

NIHAL BAKIRKALAY AYDIN^{1, A-C, F}, TURGUT TEKE^{1, A, D-F}, HATICE TOY^{2, B, C, F}, KURSAT UZUN^{1, A, C-F}

The Effect of Theophylline on the Prevention of Mechanical Ventilation-Induced Diaphragm Atrophy in Rats

¹ Department of Chest Diseases, Necmettin Erbakan University, Meram Medical Faculty, Konya, Turkey

² Department of Pathology, Necmettin Erbakan University, Meram Medical Faculty, Konya, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. Movement disorders and atrophy occur in the diaphragm, the most important muscle of respiration, because of mechanical ventilation (MV).

Objectives. In this animal model, we aimed to evaluate the effect of intravenous theophylline administration on the prevention of mechanical ventilation-induced diaphragmatic atrophy.

Material and Methods. In our study, 30 healthy male Sprague-Dawley rats were used. They were divided into 3 equal groups. Group 1: the control group (no MV); group 2: the placebo group that received MV; Group 3: the theophylline group composed of rats that received both MV and theophylline therapy. In all 3 groups, the diaphragmatic atrophy was evaluated histopathologically.

Results. In the histopathological examination, no macroscopic thickening and microscopic atrophy were observed in the diaphragm in the control group. In the placebo group (group 2), macroscopically definite thickening was observed in all rats, and microscopically, heavy (+++) atrophy was observed. In the theophylline group (group 3), there was no atrophy in one rat. In 8 rats, light (+), and in 1 rat medium (++) atrophy was observed.

Conclusions. In our study, it was shown that atrophy occurred in the diaphragms of rats after MV, and the atrophy was decreased after theophylline administration (*Adv Clin Exp Med* 2014, 23, 1, 33–38).

Key words: mechanical ventilation, diaphragmatic atrophy, theophylline, rat.

Mechanical ventilation (MV) is a lifesaving method that is used to sustain the blood gas balance of patients who are unable to maintain alveolar ventilation. However, 25% of the patients are in need of prolonged ventilation support and this causes a rise in infection rates, length of stay in the intensive care unit, morbidity and mortality [1]. Prolonged ventilation causes a decrease in the contraction strength of diaphragmatic muscles and leads to atrophy. In most of the studies, it is stated clearly that MV causes diaphragmatic atrophy and loss of isometric strength production, diaphragmatic mass and the protein content of diaphragmatic muscle [2–4].

Therefore, in order to maintain the mass and function of respiratory muscles during ventilation, strategies have been developed. These are: MV modes that help the diaphragm contribute to

respiration in an active way; various exercise methods, prevention of catabolic processes and pharmacotherapy [5]. Studies on both humans and animals have shown that the application of prophylactic theophylline in pharmacotherapy practices prevents diaphragmatic weakness and reverses diaphragmatic fatigue [6–17]. However, in most of these studies, the effect of theophylline on the contraction strength of diaphragmatic muscles has been evaluated, but the effect on diaphragmatic atrophy was never mentioned. According to the records the authors have, there is no histopathological study on the effects of diaphragmatic atrophy development due to mechanical ventilation. In this study, the authors aimed to study the effects of theophylline therapy on diaphragmatic atrophy, which improves depending on mechanical ventilation, in animal models.

Material and Methods

Animals

The rat was chosen as the experimental animal because of its being favored for biomedical studies, its small dimensions and its maintainability. For this purpose, four-month-old male Sprague-Dawley 30 rats with an average weight of 315–375 g were housed at the Selcuk University Experimental Medicine Research and Practices facility and fed rat pellet feed and water ad libitum and maintained on a 12 : 12 h light-dark cycle for 3 weeks before initiation of this study. This study was approved by the University of Selcuk Experimental Animals Ethics Committee.

