American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

Exercise Training Prevents Age-induced Insulin Resistance in Rats: Effect on Circulating Catecholamines, Inflammatory Cytokines and Skeletal Muscle Glut4 Transporters

Ola A. EL-Gohary

Department of Physiology
Faculty of Medicine, Benha University, Benha, Egypt.
*Corresponding Author
MDr. Ola Ahmed El-Gohary
Physiology department
Benha Faculty of Medicine
Benha, Qualubia
Egypt
E.Mail: olaahmed202020@gmail.com
Mobile: 00201284499665.

Received: 03 July 2017; | Revised: 02 August 2017; | Accepted: 13 August 2017

Abstract

The incidence of insulin resistance increases with age. This work tested the effect of exercise on age-induced insulin resistance and the possible involved mechanisms. Six groups of rats were used: 4 months old, 14 months old and 24 months old, either with and without exercise training. Swimming exercise training was performed 2h/day, 5days/week for 6 weeks. Insulin resistance was assessed by measuring serum glucose, serum insulin and homeostasis model assessment of insulin resistance (HOMA-IR) index. Serum levels of catecholamines, inflammatory cytokines and total antioxidant capacity (TAC) together with skeletal muscle Glut4 mRNA expression were assessed. Aged rats developed insulin resistance associated with increased serum catecholamines and inflammatory cytokine levels, decreased serum TAC and suppression of skeletal muscle Glut4 expressions. Exercise training reversed the developed insulin resistance and restored the values of catecholamines, inflammatory cytokines and skeletal muscle Glut4; however, it did not modify TAC. It was concluded that exercise could reverse aging-induced insulin resistance in rats by decreasing catecholamines and inflammatory cytokines production and also by increasing Glut4 expression in skeletal muscles.

Keywords: Exercise, Insulin resistance, Aging, Catecholamines, Pro-inflammatory cytokines, Total antioxidant capacity, Glut4 transporters

1. Introduction

Aging can be simply defined as a process of gradual reduction in the functional reserve of the body [1,2]. It is characterized by a progressive decline in the ability of the body to maintain homeostasis [3,4]. Aging is associated with an increase in insulin resistance [5]. Some researchers [6,7,8] have suggested assigning insulin resistance to aging-associated obesity, instead of to aging itself. However, Fink et al. [5] showed development of insulin resistance in non-obese elderly humans, suggesting that the increase in insulin resistance was a function of age.

Several mechanisms involved in aging-induced insulin resistance, specially oxidative stress [9]. Antioxidant enzymes were significantly decreased in old rats compared to young rats, suggesting a reduction in the capacity of the body to remove free radicals in old rats. [10]. Ageing is also associated with increased inflammatory activity, including elevated circulating levels of tumor necrosis factor (TNF)-α [11,12] and interleukin (IL)-6 [11,13,14]. These cytokines are closely linked to insulin resistance [15,16]; so increased inflammatory activity in the elderly may reflect age-related insulin resistance. In addition, plasma norepinephrine concentration was found to be ~66% higher in older than in younger males. The enhanced circulating catecholamines ultimately lead to insulin resistance [8].

The ability to control the blood glucose level progressively reduces during the life span [17,18]. A gradual decline in skeletal muscle glucose uptake was observed during the phase of growth [18,19]. Variations of glucose transporters (GLUTs) in the skeletal muscle have been reported, namely, the glucose transporter type 4 (GLUT4). GLUT4 is a protein that exists in many tissues specifically in skeletal muscle. This protein is required for glucose uptake from plasma and reduction of blood glucose [20,21].

Exercise training is recorded as an effective method for both the prevention and the reversal of insulin resistance; however, how exercise training ameliorates insulin resistance is still unknown [22,23]. Swimming exercise is an aerobic exercise that uses large-muscle groups [24,25]. Aerobic exercise is generally prescribed to diabetic patients, as it treats associated glucose abnormalities [26].

Physical exercise has possessed a key role in tissue homeostasis, associated with increased antioxidant defenses as well as decreased systemic inflammation [27,28,29]. In addition, although exercise training can be considered as a stressor that is able to increase plasma catecholamine concentrations; [30] however, catecholamine concentrations are influenced by many factors as gender and age in response to exercise [31].

There is evidence that exercise training has a role in skeletal muscle regulation of glucose metabolism. Muscular glucose uptake during exercise can increase up to 50-fold [32]. Glucose uptake by contracting skeletal muscle depends on the presence of GLUT4, which is the main glucose transporter existed in skeletal muscle [33].

The goal of the present study was to characterize the development of insulin resistance during the natural aging process in 4-month-old (young), 14-month-old (middle-aged), and 24-month-old (old) male Wistar rats, assessed by measuring fasting serum glucose level, fasting serum insulin level and homeostasis model assessment of insulin resistance (HOMA-IR) index. Also, the potential effect of physical exercise was evaluated in order to develop strategies that allow old individuals who developed insulin resistance to perform exercise in a safe way that contributes to the glycemic control. With a trial to clarify some of the possible involved mechanisms, several parameters were assessed including serum catecholamines, inflammatory markers and total antioxidant capacity (TAC) in addition to skeletal muscles GLUT4 transporters.

2. Materials and Methods

2.1 Animals

The present study was conducted on 48 adult male albino Wistar rats. Animals were housed in the animal laboratory at the medical research center of the Faculty of Medicine, Benha University. They were housed at room temperature (25 °C) with a 12 h light: 12 h dark cycle. Animals were fed with a balanced diet and tap water. The animal procedures were performed in accordance with the guidelines of the Ethics Committee, Faculty of Medicine, Benha University.

