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**RESEARCH ARTICLE**

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# Histological and cytoskeletal age-related changes of rabbit skeletal muscle fibres and the preventive role of vitamin E

**ABSTRACT:**

The current study is conducted to study the adverse effect of aging on the immunohistochemical (IHC) and histological architecture cytoskeleton of rabbit's skeletal muscle fibres and the preventive role of vitamin E on elderly animals. Twenty-one male New Zealand rabbits were divided into three groups. Group I: represented the adult rabbits (6 months of age, weighing 4 ± 0.5 kg), group II included aged rabbits (24-months of age, weighing 7.5 ± 0.5 kg), and group III consisted of aged animals given a daily therapeutic dose of vitamin E orally at 14 mg/kg b.w /day for 60 days. The histological structure of skeletal muscles of aged rabbits revealed variety of changes in the form of disorganization of muscle fibres with loss of transverse striations, sarcoplasmic degeneration, infiltration of inflammatory cells, congestion and dilation of blood vessels and nuclear pyknosis. IHC study revealed an increase in the intensity of both desmin and vimentin filaments immunoreactivity in skeletal muscle fibres of aged animals with irregular distribution of desmin immunostain within the aged myocytes. Vitamin E supplementation to senescent animals showed a remarkable effectiveness in restoring the normal histological structure of skeletal muscles, as well as the cytoskeletal proteins structure of either desmin or vimentin and became more or less like the adult ones. Therefore, the present work recommends using vitamin E to relieve the changes in skeletal muscles that accompany aging. In addition, the present study recommends that senescent New Zealand rabbits should be given vitamin E to improve their skeletal muscles quality and antagonize the deleterious effects of aging. So, rabbits will have a higher quality of meat which people consume in their daily life.

**KEY WORDS:**

Skeletal muscles, Aging, Vitamin E, Histology, Immunohistochemistry, Intermediate Filaments, Desmin, Vimentin, Rabbit.

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## INTRODUCTION:

Aging represents an unavoidable and complex biological process which is characterized by a general time-dependent decline in the major system's functions (Buonocore *et al.*, 2011). There are various changes in aging muscle, some of which are normal (age-associated sarcopenia) and others are not; e.g. cachexia syndrome and cancer-related anorexia (Roubenoff, 2000). Several alternations can be detected during aging, which encompass degeneration in muscle mass accompanied by a reduction in muscle fibre diameters (Doria *et al.*, 2012). In previous work, we recorded changes in the cardiac muscle structure of aged rabbits associated with degeneration of many cardiomyocytes and the appearance of pyknotic nuclei (El-Desouki *et al.,* 2018).

Reduction of muscle strength is highly notable of incident disability and gives rise to mortality in older persons (Metter *et al.*, 2002). In humans, the structural adjustments that are accountable for age-related atrophy and muscle strength decline are correlated to

the cumulative impairment of the cross-sectional fibre areas (Porter *et al.*, 1995), and to the decline in fibres number and fibres denervation (Faulkner and Brooks, 1995).

During aging, there is a defect in the balance between free radical production and neutral defence system which resulted in the progressive production and accumulation of harmful changes on the cell and tissue, generating severe functional disorder. This will be the causality of normal aging (Crastes de Paulet, 1990). Free radical increase may be used to explain many of the structural alternations that evolve with aging including membranes lipid peroxidation, cross-linkage of proteins, DNA damage and reduction of mitochondrial function (Wickens, 2001). Therefore, one of the factors that would play an essential role in triggering aging is the build-up of reactive oxygen species (ROS) that have been generated throughout one's lifetime. ROS, which are formed by a single electron addition to the oxygen molecule, are produced in all tissues including muscle fibres and, specifically, in the mitochondrial respiratory chain (Fielding *et al.*, 2011; Doria *et al.,* 2012).

Intermediate filaments (IFs) play a substantial role in the structure integration and function of striated muscle myonuclei, mitochondria, and the sarcolemma. The connection of all these membranous structures to the contractile apparatus, mainly via the Z-discs, supports the growth integration and energy needs of the working myocytes (Capetanaki *et al.*, 2007). The cytoskeleton properly includes actin filaments, microtubules, and intermediate filaments, such as desmin for muscle cells and vimentin for interstitial cells (Schaper *et al.*, 1991; Vaittinen *et al.,* 2001).

