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Original Article

Effects of Omega 3 on Testosterone Hormone Levels and Quality of Spermatozoa in Obese Rattus Norvegicus Wistar Albino Strain

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Chronic obesity can lead to inflammation of the testes which can affect the production of testosterone and spermatogenesis. As omega 3 is acting as an anti inflammatory, this study aimed to investigate the effects of omega 3 on serum testosterone levels and quality of spermatozoa in obese rats. 25 male rats, 2-3 months old and weighting 160-240 g were divided into 5 groups. The control group was given a standamrd diet. The treatment 1 group received high fat diet and cheese while the treatment 2, 3, and 4 groups were given high fat diet and cheese for 6 weeks followed by omega 3 administration at doses of 28.8, 46.8, and 64.8 mg, respectively during the next 51 days . At the end of treatment period, serum testosterone levels were analyzed by ELISA, and sperm amount, motility, viability, and morphology were observed under a digital microscope. Data were analyzed using one way Anova test followed by Bonferroni post hoc test to analyze the differences of influence between groups. Results showed that serum testosterone levels as well as the amount and viability of spermatozoa were not significantly different between studied groups (P= 0.230, P= 0.071, and P= 0.241, respectively). The mobility of spermatozoa (P= 0.039), and their morphology (P= 0.047) were significantly affected by omega 3 treatment. Omega 3 may affect fertility by improving the motility and morphology of spermatozoa.

Keywords: Omega 3, testosterone, quality of spermatozoa, obesity

Obesity is one of the most common nutritional problems (1). Generally, obesity occurs due to a much larger intake of energy within a certain time. The cause of obesity is complex and can be linked to behavioral, environmental, metabolic, and genetic factors (2).

The World Health Organization data (WHO) suggests that by 2014 more than 1.9 billion adults aged 18 years and older were overweight and more than 600 million of these people were obese, 39% of adults over the age of 18 were overweight and 13% were obese (3). In addition, data from Basic Health Research Indonesia 2013 reported that in Indonesia the obesity rate increased from 15% in 2010 to 20% in 2013 for men, and for women the obesity rate increased from 26% to 35% (4).

Obesity can affect the body's functional systems including the reproductive system by inhibiting steroidogenesis (5). In addition, obesity induces infiltration of immune cells and inflammation, with large adipocytes producing more reactive oxygen species (ROS) (6). In obese men there will be a

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reduction in leydig cells and an increase in pro-inflammatory markers in the testes. Chronic inflammatory conditions will negatively impact steroidogenesis by leydig cells (7).

Omega 3 is an essential fatty acid. Fatty acids can effect on obesity by reducing inflammation through the constraints of arachidonic acid formation on the phospholipid membranes that activate pro-inflammatory cytokines. In addition, omega 3 may also inhibit nuclear factor kappa beta (NF-kb), which is a key factor for transcription of pro-inflammatory cytokines (8). The aim of this study was to investigate the effect of omega 3 administrations on testosterone levels, and the quality of spermatozoa in obese rats.

Materials and methods

Animals and study design

This research was an experimental study with post test only design. 25 adult male Rattus norvegicus starin wistar albino weighting 160-240 g and aged 2-3 months old were divided into 5 groups. The control was given a standard diet, the treatment 1 group was given high fat diet and cheese while the treatment 2, 3 and 4 groups were given high fat diet and cheese for 6 weeks and then received omega 3 doses of 28.8, 46.8, and 64.8 mg, respectively for 51 days. On the 52nd day, testosterone levels and spermatozoa features were analyzed in all groups. This study has obtained ethical approval from the Research Ethics Committee at the Faculty of Medicine, Andalas University

Hormonal analysis

Examination of rats serum testosterone levels was performed using ELISA method. Results were expressed as mean± SD.

Characterization of spermatozoa

The amount, motility, viability, and morphology of spermatozoa were determined under a digital microscope. Spermatozoa viability shows the proportion of sperm that live normally after exiting the testes. Spermatozoa viability was obtained from the observation of the structure of the spermatozoa in the head area. The presence of a transparent color upon eosin staining means that the spermatozoa are still alive and dead spermatozoa present a colored head area. The surviving sperm has an acidic cover layer. In live spermatozoa, eosin solution cannot enter the sperm's body because they both are acidic. But in dead sperm the outer layer is damaged and alkaline, so it can absorb eosin (3). Viability was expressed as percentage. In normal morphological situation, the head is shaped like a sickle or hook, the middle part is called short (middle piece), and has a long tail (9, 10). The morphology of spermatozoa is said to be abnormal when there are several abnormalities such as size abnormalities including large or small head, abnormal shape like twin heads, neck disorders, the middle part presenting a curved middle shape or being thin, and abnormalities in the tail such as short, double tail, rolled tail, no tail, and bent tail (11).