2.2 Experimental design

Rats were randomly divided into six equal groups (n:8). Group I (4M group): 4 months old rats (similar to 14 years of age in humans) [34] without swim exercise training. Group II (4ME group): 4 months old rats (similar to 14 years of age in humans) with swim exercise training for 6 weeks [35]. Group III (14M group): 14 months old rats (equivalent to 50 years of age in humans) [34] without swim exercise training. Group IV (14M-EX group): 14 months old rats (equivalent to 50 years of age in humans) with swim exercise training for 6 weeks [35]. Group V (24M group): 24 months old rats (approximately 84 years of age in humans) [34] without swim exercise training. Group VI (24M-EX group): 24 months old rats (approximately 84 years of age in humans) with swim exercise training for 6 weeks [35]. Body weight was recorded weekly during the experimental period, and before decapitation for all groups.

At the end of the experiment period, animals were anaesthetized with urethane (1.5 g/kg; i.p.) after 12 hour fasting. Rats were then sacrificed by decapitation and blood samples were collected through cardiac puncture for serum separation and estimation of fasting glucose level, fasting insulin level, HOMA-IR index, TAC, inflammatory markers (TNF-α and IL-6) and catecholamines. The hindlimb gastrocnemius muscle was rapidly removed from each rat, washed with ice-cold normal saline and stored at -80°C for further analysis of GLUT4 mRNA expression using real time RT-PCR.

2.3 Exercise protocol

Swimming exercise was done in a circular tank 80 cm in diameter and 90 cm in height, filled to 60 cm mark with 32- 35°C water. The protocol of swim – training (equivalent to moderate training) was 2 hours/day, 5 days/week for 6 weeks. In the first week, the duration of the swimming session was slowly increased from 20 minutes in the 1st day to reach 120 minutes by the 5th day for adaptation (progressive training) [35]. To serve as controls for the effects of handling and exposing to water, sedentary control rats were immersed in water for 5 minute at the beginning of every swimming session.

2.4 Biochemical analysis

Blood samples were allowed to clot and serum was separated by centrifugation at 3000 revolution per minute (rpm) for 15 min and stored at 20°C in dark containers for biochemical assessment;

Determination of Fasting Serum Glucose, fasting serum Insulin and HOMA-IR:

Fasting serum glucose was estimated by the glucose oxidase–peroxidase method (GOD–POD kit). Fasting serum insulin level was carried out using an enzyme linked immunosorbent assay kit (ELISA, Boehriger Mannheim Immunodiagnostics, and Mannheim, Germany). Insulin resistance was assessed by HOMA (homeostatic model assessment) using the following formula: HOMA-IR = fasting glucose value (mg/dl) × fasting insulin value (μU/ml)/405. A HOMA value that is > 2 was used to identify significant insulin resistance [36].

Determination of serum TAC:

To measure TAC of serum, Ferric reducing antioxidant power (FRAP) assay developed by Benzie and Strain [37] was carried out on serum samples and the reduction of the ferric to ferrous ion by antioxidants was assessed using spectrophotometry at 593 nm wavelength.

Determination of serum TNF-α and IL-6:

Serum TNF-α and IL-6 was determined using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota, USA).

Determination of serum catecholamines:

The quantitative determination of serum catecholamines was performed using rat adrenaline and Noradrenaline ELISA kits (Labor Diagnostica Nord GmbH & Co. KG, Germany), according to the manufacturer's instructions.

2.5 RT-PCR analysis

The tissue was homogenized using a Mixer Mill MM400 (Retsch, Germany). Total RNA was isolated from 25 mg tissue using total RNA purification kit(Jena Bioscience Germany). The concentration and purity of the RNA were determined by measuring the absorbance at 260/280

nm. 2 μg mRNA was reverse transcribed into cDNAs by using the iScript cDNA kit (Bio-Rad, Hercules, CA, USA). The amount of GLUT4 mRNA was determined with ABI Prism 7900HT quantitative real-time PCR (Applied Biosystems, Foster City, CA). The primers were as follows: GLUT4 (forward: 5′-GTGTGGTCAATACCGTCTTCACG-3′; reverse: 5′-CCATTTTGCCCCTCAGTCATTC-3′). PCR amplification was carried out in a 20μl reaction mixture (2 μl of cDNA and 200 nmol/l primers for GLUT4 and 1 μl SYBR green). The temperature program was as follow: inactivation of reverse transcriptase at 95°C for 15 min, followed by 40-cycles of 95°C for 15 s, 55°C for 30 s, and 76°C for 30 s. The specificity of the PCR results was confirmed by dissociation curve analysis. According to the RQ manager program ABI SDS software (ABI 7900), the data are produced as sigmoid shaped amplification plots in which the number of cycle is plotted against fluorescence (when using linear scale). The housekeeping gene β-actin was used as a control with the following primers: (forward, 5′-TGG CAC CAC ACC TTC TAC AA-3′ and reverse, 5′-TCA CGC ACG ATT TCC CTC TC-3′) Fluorescent emission data were captured and mRNA levels were analyzed using the critical threshold (CT) value. The ΔCT was calculated by subtracting the CT for β-actin from the CT for the gene of interest and divided by the ΔCT of a control sample on every plate to control for any plate-to-plate variation. The relative expression of the gene is calculated using the expression 2−ΔCT and reported as arbitrary units.

2.6 Statistical analysis

All analyses were performed using the program "Statistical Package for Social Sciences (SPSS) version 16" (SPSS Inc, Chicago, IL, USA). The data were presented as mean ± standard deviation (SD). Student's t-test was used for comparison between individual groups. Probability $p < 0.05$ was considered statistically significant.