Desmin is one of the most abundant and intensively studied muscle intermediate filament proteins. Moreover, it is related to proper costamere coordination, myoblast and stem cell fusion and differentiation, nuclear shape and positioning, as well as mitochondrial structure, shape, positioning and function (Capetanaki *et al.*, 2007). Desmin scarcity prevents myoblast fusion and myotube formation. So, it has proposed that myoblast fusion can occur only between desmin positive cells (Camargo *et al.*, 2003).

Vimentin is the dominant type III protein in myoblasts. It is known to be less expressed with development (Capetanaki *et al.*, 2007). During the early phases of skeletal muscle development, vimentin and desmin are co-expressed and participating in muscle fibres regeneration in activated satellite cells. In some congenital myopathies such as myotubular myopathy, vimentin-reactive fibres found to be more abundant (Sarnat, 1990). Vimentin in normal myocardium expressed in fibroblasts and endothelial cells (Heling *et al.,* 2000; El-Desouki *et al.,* 2014). Moreover, vimentin is present in the interstitial cells and is increased in relevance to fibrosis which accompanied degeneration of myocardial cells (Fraccarollo *et al.*, 2017; El-Desouki *et al.,* 2018).

Vitamin E (α-tocopherol) is a powerful peroxyl radical scavenger that prohibits propagation of free radicals in cell membranes and in plasma lipoproteins (Buettner, 1993). Vitamin E may be useful for prevention of aging signs and treating different illnesses like atherosclerosis, cardiovascular diseases, cancer, diabetes mellitus and neurodegenerative diseases (Mocchegiani *et al.*, 2014). In the presence of enough vitamin E levels, skeletal muscle survived, even though there is a large ROS production during muscle contraction because vitamin E assists in repairing the myoblasts membrane. Moreover, α-tocopherol has been mentioned to be more effective than other antioxidants as it acts as a membrane stabilizer due to its lipid soluble properties nature that give its ability to enter the hydrophobic core of plasma membrane. In addition, its capability to scavenge the ROS effectively related to the presence of chromanol-head group which can bind to phospholipids head on the membrane surface (Khor *et al.*, 2014).

The present study was planned to study the age-related alternations in the histological and cytoskeletal intermediate filament proteins desmin and vimentin of skeletal muscle of rabbits at different ages (adult and aged) and after administration of vitamin E as an antioxidant to elderly animals.

## MATERIAL AND METHODS:

### Animals:

Twenty-one male New Zealand rabbits of two different ages (6 and 24 months), weighing from 4 to 8 kg were collected during autumn and housed in environmentally controlled optimal conditions for one week. Diet and water were allowed *ad-libitum.* During this study all care and procedures are carried out according to the guidelines and approval of the Institutional Animal Ethics Committee of National Research Centre.

### Experimental design:

The animals were divided into three groups; 1) a group of adult rabbits (6 months of age, weighing 4 ± 0.5 kg), 2) a group of aged (senescent) animals (24 months of age, weighing 7.5 ± 0.5 kg), and 3) a group of aged rabbits administered daily with vitamin E orally (E Viton, Kahra Pharm & Chem. Ind. Co) at a dose of 14 mg/kg b.w /day for 60 days. Vitamin E (Alpha tocopheryl acetate) dosage was estimated according to Baydas *et al.* (2002).

The animals of adult and aged groups were sacrificed after one week, while the aged vitamin E-supplemented animals were sacrificed after 60 days. The skeletal muscle specimens from *Vastus lateralis* were fixed in 10% neutral buffered formalin for 24 hrs for histological and IHC studies.

**Histological study:**

Fixed specimens were washed and transferred to 70% ethanol, dehydrated in ascending ethanol series, cleared in xylene, embedded in paraffin wax and sectioned at 5µ thickness using a microtome. Paraffin sections were further processed by haematoxylin and eosin (H & E) staining according to Bancroft and Gamble (2002).