Experimental Design

The rats were randomly assigned to one of 3 experimental groups. Group 1 (control group): Intraperitoneal anesthesia was applied as acutely. Group 2 (MV group): After reaching a surgical plane of intraperitoneal anesthesia, the animals were tracheostomized and mechanically ventilated and monitored for 24 h. Group 3 (MV + Theophylline Group): After reaching a surgical plane of intraperitoneal anesthesia, animals were tracheostomized and mechanically ventilated and monitored for 24 h and theophylline infusion was given (15 mg/kg as a loading dose; 0.05 mg/kg/h infusion as maintenance).

Protocol for Control Animals

The animals in the control group were not mechanically ventilated or exposed to long-term anesthesia before study. These animals were weighed and then sodium pentobarbital was given intraperitoneally (50 mg/kg). After a surgical plane of anesthesia was achieved, the diaphragm was rapidly removed, and its weight was measured on a precision scale. The largest dimensions of the diaphragm and membranous region were measured and segments from the costal region were fixed in a buffered 10% formalin solution for histopathological evaluation.

Mechanical Ventilation Protocol

All surgical procedures were performed using aseptic techniques. After weighing and reaching a surgical plane of anesthesia (sodium pentobarbital, 50 mg/kg, intraperitoneally), the animals were tracheostomized and mechanically ventilated with a volume-controlled small animal ventilator (SAR-830, IITC Life Science, USA). The tidal volume was 1 mL/100 g of body weight, with a respiratory rate of 80 breaths/min. This respiratory rate was selected to

mimic the breathing frequency of adult rats at rest. Additionally, positive end-expiratory pressure (PEEP) of 1 cm H₂O was used throughout the protocol.

An arterial catheter was placed in the carotid artery to permit the continuous measurement of blood pressure. A venous catheter was inserted into the femoral vein epigastric branch for continuous infusion of isotonic saline. A surgical plane of anesthesia was maintained with sodium pentobarbital infusion (10 mg/kg/h) over the entire period of MV. The level of anesthesia was monitored with methods such as heart rate, blood pressure and corneal-lid reflexes. Body temperature was maintained at 37°C using a heating blanket. In addition to this, the heart rate and electrical activity of the heart were monitored placing subcutaneous electrodes through an EKG. The fluid balance of the body was maintained with the continuous application of 2 mL/kg/h IV isotonic saline. Continuous care during the MV protocol included expressing the bladder, removing airway mucus, moistening the eyes, passive movements of the limbs and rotating the animal. This care was maintained throughout the experimental period at hourly intervals.

On completion of MV, the body weight of the animals was reweighed and the diaphragm was removed under abdominal surgery. The existence of macroscopic abnormalities (color change, thickening, and hemorrhage) was checked and segments of the costal diaphragm were fixed in a buffered 10% formalin solution for histopathological evaluation.

Histopathological Evaluation

Costal diaphragm samples 1 cm in length and 0.5 cm in width were taken. These samples were stained with hematoxylin eosin and were examined with a light microscope.

Histopathological evaluations were done by a pathologist who had no previous knowledge of the protocol and procedures of this study. According to these histopathological evaluations, the atrophy of the diaphragm muscle, neutrophil infiltration, fibrosis and steatosis were analyzed. Diaphragmatic atrophy was scored that 0–33% was light (+), 34–66% was medium (++) and more than 67% was heavy (+++).

Statistical Analysis

The data was expressed as mean \pm SD and analyzed using SPSS 11.0 for Windows. A Kruskal-Wallis variance analysis test was used among the

groups and a Mann-Whitney *U* test was used for binary comparisons if a significant difference was observed among the groups. A value of $p < 0.05$ was considered significant.

Results

None of the animals were eliminated due to infection. There was no significant difference between initial and final the body weight of the animals between the experimental groups ($p > 0.05$). These results confirm that our program of nutrition and hydration was adequate. To determine whether our MV protocol was successful at sustaining homeostasis, we monitored blood pressure and heart rate during the MV period. It was maintained within the physiological range.

