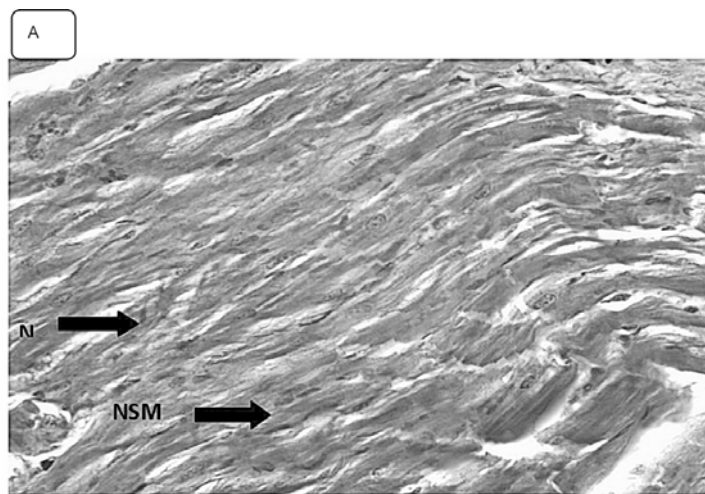
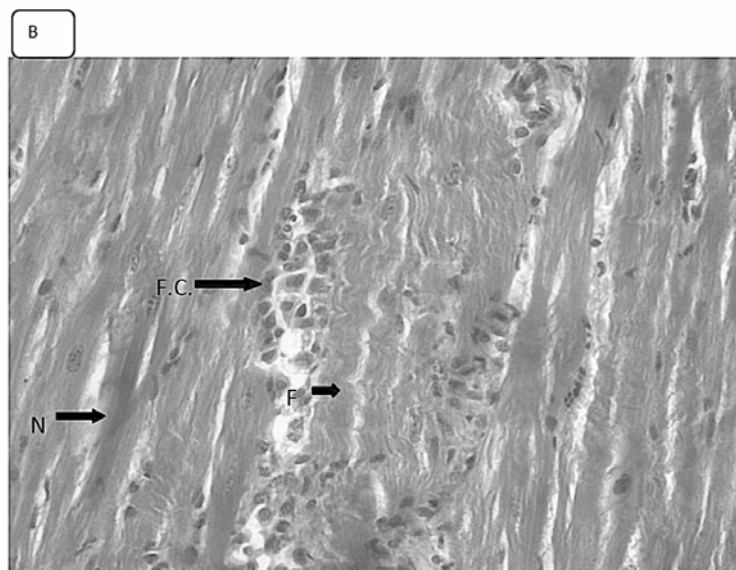


Figure 3. PWV of control vs. LVH. (n = 6 in each group) \* represents  $p < 0.05$  compared to control group

group as compared to control WKY group ( $p < 0.05$ ) while urinary  $K^+$  was higher in the LVH compared to the control WKY group (Table 4b). The  $U_{Na}/U_K$  ratio was significantly lower in the LVH ( $p < 0.05$ ) compared to the control WKY group as shown in Table 4b. Absolute Na excretion and absolute potassium excretions in the control groups were significantly (both  $p < 0.05$ ) lower than in the LVH group. Significantly elevated creatinine in plasma was observed in LVH WKY as compared to control WKY ( $p < 0.05$ ). The fractional excretions of sodi-



N: Normal nuclei      NSM: Normal cardiac striated muscle



N: Necrotic tissue      F: Fibrosis      F.C.: Fibroblast cells

Figure 4. Histopathological slides of heart muscles of control WKY and LVH rats. Fig. A showing normal nuclei, no breakdown of muscle fibre and striated muscles while figure B showing the breakdown of myofibers, no cardiac muscle characteristic striation. Necrotic tissue seen and the presence of fibroblast and fibrosis observed in LVH WKY group

Table 4a. Body weight gain, daily water intake, daily urine output of control and LVH. \* denotes significant difference  $p < 0.05$  between control vs. LVH.

Groups	Wt. D-0	Wt. D-14	Wt. Gain	Water intake (mL/24 h)	Urine output (mL/24 h)
Control group	192	298 ± 5	106	45	10
LVH group	184	273 ± 3	89*	50	15*

Wt. represents weight, LVH is left ventricular hypertrophy and D represents day.

Table 4 (b). Electrolytes data of control WKY and LVH WKY groups. \* denotes significant difference  $p < 0.05$  in comparison of control WKY and LVH WKY.