3. Results

Effect of exercise training on body weight, serum glucose level, serum insulin level and HOMA-IR index in 4, 14, and 24 month-old rats (Table 1):

The rats of the two experimental groups at the same age had nearly the similar initial body weights. Swim exercise training for 6 weeks did not significantly modify body weight within groups of the same age (Table 1).

The analysis of insulin resistance-related parameters revealed significant elevation of serum glucose level, serum insulin level and HOMA-IR index in 14 M and 24 M rats as compared to 4 M rats ($p < 0.05$). Exercise training did not modify serum glucose level, serum insulin level or HOMA-IR index in 4 M rats, but completely reversed the aging-induced insulin resistance developed both in 14 M and 24 M animals (Table 1).

Effect of exercise training on serum catecholamines, serum TAC and serum pro-inflammatory cytokines in 4, 14, and 24 month-old rats (Table 2):

Aging significantly increased serum catecholamines (adrenaline and noradrenaline) in 14 M and 24 M rats compared to 4 M rats ($p < 0.05$). Exercise training did not modify plasma catecholamines in 4 M animals; however, in 14 M and 24 M rats, exercise training prevented the increase in circulating catecholamines (Table 2).

Aging significantly decreased TAC in 14 M and 24 M rats compared to 4 M rats ($p < 0.05$). Exercise training did not significantly modify TAC either in 4M rats or in 14M and 24M rats (Table 2).

As regard pro-inflammatory cytokines, TNF-α and IL-6 increased significantly in 14 M and 24 M rats compared to 4 M rats ($p < 0.05$). However, these elevated cytokines showed significant reduction in rats subjected to exercise training ($p<0.05$) compared to sedentary rats. (Table 2).

Effect of exercise training on skeletal muscles GLUT4 mRNA expression in 4, 14, and 24 month-old rats (Fig. 1):

The expression of Glut4 transporters in skeletal muscle significantly decreased by ~ 50% and ~ 70% in 14 M and 24M respectively compared to 4 M rats ($p < 0.05$). Exercise training restored Glut4 expression to control levels,Fig. 1

Table 1: Effect of exercise training on body weight, serum glucose level, serum insulin level and HOMA-IR index in 4-, 14-, and 24-month-old rats.

	4- month-old group		14- month-old group		24- month-old group	
	Without exercise	With exercise	Without exercise	With exercise	Without exercise	With exercise
Body weight (g)	392±13	395±16	503±22*	508±28	711±34*	715±45
Serum Glucose (mg/dl)	96.6±2.71	97.5±4.63	127.2±3.87*	98.4±4.69#	129.2±3.24*	97.1±2.68#
Serum insulin (μIU/ml)	4.2±0.86	4.4±0.79	5.3±0.34*	4.7±0.18#	5.5±0.45*	4.6±0.29#
HOMA-IR index	1.02±0.53	1.04±0.37	2.13±0.51*	1.03±0.20#	2.18±0.24*	1.05±0.19#

HOMA-IR, homeostasis model assessment of insulin resistance. Data is expressed as mean ± standard deviation (n = 8 per group). $P < 0.05$ is significant tested by using One-way analysis of variance and Student's t-test. * $P < 0.05$ compared with 4-month-old rats without swim exercise training; # $P < 0.05$, compared with values without swim exercise training within groups.

Table 2: Effect of exercise training on serum catecholamines, serum inflammatory cytokines and serum total antioxidant capacity in 4-, 14-, and 24-month-old rats.

	4- month-old group		14- month-old group		24- month-old group	
	Without exercise	With exercise	Without exercise	With exercise	Without exercise	With exercise
Adrenaline (pmol/ml)	26.3±2.42	27.5±3.36	35.1±1.81*	30.5±3.53#	39.0±2.12*	33.1±4.26#
Noradrenaline (pmol/ml)	18.7±3.51	19.0±2.40	34.2±1.24*	22.5±2.62#	38.3±3.44*	27.0±3.14#
TAC (mmol/l)	1.61±0.03	1.59±0.02	1.16±0.03*	1.15±0.01	1.03±0.04*	1.01±0.03
Serum TNF-α (pmol/ml)	42.6±24	41.6±19	86.1±16*	57±12#	123±23*	89±33#
Serum IL-6 (pmol/ml)	39.2±27	40.2±20	63±18*	48±22#	85±21*	56±19#

TAC, total antioxidant capacity; TNF-α, tumor necrosis factor alpha; IL-6, interleukin -6. Data is expressed as mean ± standard deviation (n = 8 per group). $P < 0.05$ is significant tested by using One-way analysis of variance and Student's t-test. * $P < 0.05$ compared with 4-month-old rats without swim exercise training; # $P < 0.05$, compared with values without swim exercise training within groups.

