

ORIGINAL ARTICLE

Total testosterone, adiponectin and adropin in a group of men: Relation to age

*Asmaa A. Muhammed¹, Rania M.H.M. Eid¹, Wafaa Salah Mohammed², Mahmoud R. Abdel-Fadeil³

¹Departments of Medical Physiology, Faculty of Medicine, Aswan University, Aswan, Egypt.

²Department of clinical Pathology, Faculty of Medicine, Aswan University, Aswan, Egypt

³Departments of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt.

ABSTRACT

Keywords: Age, dyslipidemia, testosterone, adiponectin, adropin

***Corresponding author:**

Asmaa A. Muhammed,
Departments of Medical
Physiology, Faculty of
Medicine, Aswan University,
Aswan, Egypt.
Asmaa.ali@med.aswu.edu.eg

Background: Levels of lipid profile and total testosterone in men are affected by age. Levels of adiponectin, an adipocytokine, and adropin, a peptide hormone are altered also by the effect of age. Many age-related health consequences could be related to these changes. Aim of this study is to find out levels of lipid profile, total testosterone, adiponectin and adropin in a group of Upper Egyptian men and find out effect of age on them. **Methods:** 83 male subjects were enrolled in this study and divided into two groups: group A: aged from 18 to <30 years, and group B: aged from ≥ 30 to 50 years. Lipid profile parameters, total testosterone, adiponectin and adropin were measured. **Results:** Group B showed higher TC, TG, and LDL-c levels, and lower total testosterone, adiponectin, and adropin levels. **Conclusion:** Progressing in age is associated with dyslipidemia, lower testosterone, adiponectin, and adropin levels.

INTRODUCTION

Dyslipidemia is characterized by increased triglycerides (TG), low-density lipoprotein cholesterol (LDL-c) levels and decreased high-density lipoprotein cholesterol (HDL-c) levels. This lipid profile is atherogenic and predisposes to coronary artery disease¹. Levels of TC and LDL-c are increased with aging². Walter³ detected low HDL-c levels in adolescent and early adult males but remained constant or increased in elderly men. In addition, TG levels increase progressively with age to reach a peak at about 40 – 50 years and then decline slightly⁴. In men, testosterone levels were found to decrease despite unchanged leutinizing hormone (LH) and increased follicle stimulating hormone (FSH) levels⁵. These low testosterone

levels not only predispose to infertility but also are associated with increased insulin resistance, risk of diabetes mellitus, lower HDL-c levels, higher TG levels, hypertension, atherosclerosis, and myocardial infarction⁶. Scherer *et al*⁷ discovered adiponectin for the first time as a protein formed in adipocytes. Adiponectin plays a role in lipid and carbohydrate metabolism, it enhances glucose uptake and increases insulin sensitivity in both adipocytes and muscles⁸. It has an anti-inflammatory action, and a cardiovascular protective role in decreasing cardiomyocyte apoptosis⁹. Aging alters the number, size, proliferation, and functions of adipose tissues altering the synthesis and secretions of adipocytokines¹⁰. Adiponectin increases with progressing in age^{11, 12, 13}. Although

adiponectin is increasing with age, in older individuals aged 65 years and more it is associated with high mortality probably due to loss of function of adiponectin¹⁰. **Kumar et al**¹⁴ discovered adropin hormone for the first time in obese mice during the microarray analysis of liver gene expression. The name adropin was derived from the Latin word *aduro* (to set fire) and *pinquis* (fats or oils)¹⁴. It is encoded by the energy homeostasis-associated (*Enho*) gene and is expressed mainly in the liver and the brain¹⁴. Adropin is involved in energy homeostasis and the control of glucose and fatty acid metabolism¹⁵. It is important for normal cerebellar development and the preservation of the blood-brain barrier¹⁶. In addition, its levels are decreased in obesity and diabetes mellitus¹⁷. It has an anti-inflammatory, and an antioxidant effect¹⁸. **Butler et al**¹⁹ detected a negative correlation between adropin and age in men, persons younger than 30 years showed much higher levels of adropin compared to persons from 30-50 years and to persons larger than 50 years old. In aged rats, **Yang et al**²⁰ demonstrated lower brain *Enho* mRNA and adropin levels associated with lower serum adropin levels. In human skeletal muscle feed arteries, endothelial-dependent vasodilatory function and adropin protein expression were reduced with aging while adropin incubation in aged skeletal muscle feed arteries restored the vasodilatory function through increasing nitric oxide bioavailability²¹. Aged mice showed lower arterial and circulating adropin levels with decreased arterial nitric oxide (NO) production²².