**Immunohistochemical (IHC) study:**

Paraffin sections of skeletal muscle tissues were used for IHC study. The primary monoclonal antibody against desmin (RD301) which is a rabbit monoclonal IgG2b antibody that reacts exclusively with desmin was used. Also, monoclonal antibodies anti-vimentin (V9) (received from Dako Carpinteria, CA 93013, USA) was applied. These two primary antibodies were obtained from Thermo Fisher Scientific Industries. Avidin-Biotin immunoperoxidase technique was performed in which a biotinylated secondary antibody reacts with peroxidase conjugated streptavidin molecules. Colour reaction was developed by using 3, 3' diamino-benzidine (DAB) that gave a brown colour. Finally, haematoxylin was used for counter-staining (Hsu *et al.,* 1981).

## RESULTS:

**Histological observations:**

In adult animal group (6-month-old), skeletal muscle fibres appeared elongated, cylindrical and parallel with regular transverse striations. Closer observations demonstrated multiple pale elongated and peripheral nuclei just beneath the sarcolemma (Fig. 1).

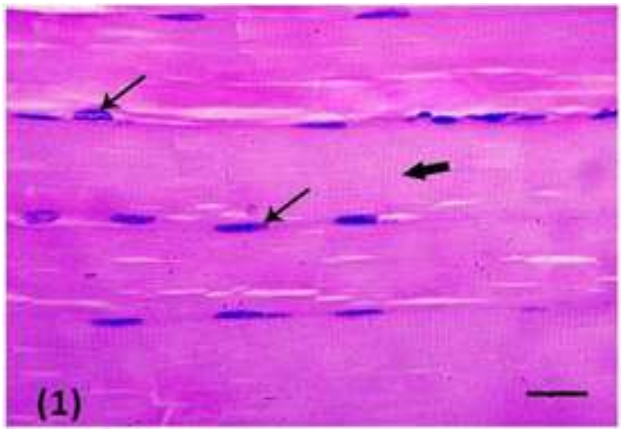


Fig. 1. L.S. of skeletal muscle of an adult rabbit showing regularly arranged long cylinder myofibers with apparent transverse striations (thick arrow) and normal oval multi peripheral nuclei (thin arrows).

Longitudinal sections of skeletal muscles of aged animal group (2-years-old) demonstrated variety of histological changes. These changes included irregular organization of muscle fibres with the absence of the regular transverse striations, focal degeneration of the contractile material that displayed variable staining density of sarcoplasm, wide separation between myofibers, pyknotic nuclei dilated and congested blood vessels as well as leucocytic inflammatory infiltration (Fig. 2).

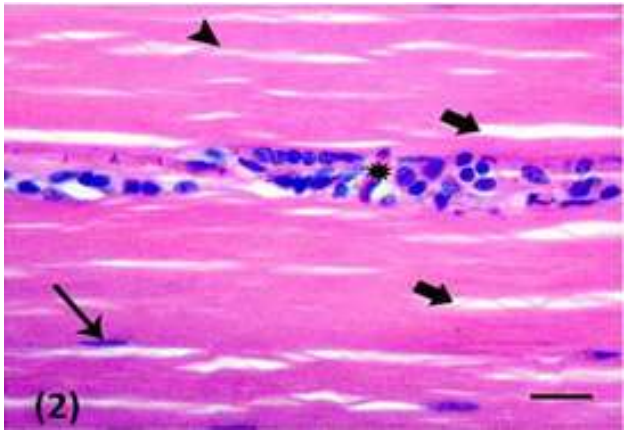


Fig. 2. L.S. of skeletal muscle of an aged rabbit demonstrating leucocytes infiltration (*), dilatation of endomysium space between myofibrils (thick arrows) and absence of striations with disarrangement of muscle fibres (arrow head) with clearly seen pyknotic nuclei (thin arrow).

In aged animals treated with vitamin E at a dose 14 mg/kg b.w /day for 60 days, the skeletal myofibers illustrated restoration of the relatively normal histological architecture. The muscle fibres showed tighter intercellular spaces except for some areas. Transverse striations were restored and detected in some locations of the sarcoplasm with peripherally located normal-shaped nuclei. The blood vessels were also seen with less congestion (Fig. 3).

