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Respiratory Muscles: Structure, Function and Relationship with the ACE Gene. A Brief Morphofunctional Communication

Músculos Respiratorios: Estructura, Función y Relación con el Gen de la ECA. Una Breve Comunicación Morfofuncional

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SUMMARY: Pulmonary ventilation is a mechanical process in which the respiratory muscles act in coordination to maintain the oxygenation of the organism. Any alteration in the performance of these muscles may reduce the effectiveness of the process. The respiratory muscles differ from the other skeletal muscles in the vital support that they provide through rhythmiccontractions. The structure and energy system of the muscles are specially adapted to perform this function. The composition of the respiratory muscles is exceptional; they are small, and present an abundant capillary network, endowing them with a high aerobic level and resistance to fatigue. Coordinated regulation of the local renin-angiotensin system provides proper blood flow and energy supply in the myofibrils of the skeletal muscle tissue. Specifically, this performance will depend to a large extent on blood flow and glucose consumption, regulated by the renin-angiotensin system. The angiotensin converting enzyme is responsible for degrading kinins, which finally regulate muscle bioenergy and glucose between the blood vessel and the skeletal muscle. The objective of this review is to describe the structure of the respiratory muscles and their association with the angiotensin converting enzyme gene.

KEY WORDS: Respiratory muscles; Embryology; Histology; Anatomy; Energy support; Renin-angiotensin system; Angiotensin converting enzyme.

INTRODUCTION

Lung ventilation is a mechanical process dependent on the respiratory muscles, both inspiratory and expiratory, which direct the processes of thorax expansion and retraction (Ratnovsky *et al*., 2008). Their structure helps to support their action in these different functions (Polla *et al*., 2004). They differ in the type, size and vascularisation of their muscle fibres (Luce & Culver, 1982), which can vary considerably between one subject and another depending on environmental, genetic and other factors (Ostrander *et al*., 2009).

Among the genetic factors, the predominance of specific polymorphisms will influence enzyme activity and energy production at muscle level, which may vary between different muscles, and even between the same muscle in different subjects (Caló & Vona, 2008). A clear example of this is the variability in the angiotensin converting enzyme (ACE) gene, found in striated muscle; this enzyme is important in regulation of the blood volume, arterial pressure and vascular function (Colakoglu *et al*., 2008), all factors which can affect and differentiate muscle performance

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between individuals. In view of the above, the object of the present review was to describe the morphology of the respiratory muscles and their association with the ACE gene.

HISTORICAL DEVELOPMENT

Respiration is a fundamental biological process in which exchange occurs between elements in order to obtain the energy necessary for basic processes. *Aristotelis* (Aristotle) condensed most of what we know about *Empedocles*' theory of respiration into 25 lines of poetry. He described respiration as an inward and outward movement of air through small pores in the skin (Derenne *et al*., 1994). This shows that Greek philosophers were interested in investigating respiration and its relation with the muscles. Although their questions seem basic, they are still being investigated today (Derenne *et al*., 1994; Lessa *et al*., 2016).

Under the aegis of the Roman Empire, the Greeks achieved great progress in all the scientific disciplines, and among other achievements they reached an understanding of the part played by the muscles in the respiratory mechanism (Derenne *et al*., 1995). Unfortunately, a series of external events resulted in their knowledge being lost, to be rediscovered only centuries later (Derenne *et al*., 1994). *Aristotelis* was one of the first to recognise that respiration involves a specific organ and mechanism. He introduced the idea that the function of the respiration was to cool the body's innate heat, sited in the heart. According to this theory, known as "cardiocentric", the lungs expand due to the heat produced by the heart (Lessa *et al*., 2016; Derenne *et al*., 1995). Not long afterwards, *Plato* (Plato) proposed that oral and cutaneous respiration work in combination; this theory has the advantage of explaining the permanence of the respiratory movement and the integration of a cold-heat cycle (Derenne *et al*., 1994).

Herophilus and *Erasistratus* (340 BC) introduced two new concepts. The first dealt with the brain and how it was the centre of the nervous system, connected by nerves to the different organs of the body. The second was that the muscles were responsible for the generation of force (Derenne *et al*., 1994; Derenne *et al*., 1995). Thus, according to *Herophilus*, respiration consisted of the expansion and contraction of the thorax and lungs, together ensuring the entry and exit of air. *Erasistratus* recognised that the respiratory muscles were the structures which generated the respiratory movement, at the same time identifying the diaphragm as the active muscle in respiration (Lessa *et al*., 2016; De Vito, 2001).

Years later *Galenus* (Galen) experimented with animals, carrying out systematic clinical studies. This enabled him to reach conclusions which are still considered to be largely correct. He showed conclusively that the muscles were the principal source of movement, generated force in the direction of their fibres by contraction, could not be actively extended autonomously when their nerves were cut (Derenne *et al*., 1994). He managed to describe specifically the physiology of the respiratory muscle, explaining the movements of the thoracic wall and the function of the diaphragm. He discovered two layers of intercostal muscles, and enumerated the respiratory muscles together with their innervation (Derenne *et al*., 1994; De Vito, 2001). In "*De Motu Musculorum*" he considered whether expiration was controlled passively or actively, deciding in favour of completely passive expiration. Just as an assumption, we may think that *Galenus* supposed an expiratory effect from the contraction of the diaphragm, or of an action to brake the inspiration during expiration. In all his studies of the respiratory muscles, Galenus clearly understood the function of a muscle *per se*. Finally, he also made progress in explaining the relation between the diaphragm and the abdominal wall, observing that they were joined by “a seam” along the lower margin of the ribs. To contract, the muscles apply a force in the direction of the abdominal contents, which are expelled downwards. Today we know that contraction of this muscle changes the abdominal pressure. *Galenu*'s explanation of the “expulsive forces” (expiration) indicates that he did not fully understand this concept (Derenne *et al*., 1994; Lessa *et al*., 2016; De Vito, 2001).