Groups	Na in plasma (mmol/L)	K in plasma (mmol/L)	Na in urine (mmol/L)	K in urine (mmol/L)	Absolute Na excretion	Absolute K excretion
WKY	135 ± 3	6 ± 1.2	122 ± 7	60 ± 9.3	0.05 ± 0.01	0.14 ± 0.01
LVH	120 ± 3*	4 ± 1*	146 ± 9*	108 ± 5.1*	0.09 ± 0.01*	0.19 ± 0.02*

Na: Sodium; K: Potassium.

Table 4 (c). Renal functional parameters investigated on day 14<sup>th</sup>. \* denotes significant difference  $p < 0.05$  in comparison of control WKY vs. LVH WKY.

Renal function parameters	WKY	CONTROL
<b>Creatinine in Plasma</b>	1.05 ± 0.33	1.38 ± 0.03*
<b>Cr.Cl (mL/min/kg b.w.)</b>	1.14 ± 0.22	1.34 ± 0.10
<b>FE Na (%)</b>	2 ± 0.2 3	± 0.2*
<b>FE K(%)</b>	22 ± 4.5	79 ± 12.3*
<b>Urine flow rate (µL/min/100 g b.w.)</b>	2.38 ± 0.5	3.54 ± 0.3*
<b>U<sub>Na</sub>/U<sub>K</sub></b>	2 ± 0.33	1.36 ± 0.10*

Cr.Cl: Creatinine clearance, b.w.: body weight, mL/min/kg: millilitre per minute per kilogram  
FE Na: Fractional excretion of sodium; FE K: Fractional excretion of potassium; U<sub>Na</sub>/U<sub>K</sub>: Urinary sodium to urinary potassium ratio.

um, potassium and urine flow rate is significantly greater in LVH WKY group as compared to control WKY ( $p < 0.05$ ) as shown in Figure 4c.

#### Oxidative stress parameters

In LVH disease model, the level of oxidative stress was measured as LVH is chronic inflammatory process. It was observed that enzymatic SOD, NO, GSH and T-AOC levels were significantly lower in LVH WKY group as compared to control WKY (all  $p < 0.05$ ) while enzymatic oxidative parameter was higher in LVH WKY group as compared to control WKY ( $p < 0.05$ ) as shown in Table 5.

#### Effect on pulse wave velocity (PWV)

Pulse wave velocity was significantly lower ( $6.3 \pm 1.01$  m/s) in control WKY as compared to

LVH WKY ( $8.38 \pm 1.04$ ) indicating a higher arterial stiffness in LVH WKY group as shown in Figure 3.

#### Histopathology studies of left ventricles of Control WKY and LVH WKY

Histopathology observations are shown in Figure 4. The arrangement of the perimysial fibres in control (A, B) and LVH tissue (C, D) as can be seen with the straight arrows indicating the organized and uniform direction of these fibres while the circle in D slide shows distorted and deranged fibres which are indicative of collagen deposition.

#### Measurement of baseline value of renal cortical blood perfusion

The renal cortical blood perfusion was significantly lower in LVH WKY as compared to control

WKY indicating decreased blood perfusion in LVH WKY group ( $p < 0.05$ ) local vasoconstriction in kidney as shown in Figure 5.

## DISCUSSION

The present study was aimed at undertaking a detailed investigation into the cardiovascular and renal functional changes that develop during the chronic administration of isoprenaline and caffeine (I/C) to induce left ventricular hypertrophy (LVH). Moreover, a somewhat different regime of I/C administration was used like isoprenaline alone and in combination with methylxanthines (20) for 7 days. This I/C model was used for 2 weeks by giving 5 injections 72 h apart as we reported earlier (21) by modifying the original I/C model (4). Present studied modified this model by administering 5 injections of isoprenaline (5 mg/kg 72 h apart s.c.) and caffeine (62 mg/L) to make this model more valid for LVH and cardiac hypertrophy. Although previously reported data successfully described the LVH model but further modification

definitely gave more confidence about reproducibility of data and model can be studied closer to heart failure. This model of LVH may be considered as a transition state between LVH and heart failure. Furthermore, a detailed examination was undertaken of the effect of I/C administration on cardiac geometry, especially the arrangement and breakdown of myofibers. A third area of consideration was the effect of I/C administration on renal and systemic hemodynamics including physiological parameters as reflected by electrolyte homeostasis, renal excretion, renal cortical blood perfusion and arterial elasticity.