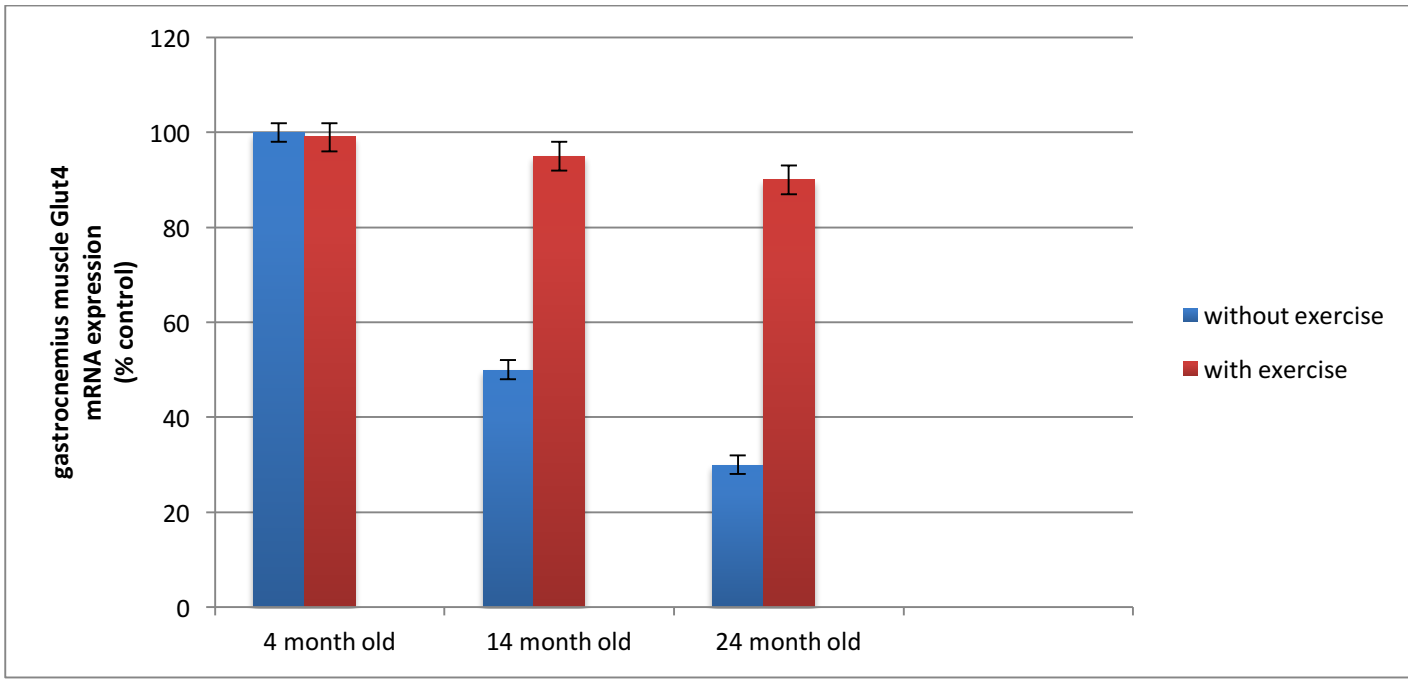

Figure 1: Effect of exercise training on skeletal muscle GLUT4 mRNA expression in 4-, 14-, and 24-month-old rats.

GLUT4, glucose transporter 4. Data is expressed as mean ± standard deviation (n = 8 per group). $P < 0.05$ is significant tested by using One-way analysis of variance and Student's t-test. * $P < 0.05$ compared with 4-month-old rats without swim exercise training; $^{\#}$ $P < 0.05$, compared with values without swim exercise training within groups. The values represent the mRNA expression levels relative to control (Control group set as 100%).

4. Discussion

The interaction of multiple factors associated with aging contributes to age-related changes in glucose tolerance found in elderly population. [39,40]. The current study examined the development of insulin resistance in a naturally aging rat model. It was found that aged rats developed an increase in insulin resistance represented by, significant elevation of serum glucose, serum insulin and HOMA-IR index. This finding is in agreement with previous observations [41,42]. Swim exercise training for 6 weeks was seen to reverse insulin resistance induced by aging in rats. These findings are in line with recent studies reported that exercise could ameliorate insulin resistance [43,44,45,46]. To understand the mechanisms underlying the impact of exercise on age- induced insulin resistance, serum levels of catecholamines, pro-inflammatory cytokines and TAC together with Glut4 mRNA expression level in skeletal muscles were assessed.

The present data showed increased level of circulating catecholamines in aged animals, an effect that is supported by a previous report of Di Nardo's group [47]. The elevated levels of catecholamines could be explained by a significant reduction in catecholamines clearance from plasma [48]. Catecholamines play an important role in carbohydrate metabolism. Their hyperglycemic actions are exerted directly (by stimulating both hepatic gluconeogenesis and glycogenolysis, leading to increased hepatic glucose production) [49], and indirectly (by inhibition of pancreatic insulin secretion) [50]. Nevertheless, their main effect is inhibitory on glucose uptake by the muscles. Exercise training restored circulating adrenaline and noradrenaline in aged animals to values observed in young 4M control rats. This reduction in catecholamines concentration during exercise can be explained by the increase in their clearance, which according to Sacca et al. [51] due to increased blood flow in the tissues that are responsible for elimination of these hormones.

Aging is also associated with increased levels of serum inflammatory cytokines in the examined rats models. The 14 M and 24 M animals showed elevated levels of TNF-α and IL-6 compared to the 4 M control group. Abood and Alghamdi [52] recently showed a highly significant positive correlation between insulin resistance index (HOMA-IR) and proinflamatory cytokines in aged rats. They attributed the state of age related insulin resistance to the state of chronic inflammation displayed in old rats. Inflammatory cytokines such as TNF-α and IL-6 have been related to reduce GLUT4 expression [53,54], consequently lowering glucose uptake by muscle [55,56,57]. Plomgaard [58]recently reported that an increase in plasma levels of TNF-α down regulates insulin signaling and whole body glucose uptake because of a decreased insulin-mediated glucose uptake in the skeletal muscle with an unchanged endogenous glucose production. Exercise significantly reduced levels of TNF-α and IL-6 in 14-month and 24-month-old rats, supporting the role of exercise in the improvement of age-induced insulin resistance. Several interventional studies report that exercise reduces inflammatory markers such as TNF-α and IL-6, in the elderly [59,60,61].