The measurements done on the diaphragmatic tissue of the control, placebo (MV only) and theophylline groups are summarized in Table 1. Compared with the control, the application of MV resulted in a statistically significant

decrease in diaphragm weight, large diameter of the diaphragm (Fig. 1A), large diameter of the membranous portion (Fig. 1B) and the ratio of diaphragm weight to body weight (respectively; $p < 0.01$, $p < 0.01$, $p < 0.01$ and $p = 0.05$). Theophylline administration restored these values to the control values. Compared with the placebo, the administration of theophylline resulted in a statistically significant increase in diaphragm weight ($p < 0.05$), large diameter of the diaphragm ($p < 0.05$), large diameter of the membranous portion ($p < 0.01$) and the ratio of diaphragm weight to body weight ($p = 0.05$).

The histopathological evaluation results are shown in Table 2. As a result of this histopathological evaluation, it is seen that macroscopic thickening and microscopic atrophy were observed in the diaphragm of rats in the control group and all are normal (Fig. 2A). Macroscopically evident thickening and heavy (+++) atrophy microscopically were observed in the diaphragm of the rats in the MV applied placebo group (Group 2). An increase in the distance between the muscle fibers was detected and

Table 1. Measurements of diaphragm tissue in control, placebo and theophylline groups

	Control (mean \pm SD)	MV (mean \pm SD)	MV plus Theophylline (mean \pm SD)
Weight	122.20 \pm 1.92	116.50 \pm 4.71 ^a	119.90 \pm 3.03 ^c
Large diameter	4.90 \pm 0.15	4.44 \pm 0.21 ^a	4.73 \pm 0.14 ^c
Large diameter of the membranous portion	2.42 \pm 0.08	2.19 \pm 0.11 ^a	2.36 \pm 0.08 ^d
DW/BW	0.34 \pm 0.00	0.33 \pm 0.01 ^b	0.34 \pm 0.01 ^e

MV – Mechanical ventilation, DW/BW – ratio of diaphragmatic weight to body weight, ^a $p < 0.01$ – compared with the control group, ^b $p = 0.05$ – compared with the control group, ^c $p < 0.05$ – compared with the MV group, ^d $p < 0.01$ – compared with the MV group, ^e $p = 0.05$ – compared with the MV group.

