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RESEARCH ARTICLE

Aging induced Hematological, Anthropometrical, Biochemical, and Reproductive Changes in Male Rats

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ABSTRACT

Aging is a complex biological process characterized by the accumulation of changes over time with loss of physiological integrity. Age-related changes occur in all tissues of the body and affect the functioning of all body systems. The purpose of this study is to investigate the effect of aging on male reproductive function, different body fat, skeletal muscles weight and hematological parameters in male Sprague–Dawley rats. Fifty male rats were randomly divided into two groups (n= 25/group); young and old groups. Rats of the old group were reared for 12 months. Changes in body weight were monitored weekly. The results showed that body mass index (BMI), mean abdominal circumference and testes, epididymis, seminal vesicle, and prostate weights were increased in the old group. Additionally, pre-renal, epididymal, inguinal fat, and brown adipose tissue (BAT) weights were enlarged in the aged group. Weights of gastocnemius, soleus, and extensor digitorum longus (EDL) muscle were also significantly increased in old rats. The blood glucose and corticosterone levels were significantly declined by age. Erythrocytes count (RBCs, 4.113 ×106/cmm ± 0.316), hemoglobin concentration (Hb, 12.261 ± 0.817 g/dL), the percentage of packed cell volume (PCV, 35.77 ± 1.806%), and mean corpuscular hemoglobin concentration (MCHC, 34.161± 0.805%) were decreased, while the mean corpuscular volume (MCV, 88.001± 3.483 fl) and mean corpuscular hemoglobin (MCH, 29.958± 0.812 pg) were increased with aging. Both sperm cell concentration (45.5 ± 9.3 ml X 125 X 10) and sperm abnormalities (41.5 ±2.7%) were increased, while sperm motility (44.166 ± 13.8%) decreased in the old group.

In conclusion, aging has adverse effects on some hematological and reproductive parameters in male rats. Therefore age is a fundamental factor that should be carefully considered.

***Keywords*:** Aging, Glucose, Corticosterone, BMI, Sperm

Introduction

Aging is a complicated process of tissue deterioration that is associated with a reduction in the organs' ability to operate physically. A significant body of research indicates that aging is closely linked to various chronic diseases, including diabetes, hypertension, Parkinson's disease, Alzheimer's disease, and atherosclerosis [1]. Age-related changes may originate from a variety of sources, including genetic, metabolic, or environmental factors. Typically, these causes interact throughout an individual's lifetime [2].

Numerous physiological changes, such as alterations to body weight and composition, are linked to aging. It is especially concerning when body fat and excess weight build up [3]. Unaffected by

typical and physiological fluctuations in weight and body mass index (BMI), the aging process is marked by a rise in total fat mass in the body and a corresponding decrease in lean mass and bone density. Age-related changes in body adiposity also lead to a general rise in trunk fat (mostly abdominal fat) and a decrease in appendicular fat (primarily subcutaneous fat). To maintain metabolic homeostasis and a state of health, it is essential to maintain a balanced rate of fat, muscle, and bone [4]. A high BMI has a detrimental impact on healthy life expectancy and is linked to a higher risk of cancer [5]. A major connection between obesity, insulin resistance, and age-related disorders is inflammaging, which is marked by a significant increase in chronic low-grade inflammation [6]. Numerous diseases are associated with age, such as cancer, immune system problems, musculoskeletal disorders, cardiovascular diseases, and neurological diseases [7].

In many mammalian species, aging is accompanied by a steady decline in reproductive function, which is linked to a drop in testosterone [8]. Aging also results in genetic and epigenetic alterations in spermatozoa, which negatively impact sperm quality and quantity, sexual organs, and the hypothalamic-pituitary-gonadal axis, all of which are factors that affect male reproductive capabilities. Testes, spermatogenesis, and hormone production are altered. Spermatozoa quantity and quality decline as a result of these minor modifications [9]. Several studies examined the structural alterations that take place in the rat testis during maturation [10]. It's critical to distinguish between aging, healthy males without concurrent diseases and those suffering from chronic disorders that have a substantial impact on male gonadal function [11].