Oribasius explained the concept of interaction between the muscles of the abdominal walls and the thorax. This indicated that during forced expiration, when the muscles contract the thorax violently, the lungs are strongly compressed against the thoracic walls, creating a strong downward pressure on the diaphragm and displacing the abdominal content towards the hypochondria. (Derenne *et al*., 1994). He also divided the respiratory cycle into four phases: thoracic expansion, lung expansion, lung contraction and thoracic contraction (Derenne *et al*., 1994; Derenne *et al*., 1995).

At the end of the 19th century, Le Double *et al*., (1897) described the anatomical variations of the respiratory muscle group as a contribution to the complete description of human myology intended to facilitate the solution of surgical problems. Later, in the 20th century, Testut & Latarjet (1969) described the anatomy of the respiratory muscles considering six points: i) origin and insertion, ii) diaphragmatic foramina, iii) relations, iv) innervation, v) vessels and vi) action. Recently, investigators like Ratnovsky *et al*., (2008) and De Troyer & Boriek (2011) have implemented techniques which combine electromyography

with clinical and physiological tests from which to develop mechanical and mathematical models of thoracic movement and the components of respiration (Ratnovsky *et al*., 2008; De Troyer & Boriek, 2011).

EMBRYOLOGY OF THE RESPIRATORY MUSCLES

The embryonic origin of the skeletal muscles involved in ventilation are the somites, structures that appear in the mesoderm of the human embryo between days 20 and 21 post-fertilisation on both sides of the axis. The cells of the somitic mesoderm are divided into different cell groups: sclerotome, dermatome and myotome; it is from the latter group that the skeletal muscles will originate (Krück *et al*., 2013). The myotome consists of cells, called myogenic cells, with mesenchymal characteristics but with a restricted gene expression which commits them to muscle formation. They pass through a series of mitoses until they differentiate into post-mitotic myoblasts, regulated by the Fibroblast Growth Factor (FGF) and the Transforming Growth Factor-b (TGF- b); this continues until synthesis of the protein p21, which stops mitosis. This post-mitotic myoblast, mediated by the Insulin-like Growth Factor (IGF), transcribes mRNA from the contractile protein's actin and myosin, and starts to fuse with other post-mitotic myoblasts. They form a multi-nucleate myotube which is capable of producing proteins that regulate muscular contraction, such as troponin and tropomyosin. The organisation of these proteins forms the sarcomere. As it forms, the cell nuclei become peripheral, with their mitochondria and organelles, and are differentiated into muscle fibre (Endo, 2015).

The muscles of the thoracic wall originate from two groups of cells which develop from the somitic mesoderm: the medial myotome, which forms the dorsal musculature (epaxial muscles); and the lateral myotome from which the lateral and ventral muscles of the thorax develop. The tendons develop from the sclerotome (Musumeci *et al*., 2015). The myogenic cells from which the diaphragm is formed come from more cranial segments, which originate from somites located at the cervical level (Merrell & Kardon, 2013).

The diaphragm is considered a complex muscle derived from four embryonic components: the *septum transversum*, the pleuroperitoneal membrane, the dorsal mesentery of the oesophagus and the lateral body walls. The central tendon of the diaphragm is formed from the *septum transversum*, which grows to dorsal of the ventrolateral body wall; it forms a semi-circle capable of separating the heart from the liver, and is initially located to caudal of the pericardial cavity (Greer, 2013). Subsequently, in the fourth week of human embryo development, the *septum transversum* expands due to cephalocaudal folding and fuses with the mesenchymal tissue located in the ventral region of the oesophagal membrane and the pleuroperitoneal membrane. The pleuroperitoneal membrane fuses with the dorsal mesentery of the oesophagus and the septum transversum to form a primitive diaphragm. Subsequently the dorsal mesentery forms the medial segment of the diaphragm and the diaphragmatic pillars. Between the ninth and twelfth week of development, both the lungs and the pleural cavity increase in size and the peripheral portion of the diaphragm is formed (Merrell & Kardon, 2013). As the lungs develop throughout foetal development, the pleural cavity continues to grow larger, forming the left and right costodiaphragmatic recesses, while the diaphragmatic cupula becomes established (Merrell *et al*., 2015).