This study aims to find possible relations between age and lipid profile, testosterone, adiponectin, and adropin serum levels in a group of men.

Subjects and methods

This study was approved by the ethics committee of the Faculty of Medicine, Aswan University, Institutional

Review Board (IRB) approval No: 287918. Aim, steps of the study and the risks of blood sample withdrawal were explained to the participants. They had the right of withdrawal from the study at any time without giving any reasons. Participants data were kept confident by coding every participant, and analyzing data without identifying participant names. All participants were notified by end results of this research. 83 male subjects were enrolled in this study, they were divided into two groups group A (no. 42; age from 18 to <30 years), and group B (no. 41; age from ≥ 30 to 50 years). Diabetic, hypertensive and smokers were excluded. Persons with heart failure, kidney diseases and obstructive sleep apnea were excluded.

Blood samples were collected after 8-12 hours fasting, serum was separated by centrifugation at 3000 rpm for 15 minutes. The clear, non-hemolysed supernatant was separated and stored at -20°C until analysis. Enzymatic colorimetric method was used to detect serum TC, TG, HDL-c and LDL-c by kits purchased from Biotecnica instruments S.p.A, Italy. The equation described by **Friedewald et al**²³ was used to determine serum LDL-c levels. VIDAS Testosterone kits (catalog No. 414320) purchased from Biomerieux, SA, France was used to measure total testosterone levels by the Enzyme-linked fluorescent assay (ELFA) technique. Adropin levels were measured by Enzyme-Linked immunosorbent assay (ELISA) technique using kits purchased from SinoGeneClon Biotech Co., Ltd., China. (Catalog No. SG-11595) with sensitivity: 0.5 ng/ml, Adiponectin levels were measured by ELISA technique using kits purchased from SinoGeneClon Biotech Co., Ltd., China. (Catalog No. SG-10421) with sensitivity: 5 ng/ml.

SPSS ver. 23 was used to analyze the data, Shapiro-Wilk test was used for testing normality. Normally distributed data were expressed as mean \pm standard

deviation, an independent t test and Pearson correlation tests were used. In non-normally distributed data, they were expressed as median and interquartile range, Mann–Whitney U test and Spearman correlation test were applied. The threshold for statistical significance was (p value < 0.05) and for correlation (if $r = 0$ no correlation, $0 < r \leq 1$ positive correlation, $-1 \leq r < 0$ negative correlation).

Results

Descriptive results of age, BMI and lipid profile parameters are showed in table 1. Age and BMI was significantly increased in group B when compared to group A. TC, TG and LDL-c levels were significantly increased in group B compared to group A. No significant change of HDL-c levels was detected between both groups. Figure 1 shows a significantly decreased total testosterone levels in group B compared to group A. significant decreased levels of adiponectin (figure 2) and adropin (figure 3) were detected in group B compared to group A subjects.

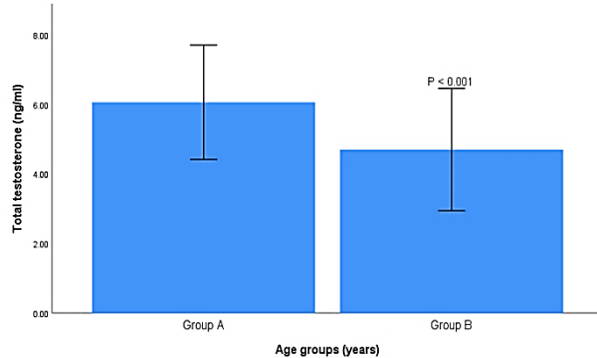


Figure 1: Total testosterone levels in both groups. Data are presented as mean \pm SD. nanogram/milliliter (ng/ml). P: significance versus normal weight group, P-value < 0.05 is considered significant.

