



Original article

Alterations in biogenic amines levels associated with age-related muscular tissue impairment in *Drosophila melanogaster*Iman M. El Husseiny^a, Samar El Kholy^a, Amira Z. Mohamed^b, Wesam S. Meshrif^{a,*}, Hanaa Elbrense^a^a Department of Zoology, Faculty of Science, Tanta University, 31527 Tanta, Egypt^b Faculty of Medicine, Tanta University, 31527 Tanta, Egypt

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ABSTRACT

While holding on youth may be a universal wish, aging is a natural process associated with physical and physiological impairment in living organisms. *Drosophila* provides useful insights into aging-related events. Hence, this study was conducted to investigate the age-related changes in muscle function and architecture in relation to the biogenic amine titers. To achieve this aim, visceral and skeletal muscles performance was tested in newly-eclosed, sexually mature and old adult flies using climbing and gut motility assays. In addition, age-related ultrastructural alterations of muscular tissue were observed using transmission electron microscopy (TEM). The titer of selected biogenic amines was measured using high-performance liquid chromatography (HPLC). The results demonstrated that old flies were dramatically slower in upward movement than either newly-eclosed or sexually mature flies. Similarly, gut contraction rate was significantly lower in old flies than the sexually mature, although it was markedly higher than that in the newly-eclosed flies. In TEM examination, there were several ultrastructural changes in the midgut epithelium, legs and thorax muscles of old flies. Regarding biogenic amine titers, the old flies had significantly lower concentrations of octopamine, dopamine and serotonin than the sexually mature. We concluded that aging has adverse effects on muscular system function and ultrastructure, synchronized with biogenic amine titers changes. Our results highlighted the need for more researches on therapeutics that may balance the levels of age-related alterations in biogenic amines.

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1. Introduction

Human life expectancy has been prolonged due to increasing health care and hygiene (Lubitz et al., 2003). Consequently, humans have become more exposed to the risk factors for physical impairments such as neurodegenerative diseases and aging (Firoz et al., 2015). Skeletal muscles represent about 40% of the total adult human body weight (Reid and Fielding, 2012). This tissue is indispensable for vital functions such as respiration, locomotion, and voluntary movements, and is among the most age-sensitive in mammals. Aging affects skeletal muscle quantity and quality in ways that may cause weakness in old people. After the age of

30 years, about 0.5–1% of muscle mass is being lost per year in humans, with a dramatic acceleration of the rate of decline after the age of 65 years (Nair, 2005).

Aging studies on humans are limited because of ethical issues, genetic heterogeneity, environmental sensitivity and long life span (Mitchell et al., 2015). Thus, experimental studies have used various animal models such as mice (Köks et al., 2016), nematodes (Tissenbaum, 2015) and flies (He and Jasper, 2014; Piper and Partridge, 2018; Sun et al., 2013). *Drosophila melanogaster* is an ideal model organism for aging research due to its short lifespan, low maintenance requirements, rich genetic resource, and ease of performing genetic manipulation (Helfand and Rogina, 2003; Tsurumi and Li, 2020). More importantly, the *Drosophila* genome is fully sequenced (Adams et al., 2000; Myers et al., 2000); also, more than 75% of known human disease genes have fly homologs (Reiter et al., 2001).

The consequences of aging on muscular system activity and performance have been reported before, for example, Sohal (1976) observed a dramatic decline in flight activity of *Musca domestica* with aging, Miller et al. (2008) noted flight impairment in 49-day-old *Drosophila*, while 56-day old flies were unable to

* Corresponding author at: Department of Zoology, Faculty of Science, Tanta University, 31527 Tanta, Egypt.

E-mail address: wmeshrif@science.tanta.edu.eg (W.S. Meshrif).

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beat their wings due to their deteriorated ultrastructure of flight muscles. He and Jasper (2014) reported that aging induces a progressive decline in muscular integrity and function. Despite the plethora of information regarding age-related muscular system function changes, the underlying mechanisms of those changes remain unclear. The age-related changes in biogenic amines level, which have been reported before (Rauschenbach et al., 2011, Seid and Traniello, 2005), maybe one of the possible underlying mechanisms.

Biogenic amines are important messenger substances and regulators of cell functions. They act as neurotransmitters, neuromodulators and neurohormones (Baumann et al., 2009). Both vertebrates and invertebrates have several common biogenic amines including dopamine, serotonin, acetylcholine and histamine. In contrast, invertebrates exclusively have octopamine and tyramine, presumably considered the counterparts of vertebrates' adrenaline and noradrenaline (Roeder, 1999). Octopamine is the invertebrates stress hormone that prepares animals for energy-demanding behavior such as aggression and escapes via stimulating glycogenesis, consequently providing more energy for muscles contractions (Brembs et al., 2007). Although tyramine is the precursor for octopamine synthesis, balanced level of octopamine/tyramine is important for proper locomotion, consequently, *Drosophila* mutants in tyramine β hydroxylase (the enzyme catalyzes the hydrolysis of tyramine to synthesize octopamine) accumulated tyramine meanwhile lacking octopamine synthesis have severe locomotion (Saraswati et al., 2004, Selcho et al., 2012). Some biogenic amines have similar functions in vertebrates and invertebrates. Dopamine, for example, is involved in locomotion, cognition and development (Civelli, 1993, Civelli et al., 1993), acetylcholine is a neurotransmitter released by neurons, to transfer action potentials between them to communicate with specialized cells such as muscles (Hebb, 1957) and serotonin is involved in regulating many physiological functions including mood stabilization, appetite, digestion, sleep (<https://www.medicalnewstoday.com/articles/232248>). In contrast, the neurotransmitter histamine exerts different functions in vertebrates and invertebrates. In vertebrates, histamine is involved in local immune reactions and related inflammatory responses (Nieto-Alamilla et al., 2016). While in invertebrates, histamine is the main photoreceptor (Alejevski et al., 2019) and is also involved in modulating temperature preference, controlling the tolerance of low and high temperature (Hong et al., 2006).