During the foetal stage, once the respiratory muscles become differentiated, muscular contractions occur cell by cell, necessary for lung growth and cell differentiation. These intermittent contractions, called foetal respiratory movements, are generated by rhythmic neuronal impulses from the respiratory centre located in the encephalic trunk. It was found in a transgenic mouse model Myf5-/-:MyoD-/- that the embryos were not capable of developing respiratory musculature. This caused pulmonary hypoplasia with a diminution of cell proliferation and an increase in the apoptotic index, as well as lower expression of the Platelet-Derived Growth Factor (PDGF) and IGF-I. The use of glycogen, and the storage and secretion of surfactant by the type II pneumocyte, were also diminished. For type I pneumocytes, although early differentiation occurred, late differentiation did not (Inanlou & Kablar, 2005).

HISTOLOGY OF THE RESPIRATORY MUSCLES

The respiratory muscles are formed histologically by striated myocytes (striated muscle fibres), associated with each other and with myosatellite cells (satellite cells) and packaged in connective tissue which forms their fasciae (Junqueira & Carneiro, 2015). Myocytes are characterised by their elongated shape and a series of transverse striations from which they gettheir name. The peripheral fibre is multi-nucleate (Junqueira & Carneiro, 2015). In humans these muscles are formed of a heterogeneous variety of muscle fibre types (Scott *et al*., 2001), giving them great ability to adapt to the constant demands made on them by changing the size or composition of their fibres (Scott *et al*., 2001; Schiaffino & Reggiani, 2011).

Inspiratory and expiratory muscles are composed mostly of slow fibres (red or type 1) (Table I). However, the

sternocleidomastoid muscle, which belongs to the cervical region and is involved, among its other functions, in forced or emergency inspiration, differs from this norm, having only 35 % of slow fibres consistent with its primary function of head and neck movements (Derenne *et al*., 1978). The diaphragm possesses 55 % slow fibres, 21 % type 2A fast fibres (white) and 24 % type 2B fast fibres (Polla *et al*., 2004). Mizuno (1991) observed that the costal part of the diaphragm was composed of 50 % slow-twitch fibres and 25 % type A and B fast-twitch fibres. Saudela *et al*. (1998) observed an increase to up to 56 % slow fibres in this muscle, close to the data reported by Meznaric *et al*. (2016) of around 50 % of slow fibres.

If we compare the area of muscle fibres in cross section, the diaphragm presents a smaller area than the intercostals and the muscles of the upper limbs, with an average of 800 at 2500 μm^2, despite containing the same number of blood vessels (on average between 1.5 and 2.4 per fibre) (Table II). This ratio explains the diaphragm's great resistance to fatigue and its ability to maintain its function over time (Polla *et al*., 2004).

The internal and external intercostal muscles possess a higher percentage of slow fibres than the rest of the respiratory muscles, 60 % versus the 50 % of the diaphragm. They also contain different percentages of type A fast fibres and different numbers of capillaries per fibre: while the internal intercostal muscle has 35 % and a ratio of 2.3 capillaries per fibre,the external intercostal muscle has 22 % and a ratio of 1.6 (Table I). This distribution would be useful in the event of ventilatory dysfunction; according to electromyographic records, the intercostal muscles are the first to assist the insufficient gas exchange characteristic of this situation. If this is not successful, the next to assist this deficiency will be the scalene and sternocleidomastoid muscles (Luce & Culver, 1982).

Although the primary function of the anterior lateral abdominal muscles in humans is trunk flexion, inclination and rotation, increased intra-abdominal pressure, urination, defecation, vomiting and labour, they may assist expiration in particular situations. As noted above, this group includes the recto-abdominal, the external and internal oblique and the transverse abdominal muscle. In their macrostructure they consist of 54 % slow fibres, 20 % type A fast fibres and 26 % type B fast fibres. The mean fibre diameter of the slow and type A fast fibres in the different abdominal muscles is approximately 52 μm^2; the type B fast fibres form an exception, with a diameter of 45 μm^2. It is this size that enables them to fulfil their double function: maintaining posture (permanent tone) and complementing expiration in exercise or pathological situations (Luce & Culver, 1982; Derenne *et al*., 1978). In this context, if we consider the morphology of the respiratory muscles in terms of fibre distribution and vascularisation, in conjunction with their main function (system ventilation), their energy support must be known (Dimitriou *et al*., 2010; Muñoz *et al*., 2017).

These structural and metabolic characteristics would explain the changes experienced by the inspiratory and expiratory muscles in function and dysfunction, and their dependence both on the action carried out and on the work load to which they are subjected (Mizuno, 1991).

Table I. Composition of the inspiratory and expiratory musculature by type of muscle fibre.

	Muscle	**Fibre type I**	**Fibre type IIa**	**Fibre type IIb**	**References**
Inspiration	Diaphragm	54 %	21 %	24 %	Meznaric & Cvetko, 2016
	Sternocleidomastoid muscle	35 %	-	-	Mizuno, 1991
	Scalene muscles	59 %	22 %	17 %	Mizuno, 1991
	External intercostal muscles	62 %	14 %	24 %	Sauleda *et al*., 1998
	Internal intercostal muscles	64 %	35 %	1 %	Polla *et al*., 2004
Expiration	Rectus abdominis muscle	-	-	-	-
	External abdominal oblique muscle	-	-	-	-
	Internal abdominal oblique muscle	54 %	20 %	26 %	Mizuno, 1991
	Transversus abdominis muscle	-	-	-	

Table II. Cross section area and number of capillaries in the fibres of the ventilatory muscle.

