THE EFFECT OF TAURINE ON REPRODUCTIVE EFFICIENCY IN MALE RATS FED HIGH CHOLESTEROL DIET

Nameer A.Kareem Alzubaidi Mohammed Ali Al Diwan

Departement of Physiology, College of Veterinary Medicine, University of Basrah, Basrah- Iraq

(Received 30 October 2012, Accepted 10 December 2012)

Keywords: cholesterol, taurine, hypercholesterolemia

ABSTRACT

The study designed to use high cholesterol diet to male rats(*Rattus norvegicus*) and asses the ability of the different concentrations of taurine to protect the reproduction from the harmful effect of hypercholesterolemia during 4 weeks of treatment .Thirty six adult male rats were used, randomly divided into six equal groups (six for each)as control and five treatment groups. Control of animals were fed on the standard ration. First treated group was supplied with the standard ration in addition to 1.5% taurine . Second treated group was supplied with standard ration in addition to 1.5% cholesterol .Third, Fourth and Fifth treated groups were supplied with standard ration in addition to 1.5% cholesterol and 2,3and 4% taurine /kg ration respectively and were handled for four weeks. At the end of the experiment the blood serum samples were collect and FSH, LH, testosterone and estrogen levels were taken and sperm vitality was recorded in addition to the weight of testis and epididymis were recorded. The results revealed to the positive role of taurine in protection of reproductive from the pad effect of hypercholesterolemia in male rats .The taurine led to increase in Gonadotropin hormones FSH and LH in addition to the testosterone after it has been reduced due to cholesterol in the ration and as a results to gonadotropin and testosterone hormone improvement ,the sperm viability was improved as well after it was decline due to high cholesterol addition in experimental diet .

INTRODUCTION

Abnormality of lipid metabolism is known to be associated with life style –related diseases such as metabolic syndrome .It is necessary to normalize cholesterol metabolism in blood for prevention and treatment atherosclerosis (1) .

Hypercholesterolemia was found to cause an increase in oxygen radicals production and lipid peroxidation levels in different tissues(2).Lipid peroxidation is an important factor that may induce morphological changes in the spermatozoa(3).Feeding rats with diet supplemented with fat leads to increase in testes cholesterol level and degeneration of some gonadal cells (4).The feeding of male rabbits on a diet rich in fat results in a functional disorder of hypothalamo-pituitary gonadal axis associated with short penis and damage of spermatogenesis (5).It was found that the decrease in HDL and elevation of total cholesterol resulted in erectile dysfunction in men(6). Taurine, 2-aminoethanesulfonic acid, conditionally essential nutrient, is synthesis from amino acids cysteine and methionine and it is the major free amino acid that found in many animal tissues (7,8). There are many studies on physiological role of taurine and most of the researchers suggested physiological functions of taurine include conjugation with bile acid and fat metabolism (9), inhibitory neurotransmitter (10,11) and brain and retinal development (12) cell membrane stabilization (13), detoxification (14), antioxidation and protecting blood vessels and blood cells from oxidative damage (15), controlling osmoregulation through membrane transport (16),. Degradation and reduce high cholesterol level in serum and liver (17). It has been reported that taurine can be biosynthesized by male reproductive organs (18). In the male reproductive system, taurine has been detected in Leydig cells, vascular endothelial cells, and some other interstitial cells of testis and epithelial cells of efferent ducts in rats (19). Taurine may act as an antioxidant (20), capacitating agent(21,22), membrane-stabilized factor (23) and motility factor (24) of sperm.The main aim of present study was to investigate the role of dietary taurine supplementation to reduce the oxidative stress induced by high cholesterol diet on the male reproduction in rats .

MATERIAL AND METHODS

Thirty six adult male (*Rattus norvegicus*) , weighting about 120-125g were maintained at standard experimental condition . Rats were housed in stainless steel cages in a room with controlled temperature and humidity for 4 weeks .They were given free access to the experimental diet and water. The rats were allowed to use the experimental diet for one week before the start of experiment . The composition of the experimental diet are shown in Table 1.After the accommodation period , laboratory animals were randomly divided into six groups (each of six) and were

handled as follows for four weeks .Group one control group were fed normal diet ,T1 animals were fed normal diet with taurine (1.5gm/kg diet) .HC group were fed normal diet and 1.5% cholesterol(25) .T2,3 and 4 were fed normal diet and 1.5% cholesterol supplemented with taurine (2 ,3,and 4%) respectively, in the diet .

Testes and epididymis were removed and weighed with an electronic balance . The tail of epidydimis was kept in concave watch glass contain 5 ml normal saline to be used for total sperm account and sperm availability . Blood samples were collected at the end of experiment via cardiac puncture by using 5ml disposable syringe according to the method of (26) .Then the blood put in plan tube to be centrifuged at (3000 rpm for 15 minute) to obtain the serum which then transferred into numerous ependorf tube and stored at -4 c° to measurement Follicle stimulating hormones (FSH) , Luteinizing hormone LH ,testosterone and estrogen Assay by using enzyme-liked immune sorbent assay(ELISA) kit manufactured by Human company for diagnostic and biochemical-Germany.

