

ORIGINAL RESEARCH ARTICLE

Impact of diet and physical activity on reproductive profile: A comparison of middle-aged and elderly men

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Abstract

Age is a known determinant of reproductive health and fertility in both genders. The present work aims to assess the reproductive hormone profile of a middle-aged and elderly man. For this descriptive cross-sectional study, healthy male subjects (n=77) were recruited from the valley. Any individual suffering from any acute or chronic diseases and on drugs was ruled out from the study. Group A consisted of 40 elderly men between 60-70 years of age, and Group B comprised 37 men between 35-46 years of age. Blood samples were taken to estimate the reproductive hormone profile. Level of oxidant and antioxidant: Malondialdehyde and Glutathione. The demographic variables, which included retrospective and prospective questions, helped to assess the physical activity and diet intake behaviour of all inducted individuals. The analysis of the reproductive profile of both groups was similar and within the normal range of standards. However, the median level of LH was higher in group A than in group B: 6.7 mIU/ml versus 3.4 mIU/ml, respectively, and $p < 0.003$. Both groups showed predominantly involvement in physical activity, >90%. The correlation of biochemical variables gives an insight into the fact that the Mediterranean diet and physical activity help to maintain a normal BMI. These implicate the normal secretion of various hormones, leading to intact spermatogenesis. We can safely deduce from this study that physically active lifestyles and a healthy diet are crucial factors in maintaining an endocrine profile. (*Afr J Reprod Health* 2024; 28 [4]: 22-29).

Keywords: Body mass index; elderly men; healthy diet; physical activity; reproductive hormones

Résumé

L'âge est un déterminant connu de la santé reproductive et de la fécondité chez les deux sexes. Le présent travail vise à évaluer le profil hormonal de la reproduction d'un homme d'âge moyen et âgé. Pour cette étude transversale descriptive, des sujets masculins en bonne santé (n = 77) ont été recrutés dans la vallée. Toute personne souffrant de maladies aiguës ou chroniques et prenant des médicaments a été exclue de l'étude. Le groupe A était composé de 40 hommes âgés de 60 à 70 ans et le groupe B de 37 hommes âgés de 35 à 46 ans. Des échantillons de sang ont été prélevés pour estimer le profil des hormones reproductives. Niveau d'oxydant et d'antioxydant : Malondialdéhyde et Glutathion. Les variables démographiques, qui comprenaient des questions rétrospectives et prospectives, ont permis d'évaluer l'activité physique et le comportement alimentaire de tous les individus intronisés. L'analyse du profil reproducteur des deux groupes était similaire et se situait dans la fourchette normale des normes. Cependant, le taux médian de LH était plus élevé dans le groupe A que dans le groupe B : respectivement 6,7 mUI/ml versus 3,4 mUI/ml et $p < 0,003$. Les deux groupes présentaient une participation prédominante à l'activité physique, > 90 %. La corrélation des variables biochimiques donne un aperçu du fait que le régime méditerranéen et l'activité physique contribuent à maintenir un IMC normal. Celles-ci impliquent la sécrétion normale de diverses hormones, conduisant à une spermatogénèse intacte. Nous pouvons déduire de cette étude qu'un mode de vie physiquement actif et une alimentation saine sont des facteurs cruciaux pour maintenir un profil endocrinien. (*Afr J Reprod Health* 2024; 28 [4]: 22-29).

Mots-clés: Indice de masse corporelle ; hommes âgés; régime équilibré; activité physique; hormones de reproduction

Introduction

Genetic or environmental factors can alleviate the reproductive capacity of an individual. Compared to females, the reproductive capacity does not

abrogate in men, and spermatogenesis is known to continue even in advanced age, enabling some men to reproduce throughout their life spans¹. However, the libido factor is markedly reduced in elderly men. Generally, this is linked to the reduced level

of testosterone, social, psychological, and physical changes, and the use of medication for chronic illnesses².