Biogenic amines exert their functions by binding to transmembrane receptors which belong to the superfamily G protein-coupled receptors (Borowsky et al., 2001). Although biogenic amine receptors in humans and *Drosophila* are not completely similar, the homology in certain G protein-coupled receptors was documented before. For example, El-Kholy et al. (2015) stated that three octopamine β -receptors are homologs to human β -adrenergic receptors, the close relation between *Drosophila* serotonergic receptors and human dopamine receptors belong to D2-like subfamily as well as human α 2-adrenergic receptors, the close relationship between human α 1-adrenergic receptors and D1-like receptor of *Drosophila*. Therefore, data obtained using *Drosophila* as a model organism will no doubt increase our understanding of age-related adverse consequences.

In this study, we hypothesized that the changes in biogenic amine(s) titers may be correlated with muscles' weakness in the elderly and whether this weakness is accompanied by alterations in the architecture of the muscular tissue. To address this hypothesis, muscular tissue performance and architecture in *D. melanogaster* as an animal model and their relation to the levels of the biogenic amines were tested in three chosen ages representing different life stages.

2. Materials and methods

2.1. *Drosophila melanogaster*

The Canton-S wild-type strain obtained from Bloomington *Drosophila* stock center (#64349) was used in all experiments. *Drosophila* was reared on standard cornmeal-yeast food media in the Animal facility, Faculty of Science, Tanta University at $25 \pm 2^\circ\text{C}$ and 50–70% RH under a 12:12 h dark/light cycle (Hoffmann et al., 2013). Adults were transferred to new vials every 5 days to prevent the overlap of generations. Three ages of adult *Drosophila* were chosen for all subsequent experiments (Fig. 1): newly-eclosed representative of childhood within 8 h after emergence (Tauber Research Lab, University of Leicester), sexually mature within the range of 3 to 5 and 45 to 55 days post-emergence, respectively (-Bednářová et al., 2018, Vassy et al., 2003), where the average longevity of a *Drosophila* adult is approximately 70 days (Piper and Partridge, 2018).

2.2. Climbing assay

Locomotion activity of newly-eclosed, sexually mature and old male flies ($N = 3$) was tested using a negative geotaxis assay as described by Feany and Bender (2000) with some modifications. In brief, nine male adult flies were placed into a 100 ml clean glass cylinder. After 10 min, the flies were tapped to the bottom of the cylinder and let climb. The upward movement was videotaped. The speed of individuals involved in the experiment was analyzed by using Image J (Version 1.2) (Schneider et al., 2012). The speed of the flies that did not climb at all was calculated as zero. The experiment was repeated three times under normal light. We only use male flies in this assay to avoid data inconsistency due to the effect of oogenesis/ovulation on female body weight (Stephano et al., 2018) because the skeletal muscles performance is sensitive to body weight variation in *Drosophila* (Schilder, 2016).

2.3. Gut peristalsis assay

To test the effect of aging on spontaneous adult gut muscle performance, a gut assay was done according to Palmer et al. (2007). Briefly, cold-anesthetized adults ($n = 15$) of each age were pinned dorsal side down onto a dissecting Petri dish; the sample was covered with physiological saline (5 mM HEPES, 128 mM NaCl, 36 mM sucrose, 4 mM MgCl_2 , 2 mM KCl, and 1.8 mM CaCl_2 , pH 7.1). The insect was dissected to show the gut and the number of contractions was recorded for a 30-s period followed by a 30-s waiting period, repeating this paradigm for 5-min.

2.4. Transmission electron microscopy (TEM) of visceral and skeletal muscles

Samples ($n = 3$) of thoracic region, abdominal region and hind legs dissected from newly-eclosed, sexually mature and old adult *Drosophila* were fixed in 2.5–3 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2–7.4) at 4°C for 2 h, then 1% osmium tetroxide was added as a post fixative (4°C , 1.5 h). Then the sample was immersed in serial dilutions of ethanol (50, 70, 90, 95 and four times 100%, each for 15 min) for dehydration, and the specimen was further dehydrated by acetone for 30 min. Finally, the fixed specimens were embedded in epoxy resin (Epoxy Embedding Medium Kit, Sigma, Germany). Semi- and ultra-thin sections were cut on an ultramicrotome (RMC PT-XL PowerTome Ultramicrotome). Semi-thin (1 μm) sections were stained with 1% toluidine blue and examined using an Olympus BX61 light microscope. At thickness 70–90 nm, ultra-thin sections were cut and then stained with

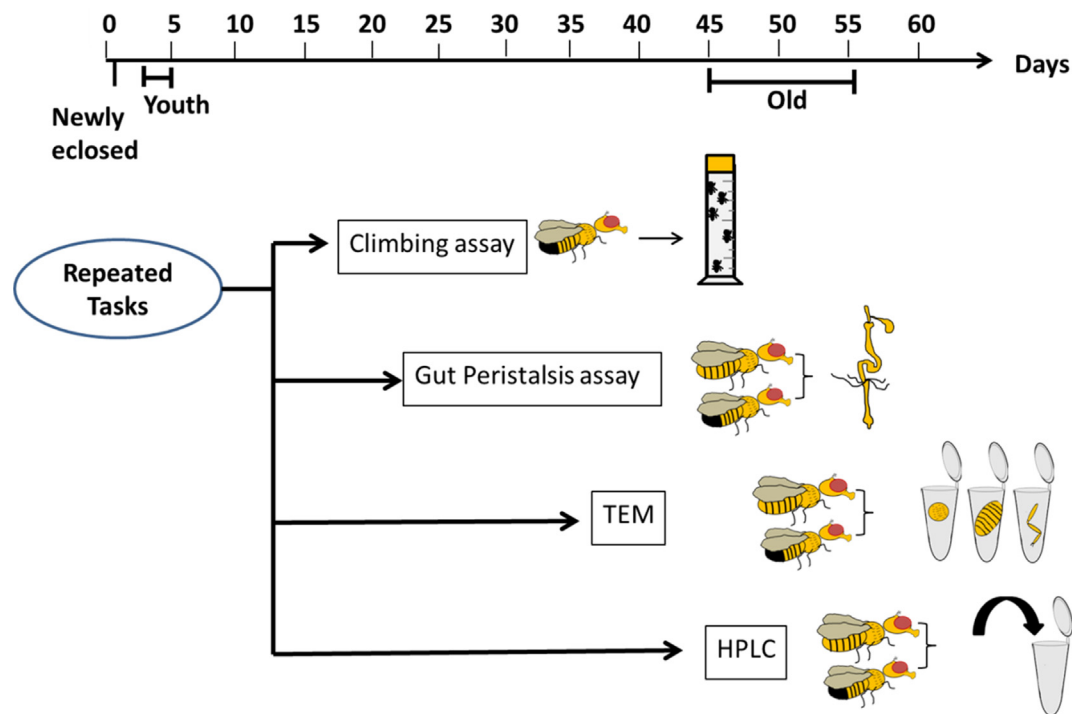


Fig. 1. Schematic diagram representing the time scale of the entire experimental design components. The repeated tasks were: to evaluate male climbing ability (see text), to measure males and females' gut peristalsis, to examine ultra-structural alterations in thoracic, abdominal and gut muscles of both males and females and finally, to measure the titer of biogenic amines level in both males and females. This full scheme was repeated at least three times unless other replicate numbers were mentioned in the text.