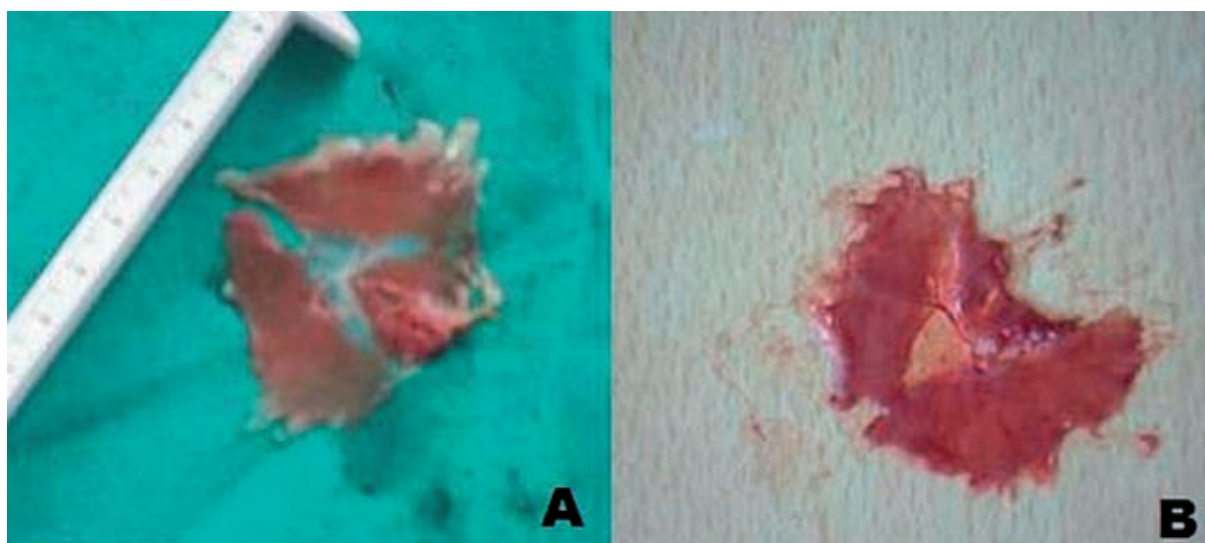


Fig. 1. The diaphragm (A) and the membranous portion (B) larger in diameter

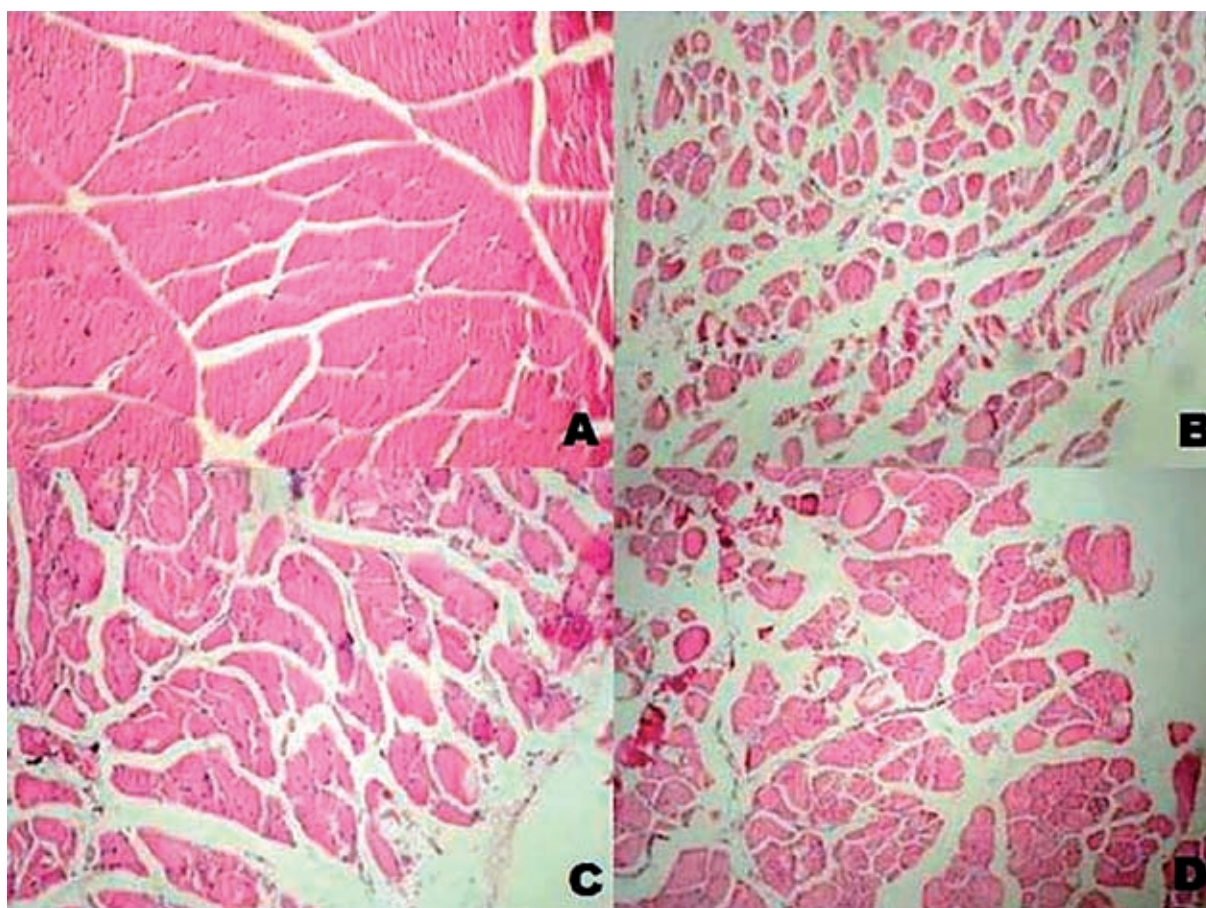


Fig. 2. Histopathological evaluation (HE, 10 × 10), A; the normal appearance of the diaphragm in the control group, B; diaphragm +++ atrophy in group 2, C; + atrophy of diaphragm in group 3, D; ++ atrophy of the diaphragm in group 3