Muscle	**Area in μm^2**			**Number of capillaries per fibre**	**References**
	Fibre Type I	Fibre Type IIa	Fibre Type IIb		
Diaphragm	2200	2200	1800	1.9 (1.5-2.4)	Meznaric & Cvetko, 201
Internal intercostal muscles	4300	-	-	2.3	Mizuno, 1991
External intercostal muscles	2900	-	-	1.6	Sauleda *et al*., 1998
Scalene muscles	1900	-	-	-	Mizuno, 1991
Rectus abdominis muscle	45	52	52	-	Mizuno, 1991

GENERAL ANATOMICAL CONSIDERATIONS

The function of the respiratory muscles is essential for life (Polla *et al*., 2004); during ventilation in repose, inspiration is carried out principally by the diaphragm and assisted by the external intercostal, sternocleidomastoid and scalene muscles (De Troyer & Boriek, 2011). Passive expiration is produced by an eccentric contraction of the diaphragm. When expiration changes from passive to forced, the internal intercostal muscles, the recto, and the external oblique, internal oblique and transverse muscles of the abdomen come into play (Ratnovsky *et al*., 2008).

Inspiratory muscles: The diaphragm is the principal inspiratory muscle; it presents tendons in its various insertions, as well as having a tendinous centre. It has a foramen (formed by arched oblique bands) and two hiati (aortic and oesophagal) allowing the passage of the aorta and the oesophagus respectively. Its fibres originate from the centre and divide into three parts, sternal, costal and lumbar. It is inserted into the inferior margin of the six last costal cartilages, the posterior face of the xiphoid process and the first three lumbar vertebrae by tendinous pillars. When the diaphragm contracts, the abdominal content is displaced to inferior and anterior, increasing the vertical diameter of the thorax. At the same time, the margins of the ribs are elevated and displaced to lateral, increasing the transverse diameter of the thorax (Wilson & De Troyer, 2010). These structural changes create a negative intrathoracic pressure, which will modify the lung volumes (De Troyer & Boriek, 2011; Wilson & De Troyer, 2010).

The external intercostal muscles consist of thin layers of fibres lying obliquely to anterior and lateral; they originate in the inferior margin of the rib and are inserted into the superior margin of the rib below. Their origin is further away from the rotational axis of the insertion. Thus, the contraction of this muscle exercises torque on the rib below, raising the thorax. It has been seen in the dorso of the thorax that this expansion is the result of the action of muscles on the ribs, which act as a rigid support to increase the tension in the external intercostal muscles (Ratnovsky *et al*., 2008; De Troyer & Boriek, 2011; Drake *et al*., 2005).

Other muscles, such as the sternocleidomastoid and the scalene, are called accessory inspiratory muscles because their primary functions are head and neck movements; however, they assist ventilation when an increase in total lung capacity is needed. The sternocleidomastoid muscle originates in the lateral surface of the apex and superior margin of the mastoid process, and is inserted by means of two tendinous heads: one in the upper part of the anterior surface of the manubrium of the sternum, and the other in the superior face of the middle third of the clavicula. The scalene muscles are divided into anterior (ASM), middle (MSM) and posterior (PSM). The ASM originates in the anterior tubercles of the transverse processes of the third to sixth cervical vertebrae and is inserted into the scalene tubercle and of the first rib. The MSM originates in the transverse process of the axis and the anterior part of the posterior tubercles of the transverse processes of the five lower cervical vertebrae and is inserted into the superior face of the first rib. The PSM originates in the two tubercles of the transverse processes of the fourth to sixth cervical vertebrae and is inserted into superior margin and the external superior surface of the second rib. In humans at maximum inspiration, the contraction of the sternocleidomastoid and scalene muscles expands the thorax, increasing the inferior superior and anterior posterior diameters and displacing the sternum, and therefore also the thorax, towards the superior (Ratnovsky *et al*., 2008; De Troyer & Boriek, 2011; Koulouris & Dimitroulis, 2001).

Expiratory muscles: The internal intercostal muscles are described as layers of fibres lying obliquely to superior and posterior; they originate in the inferior and lateral margin of the costal groove of the rib and are inserted into the superior margin of the rib below. The insertion of this muscle, unlike the external intercostal, is less distant from the rotational axis of the superior rib, lowering the ribs during contraction (De Troyer & Boriek, 2011; Drake *et al*., 2005; Koulouris & Dimitroulis, 2001).

The rectus abdominis muscle is long and segmented, presenting three or four aponeurotic intersections. It originates from two tendons, one lateral from the crest of the pubis, and one medial which becomes interwoven with its contralateral pair, merging into the ligament fibres which cover the anterior face of the pubic symphysis. The superior insertion of the muscle is through three bellies in the 5th, 6th and 7th costal cartilages and the lateral margin of the xiphoid process. The external abdominal oblique muscle originates from the external surface and the inferior margin of the eight lower ribs, covering the ribs and the internal and external intercostal muscles. Its fibres are inserted into the outer lip of the three anterior quarters of the iliac crest (Drake *et al*., 2005; Koulouris & Dimitroulis, 2001). The fibres of the internal abdominal oblique muscle originate from the lateral two thirds of the superior surface of the inguinal ligament and the anterior two thirds of the intermediate zone of the iliac crest, and also merge through an aponeurosis with the latissimus dorsi muscle and are inserted into the inferior margin of the cartilages of the last three or four ribs. Finally, the transversus abdominis muscle of the abdomen is the deepest of the muscles which make