Various lifestyle factors are known to influence reproductive health, such as age at the time of marriage, body mass index (BMI), dietary patterns, physical activity, occupational and environmental exposures. Similarly, the use of caffeine, alcohol, and smoking has a pronounced effect on spermatogenesis³. Inhabitants of highly polluted areas have a poor quality of semen compared to men living in less polluted places⁴.

The Mediterranean diet, rich in vegetables, fruits, legumes, nuts, seafood, and olive oil, and physical activity are related to good semen quality in men⁴. A healthy diet and physical activity increase the concentration of sperms and their normal morphology and motility in healthy men⁵. Besides, diet and physical activity help to improve hypogonadism in men, irrespective of age⁶. In addition, various phytochemical compounds rich in the Mediterranean diet help counter environmental contaminants' adverse effects⁷.

The aging process alters the metabolic and endocrine milieu, changing hormonal patterns⁸. Age-related changes have been shown to result in variations in the levels of reproductive-associated parameters like Sex Hormone-Binding Globulin (SHBG), Total and Free Testosterone, Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH). Furthermore, Age-related oxidative stress (OS) can cause ROS overproduction and increase the serum's Malondialdehyde (MDA) level^{9,10}, and decrease the anti-oxidant, like Glutathione (GSH). Similarly, ROS production induces apoptosis of many spermatozoa^{11,12}. It is well documented in the literature that about 35% of the changes in Inhibin B level are with age in males. During OS, these inhibins and testicular estradiol are synthesized intensely and block the release of testosterone¹³. Inhibin B is an essential endocrine regulator of FSH secretion, whereas gonadotropins regulate inhibin B secretion^{14,15}.

The fertility rate is reduced with increasing age, along with adverse effects on semen parameters and increased chances of genetic mutations that may affect offspring^{16,17}. Thus, the impact of aging on male fertility is a significant health issue. However, the outcomes of aging on the reproductive potential of older men are poorly understood, particularly in the loco-regional

context. Anecdotal evidence points to high fertility and reproductive effects in older men in the mountainous northern areas of Pakistan. Therefore, the present study aims to determine if elderly men from rural hilly areas have unaltered reproductive health parameters compared to younger men.

Methods

Study protocol

Study design

It is a descriptive cross-sectional study. The data was collected at a single point to provide a brief detail of the investigated variables. Here, we focused on assessing the differences in hormone levels and other parameters between the two age groups.

Study population

Participants: The study included a total of 77 male subjects from the mountainous valley area of Pakistan. Inducted individuals were categorized into two groups;

Group A (study subjects): This group comprised 40 older men aged between 60-70.

Group B (control subjects): Group B comprised 37 men aged between 35-46 years.

Inclusion criteria & exclusion criteria. A convenient designed questionnaire was used to assess the demographic single point variable All recruited participants were healthy and those who were on any hormonal therapy, suffering from acute or chronic diseases, on drugs and having family history of infertility in their direct relationship was also excluded from the study. Few individual reported to have thyroid complications were also excluded from the study. The structured study proforma recorded basic demographic details, clinical history, and other baseline investigations.

Data collection

Demographic data: Anthropometric measurements: Height, weight, and body mass index (BMI) were recorded for all participants. These measurements are used to assess the physical characteristics of the participants.

Assessment of dietary patterns and activity levels: Dietary assessment were carried out using and

modified “validated questionnaire” by Sotos-Prieto *et al*¹⁸ and used Mediterranean Diet were given the score as describer earlier by Ruggeri *et al*¹⁹. Open-ended questionnaire helped in the assessment of dietary patterns, through a surveys designed specifically for this study. Participants' dietary habits were recorded to understand their nutritional intake. The dietary pattern was considered Mediterranean if it included routine intake of plant-based foods like whole grains, legumes, vegetables, nuts, seeds, fruits, herbs, milk, and boiled meat.

Activity assessment: At large open-ended and self reported physical activity assessment was employed, and for the convenient purpose modified IPAQ-L questionnaire^{20,21} was used to subjectively assess to gain insight into participants' physical activity patterns and lifestyles. Physical activity was determined based on routine engagement in physically challenging chores in the mountainous regions as equivalent to moderate to high aerobic cardio and resistance exercises.