The availability of antioxidants has become altered with age. The current study indicated that antioxidant enzyme activity was significantly reduced in old rats as compared with young rats, suggesting a decrease in the capacity of the body to remove free radicals in old rats, an effect that is in accordance with the work of other investigators [34]. In the absence of an appropriate compensatory response from the endogenous antioxidant network, deregulation in intracellular signaling will develop, and ultimately resulting in a pathological situation including insulin resistance [62]. In the present study, exercise training did not significantly modify TAC in aged animals. This result suggested that acknowledged mechanism of the action of exercise through its antioxidant activity could not be involved in the reversal of age-related insulin resistance. There are few data concerning the exercise response of antioxidant enzymes at old age. Ji [63], in accordance with our results found no significant alteration in antioxidant enzymes in old rats after 1 h treadmill running. On the contrary, Teixeira-Lemos et al. [64] has recently found exercise to improve glucose metabolism and TAC. The effect of exercise depends mainly upon the type of exercise, its intensity, frequency, and duration [65]. Low-intensity treadmill exercise for 4 weeks is effective for improving TAC in diabetic rats [66]. Also, eight weeks of moderate- intensity treadmill exercise is an appropriate method for enhancement of TAC [67]. On the other hand, swimming exercise with moderate intensity for 10 weeks has no effect on serum TAC [68]. Also, there were no significant changes in TAC in the plasma of rats after swimming exercise at 2 different intensities; low intensity or high intensity [69]. Although it appears contradictory, these data suggest that for this specific variable (TAC), exercise type is more important than intensity.

An age-related reduction of skeletal muscle GLUT4 expression in the present study could be expected. It was reported that 24-month-old animals that developed insulin resistance, had lower skeletal muscle Glut4 protein expression compared to 3-month control group [70]. Insulin resistance may result from diminished capacity for GLUT4 synthesis [71]. Exercise training increased skeletal muscle Glut4 expression in 14M and 24 M animals. This conforms to the data published by Chibalin et al. [72] regarding the stimulatory effect of swimming exercise on skeletal muscles Glut4 expression. Exercise training increases transcription factors involved in GLUT4 gene expression [73].

The present study suggested that regular physical exercise seems to play a beneficial role by which the age-associated insulin resistance can be efficiently prevented. The protective effect of exercise could be explained on the basis of suppression of circulating catecholamines and inflammatory cytokines as well as stimulation of skeletal muscle Glut4 expression. Other mechanisms might also be involved. Complementary studies are necessary to elucidate the effects of different types of exercise with variable intensity, frequency, and duration on age-induced insulin resistance and other possible involved mechanisms as well.

5. Conclusion

In conclusion, the present study revealed that aging is associated with development of insulin

resistance in rats and it seems reasonable to propose that exercise may play a beneficial role. This protective effect of exercise could be explained on the basis of suppression of circulating catecholamines and inflammatory cytokines as well as stimulation of skeletal muscle Glut4 expression.

Acknowledgments

The assistance of Dr. Naglaa Yahia Professor of Biochemistry and Dr. Hanan soliman Professor of Pharmacology, Benha Faculty of Medicine is gratefully acknowledged.

Declaration of interest statement

The author declares that there is no conflict of interest.

References

1. DeFronzo, R.A. 1981. Glucose intolerance and ageing. ***Diabetes Care.*** 4: 493–501. doi: 10.2337/ 4.4.493.
2. Rowe, J.W., Minaker, K.L., Pallotta, J.A., and Flier, J.S. 1983. Characterization of the insulin resistance of ageing. ***J Clin Invest.*** 71:1581–1587. doi: 10.1172/JCI110914.
3. Gad, S.B., and Zaghloul, D.M. 2013. Beneficial Effects of Green Tea Extract on Liver and Kidney Functions, Ultrastructure, Lipid Profile and Hematological Parameters in Aged Male Rats. ***Global Veterinaria.*** 11(2): 191–205. doi: 10.5829/idosi.gv.2013.11.2.7472.
4. Salmon AB, Richardson A, Perez VI. Update on the oxidative stress theory of aging: does oxidative stress play a role in aging or healthy aging? ***Free Radic Biol Med*** 2010; 48(5): 642-655. doi: 10.1016/j.freeradbiomed.2009.12.015.
5. Fink, R.I., Kolterman, O.G., Griffin, J., and Olefsky, J.M. 1983. Mechanisms of insulin resistance in ageing. ***J Clin Invest.*** 71:1523–1535. doi: 10.1172/JCI110908.
6. Bartke A. Insulin and aging. ***Cell Cycle*** 2008; 7(21): 3338-3343 doi: 10.4161/cc.7.21.7012.
7. Carrascosa, J.M., Andrés, A., Ros, M., Bogónez, E., Arribas, C., Fernández-Agulló, T., et al. 2011. Development of insulin resistance during ageing: involvement of central processes and role of adipokines. ***Curr Protein Pept Sci.*** 12: 305–315. doi: 10.2174/138920311795906655.
8. Seals, D.R., and Bell, C. 2004. Chronic sympathetic activation: consequence and cause of age-associated obesity? ***Diabetes.*** 53: 276–284. doi: 10.2337/diabetes.53.2.276.
9. Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C, Ghirlanda G. Oxidative stress, nitric oxide, and diabetes. ***Rev Diabet Stud*** 2010; 7(1): 15-25 doi: 10.1900/RDS.2010.7.15.
10. Miyazawa M, Ishii T, Yasuda K, Noda S, Onouchi H, Hartman PS, Ishii N. The role of mitochondrial superoxide anion (O2(-)) on physiological aging in C57BL/6J mice. ***J Radiat Res*** 2009; 50(1): 73-83. PMID: 19218782.
11. Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK. A high plasma concentration of TNF-alpha is associated with dementia in centenarians. ***J Gerontol A Biol Sci Med Sci*** 1999; 54(7): M357-364. PMID: 10462168.
12. Paolisso G, Rizzo MR, Mazziotti G, Tagliamonte MR, Gambardella A, Rotondi M, Carella C, Giugliano D, Varricchio M, D'Onofrio F. Advancing age and insulin resistance: role of plasma tumor necrosis factor-alpha. ***Am J Physiol*** 1998; 275(2 Pt 1): E294-299. PMID: 9688632.
13. Cohen HJ, Pieper CF, Harris T, Rao KM, Currie MS. The association of plasma IL-6 levels with functional disability in community-dwelling elderly. ***J Gerontol A Biol Sci Med Sci*** 1997; 52(4): M201-208. PMID: 9224431.
14. Ershler WB, Sun WH, Binkley N, Gravenstein S, Volk MJ, Kamoske G, Klopp RG, Roecker EB, Daynes RA, Weindruch R. Interleukin-6 and aging: blood levels and mononuclear cell production increase with advancing age and in vitro production is modifiable by dietary restriction. ***Lymphokine Cytokine Res*** 1993; 12(4): 225-230. PMID: 8218595.
15. Borst, S.E. 2004. The role of TNF-alpha in insulin resistance. ***Endocrine.*** 23: 177–182. doi: 10.1385/ENDO:23:2-3:177.
16. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. ***J Clin Invest*** 2005; 115(5): 1111-1119. doi: 10.1172/JCI25102.