up the abdominal wall; its fibres originate from the anterior two thirds of the inner lip of iliac crest and the internal face of the last five or six ribs, and are inserted into the two upper thirds of the linea alba (De Troyer & Boriek, 2011; Koulouris & Dimitroulis, 2001). The abdominal muscle group has a number of tasks, including maintaining biped posture and carrying out flexion, inclination and rotation movements of the spine; in addition, its contraction fixes the abdominal content and displaces it to posterior and superior, causing the diaphragm and lower ribs to be displaced in the same direction, a process which finally assists expiration. It has also been observed that separate compression of the thorax and the abdomen, in that order, produces greater diminution of the lung volumes than simultaneous compression. Simultaneous action may be limited by the premature closure of the airways in expiration. Another important point is that the lengths of theintercostal and abdominal muscles are interdependent, thus isolated contraction of the internal intercostal muscles causes relaxation of the abdominal muscles (Ratnovsky *et al*., 2008; Koulouris & Dimitroulis, 2001).

FUNCTIONAL IMPLICATIONS

Ventilation is a highly coordinated process; once inspiration is complete, the inspiratory musculature relaxes and expiration occurs by elastic retraction of the lung parenchyma (Ratnovsky *et al.*, 2008). In maximum exercise (≥ 80 % of maximum oxygen consumption), the rise in lung flows and volumes results from use of the supraclavicular accessory inspiratory musculature; the accessory expiratory musculature is activated to maintain the equilibrium of the additional volumes and flows (De Troyer & Boriek, 2011; Meznaric & Cvetko, 2016). In the presence of weakness of the ventilatory muscles, the system responds with polypnea or hypoventilation, resulting in a limitation of capacity for exercise or incompatibility with life respectively (Terzi *et al*., 2008).

Therefore, correct evaluation of the strength and resistance of the ventilatory muscles is fundamental for selecting specific training programmes to reverse these conditions (Enright & Unnithan, 2011). Muscle strength and resistance can be measured by voluntary contraction, in which the synergistic action of the primary and accessory motor muscles of inspiration and expiration are assessed (ATS/ERS, 2002); the value obtained is an approximation at the level of the oral cavity, since in clinical practice it cannot be measured directly (Gea & Barreiro, 2008). A direct technique does exist, in which an involuntary contraction is provoked by stimulating the phrenic nerve; the pressure developed is specific for the muscle in contraction, but unfortunately special equipment and highly trained staff are needed for this technique (ATS/ERS, 2002).

Determining a diminution of the strength or resistance of the ventilatory muscles is important, due to the impact that these will have on the choice of therapies to be followed. In this context, both Lisboa *et al*. (1995) and Séron *et al*. (2005) managed to reduce clinical pictures of dyspnoea in subjects with chronic obstructive pulmonary disease (COPD) by training the inspiratory musculature. In normal subjects, Wells *et al*. (2005) trained the inspiratory and expiratory musculature of a group of swimmers for 12 weeks; they observed an increase in pulmonary function and better performance of the inspiratory and expiratory muscles in situations of hypercapnia (Wells *et al.*, 2005).

RENIN-ANGIOTENSIN SYSTEM

During physical exercise, one of the most important functions is maintaining circulatory homeostasis; the principal control pathway is the renin-angiotensin system (RAS), which regulates arterial pressure, tissue perfusion and extracellular volume (MacArthur & North, 2005). The greatest source of renin secretion is the kidney, while circulation occurs through the juxtaglomerular myocytes (granular cells). These are modified smooth muscle cells located in the juxtaglomerular terminals, which release active or inactive forms of the same cells in the initial step for RAS activation. Its action may also be triggered by: low volume states, high salt content in the distal renal tubules, activity of the sympathetic nervous system and reduction of renal perfusion (Farag *et al.*, 2015). Furthermore, prorenin may be subject to glomerular filtration and contribute to the presence of RAS components from the tubular fluid (Chappell, 2012), which become joined to the angiotensinogen in the bloodstream to generate angiotensin I (ANG I). The latter is hydrolised by ACE and converted into angiotensin II (ANG II) **(Figure 1)**, which has a strong vasoconstrictor effect mediated by its receptors, located in the supra-renal glands, blood vessels of smooth muscles, kidney and heart (Jones & Woods, 2003). ACE also degrades bradykinin (BK), associated with the secretion of ANG II, which facilitates vasoconstriction (Caló & Vona, 2008).

Thus, the effects of the ANG II at the cardiac, renal, vascular and adrenal levels are mediated by two receptors: type I (AT1) and type II (AT2), which have totally opposed forms of action (Jones & Woods, 2003). AT1 is related with vasoconstriction, aldosterone secretion, sodium (Na^+) resorption in the renal tubules, activation of the sympathetic nervous system, cardiovascular inflammation, hypertrophy and fibrosis (Farag *et al*., 2015). AT2 mediates the activation of the protein tyrosine phosphatase, generation of nitric oxide (NO), vasodilation, and inhibition of growth, anti-inflammatory and antifibrotic cells (Dinh & Touyz, 2011).