2.5% uranyl acetate as a principal stain and lead citrate as a counterstain. Finally, the examination and capture of ultrathin sections were conducted using a JEM-2100 (JEOL, Japan) transmission electron microscope.

2.5. Biogenic amines titer measurement (HPLC)

The whole body concentration of dopamine, serotonin, histamine, octopamine, tyramine and acetylcholine in *D. melanogaster* was measured in newly-eclosed, sexually mature and old *Drosophila* adults using HPLC as described previously (Borycz et al., 2000, Hardie and Hirsh, 2006). Briefly, 5 pairs of adult *Drosophila* were homogenized in a mixture of acetonitrile and methanol (1/1) followed by sonication (Ultrasonic, Frequency 75 kHz) for 15 min. The samples were centrifuged for 5 min at 5000 rpm. This procedure was replicated 3 times. The resultant supernatants were dried to concentrate the samples. Quantitative HPLC was carried out using a system equipped with a binary pump (LC 1110; GBC Scientific Equipment, Hampshire, USA) and a C18 column (Kromasil C18, 5 μ m, 150 $\times$ 6.4 mm Sigma Aldrich, St Louis, USA). A volume of 20 μ l was injected into the column with an isocratic procedure where the mobile phase was methanol–acetonitrile–sodium pentane sulphonate (7.5:7.5:85, v/v/v) and the flow rate 0.85 ml/min. The ultraviolet detector (LC 1200; GBC Scientific Equipment, Hampshire, USA) with the wavelength of 275 nm was used to detect and measure dopamine, serotonin, histamine, octopamine, tyramine and acetylcholine, where individual standard curves for each biogenic amine were prepared using different concentrations of the standard corresponding one (obtained from Sigma-Aldrich, USA) used for calculation of each biogenic amine in samples.

2.6. Statistical analysis

Data were expressed as Mean $\pm$ standard deviation (M $\pm$ SD). The response variables were checked for normality using the

Darling-Anderson test and for homogeneity of variances using Levene's Test. The effect of aging on biogenic amines and gut peristalsis was analyzed using one-way ANOVA with multiple comparisons. The P-value was adjusted according to Bonferroni correction to control the familywise error rate. For non-normal data (locomotion activity), the Kruskal-Wallis analysis with Dunn's multiple-test was adopted. Data were expressed as a median, 25 and 27% percentiles. These analyses were carried out in GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com".

3. Results

3.1. Climbing

To assess the locomotion activity of adult *D. melanogaster* with age progress, a negative geotaxis assay was used. Fig. 2 demonstrates the effect of age progress on the climbing speed (distance of upward movement in cm/sec) of newly-eclosed, sexually mature and old adult flies. Statistical analysis indicated that age significantly ($H = 45.28$, $df = 2$, $P < 0.001$; Kruskal-Wallis test) affects the speed of upward movement. Specifically, the climbing speed of newly-eclosed adults was 0.328 (Median). The climbing speed of sexually mature adults significantly ($P < 0.0001$) increased, compared to newly-eclosed adults, to 2.2 (Median), whereas, the climbing performance of old flies was dramatically ($P < 0.0001$) reduced to zero (median).

3.2. Gut peristalsis

To test the function of gut muscles, the number of gut contractions/30 sec in adult *D. melanogaster* was estimated (Fig. 3). In the newly-eclosed adults, the gut peristalsis was 2.93 ± 1.71 (M $\pm$ SD). With age progress, the gut peristalsis significantly increased in the mature ($P < 0.0001$) and old adults ($P = 0.0043$) compared with the

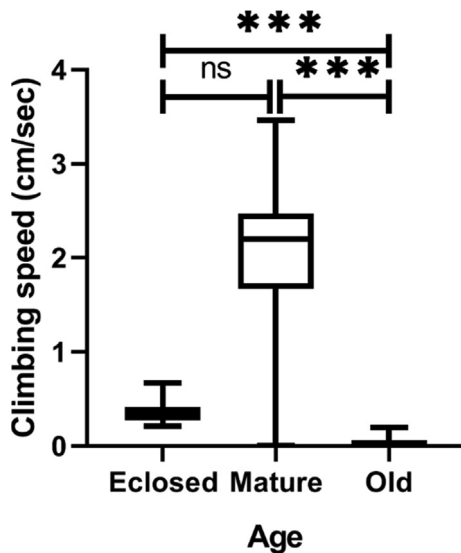


Fig. 2. Climbing speed (Median, 25%, and 75% percentiles) of newly-eclosed, sexually mature and old adult *Drosophila melanogaster*. $n = 27$ replicates. The bars are significantly different when $P < 0.05$ (Kruskal-Wallis test). *** refers to a significant difference between means when $P < 0.0002$ (Bonferroni correction on multiple comparisons). ns refers to a non-significant difference between means when $P \geq 0.033$.