Statistical analysis

The data obtained were tested for normality by the shapiro-wilk test, and the data were processed by parametric one-way Anova followed by post hoc Bonferroni differences of influence test between groups, with a significance level of $P < 0.05$.

Results

Evaluation of testosterone levels

Table 1 shows the levels of testosterone in different groups. The highest average testosterone level was observed in the treatment group 4 while the lowest level was observed in the treatment group

Table 1. Testosterone levels in controls and omega 3 treated groups

Group	Testosterone (ng/ml)		
	N	Mean± SD	P
Control	5	13.33±6.88	
Treatment 1	5	15.43±2.74	0.230
Treatment 2	5	10.39±5.04	
Treatment 3	5	14.84±9.06	
Treatment 4	5	23.21±14.36	

2. There was no significant difference in testosterone levels between the control and treatment groups after administration of omega 3 to Rattus norvegicus strain wistar albino obese rats (P= 0.230).

Characterization of spermatozoa

Table 2 shows the amount of spermatozoa determined for each tested group. The highest average amount of spermatozoa was found in the control group while the lowest average was found in the treatment 1 group. There was no significant difference in the amount of spermatozoa between the control and treatment groups after administration of omega 3 to Rattus norvegicus wistar albino obese animals (P= 0.071).

Table 3 shows the percentage of motile spermatozoa in different groups. The highest sperm motility percentage was observed in the control group while the lowest percentage was present in the treatment group 1. There was a significant difference in sperm motility between the control and treatment groups after the administration of omega 3 to Rattus norvegicus wistar albino obese strain (P= 0.039).

Based on the results of the post hoc bonferroni test in table 4 it can be concluded that there was a significant difference in sperm motility between the control group and treatment 1 (P= 0.036), while the omega treated obese rat groups did not show a significant difference (P > 0.05).

Sperm viability was determined as the ability of live spermatozoa outside the body within a time range of 20-30 min after being expelled (3). In table 5 the highest spermatozoa viability was found in the control group while the lowest rate was observed in the treatment group 2. There was no significant difference in spermatozoa viability between the control and treatment groups administered with omega 3 (P= 0.241).

In table 6 the highest amount of normal spermatozoa morphology was found in the treatment group 4 while the lowest average was in the treatment group 2. There was a significant difference in sperm morphology between the control

Table 2. Amount of spermatozoa in controls and omega 3 treated groups

Group	Amount of spermatozoa (milion/ml)		
	N	Mean± SD	P
Control	5	44.30±14.33	
Treatment 1	5	26.80±9.19	0.071
Treatment 2	5	41.60±6.41	
Treatment 3	5	34.90±7.48	
Treatment 4	5	35.60±7.94	

Table 3. Motility of spermatozoa in controls and omega 3 treated groups

1Group	Spermatozoa motility (%)		
	N	Mean ± SD	P
Control	5	61.28±10.31	
Treatment 1	5	28.96±10.16	0.039
Treatment 2	5	40.30±13.97	
Treatment 3	5	51.09±19.58	
Treatment 4	5	49.55±20.42	

Table 4. Post hoc bonferroni multiple comparison test on spermatozoa motility

Group	Significance level of spermatozoa motility (P)				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Control	-	0.036	0.452	1.000	1.000
Treatment 1	0.036	-	1.000	0.356	0.489
Treatment 2	0.452	1.000	-	1.000	1.000
Treatment 3	1.000	0.356	1.000	-	1.000
Treatment 4	1.000	0.489	1.000	1.000	-

and the groups administred with omega 3 (P = 0.047).

Based on the results of the post hoc bonferroni test in table 7 it can be concluded that regarding sperm morphology there was no significant difference between groups after administration of omega 3.

Table 5. Viability of spermatozoa in controls and omega 3 treated groups

Group	Spermatozoa viability (%)		
	N	Mean ± SD	P
Control	5	87.67±7.76	
Treatment 1	5	77.87±7.71	0.241
Treatment 2	5	76.71±5.78	
Treatment 3	5	82.47±10.85	
Treatment 4	5	81.14±6.43	

Table 6. Morphology of spermatozoa in controls and omega 3 treated groups

Group	Spermatozoa with normal morphology (%)		
	N	Mean ± SD	P
Control	5	73.99±13.95	
Treatment 1	5	60.12±8.34	0.047
Treatment 2	5	57.79±19.4	
Treatment 3	5	64.2±8.81	
Treatment 4	5	81.16±10.57	

Table 7. Post hoc bonferroni multiple comparison test on spermatozoa morphology

Group	Significance level of spermatozoa morphology (P)				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Control	-	1.000	0.606	1.000	1.000
Treatment 1	1.000	-	1.000	1.000	0.178
Treatment 2	0.606	1.000	-	1.000	0.095
Treatment 3	1.000	1.000	1.000	-	0.505
Treatment 4	1.000	0.178	0.095	0.505	-