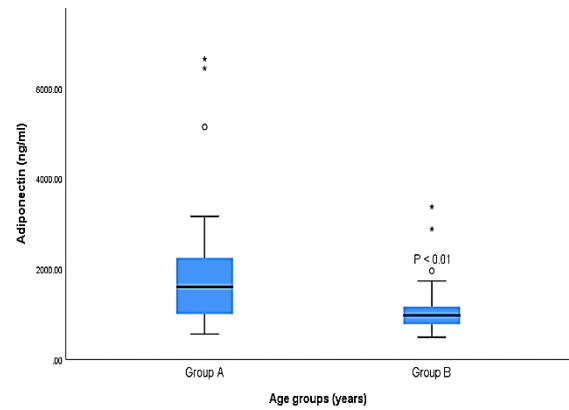


Figure 2: Serum adiponectin levels in both age groups

Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 90th percentile; lower horizontal bar outside box, 10th percentile. Circles represent outliers. Stars represent extreme outliers. P: significance of group B versus group B, P-value < 0.05 is considered significant. nanogram/milliliter (ng/ml).

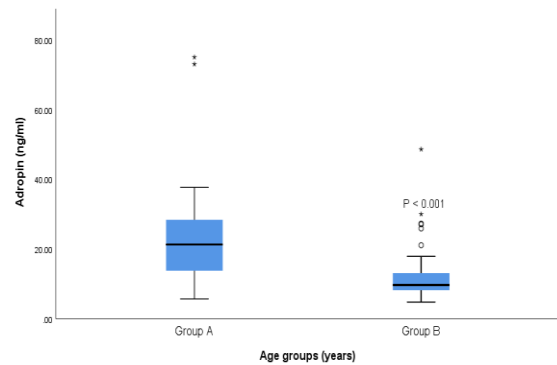


Figure 3: Serum adropin levels in both age groups

Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 90th percentile; lower horizontal bar outside box, 10th percentile. Circles represent outliers. Stars represent extreme outliers. P: significance of group B versus group B, P-value < 0.05 is considered significant. nanogram/milliliter (ng/ml)

Age correlation analysis in table 2 shows a significant positive correlation between age and BMI in group A, and a non-significant positive correlation in group B, TC showed a significant positive correlation with age in group A subjects and a positive but a non-significant correlation with age in group B subjects. TG correlated significantly with age in both age groups, While HDL-c showed a significant negative correlation in group B

and a non-significant correlation in group A subjects. Significant negative correlation was detected between age and LDL-c levels in group A subjects but a non-significant correlation was detected in group B subjects. Total testosterone, adiponectin and adropin showed significant negative correlations with age in group B subjects and non-significant correlations with age in group A subjects.

Table 1: Age, BMI, lipid profile, total testosterone, adiponectin and adropin levels in both groups

Measured parameters	Group A (n=42)	Group B (n=41)	Significance
Age (years)	21.17 ± 2.95	39.80 ± 5.42***	P < 0.001
BMI (Kg/m ²)	23.20 (5.28)	32.20 (3.85)***	P < 0.001
TC (mg/dl)	152.33 ± 27.21	198.24 ± 45.43***	P < 0.001
TG (mg/dl)	65.70 (32.88)	125.00 (92.65)***	P < 0.001
HDL-c (mg/dl)	40.31 ± 11.31	36.51 ± 6.20	P > 0.05
LDL-c (mg/dl)	98.27 ± 28.73	131.77 ± 35.21***	P < 0.001

Data were expressed as mean ± SD or median and (interquartile range); P: significance of group A versus group B; A P-value < 0.05 is considered significant. *: P < 0.05; **: P < 0.01; ***: P < 0.001. n= number of subjects/group; BMI: Body mass index TC: Total cholesterol; TG: Triglycerides; HDL-c: High-density-lipoprotein cholesterol; LDL-c: Low-density-lipoprotein cholesterol

Table 2: Correlations of age with BMI, lipid profile parameters, total testosterone, adiponectin and adropin

Measured parameters	Age (Group A)		Age (group B)	
	r	P	r	P
BMI (Kg/m ²)	0.422**	< 0.01	0.214	> 0.05
TC (mg/dl)	0.530***	< 0.001	0.122	> 0.05
TG (mg/dl)	0.321*	< 0.05	0.392*	< 0.05
HDL-c (mg/dl)	-0.042	> 0.05	-0.353*	< 0.05
LDL-c (mg/dl)	0.514***	< 0.001	0.113	> 0.05
Total testosterone (ng/ml)	-0.156	> 0.05	-0.381*	< 0.05
Adiponectin (ng/ml)	-0.011	> 0.05	-0.432**	< 0.01
Adropin (ng/ml)	-0.156	> 0.05	-0.311*	< 0.05

r: correlation coefficient; P: significance level; P-value < 0.05 is considered significant. *: P < 0.05; **: P < 0.01; ***: P < 0.001. BMI: Body mass index; TC: Total cholesterol; TG: Triglycerides; HDL-c: High-density-lipoprotein cholesterol; LDL-c: Low-density-lipoprotein cholesterol.