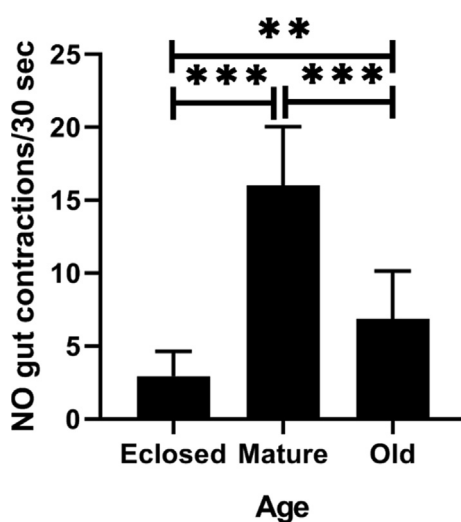


Fig. 3. Number of gut contractions (Mean $\pm$ SD) of newly-eclosed, sexually mature and old adult *Drosophila melanogaster*. $n = 15$ replicates. ** and *** refers to significant differences when $P < 0.002$ and $P < 0.0002$, respectively (Bonferroni correction on multiple comparisons).

newly eclosed ones. The sexually mature adults have a higher gut contraction rate (16 ± 4.04) than the old ones (6.87 ± 3.27).

3.3. TEM

The midgut epithelium in *D. melanogaster* is lined by a single-layered epithelium followed by a basal lamina. The epithelium layer, covered by a peritrophic membrane, acts as a barrier to the environment while allowing for nutrient uptake and related physiological processes. In newly eclosed and sexually mature ages, the ultrastructure of midgut epithelium revealed a normal intact surface epithelium with continuous long microvilli and other normal organelles such as mitochondria and rough endoplasmic reticulum (RER) (Fig. 4A & B), whereas in old age, there were several ultra-

structural changes in the midgut epithelium such as loss of junctional complex, disruption of RER and degeneration of mitochondria (Fig. 4C & D). The organization of skeletal muscle fibers in *Drosophila* is similar to that in mammals. A skeletal muscle refers to multiple bundles of cells joined together called muscle fibres. The fibers and muscles are surrounded by connective tissue. Muscle fibres are composed of myofibrils. The myofibrils are composed of actin and myosin filaments, repeated in units called sarcomeres, which are the basic functional units of the muscle fiber. In the newly- eclosed and sexually mature, the ultrastructure of thorax muscles showed more or less normal architecture of muscle (Fig. 5A & B). In old age, the muscles lose their flexibility due to lysis of fibril and distortion of the sarcomeric structure (Fig. 5C & D).

The ultrastructure of leg muscles in the newly-eclosed showed mild indented nuclei of sarcomeres with dispersed heterochromatin (Fig. 6A). In the sexually mature, the electron microscopic examination revealed a completely normal ultrastructure fibril. H line and Z line bisected dark bands and light bands respectively (Fig. 5B). The muscles undergo more dramatic age-related deterioration, presumably due to separation of the junction between sarcomeres, lysis of fibril and several abnormalities in other organelles (Fig. 6C & D).

3.4. Biogenic amines titer

Fig. 7 shows the titer of the selected bioamines in newly-eclosed, sexually-mature and old *D. melanogaster* adults. Dopamine, octopamine, and serotonin titers demonstrated significant changes along with the aging progress ($F_{2,6} = 16.85$, $P = 0.004$; $F_{2,6} = 36.57$, $P = 0.004$; $F_{2,6} = 30.34$, $P = 0.001$), respectively. The mature adults exhibited higher ($P < 0.033$) titers of dopamine, octopamine, and serotonin compared with either newly-eclosed or old ones. In the same context, the newly-eclosed showed ($P \geq 0.033$) titers of these amines close to those in the old adults. However, the titer of acetylcholine, histamine and tyramine did not show significant changes with advanced age ($F_{2,6} = 2.352$, $P = 0.176$; $F_{2,6} = 4.781$, $P = 0.057$; $F_{2,6} = 2.291$, $P = 0.182$, respectively). In these amines, neither mature nor old *Drosophila* show significant ($P \geq 0.033$) changes on multiple comparisons (Bonferroni test).

4. Discussion

Aging is a complex process having adverse consequences on the structure and function of different systems in living organisms (Kregel and Zhang, 2007). The impaired movement is one of the most important consequences. Loss of muscle mass and function is a major contributor to the physical decline that occurs with aging. In flies, muscles contribute a large percentage of body mass. The fly muscles have structural similarities with those in mammals (Piper and Partridge, 2018).

Insects are valuable models for studying the age-related consequences on locomotion activity because many of them are highly mobile on land (walking and running) and in the air (flying) (Ridgel and Ritzmann, 2005). In the current study, we documented a reduced locomotion activity in aged flies, i.e. old flies exhibited slower upward speed than either newly eclosed or mature adults. Similarly, Minois et al. (2001) and Martinez et al. (2007) showed that locomotion decreased with age in wild-type and mutant *Drosophila*.

The gut is responsible for nutrient, digestion and absorption, as well as serving as the first line of defense against pathogens. It is considered a primary source of neuronal and endocrine signals generated by the functionally important peptidergic brain-gut axis