Discussion

The results of the present study showed that the administration of omega 3 did not significantly influence the levels of the testosterone hormone of albino obese male rats. The mean testosterone levels were high, ranging from 10.39 ng/ml to 23.21 ng/ml, in comparison with the normal levels of total testosterone in white adult male rats which vary from 0.5 ng/ml to 5.4 ng/ml (12). In normal men the testosterone value ranges from 300-1100 ng/dl (13). Our data are concordant with those found by Alarcon et al. (14) where the intake of omega 3 was performed in 4 quartiles with a 1 year duration of the study. Relatively, free testosterone and total testosterone levels did not show a significant difference from the first to four quartiles (14). The results of the present study are in contradiction with the results of Risso et al. (15) in which the administration of fish oil supplements for 120 days increased the concentration of testosterone in male dogs.This difference can be attributed to the time of administration of omega 3 which was relatively unequal or shorter (ie 51 days later in our study), the composition of fish oil supplements (fish oil versus pure omega 3), as well as the type of animals (dogs versus obese rats). Increased adipose tissue in obesity cases results in hyperplasia and tissue hypertrophy, while obesity and overweight over a long period of time during prepubertal period results in reduced amounts of leydig cells and an increased level of pro-inflammatory markers in the testes. This is related to chronic inflammation which may have a negative impact on steroidegenesis by leydig cells, meaning that chronic inflammation of the testicular leydig cells results in impaired production of testosterone (7).

A study performed on Padang Indonesian Institute of Health Science students revealed that there was no significant relationship between obesity and testosterone levels (16). This supports the results of the present study where the average testosterone levels of the five studied groups did not differ significantly.

In addition to inflammation in obesity, there is also resistance to the leptin hormone which can be described as endangering male fertility through a specific mechanism such as causing steroidegenesis barriers (5).

As an anti-inflammatory, omega 3 or n-3 polyunsaturated fatty acids (PUFA) is expected to suppress inflammation. Many clinical studies have been conducted for decades to investigate the effects of dietary fatty acids on the inflammatory response in obese people (8).

Aside from being an anti-inflammatory, omega 3 also plays a role in the process of steroid hormones namely testosterone biogenesis. In this process, omega 3 as a fatty acid can control the expression of the steroidogenic acute regulatory (*STAR*) gene which encodes for a cholesterol transporter that controls the transfer of cholesterol from the cytoplasm into the mitochondria. This begins the conversion of cholesterol to produce testosterone (17).

The mean amount of spermatozoa of the five groups was not significantly different. Therefore, the administration of omega 3 has not significantly affected the amount of spermatozoa of albino obese male rats. However, the average amount of spermatozoa in the present study was relatively low and ranged from 26.8 to 44.3 million/ml. Comparatively, the normal range of spermatozoa in wistar rats is 35.5-175 x 10^6/ml (18), and the normal amount of spermatozoa in humans is about 20 million spermatozoa/ml ejaculate (3). If the amount of spermatozoa is reduced it can be said to be of no quality. One of the factors causing the lack of spermatozoa is damage to cells or tissues due to the formation of free radicals (19). Similar to our study, Mendeluk et al. (20) did not found any significant difference in sperm concentration before and after consumption of omega 3, whereas Risso et al. (15) suggested that long-term fish oil supplementation could increase sperm concentration in dogs. This discrepancy could be due to the relatively unequal time of omega 3 administration, different fish oil supplements or different animal models studied.

The provision of high-fat food can increase the accumulation of energy in the body. High-fat feeding has been shown to cause increased levels of glucose, insulin, and triglycerides and result in mitochondrial sperm cell disorders (21). The increase of glucose levels may disrupt spermatogenesis as the sperm needs energy for its survival. This is evidenced by the presence of glucose transporters from the acrosome to the sperm tail. Therefore, if glucose cannot enter the sperm cell, then the cell will lack energy and will be damaged (22). In addition, high-fat dietary feeding performed by Fan et al. showed that obesity induced in rats with high-fat foods for 10 weeks did not affect sperm concentration, but caused sperm motility decrease (23). A study from Iran reported a relationship between omega 3 fatty acids and sperm quality and fertility. This study suggested that infertile men have a lower concentration of omega 3 fatty acids in their sperm in comparison with fertile men (8, 24). Besides being an anti-inflammatory, omega 3 also acts as a sertoli cell fuel, and is also used in membrane remodeling during germ cell development (15).

The mean sperm motility of the five studied groups were significantly different. The average amount of sperm motility in the present study ranged from 28.96% to 61.28%, implying that the increase in omega 3 doses did not always improve sperm motility as witnessed by the mean spermatozoa motility values observed in treatment 3 group which was higher than that of treatment 4 group. Furthermore, the data analysis of bonferroni test revealed a significant difference in sperm motility between control and treatment 1 groups (P= 0.036)

where the administration of high fat diet in the treatment 1 group has decreased the sperm motility. This was also supported by Fan et al. who showed that obesity induced with high-fat foods for 10 weeks in rats decreased sperm motility (23). The influence of omega 3 or fish oil supplements on sperm motility increase was also demonstrated in other studies (12, 25).