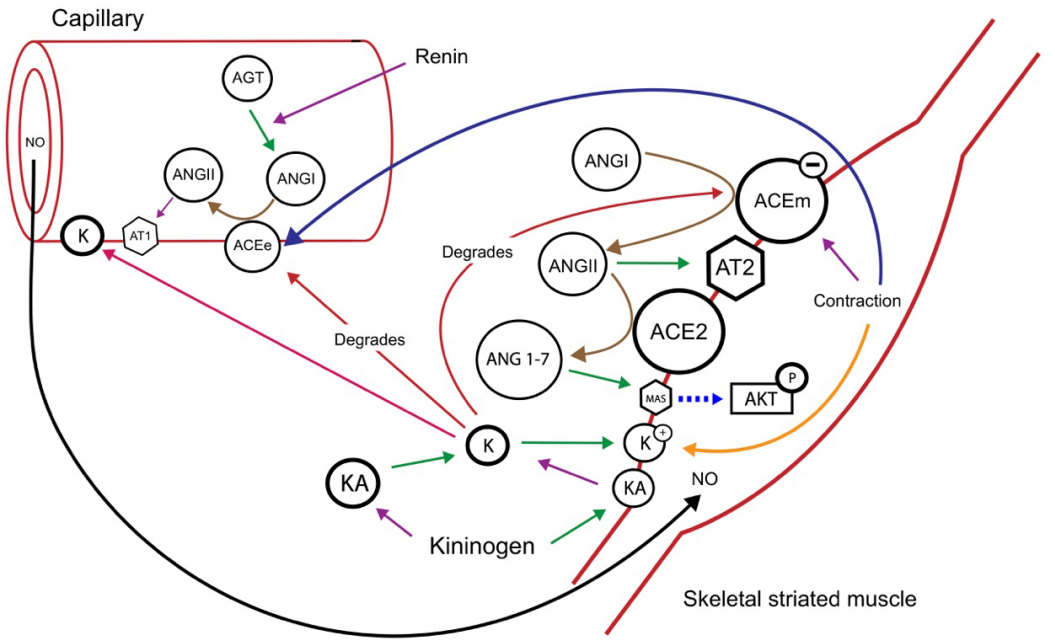

Fig. 1. Molecular control of the action of Kinin by RAS in the contraction of striated muscle. The action of the ANG I/ACEe/ANG II/AT1 axis is observed in both the capillary and the skeletal muscle, generating vasoconstriction. An increase in bioenergy demand or muscle contraction stimulates kinin generation from kininogen and inhibits the activities of ACEe and ACEm. It also generates transmitter molecules such as NO, stimulating an increase in capillary vasodilation and glucose transport. The ANG II/ ACE2/ ANG 1-7 axis, through the MAS receptor, will diminish the effects derived from ANG II, improving glucose transport.

SKELETAL STRIATED MUSCLE AND ANGIOTENSIN CONVERTING ENZYME

There is evidence of the production of ANG II from ANG I in striated muscle; this would be due to the activity of ACE in the plasma membrane and endothelial cells of the capillaries, suggesting functioning of the classic RAS axis in the latter (Dinh & Touyz, 2011). However, RAS expression is not uniform in all the muscle fibres, indicating that its function depends on their metabolism and/or biochemistry (Cabello *et al*., 2015). This is significant, since evidence exists indicating ACE inhibition associated with increases in aerobic exercise.

Energy support: Nutrition of red fibres is effected exclusively by the capillaries and their blood irrigation capacity; the kininsand their vasodilator effect play a fundamental role. We note that all the necessary components of the system, involving kinins, precursor proteins, enzymes and b-2 receptors are present in the endothelium of the vessels which supply nutrients to the red fibres, in contrast with the vessels in white muscle which are absent or less numerous. The kinins present a vasodilatory effect, under metabolic actions dependent on the endothelium and muscle contraction; when they are activated they accelerate the transfer of glucose transporter type 4 (GLUT4) to the sarcolemma, and increase the glucose phosphoinositol-3-kinase pathway (PI3K) (Jones & Woods, 2003; Puthucheary *et al*., 2011). The action of the kinins in glucose transport is linked with the flow of calcium (Ca2+) from the sarcolemma. Furthermore, this mechanism is effected by the contraction of the slow fibre of the red muscle, which is also a Ca2+-dependent process. Kinins activate enzymes like nitric oxide synthase (NOS), cyclooxigenase (COX) and cytochrome P450-2C9-monooxygenase (P450-2C9) in the endothelium, due to the release of Ca2+ in the endothelium. Finally, these enzymes generate NO and prostaglandins, favouring vasodilation and glucose transport, in this case independent of insulin and PI3K (Dietze & Henriksen, 2008) (Fig. 1).

A rise in the energy demand stimulates kinin generation, which in turn inhibits ACE activity in the striated muscle and consequently ANG II synthesis. This diminution of ACE increases the insulin concentration and favours glucose transport (Jones & Woods, 2003). Thus, muscular work increases the blood flow to the skeletal muscle and improves glucose capture in the human being (MacArthur & North, 2005). The Ins allele of the ACE gene has been associated with lower levels of ACE, impeding BK degradation and favouring vasodilation and changes in glucose availability and the efficiency of glucose use (Cabello *et al*., 2015; Dietze &

Henriksen, 2008). It would therefore be of interest to know whether muscles which participate in a movement considered principally aerobic, such as respiration, are influenced by the polymorphism of ACE.