Table 2. Histopathological evaluation results

	MV (n)				MV plus Teophylline (n)			
	0	+	++	+++	0	+	++	+++
Atrophy	-	-	-	10	1	8	1	-
Neutrophil infiltration	-	-	-	-	-	-	-	-
Fibrosis	-	-	-	-	-	-	-	-
Steatosis	8	2	-	-	9	1	-	-

MV – mechanical ventilation.

muscle fiber diameters were variable (Fig. 2B). In the theophylline group, no atrophy was observed and in only one animal it was completely normal. Eight animals had light (+) atrophy, the distance between muscle fiber increased minimally and the diameters were similar to each other (Fig. 2C). One of the animals in the theophylline group had medium (++) atrophy and the distance between the muscle fiber increased moderately (Fig. 2D). Histopathologically, no neutrophil infiltration and fibrosis was observed in the diaphragms of both the placebo and theophylline groups, and there was a similarity in both in terms of fat.

Discussion

As far as we know, this study is the first histopathological experiment on the effects of intravenous theophylline administration on MV-induced diaphragmatic atrophy. As a result of this study, it is seen that MV causes atrophy in the diaphragm muscle of rats in as little as 24 h and theophylline therapy inhibits the formation of atrophy and the reduction in diaphragm mass.

In the literature, there are many studies investigating the dysfunction of respiratory muscles after MV. Le Bourdelles et al. [3] studied the

effects of 48 h MV on both atrophy and contraction strength of rat diaphragms. As a result, a significant decrease in isometric strength production, diaphragm mass and protein content were reported. Shanely et al. [4] showed clearly that short term, controlled MV causes diaphragmatic atrophy rapidly. 18 h MV causes a 7% decrease in costal diaphragm mass and total protein destruction accelerates after MV [4]. Powers et al. [2] showed in a similar study that MV causes a significant decrease in diaphragm specific strength of the rat. Powers et al. [2] presented that diaphragm dysfunction develops fast at the beginning (12 h) and this dysfunction proceeds during the first 24 h of MV in the ventilator. Therefore, the controlled MV period is identified as 24 h in our experimental study.

Theophylline has been used for many years in the treatment of chronic obstructive pulmonary disease. Many studies have shown that theophylline increases the contraction strength of the diaphragm, decreases the weakness and has a protective effect against fatigue [6, 8, 14–18]. *In vitro* studies have shown that theophylline strengthens muscle contraction, revealed by direct and indirect electrical stimuli in isolated preparations [9]. The same effect was seen in the diaphragm preparations including isolated phrenic nerve semi-incision in rats [10]. Studies on humans show that aminophylline regulates the diaphragm contraction function of tired people significantly [11]. Aubier et al. [13] demonstrated in their study on animals that theophylline increases diaphragm contraction strength and respiratory minute ventilation. Apart from the studies indicating that theophylline increases the production of strength and decreases the weakness, there are some studies claiming that theophylline has little or no effect on diaphragm contraction strength and weakness in the diaphragm [19, 20].

There are several mechanisms mentioned in the literature about the regulatory effect of theophylline on diaphragmatic function. The first of these is the increase of diaphragmatic perfusion by theophylline. Theophylline leads to this by increasing

cardiac output and by providing vasodilation in diaphragmatic arterioles [21]. The second mechanism [22] is the adjustment of diaphragm muscle contraction even during the fatigue period when a shortening is seen in muscle fibers [7, 11, 23]. The third mechanism is by increasing the sensitivity of the respiratory center to the diaphragm motor neuron [24], and the last one is the inotropic effect on diaphragm muscle fibers [7, 11, 23, 25]. However, none of these mechanisms are clear and this subject is still controversial. Danialou et al. [22] showed that theophylline causes a significant vasodilatation on diaphragm arterioles. On the contrary, the study by Mayoock et al. [26] on animals reported that there was no change in diaphragmatic blood flow after aminophylline infusion. However, methylxantine-derived drugs are reported to weaken the intensity of diaphragmatic weakness or can delay the start. In fact, it is known that theophylline has vasodilator effects [27]. Theophylline might increase the blood flow of the diaphragm as a result of systematic vasodilatation and might improve diaphragmatic strength. One of the diaphragm function-adjustment mechanisms may be the prevention of diaphragmatic atrophy which we revealed histopathologically for the first time in our study on animals.