The motility of spermatozoa is influenced by several factors including the time of examination after ejaculation, the time between ejaculation, temperature, ionic composition, electromagnetic radiation, ROS, viscosity, pH, osmotic pressure, immunological aspects, the presence of stimulatory factors, and motility inhibition. Spermatozoa damage caused by ROS can inhibit acrosomal reactions and tail damage which is very influential on sperm motility (26). High ROS levels can damage the mitochondrial membrane causing loss of potential mitochondrial function which will interfere with sperm motility because sperm motility energy is supplied in the form of ATP synthesized by mitochondria in the tail body (27).

The administration of high-fat diets to rats caused a decrease in sperm motility. This was evidenced by Ferramosca's et al. who showed that sperm mitochondrial damage resulted in impaired sperm motility. This is due to the fact that sperm needs energy produced by mitochondria. Disruption of energy formation in sperm leads to sperm motility decrease (21).

Under obesity, the fat cells will multiply. These fat cells secrete the aromatase enzyme that converts testosterone to estradiol. Testosterone is transformed into estrogen according to weight gain. The more one gets weight gain, the lower the testosterone level, and the higher the estrogen level (28).

In addition, obesity predisposes to inflammation. Omega 3 as an anti-inflammatory compound, suppresses pro-inflammatory cytokines. Besides omega 3 can increase sperm motility. Docosahexaenoic acid (DHA) is an omega-3 fatty acid that is highly concentrated in the testicles and influences the formation of acrosomes which are enzymes acting on ovum fertilization (29).

The results of spermatozoa viability showed that the mean spermatozoa viability of all studied groups was not significantly different.. It can be interpreted that the administration of omega 3 has not significantly affected the sperm viability of albino obese male rats. The average viability of spermatozoa in this study was relatively high, varying between 76.71% to 87.67%, in comparison with normal standard sperm viability of > 58%. Our data were in contradiction with those of Risso et al. that used a long-term oil supplements among dogs (15). The epididymis plays an important role in spermatozoa viability as spermatozoa mature in the epididymis that provides food supplies, especially glucose as a substrate for spermatozoa metabolism (30). If during spermatogenesis the hormonal balance is disrupted, the viability of produced spermatozoa will not be good. Ferramosca et al. stated that obesity condition induced with a high-fat diet can cause mitochondrial spermatozoa damage (21). This is influenced by an increase in ROS in fat tissue. An increase in ROS triggers the recruitment of macrophages in adipose tissue (6). Fatty acids and especially DHA are needed for cell membrane composition. DHA is highly concentrated in the retina of the eye, cerebral cortex, testes, and sperm (31).

The morphological data of spermatozoa showed that there was an effect of omega 3 administration on obese rat spermatozoa morphology. Based on the results of the post hoc bonferroni multiple comparison test, no significant morphology difference was observed among studied groups. The mean sperm morphology in each study group was high with amounts ranging from 57.79% to 81.166%. The mean morphological value of rat spermatozoa in this study (57.79% to 81.16%) was high compared to the normal 2010 WHO standard of 40%.

The spermatozoa head functions to penetrate the

ovum wall during fertilization, and also contains a cell nucleus that carries DNA. If morphological abnormalities occur in the head, the fertility process will be disrupted and genetic information to be inherited will also be disrupted. The abnormal spermatozoa tail results in a lack of energy produced so that a lack of energy will affect the growth and movement of the spermatozoa (32).

The results of the present study showed that administration of omega 3 for 51 days affected the morphology of obese rat spermatozoa (P= 0.047). This study was corroborated by Safarinejad et al. (24) where the concentration of serum omega 3 was associated with male fertility. The higher the level of infertility, the lower serum concentration of omega 3. This was related to the sperm morphology. Besides, the study of Risso et al. on dogs showed that long-term fish oil supplementation can improve the morphology of normal spermatozoa (15). This is also in line with the findings of Attaman et al. (33) in which the consumption of omega 3 in idiopathic infertile men affected the normal sperm morphology by increasing the amount of spermatozoa with normal morphology.

In conclusion, the administration of omega 3 has an effect on increasing the motility and the amount of sperms in obese rats, and has no effect on serum testosterone levels, the amount and viability of obese mouse spermatozoa.

Further studies on obese men to examine the effect of omega 3 on testosterone levels may be performed to strengthen this study. Also investigating the effects of prolonged administration of omega 3 on obese men on improving the fertility is recommended..

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Conflict of interest

The authors declared no conflict of interest.

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