Wistar Kyoto rats were selected for this study as SD rats have been reported to develop LVH spontaneously and are not considered to be an ideal strain to investigate LVH (22). A second reason for giving priority to the WKY strain is the high mortality during acute surgical procedures in SD rats which amounted to 80% in those rats which developed LVH (22). The original I/C model was modified by using 5 injections of isoprenaline (s.c.) over 2 weeks

Table 5. Oxidative stress parameters investigated on day 14<sup>th</sup>. \* denotes significant difference  $p < 0.05$  in comparison of control WKY vs. LVH WKY.

Parameters	WKY	CONTROL
SOD ( $\mu\text{mol/mL}$ )	$5.51 \pm 1$	$1.34 \pm 0.1^*$
MDA ( $\text{nmol/mL}$ )	$21 \pm 6$	$34 \pm 2^*$
NO ( $\mu\text{mol/mL}$ )	$5 \pm 1$	$21 \pm 1$
GSH ( $\mu\text{mol/mL}$ )	$542 \pm 43$	$134 \pm 47^*$
T-AOC ( $\mu\text{mol/L}$ )	$18 \pm 3$	$10 \pm 1^*$

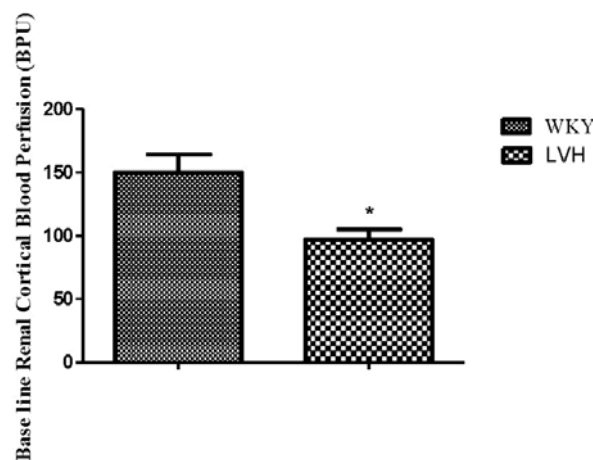


Figure 5. Comparison of renal cortical blood perfusion (RCP) in control and LVH ( $n = 6$ ). \*represents  $p < 0.05$  compared with control group



that is 72 h apart (days 1, 4, 7, 10, 13), which was different from the 4 injections as previously reported (3). The current model was found to be safe not only during the treatment period but also over the long duration protocols of the acute surgery.

To the best of our knowledge, no previous study has exclusively investigated the impact of LVH on systemic hemodynamics in WKY rats using this I/C model. There was an elevation of systolic, diastolic and mean arterial pressure in the LVH compared to the control WKY group, as shown in Table 1, which supported the view that there was an activation of the sympathetic nervous system as a result of  $\beta_1$  receptor stimulation. Our results are consistent with previous findings which suggested that LVH was due to activation of  $\beta$  ARs subtypes in this model (23). In fact, isoprenaline will activate both the  $\beta_1$  receptors of heart and  $\beta_2$  receptors in the peripheral vasculature (24). Caffeine is an adenosine receptor antagonist (25) and studies have shown that its administration will increase plasma levels of noradrenaline (26, 27). Both isoprenaline and caffeine will increase heart rate synergistically, which ultimately results in an elevation of systemic blood pressure (28). Many studies have shown elevated SBP, DBP, PP and MAP to be predictors of CV events in males (29). Pulse pressure was also elevated in the study, which also predicts LVH which is consistent with previous findings in man (30).

Surprisingly, heart rate in the LVH group was lower compared to the control WKY. This observation would be in line with previous findings generating cardiac hypertrophy models, in mice using isoprenaline (15  $\mu\text{g}/\text{g}/\text{day}$ , s.c. for 7 days) (31), in SD rats using isoprenaline and methylxanthine (4) and in male Wistar Kyoto cardiac hypertrophied anesthetized rats by using I/C model (4 injections in 2 weeks) (3). A baroreceptor mediated reflex bradycardia could be the cause of the decreased heart rate in the LVH group. Noradrenaline in isolated tissue stimulates cardiac contractility while *in vivo*, when noradrenaline stimulates the heart, it increases heart rate and ultimately blood pressure. This rise in blood pressure induces a reflex rise in vagal activity by stimulating baroreceptors, resulting in reflex bradycardia, which could be sufficient to counteract the local action of noradrenaline on the heart (32). Down regulation of adrenoceptors under these conditions has been reported in some studies (33-35) but the exact mechanism responsible for the decreased heart rate remains unclear. The cyclic duration was increased in the LVH group consistent with a prognosis which may lead to heart failure.