It has recently been discovered that the peptidase ACE2 acts on ANG II to generate ANG 1-7; this process is balanced by the actions of the ANG I /ACE /ANG II /AT1 axis. ACE2 is expressed in most mammal tissues, including striated muscle; its ligand is the MAS receptor, a receptor protein also expressed in this tissue. A limited number of investigations have described the metabolism of the ANG II/ ACE2/ ANG 1-7 axis; the conclusions are that it can activate the insulin signalling pathway/ IRS-1/ PI3K of skeletal muscle or act directly on the Akt receptor (Figure 1). This would show that ANG 1-7, through a MAS receptor-dependent mechanism, could improve the inhibiting effect of ANG II on glucose transport in the striated muscle of mammals, generating activity antagonistic to the classic RAS signalling pathway (Henriksen & Prasannarong, 2013).

MUSCLE PERFORMANCE AND POLYMORPHISM OF THE ACE GENE

The ACE gene is one of the first polymorphisms reported to improve physical performance. It is located in chromosome 17, in position q23.3; it contains 26 exons and 25 introns. Its association with physical performance is determined by its *Ins* or *Del* (Ostrander *et al*., 2009; Cabello *et al*., 2015; Dietze & Henriksen, 2008). More specifically, the *Ins* allele is associated with lower levels of ACE in circulation, which increases the availability of BK, improving energy support through the increased efficiency of glucose use predominantly in type I fibres. The *Del* allele on the other hand is associated with higher levels of ACE in circulation, and therefore of ANG II, generating greater strength through muscle hypertrophy (Colakoglu *et al*., 2005; Jones & Woods, 2003; Dietze & Henriksen, 2008).

In this context, the *Ins/Del* polymorphism of the ACE gene has been associated with changes in the type of muscle fibre. In particular, Zhang *et al*. (2003) showed the association between the *Ins* allele and an increase in type I fibres; the research team attributed this ratio to the cardio-respiratory training plan applied to the subjects in the study (Zhang *et al*., 2003). A relation has also been proposed between improvement in muscular strength and ANG II. As indicated above, it has been attributed functions in the growth of the skeletal and cardiac muscle, and of blood vessels (Gordon *et al*., 2001). To summarise, the *Del* allele has been associated with muscle hypertrophy during strength training (Folland *et al*., 2000), whereas the *Ins* allele is linked to optimisation of energy consumption in aerobic training.

Costa *et al*. (2009) reported a significant association between the *Del* allele and muscular strength in Portuguese elite athletes subjected to prehensile strength and maximum jump tests (Costa *et al*., 2009). Pescatello *et al*. (2006) subjected a North American population to a unilateral strength training programme of the elbow flexor muscle. When they compared the results with untrained subjects, they found that the subjects with the *Ins* allele recorded a greater gain in maximum voluntary contraction in the musculature of both the arm undergoing training and the untrained arm when compared to the *Del/Del* homozygote, while subjects with the *Del* allele presented greater gain in 1 maximal repetition (1RM) and the muscle size of the untrained arm (Pescatello *et al*., 2006). Charbonneau *et al*. (2008) investigated the association between the genotype of the ACE gene and the muscle response to strength training in men and women, finding that the genotype was associated with differences in muscle volume but not with muscle hypertrophy (Charbonneau *et al*., 2008).

RESPIRATORY MUSCLES AND ACE POLYMORPHISM

As we saw above, ACE is an important component of RAS and is present in different tissues such as lungs and muscles. In both, the deletion of ACE polymorphism is related with an increase in ACE activity; in this aspect the *Del* allele is associated with performance oriented towards the quality of muscular strength (Ostrander *et al*., 2009; Colakoglu *et al*., 2005; Jones & Woods, 2003; Dinh & Touyz, 2011). Thus, a union has been suggested between the *Del* allele of ACE and increase in muscular strength in healthy Caucasian subjects (Williams *et al*., 2000).

On the other hand, if we consider the information presented on the structure of the respiratory muscles together with the role of ACE in the striated muscle, we may deduce a direct relation between the *Ins* allele and the inspiratory musculature. Dimitriou *et al*. (2010) investigated the link between the levels of ACE in circulation (ACEc), the maximal inspiratory pressure (MIP) and their possible association with measurements of the pressure-time index of the inspiratory muscles (PTImus) in 110 unweaned infants on mechanically assisted ventilation. They found that the infants with *Del/Del* genotype had significantly higher levels of ACEc than those with *Ins/Ins* or *Ins/Del* genotypes. Furthermore, ACEc activity was related significantly with MIP, and inversely with PTImus (Dimitriou *et al*., 2010). These results suggest an association between the ACE genotypes and ACEc activity, but not with inspiratory muscular strength. In 2010, the same research team studied the relation between the *Ins* allele of the ACE gene and the PTImus in 132 unweaned infants. They found no significant

differences between alleles and MIP, however there was a difference in PTImus, with the infants with *Ins/Ins* genotype presenting a lower PTImus than those with *Del/Del* or Ins/Del genotypes (P=0.000007). The ACE genotype was also significantly related (P=0.005) with the resistance measurements of the inspiratory musculature (PTImus) (Dimitriou *et al*., 2010).