17. Houmard, J.A., Weidner, M.D., Dolan, P.L., Leggett-Frazier, N., Gavigan, K.E., Hickey, M.S., et al. 1995. Skeletal muscle GLUT4 protein concentration and aging in humans. ***Diabetes.*** 445: 555–560. doi: 10.2337/diab.44.5.555.
18. Kobilo T, Guerrieri D, Zhang Y, Collica SC, Becker KG, van Praag H. AMPK agonist AICAR improves cognition and motor coordination in young and aged mice. ***Learn Mem*** 2014; 21(2): 119-126. doi: 10.1101/lm.033332.113.
19. Gulve, E.A., Henriksen, E.J., Rodnick, K.J., Youn, J.H., and Holloszy, J.O. 1993. Glucose transporters and glucose transport in skeletal muscles of 1- to 25-mo-old rats. ***Am J Physiol.*** 2643: E319–E327. PMID: 8460679.
20. Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. ***Endocr Rev*** 2008; 29(3): 351-366. doi: 10.1210/er.2007-0023.
21. Ramm, G., Slot, J.W., James, D.E., and Stoorvogel, W. 2000. Insulin recruits GLUT4 from specialized VAMP2-carrying vesicles as well as from the dynamic endosomal/trans-Golgi network in rat adipocytes. ***Mol Biol Cell.*** 11(12): 4079–91. doi: 10.1091/mbc.11.12.4079.
22. Corpeleijn E, Saris WH, Blaak EE. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. ***Obes Rev*** 2009; 10(2): 178-193. doi: 10.1111/j.1467-789X.2008.00544.x.
23. Brestoff JR, Clippinger B, Spinella T, von Duvillard SP, Nindl BC, Arciero PJ. An acute bout of endurance exercise but not sprint interval exercise enhances insulin sensitivity. ***Appl Physiol Nutr Metab*** 2009; 34(1): 25-32. doi: 10.1139/H08-126.
24. Stampfer MJ, Hu FB, Manson JE, Rimm EB, Willett WC. Primary prevention of coronary heart disease in women through diet and lifestyle. ***N Engl J Med*** 2000; 343(1): 16-22. doi: 10.1056/NEJM200007063430103.
25. Kramer K, Dijkstra H, Bast A. Control of physical exercise of rats in a swimming basin. ***Physiol Behav*** 1993; 53(2): 271-276. PMID: 8446689.
26. Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B, American College of Sports M, American Diabetes A. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. **Diabetes Care** 2010; 33(12): 2692-2696. doi: 10.2337/dc10-1548.
27. Ravi Kiran T, Subramanyam MV, Asha Devi S. Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: relationship to swim intensity and duration. ***Comp Biochem Physiol B Biochem Mol Biol*** 2004; 137(2): 187-196. doi: 10.1016/j.cbpc.2003.11.002.
28. Lira FS, Rosa JC, Yamashita AS, Koyama CH, Batista ML, Jr., Seelaender M. Endurance training induces depot-specific changes in IL-10/TNF-alpha ratio in rat adipose tissue. ***Cytokine*** 2009; 45(2): 80-85. doi: 10.1016/j.cyto.2008.10.018.
29. Zanchi NE, Lira FS, de Siqueira Filho MA, Rosa JC, de Oliveira Carvalho CR, Seelaender M, Santos RV, Lancha AH, Jr. Chronic low frequency/low volume resistance training reduces pro-inflammatory cytokine protein levels and TLR4 mRNA in rat skeletal muscle. ***Eur J Appl Physiol*** 2010; 109(6): 1095-1102. doi: 10.1007/s00421-010-1456-0.
30. Botcazou M, Zouhal H, Jacob C, Gratas-Delamarche A, Berthon PM, Bentue-Ferrer D, Delamarche P. Effect of training and detraining on catecholamine responses to sprint exercise in adolescent girls. ***Eur J Appl Physiol*** 2006; 97(1): 68-75. doi: 10.1007/s00421-006-0131-y.
31. Zouhal H, Jacob C, Delamarche P, Gratas-Delamarche A. Catecholamines and the effects of exercise, training and gender. ***Sports Med*** 2008; 38(5): 401-423. PMID: 18416594.
32. Katz A, Broberg S, Sahlin K, Wahren J. Leg glucose uptake during maximal dynamic exercise in humans. ***Am J Physiol*** 1986; 251(1 Pt 1): E65-70. PMID: 3728665.
33. Bryant, N.J., Govers, R., and James, D.E. 2002. Regulated transport of the glucose transporter GLUT4. Nat. Rev. Mol. ***Cell. Biol***. 3: 267– 277. doi: 10.1038/nrm782.
34. Gu Z, Du Y, Liu Y, Ma L, Li L, Gong Y, Tian H, Li C. Effect of aging on islet beta-cell function and its mechanisms in Wistar rats. ***Age***