The physical appearance and weight of key organs like the kidney and heart showed cardiac hypertrophy to have developed. Heart weight, LV weight and their respective indices were significantly greater in the LVH compared to control WKY group as shown in Table 3. An increase in heart and left ventricle weight would suggest an increased exertion of the muscle due to increased activation of  $\beta_1$  receptors. At the same time,  $\beta_2$  receptors would also be activated in the peripheral vasculature due to isoprenaline. This would result in vasodilation, which may be a possible reason for the increase in pulse pressure. It could be argued that this vasodilation would decrease after-load resulting in a greater force of contraction by the ventricular muscle to ensure a maintained or even increased blood pressure. This increased exertion of the heart, could go part way to explaining the increased thickness of the myocardium and reduced internal diameter of the left ventricle of the LVH group as shown in Table 3 and Figure 2.

The kidney index was higher in the LVH group and the accompanying hypokalemia may be considered as a factor contributing to the renal hypertrophy (36). Plasma levels of angiotensin are increased in LVH, which may also play role in renal hypertrophy (37).

This increase in cardiac mass may result from a breakdown in the meshwork of elastin collagen membrane (ECM) in the heart, which results in structural and geometrical changes in the left ventricle as shown in Figure 4. All these changes are caused by unsymmetrical, unorganized arrangements of myofibers and breakdown of elastin and increased collagen deposition. The arrows in Figure 4A showed symmetrical and organized arrangement of myofibers while characteristic striated cardiac muscle and normal cardiac nuclei. Figure 4B showed necrotic tissues, and presence of fibroblast confirms the fibrosis in this model of LVH. These changes are indicative of changes in elastin to collagen ratio in cardiac muscle. This turnover in elastin to collagen ratio needs to be explored by evaluating the changes in activity of different matrix metalloproteinases (MMPs).

A few studies have investigated the myotoxic effects of  $\beta$  adrenergic receptors especially  $\beta_2$  receptors in soleus muscles (38). This myotoxic effect of isoprenaline is likely to be one of the reasons for the weight loss in LVH group as compared to WKY. Moreover, chronic administrations of  $\beta$  adrenergic agonists are noted for causing loss of both body weight and fat (39). Thus, chronic administration of isoprenaline and raised levels of noradrenaline due

to the caffeine together will contribute to the reduced body weight in the LVH group of rats. Another contributory factor for the blunted weight gain in the LVH group may be due to lower water intake and greater urine output in these animals. This may simply reflect energy turnover in the LVH group. These findings are in line with previous findings (3, 4).

Renal function parameters were studied to determine the status of renal functional capacity. It is well known that sympathetic nerves richly innervate the kidneys (40) and influence renal hemodynamic and fluid reabsorption to ensure both long and short term regulation of extracellular fluid volume and arterial blood pressure (41, 42). In LVH,  $\beta_1$  receptor activity is enhanced due to isoprenaline and caffeine which may impact on renal function. Creatinine clearance, which is a measure of glomerular filtration rate (GFR), was not significantly different in either the LVH or control groups indicating a satisfactory filtration capacity of the kidney. Furthermore, the elevated levels of FENa and FEK indicate decreased reabsorption of these electrolytes from proximal and distal tubules suggesting an early derangement of tubular reabsorption and excretion. One possible reason may be due to the involvement of  $\alpha$  adrenergic receptors in the kidneys which play a major role in tubular Na and water reabsorption (43). Secondly, hypernatremia is associated with caffeine administration (44). These findings of elevated fractional excretions of sodium and potassium are consistent with changes produced by caffeine in the body (45). Hypokalemia, as reported in the present study, usually results from damaged proximal tubules as a result of increased ammoniogenesis (36). Urine flow rate was higher in the LVH group as compared to the control WKY group. This increase in urine flow rate and increased urine output in the LVH group may be due to a diuretic action of caffeine, which has been widely reported (28, 45-48).