In rodents, body weight can be greatly influenced by growth and age. Also, body weight and hematological parameters, such as blood volume, cardiac output, and stroke volume, are directly correlated [12]. Hematological parameters were found to differ with age; some showed a gradual rise with age, whereas others did not. Age-related data on crucial hematologic parameters in rats would be helpful for safety pharmacological researche, which is required for drug development, as well as research of aging-related diseases utilizing this model [13]. The aim of the present study is to estimate the anthropometric, biometric-hematological, and reproductive parameters in association with the aging process in male rats.

Materials and methods:

Experimental animals

Before the experiment, 50 male, six-weeks-old Sprague-Dawley (SD) rats were allowed to acclimate for two weeks. The rats' initial mean body weight was 130 ± 10 g. They were procured from the laboratory animal unit at Zagzig University, Egypt. The rats were kept in a 12-hour light/dark cycle with free access to water, a constant temperature of 22 ± 2 °C, and a relative humidity of 50–60%. Following the acclimatization period, rats were split into two groups at random (n = 25/group).

The Faculty of Veterinary Medicine at Zagazig University's Institutional Animal Care and Use Committee Guidelines authorized this study (Approval No: ZU-IACUC/2/F/66/2020).

Experimental design

Rats were randomly divided into two groups (n= 25/group); young (2 months)

and old (14 months) groups. Rats in the young group were sacrificed after the acclimatization period while those of the old one was reared for 12 successive months. The body weights were determined once a week.

Anthropometric parameters and tissue collection

All rats were quickly decapitated after an overnight fast with unrestricted access to water [14]. Body weight and length were taken and BMI was calculated as following: BMI = body weight (g)/length*2 (cm2), then body circumference was taken. Testes, epididymis, seminal vesicle (SV), and prostate were taken out. Prerenal, epididymal, inguinal, and brown adipose tissue (BAT) were excised and weighed. Gastrocnemius, soleus, and extensor digitorum longus (EDL) muscles were also excised and weighed.

Haematological examination, blood glucose, and corticosterone estimation

Rat's trunk blood was transferred into EDTA-containing tubes for assessment of hematological parameters. The hematological parameters including red blood cells (RBCs) count, the percentages of hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets count, white blood cells (WBCs) count and differential leucocytic count were determined using a Sysmex XT-2000iV Automated Hematology Analyzer (Sysmex Corporation, Hyogo, Japan). Blood glucose levels were measured by a blood glucose meter (Lifescan, Milpitas, CA). Corticosterone concentration was estimated using rat corticosterone ELISA kits (CORT; CSB-E07014r; Cusabio Biotech Co., Ltd.) according to the manufacturer's protocol.

Epididymal sperm analysis

perm analysis

In a Petri dish that had been sterilized and contained two mL of normal saline at 37°C, one cauda epididymis was instantly removed and sliced, and the suspension was utilized as semen. On a glass slide that had been pre-warmed to 37 °C, a single drop of the semen sample was placed then covered with a cover slide, and examined under a light microscope (40x) to determine the motility of the sperm [15]. The percentage of abnormal sperm was determined by staining a glass slide with a smear of the semen sample using an eosin-nigrosine stain. An improved Neubauer hemocytometer counting chamber was used to quantify the concentration of sperm cells after the semen sample was diluted five times (v/v) with normal saline and a few drops of 40% formalin to immobilize the sperms [16].

Data analysis

Student's *t*-test was used to perform the statistical analysis for all data except the body wright was analysed by one way ANOVA. The results were expressed as the mean ± standard error of the mean (mean ± S.E.M.). A value of $p < 0.05$ was considered significant.

Results

Effect of aging on body weight, mean BMI, and abdominal circumference.