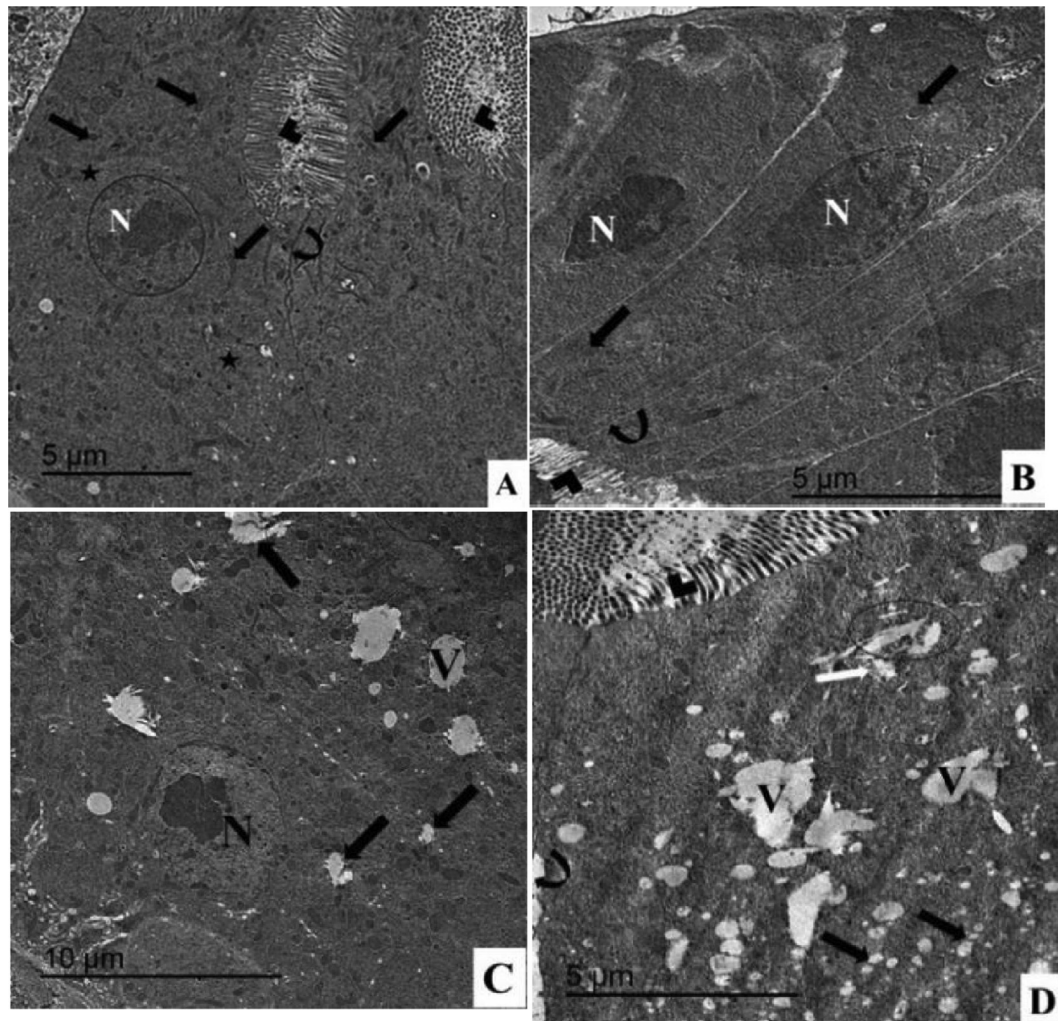


Fig. 4. Transmission electron micrograph of part of *Drosophila*'s midgut showing: A. Normal surface epithelium of midgut with continuous long microvilli (arrowhead), intact junctional complexes (curved arrow), rounded nucleus (N) surrounded by homogenous cytoplasm (asteric), mitochondria (arrow) and other normal cellular organelles in the newly-enclosed group (Bar = 5 μ m). B. Normal surface epithelium of midgut with the normal architecture of epithelium such as microvilli (arrowhead), junctional complex (curved arrow), mitochondria, cytoplasm and rough endoplasmic reticulum. Note, massive condensation of chromatin in the nucleus (N) in the sexually mature group (Bar = 5 μ m). C. Cytoplasmic vacuolation (V) of the epithelium surface, irregular nucleus (N) and a few swollen degenerated mitochondria (arrow) can be observed in the old group (Bar = 10 μ m). D. Focal loss of microvilli of the epithelium (arrowhead), disrupted rough endoplasmic reticulum (circle), dilated smooth endoplasmic reticulum (arrow), widening of intracellular space between the cells (curved arrow), cristolysis of some mitochondria (thin arrow) and cytoplasmic vacuolation (V) can be observed in the old group (Bar = 5 μ m).

(Pappas et al., 1985). Age-related changes of the human gastrointestinal system include slowing of functions and increased susceptibility to digestive system disorders (Ruiz, 2017). In this study, we tested if the rate of gut muscles contraction in *Drosophila* adults decreased with aging

The results of the current study revealed that the gut contraction rate significantly decreased in old flies compared to the sexually mature ones. However, the newly enclosed exhibited a lower contraction rate than both mature and old flies. We could attribute the lower gut contraction in the newly enclosed flies to the assumption that as they had just emerged, they had not yet reached full functionality. Saad et al. (2010) observed gastric motor changes with aging include a reduction in the gastric contractile force in humans. The changes observed in the current study in the muscular system function (locomotion activity and gut peristalsis) could yield more insights with a structural examination.

Because of the small size of *Drosophila* muscles and the difficulty in detecting changes in the muscle as it ages, there are few studies on age-related changes in muscle mass (Demontis et al., 2013). For the above-mentioned reasons, we hypothesized that

TEM could help to explain the observed changes induced by aging in *Drosophila*.

In the current investigation, the ultrastructure examination of muscles in the thorax and leg revealed that the muscles become weak with the progress of age based on several ultrastructural changes. The newly-enclosed and sexually mature *Drosophila* adults show more or less normal sarcomeric structure. Both skeletal and visceral muscles are invaginated by tracheoles and innervated by nerve axons containing synaptic vesicles. Meanwhile, in old age, several changes in the ultrastructure of muscles were observed such as separation of the junction between sarcomeres, pyknosis of the nucleus and lysis of fibrils. These results are in agreement with several previous investigators (Demontis et al., 2013, Maqbool et al., 2006, Swank, 2012).

In the present study, the ultrastructure examination of the newly-enclosed and sexually mature adult *Drosophila*'s midgut showed a normal single layer of columnar epithelial cells attached with a junctional complex. The junctional complex is composed of intact desmosome and an interdigitating process. The internal organelles of the epithelial cells, such as a regular nucleus, mito-