We chose the rat model for several reasons for this experimental study. First, adult rats are of sufficient size for surgical procedures. Second, and most importantly, human and rat diaphragms are similar in anatomical features, function and muscle fiber-type composition [28, 29].

In conclusion, although studies on both animals and humans indicate that theophylline clearly increases diaphragm contraction strength, the mechanism of this increase is not clearly explained. In our study, the preventive effect of theophylline was observed when compared to the placebo in rats with MV-induced diaphragm atrophy. The results obtained lead us to think that an increase in diaphragm contraction strength by using theophylline can be achieved, preventing muscle atrophy. However, a great number of animal and human studies are needed on the molecular level.

References

- [1] Esteban A, Frutos F, Tobin MJ, Alia I, Solsona JF, Valverdu I: A comparison of four methods of weaning patients from mechanical ventilation. *N Engl J Med* 1995, 332, 345–350.
- [2] Powers SK, Shanely RA, Coombes JS, Koesterer TJ, McKenzie M, Van Gammeren D: Mechanical ventilation results in progressive contractile dysfunction in the diaphragm. *J Appl Physiol* 2002, 92, 1851–1858.
- [3] Le Bourdelles G, Viires N, Boczkowski J, Seta N, Pavlovic D, Aubier M: Effects of mechanical ventilation diaphragmatic contractile properties in rats. *Am J Respir Crit Care Med* 1994, 149, 1539–1544.
- [4] Shanely RA, Zergeroglu MA, Lennon SL, Sugiura T, Yimlamai T, Enns D: Mechanical ventilation-induced diaphragmatic atrophy is associated with oxidative injury and increased proteolytic activity. *Am J Respir Crit Care Med* 2002, 166, 1369–1374.
- [5] Gayan-Ramirez G, Decramer M: Effects of mechanical ventilation on diaphragm function and biology. *Eur Respir J* 2002, 20, 1579–1586.