Blood sample collection: Blood samples were collected from the study participants. The specific blood parameters measured in this study included SHBG, Total and free testosterone (TT) and (FT), LH, FSH, Inhibin, MDA, and total GSH.

Assessment of the variables

Anthropometric measurements, including height and weight, were recorded. Standing height (in meters) was measured using a stadiometer, and weight (in kilograms) was measured using a weighing scale with minimum clothing and without shoes. The equipment was calibrated to zero before taking measurements. Body mass index (BMI) was calculated using the formula of weight in kilograms divided by the square of height in meters.

After informed consent and taking septic measures, blood was collected in serum, separating gel and clot activator tube from the antecubital veins. The samples were centrifuged, and the separated serum was stored at -70C. All samples were analyzed in one batch after the calibration of microplate spectrophotometer. The desired wavelength was used, as mentioned in the leaflets of the standard and commercially available enzyme-linked immunosorbent assay (ELISA) kits. Levels of FT (Cat # CSB-E05096h) and TT (Cat # CSB-E05099h), SHBG (Cat # CSB-

E08232h), LH (Cat # CSB-E12690h), and FSH (Cat # CSB-E06867h), by ELISA kit from; CUSABIO Technology LLC., Houston, Texas, USA. Inhibin (Cat # MBS040452), by ELISA kit from; MyBioSource, San Diego, CA, USA.). MDA (Cat # ab238537), and GSH (Cat # ab65322), by Elisa kit from; Abcam, UK.

To minimize the variations of test results, the intra-assay coefficients of variation were used, and replicates of all the tests were run within the same plate to measure the variance between data points of all the parameters.

Ethics statement

All subjects were informed of the purpose of the study, and they signed the consent to participate. The Ethical Committee of The University of Lahore approved the study under the Ref No. CRiMM/23/Research/28, dated 19/07/2023.

Statistical analysis

A sample size of 77 males was determined using 95% CI or confidence level, 95.0% power. The sample size was estimated using the online webpage (<https://clincalc.com/stats/samplesize.aspx>). In addition, the power analysis allowed us to estimate the sample size needed to detect the anticipated effect size with a desired level of statistical power of P=0.05 and a desired power level of 25%. The measured variables were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0. Categorical variables were expressed as frequencies and percentages. Shapiro-Wilk's statistics checked normality as the total sample size was less than 2000. Variable distribution was determined for selecting appropriate statistical tests. Mean \pm Standard Deviation (SD) was given for normally distributed quantitative variables, while median (interquartile range) was given for non-normally distributed quantitative variables. The independent sample “t” test and Mann-Whitney U test were applied to compare groups based on the distribution of quantitative variables. Correlation of biochemical parameters with BMI was carried out by Pearson correlation for normally distributed variables and Spearman correlation for non-normally distributed variables. A p-value of < 0.05 was considered statistically significant for all analyses.

Results

Distribution of the variables

Quantitative variables were separated into normally and non-normally distributed ones depending upon their distribution (W), departing significantly from the normality of the distribution of variables. Parametric or non-parametric tests were employed based on the Shapiro-Wilk outcomes for each variable. Variables were summarized as mean±SD or median for normally distributed variables or interquartile range for non-normally distributed variables. Significance variations were noted among these variables. A p-value of less than 0.05 indicates that tested data is

from a normally distributed population, and it shows some variables have significant differences in their distribution, as shown in Table 1.

Demographic parameters

Table 2 represents the men in both groups who predominantly expressed Mediterranean dietary patterns and physically active lifestyles. In group A, 90%, and in group B, 92% of the subjects were engaged in the physically active life pattern. Furthermore, physical activity has produced normal levels of BMIs in the two groups. It also confirms the measurement of other parameters, like weight, which is associated with a healthier body in both groups.