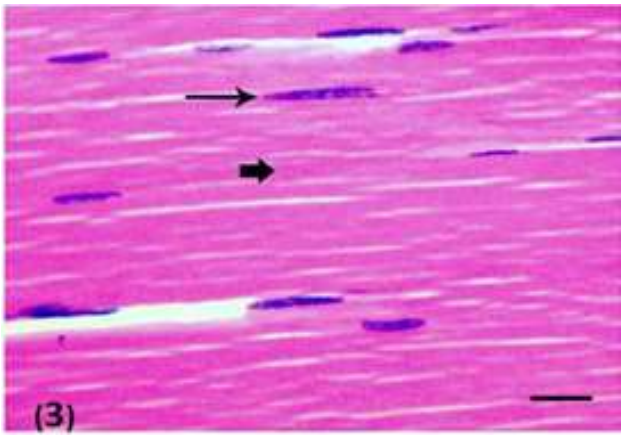


Fig. 3. L.S. skeletal muscle of an aged rabbit treated with vitamin E showing recovery of normal histological appearance of skeletal muscle fibres (thick arrow) and normal oval peripheral nuclei (thin arrow). The disappearance of dilated and congested blood vessels is also noted. H&E, Bar = 6.25 µm.

**IHC observations:**

**a. Desmin**

Desmin is expressed as a brown colour by using anti- desmin immunostain and is localized at Z-lines in skeletal muscle fibre's sarcoplasm. In adult rabbit group, the skeletal muscle fibres expressed normal moderate immunoreaction of desmin at Z-lines (Fig. 4).

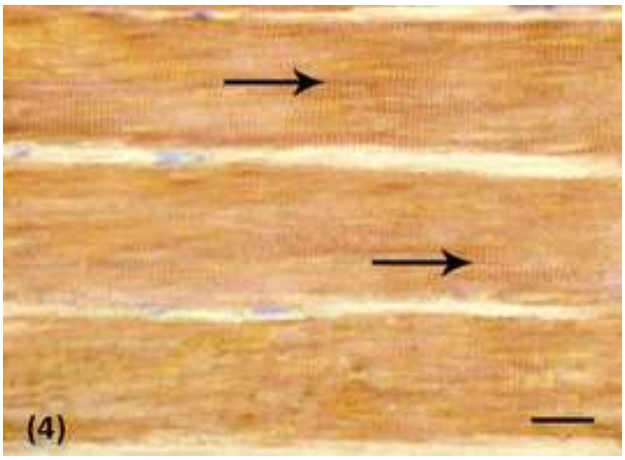


Fig. 4. L.S. of skeletal muscle of an adult rabbit expressing normal moderate immunoreactivity for desmin localized at Z-lines of muscle fibres (arrows).

The aged animals expressed more intense desmin immunoreactivity in skeletal muscle fibres with abnormal pattern of expression where areas of intense reaction alternating with areas of faint reaction for desmin (Fig. 5). After treating the studied aged rabbits with vitamin E at a dose 14 mg/kg b.w /day for 60 days, the skeletal muscle fibres elucidated a recovery and a remarkable reduction of desmin expression in myocytes to a level almost similar to adult group (Fig. 6).

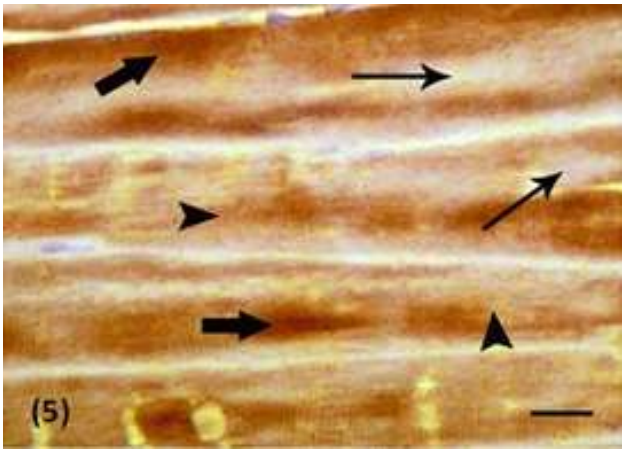


Fig. 5. L.S. of skeletal muscle of an aged rabbit expressing irregular pattern of desmin expression, densily stained areas (thick arrows) alternating with faintly stained areas (head arrows) and other areas with no stain for desmin (thin arrows).