The body weight was gradually increased with advancing age (Figure. 1A, $p < 0.001$ by ANOVA). The old group's mean BMI was much higher than the young group's (Figure. 1B, $p < 0.001$ by Student's *t*-test). The old group's abdominal circumference was significantly higher than the young

group's (Figure. 1C, $p < 0.001$ by Student's *t*-test)

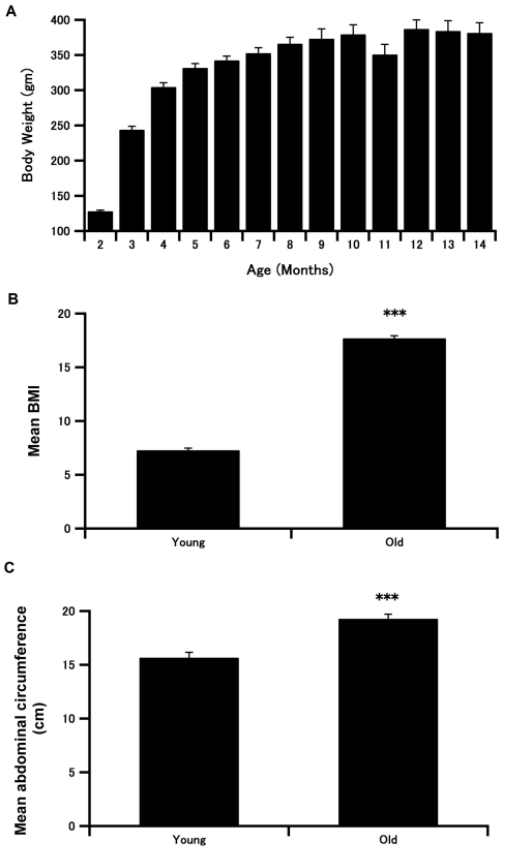

Figure 1. Changes in body weight, mean body mass index, and mean abdominal circumference. (A) Initial and final body weights (g) in the old group throughout the rearing period (1 year). Data are means ± standard error of the mean (n = 25); (B) mean body mass index of the young and old rats; and (C) mean abdominal circumference (cm) of the young and old groups. Data are means ± standard error of the mean (n = 25 in each group). ***$p \leq 0.001$ by Student's *t*-test.

Effect of aging on reproductive organs, body fat, and different muscle weight.

The old group exhibited a substantial increase in testes, epididymis, SV, and prostate weight in comparison to the young group (Figures 2A-D, $p < 0.001$ by Student's *t*-test).

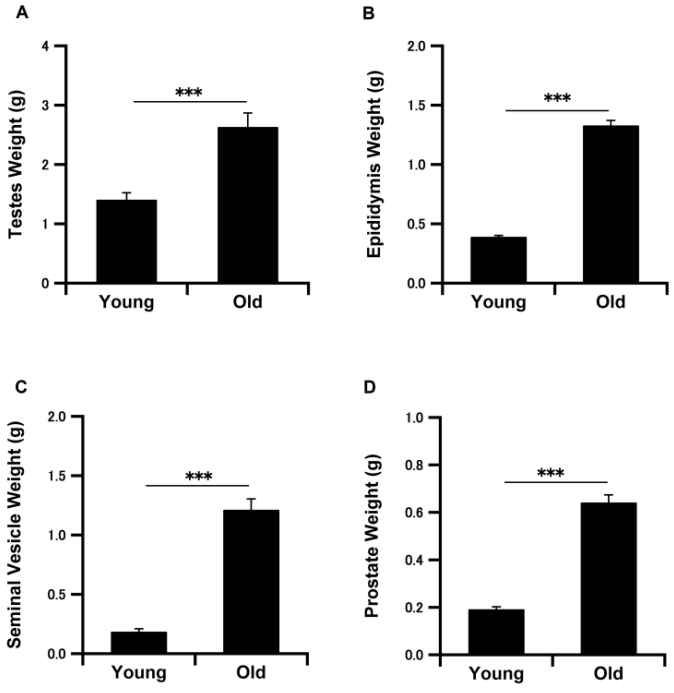

Figure 2. Weight of reproductive organs in the young and old groups. (A) Testes weight (g); (B) epididymis weight (g)**;** (C) seminal vesicle weight (g); and (D) prostate weight (g). Results are means ± standard error of the mean (n = 25 in each group). ***p ≤ 0.001 by Student's *t*-test.

The old group exhibited a substantial increase in prerenal, epididymal, inguinal fat, and BAT weight in comparison to the young group (Figures 3A-D, $p < 0.001$ by Student's *t*-test).