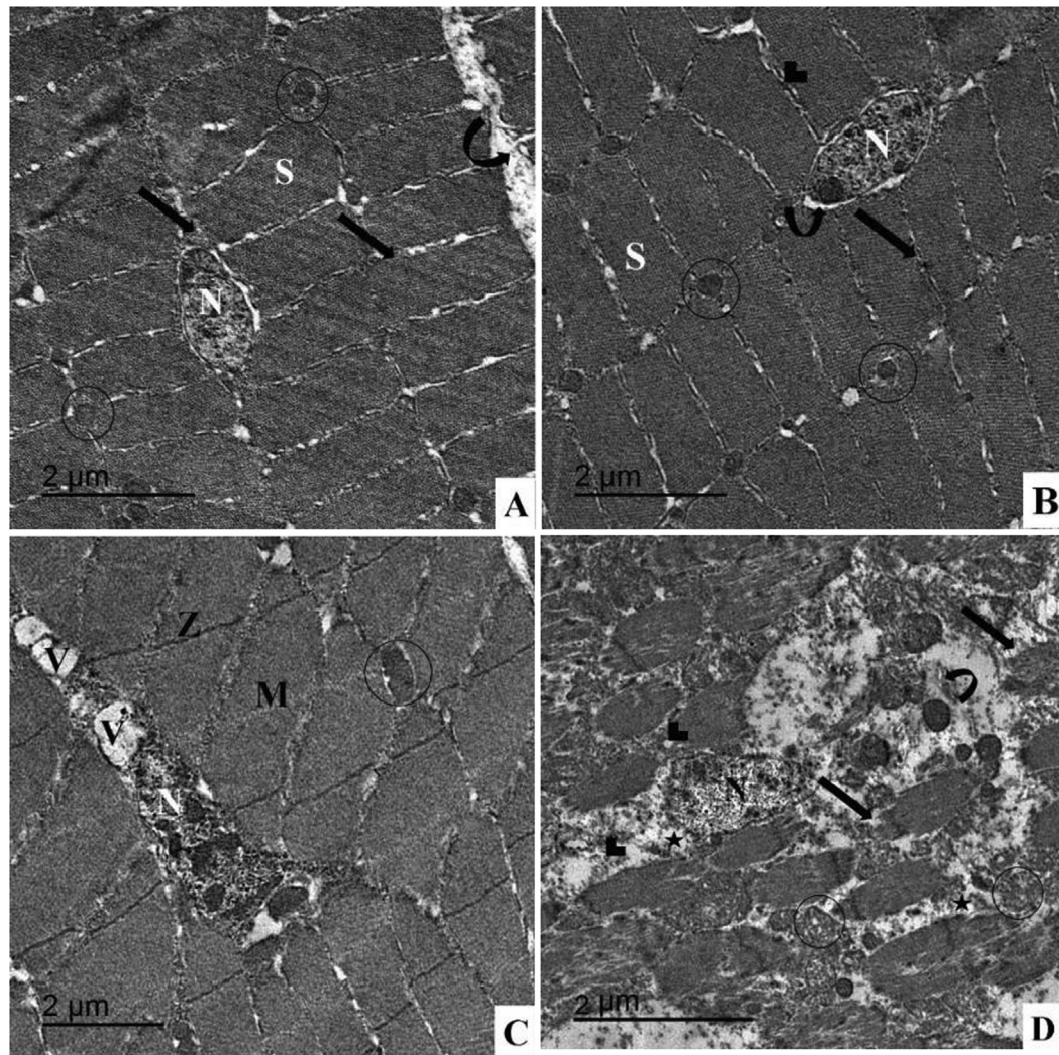


Fig. 5. Transmission electron micrograph of part of *Drosophila*'s thorax muscle showing: A. More or less normal oval nucleus (N), sarcoplasm (arrow), mitochondria (circle) with abundant cristae are distributed between sarcofibrils (S) in an organized sarcomeric structure. Note, slightly widening with a decrease of electron density of connective tissue between sarcomeres (curved arrow) in the newly-eclosed group (Bar = 2 µm). B. Normal architecture of sarcomere in an organized form of fibrils (S), oval regular nucleus (N) surrounded by a mild dilated perinuclear membrane (curved arrow), mitochondria (circle), sarcoplasm (arrow) and rough sarcoplasmic reticulum (arrowhead) in the sexually mature group (Bar = 2 µm). C. Vacuolated sarcoplasm (V) surrounded irregular nucleus (N) with fragmented chromatin. Oblique section of fibrils showing Z line and M line, mitochondria (circle) in the old group (Bar = 2 µm). D. Disorganized sarcomeric structure with lysis of most of the sarcofibrils (arrow), massive rarification of sarcoplasm (asteric), a decrease of electron density of nucleus (N), partial lysis of crista of mitochondria (circle), dilated SER (arrowhead) and disrupted RER (curved arrow) in the old group (Bar = 2 µm).

chondria, cytoplasm and rough endoplasmic nucleus are normal. The surface epithelium was lined with continuous microvilli. These results were also similar to the observation of other studies (Bonfanti et al., 1992, Hecker et al., 1971, Thomas and Bockeler, 1994).

In contrast, the examination of the old age ultrathin section in *Drosophila*'s midgut showed slight disruption in the rough endoplasmic reticulum and loss of junctional complex in addition to cytoplasmic vacuolation. These findings agree with Gartner (1985) and Jimenez and Gilliam (1989). We also observed that most of the mitochondria of old age samples were swollen and degenerated. This result was in line with Anton-Erxleben et al. (1983) who found an enlargement in mitochondrial structure with increasing age in *Drosophila* midgut. Mitochondrial enlargement affects the production of ATP which is necessary for most cellular activities. Ultrastructural studies show that with increasing age, the mitochondria become more osmiophilic, their cristae disarranged, cristae-free areas can also be found, and there is an accu-

mulation of homogeneous, granular and lamellar dense bodies as well as several abnormalities in other organelles. These ultrastructural changes may explain the lower gut contraction rate in the old *Drosophila*, however, the underlying mechanisms that explain the observed age-related changes either in structure and function remain unclear.

Biogenic amines exert their functions as neurotransmitters via binding with GPCR as endogenous ligands. Thus, biogenic amines activate the downstream GPCR pathways, regulate intracellular second messengers through coupling to heterotrimeric G-proteins adenylate cyclase (AC), cyclic adenosine monophosphate (cAMP), and protein kinase A (PKA). This in turn regulates many different physiological processes in the organism (Gardner et al., 2012, Gether, 2000, Millar and Newton, 2010, Oldham and Hamm, 2008, Verrier et al., 2011, Wise et al., 2002). GPCRs either increase or decrease the level of phospholipase C beta (PLC-β) that induce Ca^{2+} oscillations due to Ca^{2+} release from intracellular stores (Balfanz et al., 2005). Calcium-dependent pathways control