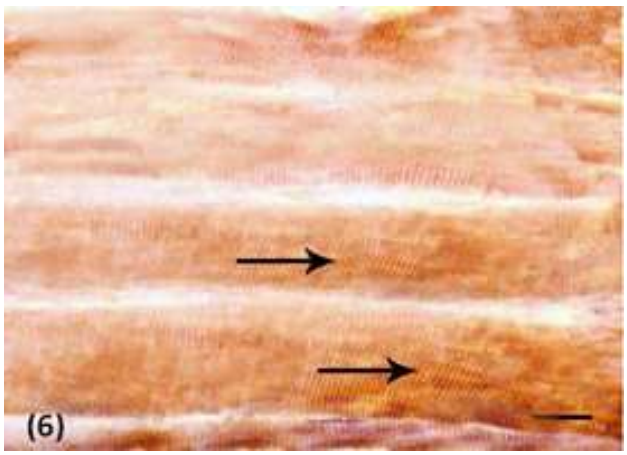


Fig. 6. L.S. of skeletal muscle of an aged rabbit treated with vitamin E showing partial recovery of desmin expression with more regular its distribution (arrows). Desmin immunostain, Bar = 6.25 µm.

**b. Vimentin**

Immunohistochemical expression of vimentin demonstrated as brown filaments localized in endomysium between skeletal myocytes and around blood vessels. The skeletal muscles of adult rabbits group exhibited normal weak to moderate vimentin immunoreactivity that expressed as faint brown reaction in endomysium (Fig. 7).

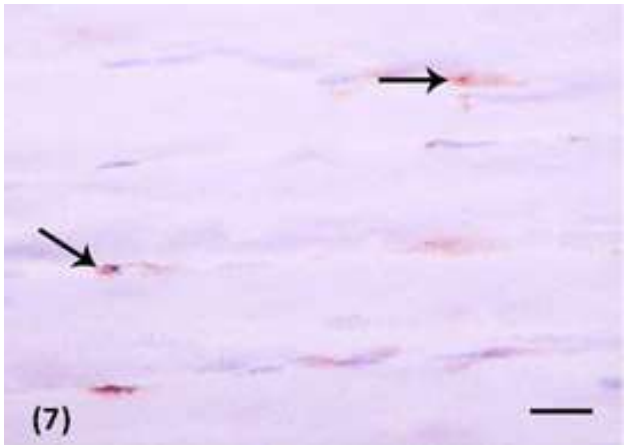


Fig. 7. L.S. of skeletal muscle of an adult rabbit expressing weak to moderate expression of vimentin immunoreactivity in endomysium between myofibers (arrows).

Sections of skeletal muscle fibres of aged animals expressed a marked increase of immunoreaction of vimentin in the endomysium and in the periphery of the dilated blood vessels. Also, vimentin was readily visible not only around the myofibers in endomysium but also in the muscular area itself (Fig. 8). The senescent animals supplemented with vitamin E at a dose 14 mg/kg BW/ day for 60 days displayed a remarkable reduction of vimentin immunoreactivity in endomysium and expressed almost negative reaction in sarcoplasm resembling the adult ones (Fig. 9).

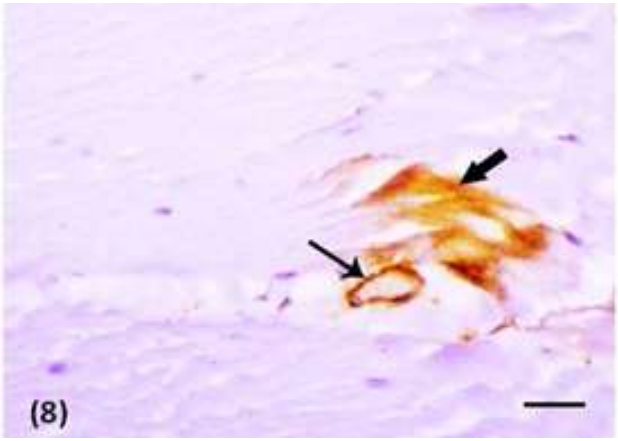


Fig. 8. L.S. of skeletal muscle of an aged rabbit expressing intense vimentin immunoreaction in the endomysium and around blood vessel (thin arrow). Also, a positive vimentin immunoreaction can be seen in sarcoplasm of aged skeletal muscle fibres (thick arrow).