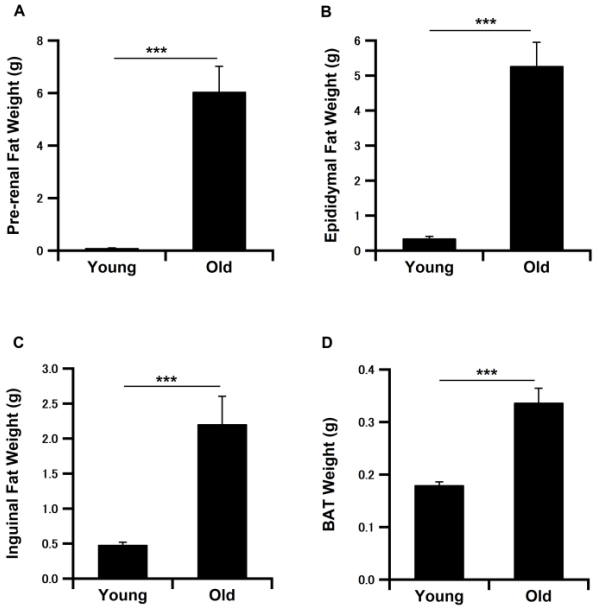

Figure 3. Weight of different body fat in the young and old groups. (A) prerenal fat weight (g); (B) epididymal fat weight (g)**;** (C) inguinal fat weight (g); and (D) brown adipose tissueweight (g). Results are means ± standard error of the mean (n = 25 in each group). ***p ≤ 0.001 by Student's *t*-test.

The old group's muscles, namely the gastrocnemius, soleus, and EDL muscles, had much higher weights (Figures. 4A-C, $p < 0.001$ by Student's *t*-test).

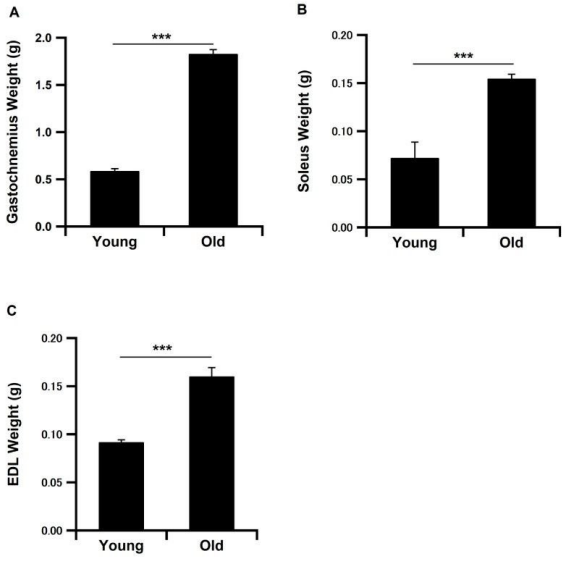

Figure 4. Weight of different skeletal muscles in the young and old groups. (A) Gastrocnemius weight (g); (B) soleus weight (g); and (C) extensor digitorum longus weight (g). Data are means ± standard error of the mean (n = 25 in each group). ***p ≤ 0.001 by Student's *t*-test.

Effect of aging on glucose and corticosterone concentrations, hematological and semen parameters

Glucose concentration significantly declined in the old group when compared to the young group (Figure. 5A, $p < 0.001$ by Student's *t*-test). The levels of corticosterone were significantly decreased in the old rats as compared with young rats (Figure. 5B, $p < 0.001$ by Student's *t*-test).