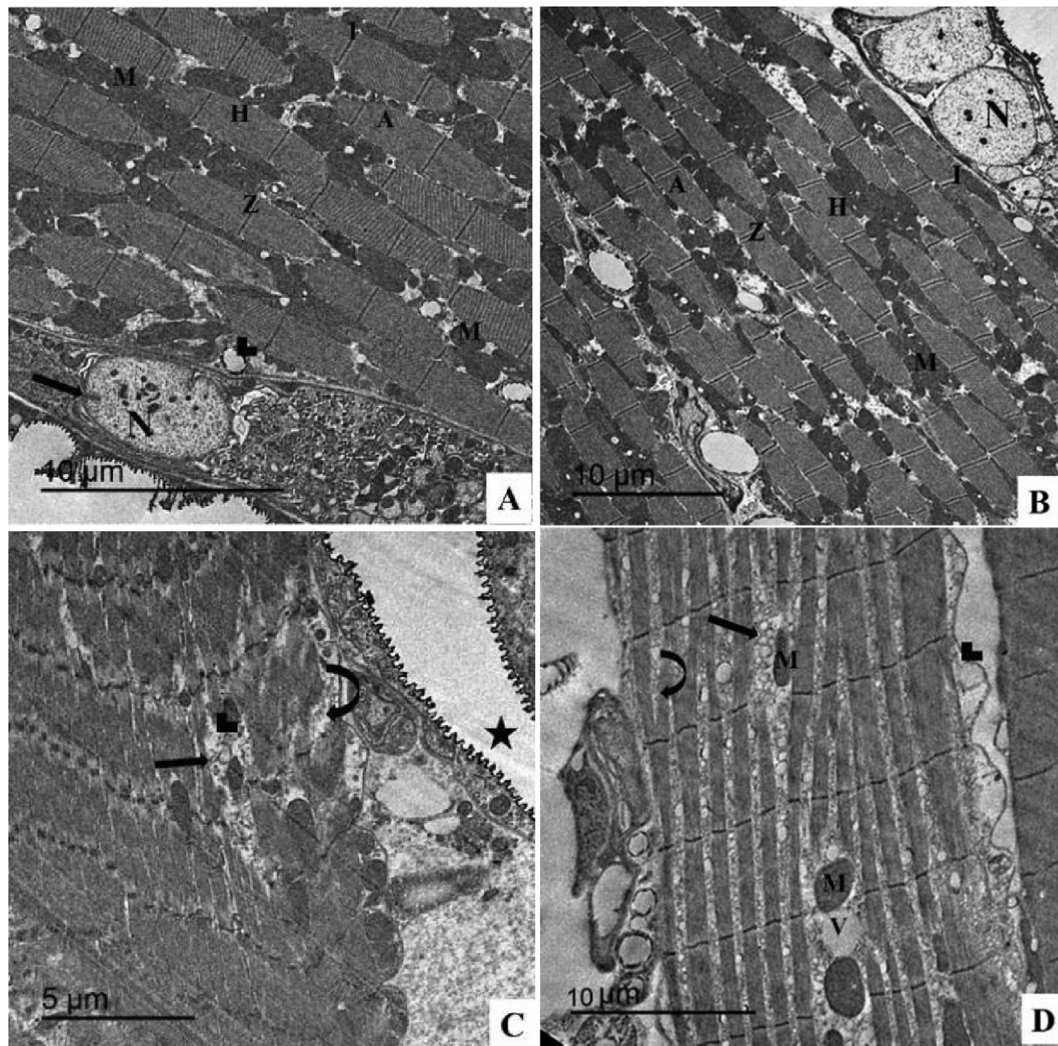


Fig. 6. Transmission electron micrograph of part of *Drosophila*'s legs muscle showing: A. mild indented nucleus (arrow) with dispersed heterochromatin (N). Sarcoplasm between fibrils rich with vessels (arrowhead) and the fibrils showing alternates dark bands (A) which bisected by (H) zone & light bands (I) which bisected by Z line. Mitochondria (M) with abundant cristae are distributed between fibrils in the newly-eclosed group (Bar = 10 µm). B. More or less normal oval nucleus with regular outline (N). Most probably normal architecture of sarcomere showing alternate dark bands (A) which are bisected by (H) zone (arrowhead) & light bands (I) which are bisected by Z line. Normal mitochondria (M) can be observed between myofibrils in the sexually mature group (Bar = 10 µm). C. Detached junction between sarcomeres (asteric), mild rarified sarcoplasm (arrowhead), dilated SER (arrow) and focal lysis area of fibril (curved arrow) in the old group (Bar = 5 µm). D. Pyknotic nucleus (N), vacuolated sarcoplasm (V), dilated SER (arrow), slightly lysis of fibril (curved arrow), dilated sarcolemma (arrowhead), rarified sarcoplasm (asteric), dense mitochondria (M) with different size in the old group (Bar = 10 µm).

muscle activity, myogenesis, and the activity of neurons (Damm and Egli, 2014). Therefore, we assumed that naturally occurring decline in certain biogenic amines levels may interpret the observed age-related changes of muscle architecture and activity. Results of this study revealed that levels of serotonin, octopamine and dopamine significantly increased in sexually mature compared to newly-eclosed adults, and these levels are further reduced in older flies these results were in consistent with those of Liao et al. (2017) who stated that the levels of stored neuromodulators decreased with aging. Also, Vermeulen et al. 2006 reported age-related dopamine variation with *Drosophila*. Dopamine requirements being increased for sexual maturity may be due to the linkage between dopamine and hyperactivity (Pendleton et al., 2002, Riemensperger et al., 2011, Ueno et al., 2012). The result is in agreement with Piccirillo et al. (2014) who showed that the concentration of dopamine was highest in flies aged 3 to 7 days. Once flies reach full sexual maturity, dopamine levels decrease with increasing age. Harano et al. (2008) stated that the dopamine level is correlated with the sexually mature hormone level of *Apis mellifera*.

In parallel, mammalian brain dopamine levels also decrease as a function of age (Goicoechea et al., 1997, Gozlan et al., 1990, Woods and Druse, 1996).

The observed high locomotion activity of sexually mature adults compared to older ones can be correlated with the high concentration of octopamine (Brembs et al. (2007), Wosnitza et al. (2013), Yang et al. (2015) reported that octopamine plays a central role in energy-demanding behaviors such as locomotion. Moreover, its absence slowed animals' walking pace. Another role played by octopamine is in the appetitive influence of courtship behavior by increasing the response to sex pheromone (Jarriault et al., 2009; Kaczner and Maldonado, 2009) as well as its role in ovulation and fertilization (Li et al., 2015). Serotonin is involved in locomotion activity and aggression (Chen et al., 2013; Majeed et al., 2016, Mohammad et al., 2016, Pooryasin and Fiala, 2015, Qian et al., 2017). The role played by serotonin in slowing locomotion was observed in other models, such as in lamprey, cat, and locust (Barbeau and Rossignol, 1991, Harris-Warrick and Cohen, 1985, Parker, 1995).