- [6] **Aubier M, De Troyer A, Sampson M, Macklem PT, Roussos C:** Aminophylline improves diaphragmatic contractility. *N Engl J Med* 1981, 305, 249–252.
- [7] **Murciano D, Aubier M, Lecocguic V, Pariente R:** Effects of theophylline on diaphragmatic strength and fatigue in patients with chronic obstructive lung disease. *N Engl J Med* 1984, 311, 349–353.
- [8] **Sigrist S, Thomas D, Howell S, Roussos CH:** The effect of aminophylline on inspiratory muscle contractility. *Am Rev Respir Dis* 1982, 126, 46–50.
- [9] **Jones DA, Howell S, Roussos C, Edwards RHT:** Low-frequency fatigue in isolated skeletal muscles and the effects of methylxanthines. *Clin Sci* 1982, 63, 161–167.
- [10] **Kentera D, Varagic VM:** The effects of cyclic N-2-0-dibutyryl-adenosine 3', 5'-monophosphate, adrenaline and aminophylline on the isometric contractility of the isolated hemidiaphragm of the rat. *Br J Pharmacol* 1975, 54, 375–381.
- [11] **Wanke T, Merkle M, Zifko U, Formanek D, Lahrmann H, Grisold W:** The effect of aminophylline on the force-length characteristics of the diaphragm. *Am J Respir Crit Care Med* 1994, 149, 1545–1549.
- [12] **Aubier M:** Effect of theophylline on diaphragmatic and other skeletal muscle function. *J Allergy Clin Immunol* 1986, 78, 787–792.
- [13] **Aubier M, Murciano D, Viires N, Lecocguic Y, Palacios S, Pariente R:** Increased ventilation due to improved diaphragmatic efficiency during aminophylline infusion. *Am Rev Respir Dis* 1983, 127, 148–154.
- [14] **Supinski GS, Deal ED, Kelsen SG:** Comparative effects of theophylline and adenosine on respiratory skeletal and smooth muscle. *Am Rev Respir Dis* 1986, 133, 809–813.
- [15] **Viires N, Aubier M, Murciano D, Marty C, Pariente R:** Effects of theophylline on isolated diaphragmatic fibers: a model for pharmacologic studies on diaphragmatic contractility. *Am Rev Respir Dis* 1986, 133, 1060–1064.
- [16] **Kolbeck RC, Speir WA:** Diltiazem, verapamil and nifedipine inhibit theophylline-enhanced diaphragmatic contractility. *Am Rev Respir Dis* 1989, 139, 139–145.
- [17] **Kuei JH, Sieck GC:** Chronic aminophylline administration: effect of diaphragm contractility and fatigue resistance in vitro. *Am Rev Respir Dis* 1991, 144, 121–125.
- [18] **Sassoon CS, Zhu E, Caiozzo VJ:** Assist-control mechanical ventilation attenuates ventilator-induced diaphragmatic dysfunction. *Am J Respir Crit Care Med* 2004, 170, 626–632.
- [19] **Derom E, Janssens S, De Bock V, Decramer M:** Theophylline minimally alters contractile properties of canine diaphragm in vitro. *J Appl Physiol* 1990, 69, 1390–1396.
- [20] **Janssens S, Derom E, Reid MB, Tjandramaga TB, Decramer M:** Effects of theophylline on canine diaphragmatic contractility and fatigue. *Am Rev Respir Dis* 1991, 144, 1250–1255.
- [21] **Dow-Edwards D, DeCrescito V, Tomasula JJ:** Effect of aminophylline and isoproterenol on spinal cord blood flow after impact injury. *J Neurosurg* 1980, 53, 385–390.
- [22] **Danielou G, Vicaut E, Aubier M, Boczkowski J:** Theophylline dilates rat diaphragm arterioles via the prostaglandins pathway. *Br J Pharmacol* 1998, 124, 1355–1362.
- [23] **Sherman MS, Lang DM, Matityahu A, Campbell D:** Theophylline improves measurements of respiratory muscle efficiency. *Chest* 1996, 110, 1437–1442.
- [24] **Bhatia J:** Current options in the management of apnea of prematurity. *Clin Pediatr* 2000, 39, 327–336.
- [25] **Eldridge FL, Millhorn DE, Waldrop TG, Kiley JP:** Mechanism of respiratory effects of methylxanthines. *Respir Physiol* 1983, 53, 239–261.
- [26] **Mayock DE, Standaert TA, Woodrum DE:** Effect of methylxanthines on diaphragmatic fatigue in the piglet. *Pediatr Res* 1992, 32, 580–584.
- [27] **Rall TW:** Drugs used in the treatment of asthma. The methylxanthines, cromolyn sodium and other agents. In: *The Pharmacological Basis of Therapeutics*. Eds.: Goodman LS, Gilman A, MacMillan Co., New York 1990, 618–630.
- [28] **Mizuno M:** Human respiratory muscles: fibre morphology and capillary supply. *Eur Respir J* 1991, 4, 587–601.
- [29] **Powers S, Demirel H, Coombes J, Fletcher L, Calliaud C, Vrabas I, Prezant D:** Myosin phenotype and bioenergetic characteristics of rat respiratory muscle. *Med Sci Sports Exerc* 1997, 29, 1573–1579.

Address for correspondence:

Turgut Teke
Necmettin Erbakan Universitesi Meram Tip Fakultesi Hastanesi
Gogus Hastaliklari Anabilim Dalı
42080 Meram-Konya
Turkey
Tel.: +90 332 2236218
E-mail: tteke@konya.edu.tr

Conflict of interest: None declared

Received: 10.08.2012
Revised: 15.05.2013
Accepted: 20.02.2014