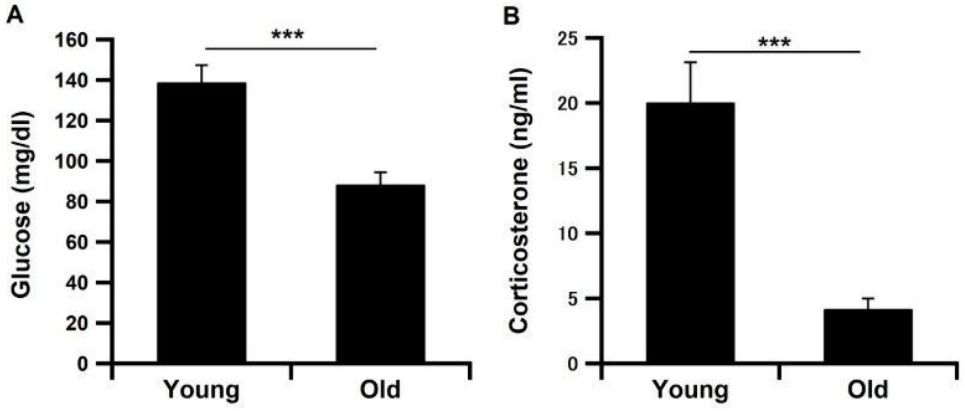

Figure 5. Blood concentrations of glucose and corticosterone in the young and old groups. (A) glucose concentration (mg/dL) and (B) corticosterone (ng/mL) concentrations in the blood of the young and old rats. Data are means ± standard error of the mean (n = 25 in each group). ***p ≤ 0.001 by Student's *t*-test.

All RBCs count (4.113 $\times 10^6$ ± 0.316 /cmm), Hb concentration (12.261 ± 0.817 g/dL), PCV % (35.77 ± 1.806%), and MCHC (34.161± 0.805%) were significantly decreased. However, MCV (88.001± 3.483 fl) and MCH (29.958± 0.812 pg) were increased in the old group while the young rats displayed significant increase in all RBCs count (5.991 $\times 10^6$± 0.158 /cmm), Hb concentration (15.8 ± 0.405 g/dL), PCV % (38.375± 0.270%), and MCHC (41.135± 0.781%). However, MCV (63.728± 1.161fl) and MCH (26.121± 0.1007 pg) were markedly decreased when compared to old rats. (Table 1, $p < 0.001$ by Student's *t*-test). The count of WBCs and differential leucocytic count revealed non-significant difference between the two groups.

Table 1: Hematologic analysis in rats (mean ± SE)

Parameter	Young	Old	Student's *t*-test *P* value
RBCs ($\times 10^6$/cmm)	5.991 ± 0.158	4.113 ± 0.316	$P < 0.001$
Platelet count ($\times 10^3$/cmm)	698.166 ± 60.167	737.833 ± 71.984	Non significant
HB g/dL	15.8 ± 0.405	12.261 ± 0.817	$P < 0.001$
PCV (%)	38.375 ± 0.270	35.77 ± 1.806	$P < 0.001$
MCV (fL)	63.728 ± 1.161	88.001 ± 3.483	$P < 0.001$
MCH (pg)	26.121 ± 0.1007	29.958 ± 0.812	$P < 0.001$
MCHC (g/dL)	41.135 ± 0.781	34.161 ± 0.805	$P < 0.001$
WBCs ($\times 10^3$/cmm)	9.808 ± 1.006	10.045 ± 1.503	Non significant
Lymphocytes (%)	85.264 ± 0.712	86.471 ± 0.561	Non significant
Neutrophils (%)	10.041 ± 0.852	9 ± 0.730	Non significant
Basophils (%)	0.132 ± 0.008	0.16 ± 0.014	Non significant
Eosinophils (%)	0.281 ± 0.014	0.271 ± 0.014	Non significant
Monocytes (%)	4.238 ± 0.149	4.181 ± 0.210	Non significant

Abbreviations: RBC, red blood cell; WBC: white blood cell; Hct, hematocrit, MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration. $P < 0.001$ by Student's *t*-test,

Old rats displayed significant higher concentrations of sperms (45.5 ± 9.3 (ml X 125 X 10)) than that of the young (2.6 ± 1.4 (ml X 125 X 10) and the percentage of abnormal sperms (41.5 ±2.7%) were significantly increased in the old group compared to the young group (18.5 ± 1.25%) (Table 2, $p < 0.001$ by Student's *t*-test), while sperm motility (44.166 ± 13.8%) was significantly reduced in the old group as compared to the young one (75 ± 3.8%) (Table 2, $p < 0.05$ by Student's *t*-test).

Table 2: Sperm parameters in the young and old groups (mean ± SE).