Discussion:

This study detected significantly higher TC, TG, and LDL-c levels in group B, the older group compared to group A, and positive correlations between age and TC, TG, and LDL-c levels were detected, while HDL-c correlated negatively with age. In agreement with this **Ericsson *et al***² detected increasing TC and LDL-c levels with aging. In addition, **Gobal and Mehta**⁴ discovered increased TG levels with age. **Walter**³ described decreasing HDL-c levels with increasing age in early adults. This age-related dyslipidemia can be caused by hepatic endothelial changes in the liver converting liver sinusoidal endothelium into capillary endothelium similar to that seen in non-fenestrated capillary beds which is called pseudocapillarization which impairs hepatic lipoprotein uptake by the aged liver²⁴. Besides, with aging, hepatic synthesis of LDL-c cholesterol increases²⁵ while its removal is decreased by decreasing LDL-c receptors and the activity of lipoprotein lipase enzyme²⁶. Lower HDL-c levels detected with aging could be explained by the effect of increased inflammation, insulin resistance and low testosterone levels associated with aging on decreasing hepatic HDL-c formation⁶. Lower total testosterone levels were found in group B compared to group A, also total testosterone correlated negatively with age. In line with this study, testosterone levels were found decreased in men with progressing in age⁵. This could be possibly caused by the decreased ability of Leydig cells to produce testosterone in response to LH stimulation²⁷, As lower testosterone levels were found in old men despite unchanged LH levels⁵. This reduced Leydig cell sensitivity is caused by decreased formation of cAMP after binding of LH with its Gs receptors, and increased cAMP destruction²⁸. Lower adiponectin levels were detected in group B than in group A subjects and a negative correlation was demonstrated between adiponectin and age. This result is opposite to previous studies which detected higher adiponectin levels in aged men^{11, 12, 13}.

However, these previous studies measured adiponectin in higher age groups than this study with mean age > 50 years old. In addition, in this study persons with higher BMI (mean BMI 27.9 Kg.m²) than these previous studies (mean BMI 23 Kg/m²) were enrolled which could affect adiponectin levels, as increased BMI decreases adiponectin levels²⁹. Adropin levels were lower in group B, and age correlated negatively with adropin levels, which is similar to **Butler *et al***¹⁹ study which detected a negative correlation between adropin and age in men. They detected also lower adropin levels in persons aged 50 years old than persons aged from 30 to 50 years than in persons younger than 30 years. The exact cause of lower adropin levels with increasing age needs further investigations.

Conclusion

Dyslipidemia, lower testosterone, adiponectin, and adropin hormones were associated with increased age.

References

1. Agrawal H, Choy H-hK, Liu J, Auyoung M, Albert MAJA, Thrombosis, Biology V. Coronary artery disease. 2020;40 (7):e185-e92.
2. Park Y-MM, Sui X, Liu J, Zhou H, Kokkinos PF, Lavie CJ, *et al*. The effect of cardiorespiratory fitness on age-related lipids and lipoproteins. 2015;65(19):2091-100.
3. Walter MJA, thrombosis, biology v. Interrelationships among HDL metabolism, aging, and atherosclerosis. 2009;29(9):1244-50.
4. Gobal FA, Mehta JLJTaicd. Management of dyslipidemia in the elderly population. 2010;4(6):375-83.
5. Beattie M, Adekola L, Papadopoulos V, Chen H, Zirkin BJEg. Leydig cell aging and hypogonadism. 2015;68:87-91.
6. Fernández-Miró M, Chillarón JJ, Pedro-Botet JJMC. Testosterone deficiency, metabolic syndrome and diabetes mellitus. 2016;146(2):69-73.
7. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HFJJoBc. A novel serum

protein similar to C1q, produced exclusively in adipocytes. 1995;270(45):26746-9.

8. Liu Y, Sweeney GJBp, endocrinology rC, metabolism. Adiponectin action in skeletal muscle. 2014;28(1):33-41.

9. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, *et al.* Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. 2011;17(1):55-63.