Fig. 9. L.S. of skeletal muscle of an aged rabbit treated with vitamin E expressing an improvement of vimentin immunoreactivity in endomysium between the parallel muscle fibres (arrows). Vimentin immunostain, Bar = 6.25 µm.

**DISCUSSION:**

At the last several decades, the scientific and medical communities have proposed that skeletal muscle dysfunction (e.g., muscle weakness, poor muscle coordination, etc.) is a life-threatening condition facing the elderly. For instance, the age-related loss of muscle strength is closely associated with both mortality and physical disability (Russ *et al.*, 2012; Distefano and Goodpaster, 2018).

In the current study, the skeletal muscles of adult rabbits demonstrated the regular architecture of normal muscle fibres. Skeletal myofibers appeared parallel, long, cylinder with regular transverse striations and peripheral multinuclei just beneath the sarcolemma. Previous studies on skeletal muscle of adult male albino rats are commensurate with the current observations (Soliman *et al.*, 2017). The authors reported that the skeletal muscle fibres appeared elongated, cylindrical, parallel and multinucleated with minimal variations in fibre sizes. The sarcoplasm of the muscle fibres appeared acidophilic and crossly striated with elongated nuclei and peripheral in position under the sarcolemma (Youssef *et al.*, 2015).

In the current work, the skeletal muscles of aged rabbits demonstrated various alternations; disorganization of muscle fibres with the disappearance of transverse striations associated with leucocytic infiltration, focally destroyed areas of myofibrils with different staining density of sarcoplasm and wide intercellular spaces between the myofibers. Similarly, Azmy and Abdallah (2013) reported focally destroyed myofibrillar areas and loss of some myofilaments, leaving wide inter-myofibrillar spaces that might be occupied by disorganized acrosomes during studying the immobilization effect on adult albino rat's hindlimb. Additionally, similar histological alternations have been detected in the cardiac muscle of albino rat under immobilization stress (El-Desouki *et al.,* 2012) and in induced skeletal muscle injury by ischemia reperfusion causing severe skeletal muscle damage (Youssef *et al.*, 2015; Abdel Hamid *et al.*, 2017).

Moreover, in the present study, pyknotic nuclei were located more internally rather than peripherally and some nuclei looked rounded in shape rather than oval. In addition, some sections exhibited dilated and congested blood vessels. In accordance, a previous study mentioned the presence of centrally nucleated muscle fibres in about 24% of the aged mice gastrocnemius fibres (Sayed *et al.*, 2016). The presence of muscle fibres with centrally located nuclei in aged animals was also reported, a finding that could not be spotted in young and middle-aged animals. The observation of muscle fibres with centralized nuclei is considered as an early sign of fibre degeneration-regeneration process and satellite cell activation (Narita and Yorifuji, 1999; Pistilli *et al*., 2014).

Aging is associated with skeletal muscle mass loss, a case known as sarcopenia (Fielding *et al.,* 2011) and it is known that actin and myosin are the abundant proteins in skeletal muscles, and the loss in these proteins is determined with aging. Hence, the aging process is characterized by an elevated oxidant production and a reduction in the ability to buffer oxidants, resulting in a continual state of oxidative stress (Doria *et al.,* 2012). Oxidative stress can destroy biomolecules (DNA, lipids, and proteins), diminish muscle protein synthesis, raise apoptotic signalling and protein degradation (Ryan *et al.*, 2010). The amount of oxidative stress progressively accumulates with aging and is considered to be the major causal factor for muscle mass and function losing

during aging (Braga *et al.*, 2008; Kovacheva *et al.*, 2010; Sinha-Hikim *et al.*, 2013).