Parameter	Young	Old	Student's *t*-test *P* value
Sperm Cell Concentration (ml X 125 X 10^4)	2.6 ± 1.4	45.5 ± 9.3	$P < 0.001$
Sperm Motility (%)	75 ± 3.8	44.166 ± 13.8	$P < 0.05$
Sperm Abnormalities (%)	18.5 ± 1.25	41.5 ±2.7	$P < 0.001$

Discussion

Aging is a complicated biological process that involves an organism's structural and functional changes throughout time [17]**.** These alterations usually start (at conception) early in life and end with physical death [2]. Rat average lifespans are typically about 3 years [18]. Rats grow quickly, and their adolescence ends by the end of the second month of ontogenesis. As a result, a rat is considered an adult at 2 months of age, at which point all of its vital systems have matured [18-20]. The signs of aging start to appear in rats after 6–7 months [21]**.** Moreover, age-related differences in telomere length and mRNA expression of telomerase between 14 months and 4 months old SD rats has been reported [22]. Therefore, in our study, rats with the age of 14 months were suitable for monitoring aging changes.

The physiologic functions that are dependent on both environmental and genetic factors gradually deteriorate with age. In reality, it is well recognized that the majority of chronic illnesses linked to aging are largely caused by age-related changes in immunological, metabolic, and stress-response functions. Adipose tissue is a significant source of proinflammatory cytokines during the aging process [23]. Lean body mass tends to be masked by an increase in fat mass, but the progressive decline in fat-free mass with aging is mostly caused by loss of muscle and bone mass so body weight rises with aging [24]. Rats body weight is enhanced by increasing age [25, 26], which supports our result. BMI is the metric currently used for defining anthropometric height/weight features in adults and additionally serves to classify them. BMI represents an indicator of an individual's level of fatness. It is also frequently employed as a risk factor for the emergence of the prevalence of a variety of medical issues. But it's becoming more and more obvious that BMI is a rather poor indication of body fat percentage [27]. As it does not distinguish between body fat and body lean mass, a person with a high BMI may also have extremely little fat mass, and vice versa, which presents a unique challenge for the BMI as an indicator of obesity [28]. A relationship between Leptin and BMI in rats was reported in rodents [29, 30]. Adipocytes act as endocrine cells and release a wide range of hormones, including leptin, adiponectin and adipsin [31]. Leptin is a hormone produced by adipocytes that controls hunger and energy expenditure. Its plasma levels and gene expression are indicators of body fat contents [31-34]. As a result, a threshold BMI is suggestive of obesity and may be used to forecast the negative effects of obesity in rats [25]. Rats that were 30 days old had decreased belly circumference and BMI; these values enhanced dramatically as the rats aged up to 90 days, after which they stayed constant [25], which goes on with our results in which, the mean BMI was significantly increased in the old group than young one.

In the present study, significant increases in the weight of male reproductive organs including testes, epididymis, seminal vesicle, and prostate were reported, which corresponds to the increase in body weight. Testicular weight and volumetric proportion showed significant changes during the maturation

phase, which were mostly attributed to the addition of spermatogenic cells [35] and the enlargement of Leydig cells [36]. At 450 days of age, a minor decline in the weight of the testes, cauda, and caput epididymis has been demonstrated [26]. Male fertility is significantly impacted by aging. The various ways that aging affects sperm motility, sperm morphology, and sperm concentration suggest that spermatozoa quality deteriorates with age [37]. Age-dependent declines in sperm quality have been shown in rats [38]. With age, male rats developed morphological changes in their prostates [39]; these alterations may have something to do with secretion production. Additionally, it has been noted that testosterone is required to maintain the weight and activity of the accessory sex glands and testes, as well as to continually generate sperm with proper motility [40]. A decrease in the testis's sperm concentration with aging has been reported [38, 41]. Spermatozoa initially appeared in the caput epididymis of 45-day-old and the cauda epididymis of 52-day-old rats. Before this time, the reproductive system was essentially free of spermatozoa. Up to 100 days of age, the number of sperm in the vas deferens and cauda epididymis kept growing. Even at 450 days, the male SD rat still contained 223.0 million spermatozoa [26]. A decrease in the proportion of motile spermatozoa occurs in old rats [38, 42], which supports our results. The lack of sperm motility was not due to sperm death but to the lack of prostatic secretions. An impairment in the prostatic function of elderly male rats may account for sperm immobility [38]. Prostate secretions are recognized to be essential for sperm motility [43]. In the present study, aging induced an increase in the number of morphologically abnormal sperms. A similar increase in the percentage of spermatozoa with cytoplasmic droplets in the cauda epididymis has been demonstrated [42]. It has been found that 99% of the young males' sperm have normal morphology [44], which coincides with our results.