Table 1: Distribution of variables based on Shapiro-Wilk statistics

Variable	W (n=77)	P-Value	Inference
Age (years)	0.892	0.000*	Non-Normal
Weight (kg)	0.928	0.002*	Non-Normal
SHBG (ng/ml)	0.937	0.005*	Normal
LH (mIU/ml)	0.782	0.000*	Normal
FSH (mIU/ml)	0.511	0.000*	Normal
Inhibin (pg/ml)	0.745	0.003	Normal
MDA (nmol/ml)	0.689	0.005	Normal
GSH (ug/dl)	0.824	0.005	Normal
Height (m)	0.957	0.052	Normal
BMI (kg/m ²)	0.948	0.061	Normal
Total T (ng/ml)	0.979	0.441	Normal
Free T (ng/ml)	0.966	0.114	Normal

*P<0.05= Significant

Table 2: Demographic, anthropometric, and lifestyle parameters

Variables	Group A; (n=40)	Group B; (n=37)	p-value
Age (years) ¹	65 (60-70)	40 (35-46)	0.000*
Weight (Kg) ¹	65 (57.5-71.25)	66 (65-68)	0.446
Height (meter) ²	1.84 ± 0.068	1.83 ± 0.069	0.719
BMI (Kg/m ²) ²	22.97 ± 3.89	23.53 ± 2.07	0.024*
Mediterranean diet ³	(36) 90 %	(34) 92%	N/A
Physical activity ³	(36) 90%	(35) 96%	N/A

¹Non-normally distributed quantitative variable given as median (interquartile range)

²Normally distributed quantitative variable given as mean ± SD

³Categorical variable given as frequency (percentage)

*P<0.05= Significant

Measurements of biochemical profile

A comparison of the studied biochemical parameters between the two groups showed a statistically significant elevation in levels of serum LH ($P<0.003$). Contrary to LH, the SHBG, FSH, Inhibin, TT, and FT were not statistically different;

similarly, no conspicuous or significant difference was seen between these two groups oxidant (MDA) and anti-oxidant (GSH) levels. It can be understood that having a normal BMI does not induce significant stress on the body and thus maintains the average hormonal level of gonadal, pituitary, and other measured biochemical parameters in

Table 3: Comparison of biochemical parameters between groups

Variables	Group A; (n=40)	Group B; (n=37)	p-value
Primary Gonads Hormone and Proteins			
Total T (ng/dl)	419.43±23.87	398.23±20.02	0.525
Free T (ng/dl)	13.50±1.7	12.92±1.5	0.585
Inhibin (pg/ml)	263.53±15.91	275.96±20	0.605
SHBG (ng/ml)	16.27±3.1	15.86±3.4	0.780
Regulatory Hormones			
LH (mIU/ml)	6.7±0.07	3.4±0.003	0.003*
FSH (mIU/ml)	8.1±0.17	6.9±0.06	0.076
Oxidant			
Malondialdehyde (nmol/ml)	2.53±0.47	2.13±0.17	0.595
Anti-Oxidant			
GSH (ug/dl)	7.3±1.7	8.4±1.1	0.745

*P<0.05= Significant

Table 4: Correlation Co-efficient of BMI with reproductive hormone profile

Variables	Group A; (n=40)		Group B; (n=37)	
	Correlation Co-efficient	P- Value	Correlation Co-efficient	P-Value
Total T (ng/ml)¹	-0.489	0.006	-0.014	0.946
Free T (ng/ml)¹	-0.168	0.374	-0.049	0.810
SHBG (ng/ml)²	-0.532	0.003	0.013	0.950
LH (mIU/ml)²	-0.176	0.352	-0.264	0.184
FSH (mIU/ml)²	0.382	0.37	-0.263	0.184

¹Pearson for Normally Distributed²Spearman's For Non-normally Distributed

Table 3. No significant association was noticed between BMI and the reproductive hormone, neither in the correlation coefficient nor in the p-values of the group's members, Table 4.