The hypokalemia observed in the LVH group was associated with the development of LVH and has been shown to be involved with stimulating myocardial growth (48). The decreased  $U_{Na}/U_K$  ratio in the LVH group would be consistent with an elevated aldosterone secretion, which would act on the collecting tubules to cause hyper-excretion of potassium. Renal cortical blood perfusion (RCBP) was decreased about 30% in the LVH which would indicate a compromised blood perfusion through kidney. Despite a normal creatinine clearance in both control WKY and LVH WKY, the RCBP was decreased in the LVH WKY group but the underlying

mechanism remains unclear. One of the possibility is due to increased regional concentration of catecholamines, especially noradrenaline, causing increased vasoconstriction leading to reduced RCBP. Increased LV mass is proportional to increased sympathetic nerve activity (49). This increased sympathetic nerve activity may cause renal sympathetic nerve to release more norepinephrine in local region thus causing increased vasoconstriction and reduced RCBP. That is fact because free radicals which are elevated in this model are involved in sympathetic neurotransmission to enhance noradrenaline production by increasing the calcium influx into nerve terminals (50).

Arterial stiffness, along with SBP, is marker of CV diseases which will elevate after load and can impact on coronary perfusion (51, 52). Arterial stiffness, as reflected by the pulse wave velocity, is considered to be an indicator of endothelial dysfunction (53). Arterial stiffness is referred to many pathological condition including altered anticoagulant and anti-inflammatory properties of endothelium, impaired modulation of vascular growth and dysregulation of vascular remodelling (54). An ample data considered the arterial stiffness as loss of vasorelaxation ability of blood vessels due to loss of NO. In present model of LVH, plasma levels of LVH WKY is reduced that may lead to arterial dysfunction. This reduced levels may be due to decreased expression of endothelial nitric oxide synthase (eNOS) (55), lack of substrate of eNOS, (56) or accelerated degradation of NO by reactive oxygen species (ROS) (57). In the present study, pulse wave velocity was significantly higher (33%) in the LVH group indicating a higher arterial stiffness as compared to the control WKY. Interestingly, vasoconstriction and elevated blood pressure is usually associated with greater arterial stiffness, which is a predictor of cardiovascular diseases (51). Although isoprenaline produces a peripheral vasodilation, this action may be counteracted by an increased noradrenaline production induced by caffeine administration. These elevated levels of noradrenaline and caffeine could cause an overall vasoconstriction and make the vasculature stiffer. Thus, the overall net effect is an increased left ventricular after-load and greater arterial stiffness ultimately leading to endothelial dysfunction.

Oxidative stress play an imperative role in cardiac and vascular abnormalities in different types of cardiovascular diseases that is why any antioxidant therapy may be beneficial for combating against these diseases (58). So, elevated level of MDA in plasma, which is enzymatic marker of oxidative

stress while attenuated level of SOD which is enzymatic marker of antioxidant mechanism, indicate of oxidative stress in LVH. This shows imbalance between prooxidants and antioxidant in LVH. Other antioxidant parameters like GSH and T-AOC is also attenuated indicating dominant role of prooxidant mechanism in LVH. The levels of NO in plasma is also reduced in LVH WKY group due to involvement of ROS (57).

There are relatively few good invasive models of LVH which produce consistent results with low mortality. One approach in the rat has been to ligate the coronary artery (59) but this has high mortality even in the hands of experienced investigators. An alternative is banding of the descending abdominal aorta (60), which results in an increase after-load but requires extensive surgery. More recently, a non-invasive vascular targeted approach using external lasers has been developed, which may have great potential (61, 62) but is dependent on extensive support in terms of staff and equipment. This has resulted in the drive to develop non-invasive models using pharmacological approaches and has included the use of compounds like caffeine and isoprenaline alone and in combination (4). There are relatively few limitations with the non-invasive models and a consistent level of cardiac dysfunction is achieved. The present model was developed and modified to achieve both objectives. This model has also shown reduced reabsorption capacity and increased excretion ability of kidney. Moreover, oxidative stress and arterial stiffness is increased while renal cortical blood perfusion is decreased in this model of LVH.

#### Acknowledgments

The Institute of Postgraduate Studies (IPS) is acknowledged for providing USM fellowship (Teaching) to Ashfaq Ahmad (No. PF-D 0067/11 (R)) and Universiti Sains Malaysia and Ministry of Science, Technology & Innovation (MOSTI) Malaysia for providing grant no. 203/PFAR-MASI/6711452 to Dr. Hassaan A. Rathore for this work.