(Dordr) 2012; 34(6): 1393-1403. doi: 10.1007/s11357-011-9312-7.
35. Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, Brooks GA, Ames BN. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. ***J Appl Physiol*** (1985) 2000; 89(1): 21-28. PMID: 10904031.
36. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. 1985. Homeostasis model assessment: insulin resistance and B- cell functionfrom fasting plasma glucose and insulin concentrations in man. ***Diabetologia.*** 28: 412-419. doi: 10.1007/BF00280883.
37. Benzie, I.F., and Strain, J.J. 1996. The reducing ability of plasma as a measure of antioxidant power – the FRAP assay. ***Anal Biochem.*** 239: 70–76. doi: 10.1006/abio.1996.0292.
38. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. ***Methods*** 2001; 25(4): 402-408. doi: 10.1006/meth.2001.1262.
39. Chang AM, Halter JB. Aging and insulin secretion. ***Am J Physiol Endocrinol Metab*** 2003; 284(1): E7-12. doi: 10.1152/ajpendo.00366.2002.
40. Patti ME, Corvera S. The role of mitochondria in the pathogenesis of type 2 diabetes. ***Endocr Rev*** 2010; 31(3): 364-395. doi: 10.1210/er.2009-0027.
41. Nakhanakup, C., Moungmee, P., Appell, H.J., and Duarte, J. 2006. Regular physical exercise in patients with type II diabetes mellitus. ***Eur Rev Aging PhysAct.*** 31:10–19. doi: 10.1007/s11556-006-0002-x.
42. Santos JM, Ribeiro SB, Gaya AR, Appell HJ, Duarte JA. Skeletal muscle pathways of contraction-enhanced glucose uptake. ***Int J Sports Med*** 2008; 29(10): 785-794. doi: 10.1055/s-2008-1038404.
43. Fisher G, Hunter GR, Gower BA. Aerobic exercise training conserves insulin sensitivity for 1 yr following weight loss in overweight women. ***J Appl Physiol*** (1985) 2012; 112(4): 688-693. doi: 10.1152/japplphysiol.00843.2011.
44. Hall KE, McDonald MW, Grise KN, Campos OA, Noble EG, Melling CW. The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced Type 1 diabetic rodents. ***Metabolism*** 2013; 62(10): 1485-1494. doi: 10.1016/j.metabol.2013.05.012.
45. Arciero PJ, Baur D, Connelly S, Ormsbee MJ. Timed-daily ingestion of whey protein and exercise training reduces visceral adipose tissue mass and improves insulin resistance: the PRISE study. J ***Appl Physiol*** (1985) 2014; 117(1): 1-10. doi: 10.1152/japplphysiol.00152.2014.
46. De Sousa MV, Fukui R, Krustrup P, Pereira RM, Silva PR, Rodrigues AC, de Andrade JL, Hernandez AJ, da Silva ME. Positive effects of football on fitness, lipid profile, and insulin resistance in Brazilian patients with type 2 diabetes. ***Scand J Med Sci Sports*** 2014; 24 Suppl 1: 57-65. doi: 10.1111/sms.12258.
47. Di Nardo F, Burattini R, Cogo CE, Faelli E, Ruggeri P. Age-related analysis of insulin resistance, body weight and arterial pressure in the Zucker fatty rat. ***Exp Physiol*** 2009; 94(1): 162-168. doi: 10.1113/expphysiol.2008.044529.
48. Xu H, Huang X, Riserus U, Cederholm T, Sjogren P, Lindholm B, Arnlov J, Carrero JJ. Albuminuria, renal dysfunction and circadian blood pressure rhythm in older men: a population-based longitudinal cohort study. ***Clin Kidney J*** 2015; 8(5): 560-566. doi: 10.1093/ckj/sfv068.
49. Exton JH, Friedmann N, Wong EH, Brineaux JP, Corbin JD, Park CR. Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and of lipolysis in adipose tissue. ***J Biol Chem*** 1972; 247(11): 3579-3588. PMID: 4337859.
50. Vance JE, Buchanan KD, O'Hara D, Williams RH, Porte D, Jr. Insulin and glucagon responses in subjects with pheochromocytoma: effect of alpha adrenergic blockade. ***J Clin Endocrinol Metab*** 1969; 29(7): 911-916. doi: 10.1210/jcem-29-7-911.
51. Sacca L, Vigorito C, Cicala M, Corso G, Sherwin RS. Role of gluconeogenesis in epinephrine-stimulated hepatic glucose production in humans. ***Am J Physiol*** 1983; 245(3): E294-302. PMID: 6614167.