Older rodents are typically obese [45, 46]. Compared to young rats, older rats were almost twice as obese [47]. Any appreciable increase in body weight or energy content above control animals is typically considered obesity [48]. Age-related alterations in anthropometric characteristics, such as an increase in visceral adipose tissue and fat mass, have been linked to the development of metabolic diseases in both humans and rats, including insulin resistance, diabetes, and obesity [49, 50]. Elevated quantities of circulating fat secretory factors, including free fatty acids, leptin, and adiponectin, may result in insulin resistance when intraabdominal fat is raised, as seen in rats during normal aging [51]. It was reported that older rats have higher pre-renal and epididymal fat weights than younger rats [47], which is in agreement with our results.

Sarcopenia is the term for this type of muscle loss, which is linked to a decline in mobility and, eventually, illness and death [52]. Studies on rats reported that skeletal muscle mass diminishes with aging [53]. Previous research suggested that the loss of muscle fibers was mostly responsible for this drop in muscle mass [54]. Nevertheless, in a subsequent study, the soleus and EDL muscles of Wistar rats did not exhibit a reduction in the total number of muscle fibers as they aged [55]. However, in the present study, we investigated three examples of skeletal muscles namely; gastrocnemius, soleus, and EDL, which showed an increase in weight with aging. The discovery that rats' muscle mass declines with age is not always true, since research on at least three distinctive skeletal muscles has not revealed a decline in weight with aging [56, 57]. Since the size of individual

muscle fibers does not change until very old age, the reported loss in muscle mass in late middle and early old age, as seen in rat muscles, must be a consequence of a drop in the total number of muscle fibers [58, 59]. Rats' gastrocnemius muscle weight gradually increased from 6 to 18 months of age and then gradually atrophied until they were 27 months old [60]. In male SD rats growing, the weight of the soleus and EDL increased almost sixfold between 25 and 165 days of age, corresponding to their increase in body weight [61]. Thus, a decrease in the overall number of fibers, a decrease in the size of individual fibers, a decline in excitation and contraction coupling, and/or a decrease in the activation of high threshold motor units could all be contributing factors to the loss of muscle function associated with age [62].

An overall deterioration in body function in aging is linked to a reduction in homeostasis maintenance. Normal glucose homeostasis is maintained by the interplay between peripheral insulin sensitivity and β cell insulin production. When β cells are unable to secrete enough insulin to keep blood sugar levels within normal range, diabetes mellitus results. The impact of aging on β-cell function has been controversial. β-cell function has been reported to either be decreased [63] or unchanged [64]. Muscle mass is the primary tissue that contributes to insulin-mediated glucose disposaltherefore, its loss leads to insulin resistance [65]. In contrast, increased muscle mass is linked to improved insulin sensitivity and a decreased chance of developing pre-diabetes mellitus [66, 67]. This may partially explain the decreased blood glucose levels in the old group in our study as increase muscle mass has been shown to significantly decrease blood glucose levels [68, 69] and muscles have a higher capacity to store blood glucose in the form of glycogen, helping in decreasing blood glucose levels. It is believed that corticosteroids play a crucial role in maintaining homeostasis [70]. Landfield proposed that excessive corticosteroid exposure could hasten the aging process of the brain [71, 72]. According to Van Eekelen *et al*., young (3 months) and old (30 months) Brown Norway rats did not differ in their basal corticosterone levels, stress-induced corticosterone response profile, or magnitude [73]. Some studies reported that basal levels of corticosterone in the blood increase with aging [71, 74]. It has been observed that in rats, extended stress causes an increase in plasma corticosterone as a function of age [75]. Contrary to our findings, the levels of corticosterone exhibited a significant decrease with aging indicating age-induced dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis. Age-produced dysfunction in the HPA axis has been previously reported [76]. It has been reported that adrenal insufficiency and low cortisol levels are associated with low blood glucose levels in human [77]. Therefore, herein, the decline in corticosterone levels may be attributed to lowering blood glucose levels in the old group as corticosterone deficiency can lead to decreased glucose production by the liver leading to hypoglycemia.