#### REFERENCES

1. Lorell B.H., Carabello B.A.: *Circulation* 102, 470 (2000).
2. Frey N., Katus H.A., Olson E.N., Hill J.A.: *Circulation* 109, 1580 (2004).
3. Flanagan E.T., Buckley M.M., Aherne C.M., Lainis F., Sattar M. et al.: *Exp. Physiol.* 93, 1058 (2008).
4. Heap S.J., Hudlicka O., Okyayuz-Baklouti I.: *Drug. Dev. Res.* 37, 249 (1996).
5. Krenek P., Kmecova J., Kucerova D., Bajuszova Z., Musil P. et al.: *Eur J. Heart Failure* 11, 140 (2009).
6. John R., Teerlink J.M.P., Pfeffer M.A.: *Circ. Res.* 105 (1994).
7. Abbas S., Munavvar A., Abdullah N., Johns E.: *Can. J. Pure Appl. Sci.* 1, 21 (2007).
8. Buckley M.M., Johns E.J.: *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R1549 (2011).
9. Nagano M., Higaki J., Nakamura F., Higashimori K., Nagano N. et al.: *Hypertension* 19, 708 (1992).
10. Nehlig A., Daval J.-L., Debry G.: *Brain Res. Rev.* 17, 139 (1992).
11. Ferdinandy P., Dhanlaxmi H., Ambrus I., Rothery R.A., Schulz R.: *Circ. Res.* 87, 241 (2000).
12. Frangogiannis N.G., Smith C.W., Entman M.L.: *Cardiovasc. Res.* 53, 31 (2002).
13. McLerie M., Lopatin A.N.: *J. Mol. Cell. Cardiol.* 35, 367 (2003).
14. Lopez-Santiago L.F., Meadows L.S., Ernst S.J., Chen C., Malhotra J.D. et al.: *J. Mol. Cell. Cardiol.* 43, 636 (2007).
15. Abdulla M.H., Sattar M.A., Khan M.A.H., Abdullah N.A., Johns E.J.: *Acta Physiol.* 195, 397 (2009).
16. Swarup K.R.A., Sattar M.A., Abdullah N.A., Abdulla M.H., Salman I.M. et al.: *Pharmacog. Res.* 2, 31 (2010).
17. Mitchell G.F., Pfeffer M.A., Finn P.V., Pfeffer J.M.: *J. Appl. Physiol.* 82, 203 (1997).
18. Gwathmey J.K., Kim C.S., Hajjar R.J., Khan F., DiSalvo T.G. et al.: *Am. J. Physiol.* 276, H1678 (1999).
19. Seeling H.P., Wust H.: *Arztl. Lab.* 15, 34 (1969).
20. Heap S., Hudlicka O., Okyayuz-Baklouti I.: *Drug. Devel. Res.* 37, 249 (1996).
21. Ahmad A., Sattar M.A., Rathore H.A., Abdulla M.H., Khan S.A. et al.: *Can. J. Physiol. Pharmacol.* 92, 1029 (2014).
22. McAdams R.M., McPherson R.J., Dabestani N.M., Gleason C.A., Juul S.E.: *Comp. Med.* 60, 357 (2010).
23. Zhao M., Fajardo G., Urashima T., Spin J.M., Poorfarahani S. et al.: *Am. J. Physiol. Heart Circ. Physiol.* 301, H1461 (2011).
24. Daly M., Farmer J., Levy G.: *Br. J. Pharmacol.* 43, 624 (1971).
25. Brown N.J., Ryder D., Nadeau J.: *Hypertension* 22, 847(1993).