Discussion

Variations in the level of reproductive hormones with age have been demonstrated previously²². Additionally, BMI, lifestyle factors, and acute or chronic diseases have been shown to modify the hypothalamic-pituitary-gonadal axis, leading to alterations in the levels of sex hormones²³. Adequate levels of androgens, particularly TT, are crucial in male well-being, lifespan, and positive health outcomes. As men age, their TT levels decline gradually²⁴. Other factors influencing the circulating TT levels are nutrition and environment.

The population for the present study was recruited from Hunza Valley in Gilgit-Baltistan, a mountainous northern area of Pakistan. To the best

of our knowledge, the present study is the first to explore the relationship of reproductive hormone profile and age in this population. This study is the first of its kind to have looked at the unique population of elderly men from a remote region where, traditionally, men are known to have well-maintained sexual function and reproductive ability even into their old age. This fact could also be postulated under the fact that these people have life longevity^{25,26}. Anecdotal evidence and local folklore suggest the preservation of reproductive function in men well into an advanced period owing to the typical dietary habits and highly active lifestyle. The present study was thus carried out to determine the levels of reproductive hormones in this unique population of elderly men, and comparisons were made with healthy middle-aged men as controls.

The results of the present study showed BMI to be similar in both elderly and middle-aged men and within the normal ranges. Expectedly, the dietary patterns were also similar to the

Mediterranean diet in elderly and middle-aged men. Moreover, as expected, elderly and middle-aged men were physically active and involved in all kinds of labor associated with people living in mountainous areas of Pakistan.

Mediterranean diet provides a large quantity of antioxidants in addition to other essential nutrients. It is known that antioxidants are crucial for male fertility, as they protect sperm from damage, improve sperm quality, and are beneficial for aging^{27,28}. Mediterranean diet, vegetables, cereals, fruits and fish, and physical activity are directly related to the quality of sperm and have been suggested to slow the aging process²⁹.

The present study showed that the levels of LH were significantly higher in older men than in middle-aged men. Still, although higher, the levels of FSH did not show statistical significance. Serum SHBG, serum TT, and FT were similar between the two groups. It has previously been suggested that a positive correlation exists between aging and serum levels of LH and FSH related to the decline in TT levels, which signals the hypothalamic-pituitary axis to release LH and FSH^{30,31}. The current findings are consistent with previous data; however, the present results do not indicate a lowering of TT in elderly men.

Additionally, the study highlighted a positive correlation between FSH levels and physical performance in men, indicating that higher FSH levels are associated with better physical performance³². Thus, consistent with these results, our findings of increased LH and similar TT, SHBG, and FT in elderly men can be explained based on their BMI being maintained within the normal range and physically active lifestyle. Moreover, TT and FT have been shown to change in men with varying BMI, and an increase in TT and FT is seen in men who maintain a stable BMI³³. Furthermore, older males with active lifestyles have been shown to maintain levels of serum TT³⁴. Another critical aspect of our study was the association of OS with inhibin and reproductive hormones; no significant correlation was observed between the study variables in group A. Additionally, it has been previously reported that exercise positively affects levels of TT and LH in men^{35,36}. Improved diet and increased physical activity have also been shown to normalize the levels of TT³⁷. These findings concord with the present results, showing that regular physical

activity improves various physiological parameters in this population.

The small sample size limits the study, restricting the derivation of generalizable conclusions. Furthermore, the lack of a comparative group of younger men is another limitation that future more extensive studies might address to inform whether the observed findings have more to do with genetics or lifestyle.

Conclusion

The present study found a well-preserved reproductive endocrine profile in aging males from the mountainous area of Pakistan, supporting the local legends of their lifelong fertility and reproductive potential. The present findings also underscore the potential synergistic benefits of the Mediterranean diet and regular physical activity in older men for improving reproductive health outcomes. Further studies are required to explore the genetic determinants of these men's reproductive profiles to gain insights into the interplay between modifiable environmental factors and genetic predispositions on aging and male reproductive health.

Conflict of interest

None of the authors have declared any conflict of interest for this work.

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