In adults, Hopkinson *et al*. (2004) wanted to test the protective effect of the allele of the ACE gene on muscular strength in patients with COPD. To do this they compared the inspiratory muscular strength and the strength of the quadriceps femoral muscle in 103 patients with COPD versus 101 healthy subjects of the same age. The results indicated that there was no correlation between MIP, strength of the quadriceps femoral muscle and genotype in the control group. The strength of the quadriceps femoral muscle was genotype-dependent in patients with COPD: 8.3 kg for *Ins* homozygotes, 10.1 kg for heterozygotes and 12.4 kg for Del homozygotes ($p<0.002$). In conclusion the researchers reported changes only in the strength of the quadriceps femoral muscle, but not in the MIP of patients with COPD (Hopkinson *et al*., 2004). In 2017, Muñoz *et al*., proposed to investigate the inspiratory and expiratory muscular strength in the different alleles of the ACE gene. To do this they assessed 83 subjects (46 men and 37 women) aged between 18 and 35 years. Their results indicated that the *Ins* homozygote women presented a greater MIP and maximum expiratory pressure (MEP) than their *Del* homozygote peers (p=0.043; p=0.0001 respectively) (Muñoz *et al*., 2017).

The studies presented show a difference between unweaned infants and adults; here the sedentary young women presented an association between MIP and the *Ins* allele of the ACE gene. This might be explained by the structure of the striated muscle of the infants. Orliaguet *et al*. (2004) proposed that the diaphragm passes through a maturing process involving morphofunctional changes; thus, in early stages the fibre distribution tends towards equilibrium with type I: 40 %; type IIa: 30 % and IIb: 30 %. Fast-twitch fibres reach 60 % of the total in the muscle (Orliaguet *et al*., 2004). As noted above, a relation exists between the *Del* allele and type II fibres, which would explain the difference in the MIP values reported. Finally, in adults the differences are associated with female sex. Minson *et al*. (2000) showed in healthy women that in the luteal phase of their menstrual cycle there is a significant increase in estradiol and progesterone, lower sympathetic nervous activity and low vascular resistance in the muscle (Minson *et al*., 2000). This would be due to the changes associated with estradiol and NO (Sudhir *et al*., 1996), facilitating vasodilation and energy support in resistance muscles.

SUMMARY AND PERSPECTIVES

The respiratory muscles fulfil a vital function, maintaining correct ventilation at systemic level. However, there is still insufficient information on its structure in humans. RAS activity has proved to be effective in the delivery of energy to red striated muscle, guaranteeing proper blood flow, as well as participating in glucose homeostasis for the organism as a whole. It is interesting to find the inclusion of the peptide ANG 1-7 in maintenance of the energy support catalysed by ACE2 from ANG II; it is able to counter the actions of the ACE/ANG II/AT1 axis in the glucose transport system of the skeletal muscle through the Akt-dependent mechanism. Although some results support an association between MIP and the *Ins* allele of the ACE gene, this information is scarce, confused and mostly limited to the pathological field. In adults the information is contradictory and sex-dependent. Progress is needed to obtain complete details of the ventilatory musculature to identify correctly the association with energy systems. Finally, more information is needed about the association between the ACE gene and the ventilatory muscle function, and observations on the influence of sex on this.

MUÑOZ-COFRÉ, R.; ROA, I.; DEL SOL, M.; CONEI, D.; LIZAMA-PÉREZ, R.; PACHECO, V. A. & ESCOBAR-CABELLO, M. Músculos respiratorios: Estructura, función y relación con el gen de la ECA. Una breve comunicación Morfofuncional. *Int. J. Morphol., 41(2)*:67-685, 2023.

RESUMEN: La ventilación pulmonar es un proceso mecánico en el que los músculos respiratorios actúan coordinadamente para mantener la oxigenación en el organismo. Así, cualquier alteración en el desempeño de estos músculos puede reducir la efectividad del proceso. Los músculos respiratorios se diferencian de otros músculos esqueléticos, debido al apoyo vital que brindan a través de sus contracciones rítmicas. La estructura y el sistema energético de estos músculos están especialmente adaptados para realizar esta función. La composición de los músculos respiratorios es especial; son pequeñas y presentan una abundante red capilar, lo que les otorga un alto nivel aeróbico y resistencia a la fatiga. La regulación coordinada del sistema renina-angiotensina local, proporciona un adecuado flujo sanguíneo y suministro de energía a las miofibrillas del músculo esquelético. En concreto, este rendimiento dependerá en gran medida del flujo sanguíneo y del consumo de glucosa, regulado por el sistema renina-angiotensina. Aquí, la enzima convertidora de angiotensina es responsable de degradar las kininas, que finalmente regulan la bioenergía muscular y la glucosa entre el vaso sanguíneo y el músculo esquelético. El objetivo de esta breve comunicación es describir la estructura de los músculos respiratorios y su asociación con el gen de la enzima convertidora de angiotensina.

PALABRAS CLAVE: Músculos respiratorios; Embriología; Histología; Anatomía; Soporte Energético; sistema renina-angiotensina; Enzima Convertidora de Angiotensina.

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