In the present research, vitamin E administration to the aged rabbit's attenuated the severity of most of the histological changes in the skeletal muscle fibres. The muscle fibres had less congested blood vessels. The myofibrils displayed an apparently normal pattern of arrangement with less intercellular spaces and more evident striations in most of the studied areas. In addition, the nuclei appeared normal in shape and were located more peripherally. In accordance, Khor *et al.* (2014) explained the protective effect of vitamin E in sarcopenia prevention in which reactive oxygen species (ROS) increase causing oxidative damage to the tissue. So, vitamin E can be used as a trend to fight sarcopenia in advanced age. Moreover, Mohamed (2015) explained the inhibitory effect of vitamin E on the harmful effect of ischemia/reperfusion on the skeletal muscle of the adult male albino rat. The skeletal muscle was markedly affected after induction of ischemia/reperfusion. The skeletal myocytes showed fragmentation, cytoplasmic lysis and degeneration. The nuclei were pyknotic and central. There were intercellular oedema and exudation. The mitochondria were damaged and there was vascular congestion. The mean value of MDA increased, while that of the GSH decreased. The administration of vitamin E or melatonin showed marked improvement in the biochemical profile as well as the histological architecture of the skeletal muscle. Vitamin E (i.e. α-tocopherol) as an antioxidant acts against ROS by reducing and preventing their oxidative damage effect. Fat-soluble vitamin E prevents lipid peroxidation chain reactions in cell membranes by acting against the propagation of lipid radicals. In addition, vitamin E effect as a robust chain-breaking antioxidant and stabilizer of membrane structure, by scavenging free radicals that may attack membrane lipids, interacting with phospholipids and increasing the organization of membrane lipid packing (Abdel Hamid *et al.*, 2017)

Desmin belongs to intermediate filament protein of the cytoskeleton is expressed in all three types of muscle cells (skeletal, cardiac, and smooth muscle). It reacts with other different cytoskeletal proteins including nestin, synemin, and lamins, constructing a well organize three-dimensional extra-sarcomeric cytoskeletal network. This network is participating in various cellular functions such as Z-disc registration maintenance, nuclei positioning, and mitochondrial activity (Nara *et al.*, 2002; Azmy and Abdallah, 2013; Mostafa *et al.*, 2016).

The present comparative immunohistochemical studies of desmin in both adult and aged skeletal muscles revealed intensity difference from normal moderate immunoreaction of desmin at Z-lines in the adult myofibers to intense desmin immunoreactivity in aged ones. Desmin staining of sections from old and young muscles have been reported in humans (Jakobsson *et al.*, 1990). Other studies reported that desmin staining pattern was changed with age as its expression was increased in the rats aging muscles, with the greatest changes observed in the gastrocnemius muscle (Ansved and Edström, 1991). These investigators suggested that the altered pattern of desmin staining in older muscles is connected to the increased desmin deposition in inter-myofibrillar spaces due to myofibrils loss (Russ and Grandy, 2011).

Additionally, in the present work, desmin exhibited irregular distribution pattern in the aged myofibers, where areas of increased immunoreaction alternating with areas of faint reaction. Such heterogeneity of staining for desmin possibly indicated that the size of intermyofibrillar spaces containing intermediate-sized filaments at the Z-line level is increased in atrophic as well as in many normally sized fibres during aging. This is probably a result of myofilaments loss (Meyer and Lieber, 2012).

Vimentin, the major subunit of fibroblastic intermediate filaments, is found in most cells of mesenchymal origin (Franke *et al.*, 1978). It is present together with desmin in myoblasts (Fürst *et al.*, 1989; Barbet *et al.*, 1991). In adult skeletal muscle, vimentin is detected in connective tissue sheaths, i.e. endomysium, perimysium and epimysium, and in the wall of blood vessels (Gabbiani *et al.*, 1981; Čížková *et al.*, 2009).

The vimentin immunoreactivity in the present study was demonstrated as threads located in endomysium between skeletal myocytes and around blood vessels. Vimentin immunoreaction intensity increased from the adult skeletal muscle fibres to the aged ones and demonstrated as an obvious intense immunoreactivity in the endomysium and in the periphery of the dilated blood vessel. These findings commensurate with other authors who confirmed that mature skeletal muscle cells themselves do not express vimentin except during regeneration after injury or in skeletal muscle cancer cells (Bornemann and Schmalbruch, 1992; Vater *et al.*, 1994; Vaittinen *et al.*, 1999 & 2001).