52. Abood, A.M. and Alghamdi, B.S. 2017. Oxytocin supplementation alleviates age-related insulin resistance through down regulation of pro-inflammatory cytokine gene expression. ***Biomedical Research.*** 28(5): 2209–2215.
53. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. ***J Biol Chem*** 2003; 278(46): 45777-45784. doi: 10.1074/jbc.M301977200.
54. Stephens, J.M., Lee, J., and Pilch, P.F. 1997. Tumor necrosis factor-alpha-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction. ***J Biol Chem.*** 272: 971–976. doi: 10.1074/jbc.272.2.971.
55. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. ***J Clin Invest*** 1995; 95(5): 2409-2415. doi: 10.1172/JCI117936.
56. Razny U, Kiec-Wilk B, Wator L, Polus A, Dyduch G, Solnica B, Malecki M, Tomaszewska R, Cooke JP, Dembinska-Kiec A. Increased nitric oxide availability attenuates high fat diet metabolic alterations and gene expression associated with insulin resistance. ***Cardiovasc Diabetol*** 2011; 10: 68. doi: 10.1186/1475-2840-10-68.
57. Gray S, Feinberg MW, Hull S, Kuo CT, Watanabe M, Sen-Banerjee S, DePina A, Haspel R, Jain MK. The Kruppel-like factor KLF15 regulates the insulin-sensitive glucose transporter GLUT4. ***J Biol Chem*** 2002; 277(37): 34322-34328. doi: 10.1074/jbc.M201304200.
58. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of Akt substrate 160 phosphorylation. ***Diabetes*** 2005; 54(10): 2939-2945. PMID: 16186396.
59. Yu Z, Ye X, Wang J, Qi Q, Franco OH, Rennie KL, Pan A, Li H, Liu Y, Hu FB, Lin X. Associations of physical activity with inflammatory factors, adipocytokines, and metabolic syndrome in middle-aged and older chinese people. ***Circulation*** 2009; 119(23): 2969-2977. doi: 10.1161/CIRCULATIONAHA.108.833574.
60. Timmerman KL, Flynn MG, Coen PM, Markofski MM, Pence BD. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? ***J Leukoc Biol*** 2008; 84(5): 1271-1278. doi: 10.1189/jlb.0408244.
61. Timmerman, K.L., Flynn, M.G., Coen, P.M., Markofski, M.M., and Pence, B.D. 2008. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? ***Journal of Leukocyte Biology.*** 84(5):1271–1278. doi: 10.1189/jlb.0408244.
62. Evans JL. Antioxidants: do they have a role in the treatment of insulin resistance? ***Indian J Med Res*** 2007; 125(3): 355-372. PMID: 17496361.
63. Ji LL. Antioxidant enzyme response to exercise and aging. Med Sci Sports Exerc 1993; 25(2): 225-231. PMID: 8450725.
64. Teixeira-Lemos E, Nunes S, Teixeira F, Reis F. Regular physical exercise training assists in preventing type 2 diabetes development: focus on its antioxidant and anti-inflammatory properties. ***Cardiovasc Diabetol*** 2011; 10: 12. doi: 10.1186/1475-2840-10-12.
65. Solberg PA, Halvari H, Ommundsen Y, Hopkins WG. A 1-year follow-up of effects of exercise programs on well-being in older adults. ***J Aging Phys Act*** 2014; 22(1): 52-64. doi: 10.1123/japa.2012-0181.
66. Hosseini, S.S., Jalili, M., Panahi, M., Naghilou, Z., and Dezhahang, M. 2014. The effect of aerobic exercise and saffron supplementation on antioxidant capacity in diabetic rats. ***International Journal of Biosciences.*** 4(8): 1–7. doi:10.12692/ijb/4.8.1-7.
67. Mohammadi, M.,Alipour, M., Alipour, M.R., and Vatankhah, A.M. 2006. Effects of High Cholesterol Diet and Parallel Chronic Exercise

on Erythrocyte Primary Antioxidant Enzymes and Plasma total Antioxidant Capacity In Dutch Rabbits. ***Int J Endocrinol Metab.*** 4: 30–40.

68. Vesali-Akbarpour, L., and Samavati-Sharif, M.A. 2016. The Effect of Endurance Swimming Plus Vitamin C Supplement on Oxidative Stress and Muscles Damage Indices in Male Wistar Rats. ***Avicenna Journal of Medical Biochemistry.*** 4(1): e34241. doi: 10.17795/ajmb-34241.
69. Ramos, D., Martins, E.G., Viana-Gomes, D., Casimiro-Lopes, G., and Salerno, V. 2013. Biomarkers of oxidative stress and tissue damage released by muscle and liver after a single bout of swimming exercise. Appl. Physiol. ***Nutr. Metab.*** 83: 507–511. doi: dx.doi.org/10.1139/apnm-2012-0302.
70. Guarino MP, Ribeiro MJ, Sacramento JF, Conde SV. Chronic caffeine intake reverses age-induced insulin resistance in the rat: effect on skeletal muscle Glut4 transporters and AMPK activity. ***Age (Dordr)*** 2013; 35(5): 1755-1765. doi: 10.1007/s11357-012-9475-x.
71. Machado UF, Shimizu I, Saito M. Reduced content and preserved translocation of glucose transporter (GLUT 4) in white adipose tissue of obese mice. ***Physiol Behav*** 1994; 55(4): 621-625. PMID: 8190786.
72. Chibalin AV, Yu M, Ryder JW, Song XM, Galuska D, Krook A, Wallberg-Henriksson H, Zierath JR. Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: differential effects on insulin-receptor substrates 1 and 2. ***Proc Natl Acad Sci USA*** 2000; 97(1): 38-43. PMID: 10618367.
73. Lima GA, Anhe GF, Giannocco G, Nunes MT, Correa-Giannella ML, Machado UF. Contractile activity per se induces transcriptional activation of SLC2A4 gene in soleus muscle: involvement of MEF2D, HIF-1a, and TRalpha transcriptional factors. ***Am J Physiol Endocrinol Metab*** 2009; 296(1): E132-138. doi: 10.1152/ajpendo.90548.2008.