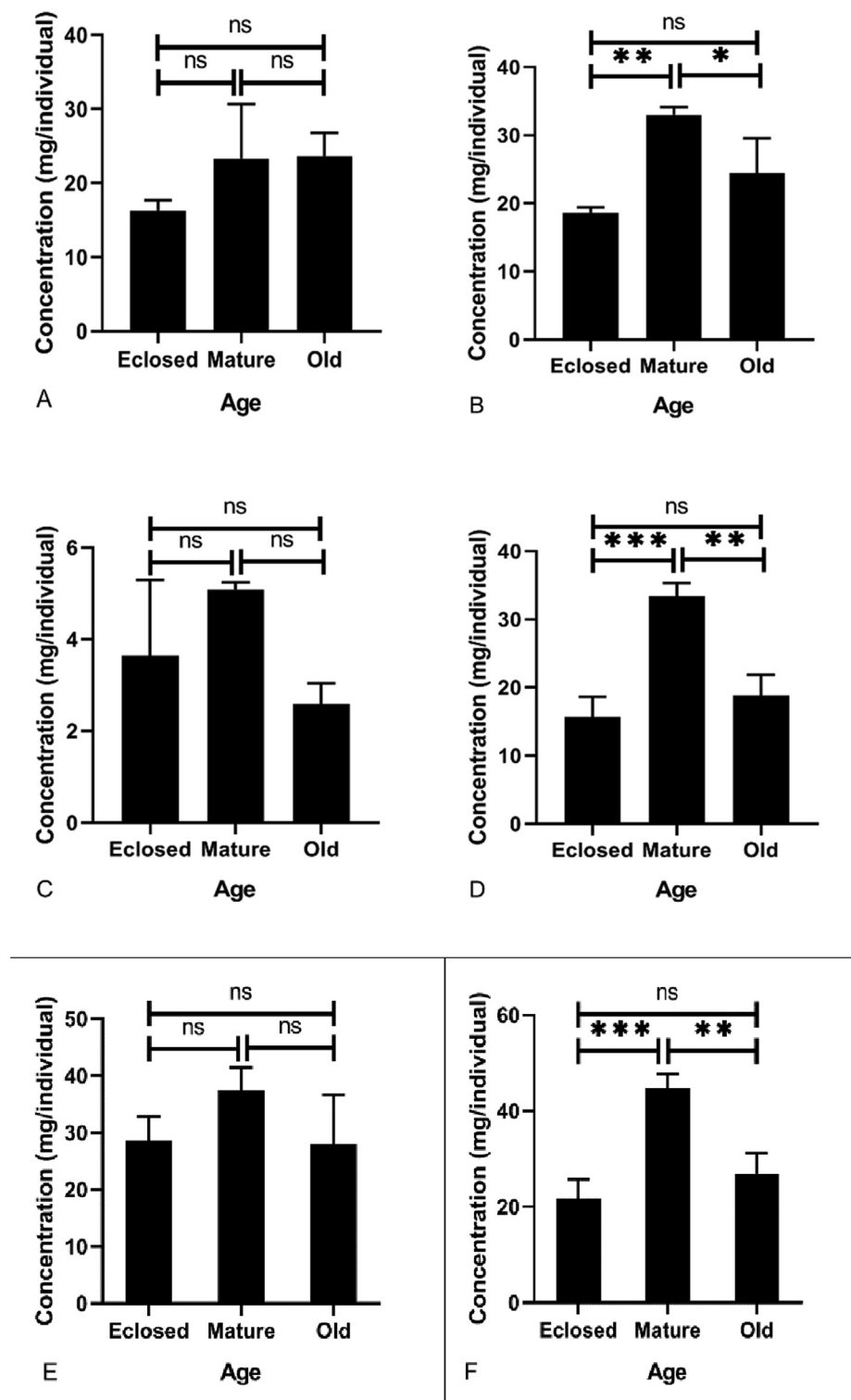


Fig. 7. Concentration (Mean $\pm$ SD) of Acetylcholine (A), dopamine (B), histamine (C), octopamine (D), tyramine (E), and serotonin (F) estimated by HPLC in newly-eclosed, sexually- mature and old adult *Drosophila melanogaster*. $n = 3$ replicates. The bars in dopamine, octopamine, and serotonin are significantly different when $P < 0.05$ (One-way ANOVA). *, **, and *** refer to significant differences between means when $P < 0.033$, $P < 0.002$ and $P < 0.0001$, respectively (Bonferroni correction on multiple comparisons). ns refers to a non-significant difference between means when $P \geq 0.033$.

Our results revealed that the titers of histamine, acetylcholine and tyramine did not change with aging. These amines play vital functions otherwise irrelevant to age-related muscles weakness. Acetylcholine plays a major role in the brain's cognitive function and is involved in neurodegenerative disorders (Vallianatou et al., 2019). In agreement with our result, the difference in acetylcholine titer occurs in *Drosophila* different

stages of the life cycle (Pant et al., 1978) rather than through adulthood because it is related more to the development of the nervous system.

Our results revealed that histamine level is not significantly changed with aging, simply because histamine signaling plays other physiological roles in modulating temperature preference and in controlling the tolerance of low and high temperature

(Hong et al., 2006) as well as photoreception (Alejevski et al., 2019). Tyramine, the precursor of octopamine synthesis (Cole et al., 2005), has a vital role in modulating metabolism to provide maximum power for energy-demanding expenditure (Li et al., 2016). Taken together, our results revealed that three (histamine, acetylcholine and tyramine) out of the six chosen biogenic amines may not participate in age-related muscular system deteriorations.

5. Conclusions

In the light of the previous results, we can conclude that aging induced several negative alterations in the architecture of visceral and skeletal muscles, with subsequent deterioration in their functions. Moreover, these changes were consistent with the titers of octopamine, dopamine and serotonin. Thus, this information opens an avenue to new studies on whether balanced biogenic amines could enhance the aging-deteriorations in the muscular system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

IE, SE and HE suggested the study idea and designed the experiments. IE, SE, AZM and HE collected the results. WSM analyzed the data. All authors wrote and approved the manuscript.

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