10. Gulcelik N, Halil M, Ariogul S, Usman AJMe. Adipocytokines and aging: adiponectin and leptin. 2013;38(2):203-10.

11. Obata Y, Yamada Y, Takahi Y, Baden MY, Saisho K, Tamba S, *et al.* Relationship between serum adiponectin levels and age in healthy subjects and patients with type 2 diabetes. 2013;79(2):204-10.

12. Zeng W-F, Li Y, Sheng C-S, Huang Q-F, Kang Y-Y, Zhang L, *et al.* Association of anthropometric and bioelectrical impedance analysis measures of adiposity with high molecular weight adiponectin concentration. 2016;11(5):e0156041.

13. Tomono Y, Hiraishi C, Yoshida HJAocb. Age and sex differences in serum adiponectin and its association with lipoprotein fractions. 2018;55(1):165-71.

14. Kumar KG, Trevaskis JL, Lam DD, Sutton GM, Koza RA, Chouljenko VN, *et al.* Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. 2008;8(6):468-81.

15. Ganesh-Kumar K, Zhang J, Gao S, Rossi J, McGuinness OP, Halem HH, *et al.* Adropin deficiency is associated with increased adiposity and insulin resistance. 2012;20(7):1394-402.

16. Yu L, Lu Z, Burchell S, Nowrangi D, Manaenko A, Li X, *et al.* Adropin preserves the blood-brain barrier through a Notch1/Hes1 pathway after intracerebral hemorrhage in mice. 2017;143(6):750-60.

17. Zang H, Jiang F, Cheng X, Xu H, Hu XJEj. Serum adropin levels are decreased in Chinese type 2 diabetic patients and negatively correlated with body mass index. 2018;65(7):685-91.

18. Chen X, Xue H, Fang W, Chen K, Chen S, Yang W, *et al.* Adropin protects against liver injury in nonalcoholic steatohepatitis via the Nrf2 mediated antioxidant capacity. 2019;21:101068.

19. Butler AA, Tam CS, Stanhope KL, Wolfe BM, Ali MR, O'Keefe M, *et al.* Low circulating adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. 2012;97(10):3783-91.

20. Yang C, DeMars KM, Candelario-Jalil EJA, disease. Age-dependent decrease in adropin is associated with reduced levels of endothelial nitric oxide synthase and increased oxidative stress in the rat brain. 2018;9(2):322.

21. Kwon OS, Andtbacka RH, Hyngstrom JR, Richardson RSJTJop. Vasodilatory function in human skeletal muscle feed arteries with advancing age: the role of adropin. 2019;597(7):1791-804.

22. Fujie S, Hasegawa N, Horii N, Uchida M, Sanada K, Hamaoka T, *et al.* Aerobic Exercise Restores Aging-Associated Reductions in Arterial Adropin Levels and Improves Adropin-Induced Nitric Oxide-Dependent Vasorelaxation. 2021;10(10):e020641.

23. Friedewald WT, Levy RI, Fredrickson DSJCC. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. 1972;18(6):499-502.

24. Cogger VC, Svistounov D, Warren A, Zykova S, Melvin RG, Solon-Biet SM, *et al.* Liver aging and pseudocapillarization in a Werner syndrome mouse model. 2014;69(9):1076-86.

25. Kolovou G, Bilianou H, Marvaki A, Mikhailidis DPJAjomsh. Aging men and lipids. 2011;5(2):152-65.

26. Li Y, Sugiyama E, Yokoyama S, Jiang L, Tanaka N, Aoyama TJL. Molecular Mechanism of Age-Specific Hepatic Lipid Accumulation in PPAR α (+/-): LDLR (+/-) Mice, an Obese Mouse Model. 2008;43(4):301-12.

27. Veldhuis JD, Liu PY, Keenan DM, Takahashi PYJAJoP-E, Metabolism. Older men exhibit reduced efficacy of and heightened potency downregulation by intravenous pulses of recombinant human LH: a study in 92 healthy men. 2012;302(1):E117-E22.
28. Sokanovic S, Janjic M, Stojkov N, Baburski A, Bjelic M, Andric S, *et al.* Age

related changes of cAMP and MAPK signaling in Leydig cells of Wistar rats. 2014;58:19-29.

29. Kim AY, Park YJ, Pan X, Shin KC, Kwak S-H, Bassas AF, *et al.* Obesity-induced DNA hypermethylation of the adiponectin gene mediates insulin resistance. 2015;6(1):1-11.