Muscle fibres with vimentin positive reaction were also detected in muscle biopsies from patients who have destructive muscular disorders: when the disease became more progressive, vimentin-positive fibres were more abundant. This increased staining in muscular disorders may relate to the more rapid turnover necrosis-regeneration cycles (Bornemann and Schmalbruch, 1993). In similar findings vimentin expression was

quantitatively measured after inducing injury by lactic acid to cultured skeletal muscles and appeared to be significantly increased at 12h after injury (Bryant *et al*., 2006).

The results obtained from the present study illustrated the protective effect of vitamin E administration to aged skeletal muscle and its effectiveness in reducing desmin and vimentin immunoreactivity most likely with a distribution pattern adult animal. Similarly, Cabet *et al.* (2015) used alpha-tocopherol, a member of the vitamin E family, as the first treatment for desminopathy, a muscular disease characterized by the presence of desmin-positive aggregates. In their study, they found that vitamin E is the most efficient inhibitor for desmin aggregation as it reduced the proportion of cells with desmin aggregation by 65 to 75% in the muscular tissue. Additionally, Choi *et al.* (2003) used vitamin E as an antioxidant to regulate vimentin expression in neuronal cells after cells oxidative damage.

In conclusion, administration of vitamin E as a curative anti-aging medicine strikingly improved the histological and cytoskeletal alternations of the elderly skeletal muscle fibres. Therefore, the present study recommends that the senescent New Zealand rabbits should be given vitamin E to improve their skeletal muscles quality and antagonize the deleterious effects of aging. So, rabbits will have a higher quality of meat which people consume on their daily life.

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## التغيرات النسيجية والهيكل الخلوي المرتبطة بالعُمر لألياف العضلة الهيكلية للأرنب والدور الوقائي لفيتامين هـ

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أجري البحث الحالي لدراسة التأثير الضار للشيخوخة على البنية النسيجية والهيكل الخلوي لألياف العضلات الهيكلية للأرانب والدور الوقائي لفيتامين هـ على الحيوانات المُسنة. تم تقسيم واحد وعشرون من ذكور الأرانب النيوزيلاندية إلى ثلاث مجموعات حسب أعمارها: المجموعة الأولى: تمثل الأرانب البالغة (6 أشهر من العمر، ويزن 4 ± 0.5 كيلوجرام)، وشملت المجموعة الثانية: الأرانب المُسنه (24 شهرًا، ويزن 7.5 ± 0.5 كج)، والمجموعة الثالثة: وشملت حيوانات كبيرة فى العمر وأعطيت يوميًا جرعة 14 ملغ / كغ من وزن الجسم / يوم من فيتامين هـ عن طريق الفم ولمدة 60 يوما. أظهرت الدراسة أن الأرانب المُسنه بها تغيرات في بنية الخلايا العضلية الهيكلية ممثلة في اضطراب بعض الألياف العضلية مع فقدان الخطوط العرضية، وفجوات في الساركوبلازم، وارتشاح الخلايا الالتهابية، واحتقان وتوسع الأوعية الدموية، وظهور مناطق ضامرة وأخرى متحللة في خلاياها وانكماش الأنوية. أما الدراسة الكيمونسيجية المناعية للهيكل البروتيني باستخدام دلالات الديسمن وكذلك الفيمنتين، فقد كشفت عن زيادة وتراكم لكل من خيوط الديسمن والفيمنتين في ألياف العضلات الهيكلية في الأرانب المُسنه. أما بعد تناول الأرانب المُسنه لفيتامين هـ فقد تراجعت التغيرات السابق ذكرها في التركيب النسيجي وتحسنت بنية البروتينات الهيكلية الخلوية ممثلاً في الديسمن والفيمنتين وأصبحت مثل تلك الموجودة في الحيوانات البالغة. لذلك يوصي العمل الحالي باستخدام فيتامين هـ لتخفيف التغيرات في العضلات الهيكلية التي تصاحب الشيخوخة. وتوصي ايضاً هذه الدراسة بإعطاء فيتامين هـ للأرانب المُسنة لتحسين حالة العضلات الهيكلية للتقليل من التأثيرات الضارة للشيخوخة، وبالتالي الحصول على أرانب تستمتع بصحة جيده مع جودة عالية من اللحوم (العضلات الهيكلية) التي يستهلكها الإنسان في حياته اليومية.