26. Collomp K., Ahmaidi S., Audran M., Chanal J.-L., Prefaut C.: *Int. J. Sports Med.* 12, 439 (1991).
27. Bell D.G., Jacobs I., Ellerington K.: *Med. Sci. Sports Exerc.* 33, 1399 (2001).
28. Robertson D., Frölich J.C., Carr R.K., Watson J.T., Hollifield J.W. et al.: *New Eng. J. Med.* 298, 181 (1978).
29. Sesso H.D., Stampfer M.J., Rosner B., Hennekens C.H., Gaziano J.M. et al.: *Hypertension* 36, 801 (2000).
30. Mitchell G.F., Moyé L.A., Braunwald E., Rouleau J.-L., Bernstein V. et al: *Circulation* 96, 4254 (1997).
31. Gava A.L., Peotta V.A., Cabral A.M., Meyrelles S.S., Vasquez E.C.: *Auton. Neurosci.* 114, 47 (2004).
32. Mycek M.J., Harvey R.A., Champe P.C., Howland R.D.: *Lippincott Illustrated Reviews: Pharmacology*. 3rd edn., p. 74, Lippincott Williams & Wilkins, Baltimore, MD 2006.
33. Hadcock J.R., Malbon C.C.: *Proc. Natl. Acad. Sci. USA* 85, 5021 (1988).
34. Sun C.-L., Hanig J.: *Pharmacology* 27, 319 (1983).
35. Hogikyan R.V., Supiano M.A: *Am. J. Physiol.* 266, E717 (1994).
36. Muehrcke R.C., Rosen S.: *Lab. Invest.* 13, 1359 (1964).
37. Wolf G., Ziyadeh F.N.: *Semin. Nephrol.* 17, 448 (1997).
38. Burniston J.G., Ng Y., Clark W.A., Colyer J., Tan L.-B., Goldspink D.F.: *J. Appl. Physiol.* 93, 1824 (2002).
39. Rothwell N., Stock M., Stribling D.: *Pharmacol. Ther.* 17, 251 (1982).
40. DiBona G.F., Sawin L.L.: *Am. J. Physiol.* 287, F1171 (2004).
41. DiBona G.F., Kopp U.C.: *Physiol. Rev.* 77, 75 (1997).
42. Salomonsson M., Brännström K., Arendshorst W.J.: *Am. J. Physiol.* 278, F138 (2000).
43. Sattar M.A., Johns E.J.: *Eur. J. Pharmacol.* 294, 727 (1995).
44. Shirley D., Walter S., Noormohamed F.: *Clin. Sci.* 103, 461 (2002).
45. Passmore A., Kondowe G., Johnston G: *Clin. Sci.* 72, 749 (1987).
46. Eddy N.B., Downs A.W.: *J. Pharmacol. Exp. Ther.* 33, 167 (1928).
47. Nussberger J., Mooser V., Maridor G., Juillerat L., Waeber B., Brunner H.: *J. Cardiovasc. Pharmacol.* 15, 685 (1990).
48. Shapiro J.I.: *Hypertension* 46, 477 (2005).
49. Burns J., Sivananthan M.U., Ball S.G., Mackintosh A.F., Mary D.A., Greenwood J.P.: *Circulation* 115, 1999 (2007).
50. Jou S.-B., Cheng J.-T.: *J. Auton. Nerv. Syst.* 66, 126 (1997).
51. Laurent S., Boutouyrie P., Asmar R., Gautier I., Laloux B. et al.: *Hypertension* 37, 1236 (2001).
52. Safar M.E.: *J. Hypertens.* 7, 769 (1989).
53. Wang Y-X., Halks-Miller M., Vergona R., Sullivan ME., Fitch R. et al: *Am. J. Physiol.* 278, H428 (2000).
54. Gimbrone M.A. Jr.: *Am. J. Cardiol.* 75, 67B (1995).
55. Wilcox J.N., Subramanian R.R., Sundell C.L., Tracey W.R., Pollock J.S. et al.: *Arterioscler. Thromb. Vasc. Biol.* 17, 2479 (1997).
56. Pou S., Pou W., Brecht D., Snyder S., Rosen G.: *J. Biol. Chem.* 267, 24173 (1992).
57. Harrison D.: *Clin. Cardiol.* 20, 11 (1997).
58. Dhalla N.S., Temsah R.M., Netticadan T.: *J. Hypertens.* 18, 655 (2000).
59. Hill J.A., Karimi M., Kutschke W., Davisson R.L., Zimmerman K. et al: *Circulation* 101, 2863 (2000).
60. Doering C.W., Jalil J.E., Janicki J.S., Pick R., Aghili S. et al.: *Cardiovasc. Res.* 22, 686 (1988).
61. Chrastina A., Schnitzer J.E.: *Exp. Lung Res.* 38, 1 (2012).
62. Chrastina A., Pokreisz P., Schnitzer J.E.: *Am. J. Physiol.* 306, H270 (2014).

*Received: 11. 03. 2015*