Despite the blood is the only component capable of self-renewal in the body, it suffers from the detrimental consequences of aging in human populations [78]. Aging has a modest impact on the hematopoietic system, and these effects generally become noticeable over the age of 65. The hematopoietic marrow's volume is continuously decreasing with age, which results in a minor drop in the population of mean Hb concentration in men but no appreciable changes in granulocyte, monocyte, or platelet count [79]. The RBCs count in male rats at 2 and 3 months, showed a significant increase,

accompanied by a parallel decrease in MCV and MCH. Subsequently, age-related declines in RBCs count, total hemoglobin, and hematocrit levels occurred. Moreover, MCV and MCH increased when the RBCs count decreased [80] which is in agreement with our findings. For SD rats (2–29 months old), the MCHC remained fairly stable [80].

Conclusion

In conclusion, the present study demonstrated changes in various physiological parameters in young and old male SD rats, which may be beneficial for further studies on the aging process for enhancing quality of life.

Declaration of competing interest

No financial or other conflicts of interest have been revealed by the authors.

Data availability

The corresponding author can provide the data that were utilized to support the study's conclusions upon request.

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الملخص العربى

التغيرات الدموية، والأنثروبومترية، والكيميائية الحيوية، والإنجابية الناتجة عن الشيخوخة في ذكور الجرذان

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الشيخوخة هي عملية بيولوجية معقدة تتميز بتراكم التغيرات مع مرور الوقت مع فقدان التوازن الفسيولوجي. تلك التغيرات المرتبطة بالعمر تحدث في جميع أنسجة الجسم وتؤثر على عمل جميع أجهزته. الغرض من هذه الدراسة هو دراسة تأثير الشيخوخة على كفاءة الذكر الإنجابية، ووزن الدهون والعضلات الهيكلية المختلفة في الجسم، والمؤشرات الدموية في ذكور فئران Sprague Dawley. تم تقسيم 50 فأراً ذكراً عشوائياً إلى مجموعتين (العدد = 25/مجموعة): مجموعة صغيرة السن ومجموعة كبيرة السن. لقد تمت تربية الفئران في المجموعة المسنة لمدة 12 شهرًا. وتمت مراقبة التغيرات في وزن الجسم أسبوعياً. وأظهرت النتائج أن مؤشر كتلة الجسم (BMI) ومتوسط محيط البطن وأوزان الخصيتين والبربخ والحويصلة المنوية والبروستاتا زادت في المجموعة المسنة. بالإضافة إلى ذلك، قد زادت أوزان الدهون الكلوية، دهون البربخ، والدهون الأربية، و الأنسجة الدهنية البنية (BAT) في المجموعة كبيرة السن. كما تم أيضًا زيادة أوزان عضلة الساق، والنعلية، والعضلة الباسطة للأصابع الطويلة (EDL) بشكل ملحوظ في الجرذان المسنة. وقد لوحظ انخفاض مستويات الجلوكوز في الدم و الكورتيكوستيرون بشكل ملحوظ بالتقدم في العمر. كما انخفض عدد كريات الدم الحمراء (RBCs, 4.113 ×106/cmm ± 0.316، و تركيز الهيموجلوبين (Hb, 12.261 ± 0.817 g/dL)، والنسبة المئوية لحجم خلايا الدم المجمعة (PCV, 35.77 ± 1.806%، متوسط تركيز الهيموجلوبين في كريات الدم الحمراء (MCHC, 34.161± 0.805%) بينما زاد متوسط حجم كريات الدم الحمراء (MCV, 88.001± 3.483 fl) ومتوسط هيموجلوبين كريات الدم الحمراء (MCH, 29.958± 0.812 pg)مع التقدم في العمر. وقد ازداد تركيز الحيوانات المنوية في المجموعة كبيرة السن (45.5 ± 9.3 ml X 125 X 10)وتشوهاتها (41.5 ±2.7%) بينما انخفضت حركتها (44.166 ± 13.8%) في تلك المجموعة. نستنتج من ذلك أن التقدم في السن له تأثيرات سلبية على بعض المعايير الدموية والإنجابية في ذكور الجرذان. ولذلك فإن العمر شئ محوري ينبغي العناية والاهتمام به.