Table (1) component of experimental diets (g/kg diet)

Ingredients / groups	**Control group**	**T . group1**	**HC group**	**T.group 2**	**T.group 3**	**T.group 4**
Casein	200	200	200	200	200	200
Corn starch	650	635	635	615	605	595
Vitamins and minerals mix.	50	50	50	50	50	50
Corn oil	50	50	50	50	50	50
Cellulose	50	50	50	50	50	50
Cholesterol	0	0	15	15	15	15
Taurine	0	15	0	20	30	40

The sperm were counted according to method of (27)by using Neubauer hemocytometer chamber which use for RBC and WBC count . The same method used to count viability of sperms , the abnormal spermatozoa and the dead spermatozoa .The results of the present study were analyzed by univalent analysis of variance (ANOVA) by using computerized SPSS(Statistical Packages for the Social Sciences') V.13 program under significant level $P<0.05$.

RESULTS

As shown in table (2),the levels of FSH ,LH and testosterone in serum of male rats were obviously increased by taurine administration compared to the control diet group ($P\leq0.05$) , but the level of above hormones were showed sever decline in their values due to cholesterol fed diet .The animals of taurine 3% group showed their hormones values close significantly to the control group value. Whereas the values of T4 was almost close to the values of taurine group .

Table (2) The effect of taurine on reproductive hormones in hypercholesterolemia male rats

parameter / groups	FSH ng/ml	LH ng/ml	Estrogen pg/ml	Testosterone ng/ml
Control group	10.20 ± 0.75 c	3.13 ± 0.33 b	19.93 ± 2.32 a	4.54 ± 0.51 c
T.group1 1.5% taurine	13.80 ± 0.93 a	4.02 ± 0.36 a	20.05 ± 2.16 a	6.45 ± 0.56 a
HC group 1.5% cholesterol	4.05 ± 0.83 d	0.92 ± 0.36 c	11.15 ± 1.73 b	0.74 ± 0.17 d
T. group 2 1.5%cholesterol 2% taurine	11.10 ± 0.78 bc	3.72 ± 0.38 a	19.66 ± 2.36 a	5.71 ± 0.53 b
T. group 3 1.5% cholesterol 3% taurine	11.62 ± 0.91 b	3.86 ± 0.48 a	19.91 ± 1.28 a	5.68 ± 0.79 b
T. group 4 1.5% cholesterol 4% taurine	13.71 ± 0.78 a	3.80 ± 0.53 a	19.56 ± 2.49 a	5.88 ± 0.28 ab
LSD	1.41	0.58	8.41	0.74

Different small letter represent significant difference at($P \leq 0.05$)

As shown in table (3) that taurine improved the sperm concentration ,sperm motility, dead sperm , and abnormal sperm to approach the control values . There were not significant differences among all tested groups supplemented with taurine compared with control diet group. It seemed that the hypercholesterolemic rats group have no live sperm in their semen, therefore, their sperm viability reach zero, and that confirmed by the ratio of testes weight / bodyweight in addition to epididymis weight / body weight were present significantly less than the weight of testes and epididymis ratio to body weight in the other experimental groups .

Table (3) the effect of taurine on seminal analysis of hypercholesterolemic male rat

Parameter / groups	Testes/B.W gm	Epididymus./B.W gm	Sperm concentration $x10^6/mm^3$	Sperm motility %	dead sperm%	abnormal sperm%
Control group	0.78 ± 0.136 a	0.28 ± 0.063 a	180.83 ± 7.049 b	81.0 ± 3.033 ab	15.83 ± 2.483 a	19.66 ± 2.160 a
T.group1 1.5% taurine	0.83 ± 0.083 a	0.31 ± 0.069 a	220.66 ± 10.953 a	86.33 ± 7.607 a	12.0 ± 2.366 b	15.33 ± 3.829 b
HC group 1.5% cholesterol	0.54 ± 0.093 b	0.17 ± 0.031 b	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c
T. group 2 1.5% cholesterol 2% taurine	0.76 ± 0.098 a	0.27 ± 0.051 a	179.33 ± 10.652 b	76.16 ± 5.115 b	17.5 ± 2.428 a	20.33 ± 5.853 a
T. group 3 1.5% cholesterol 3% taurine	0.78 ± 0.106 a	0.29 ± 0.069 a	175 ± 7.874 b	78.83 ± 9.621 ab	17.0 ± 3.687 a	18.33 ± 3.502 ab
T.group4 1.5% cholesterol 4% taurine	0.68 ± 0.179 a	0.29 ± 0.104 a	178 ± 8.921 b	83.16 ± 8.035 ab	16.83 ± 1.471 a	18.5 ± 3.563 ab
LSD	o.17	0.10	39.83	10.16	3.83	4.33

Different small letter represent significant difference at($P \leq 0.05$) .

DISCUSSION

The effect of high cholesterol diet on the development of male reproductive system was decreased the levels of testosterone , LH and FSH , in addition, reducing in nuclear diameter of degeneration Leydige cells was observed ,due to the effectiveness of high cholesterol diet on hypothalamo-pituitary gonadal axis (28). Taurine showed ameliorating effect on the reproductive hormones concentration and that may attributed to the role of taurine to stimulate secretion of LH and FSH through its effect on hypothalamo-pituitary gonadal axis and to regulate the testosterone production from testes by binding to membrane receptors on the leydige cells and stimulates them to convert cholesterol to testosterone and may also had beneficial effect on the biochemical indicators of testis such as acid phosphatase (ACP),lactate dehydrogenase (LDH) , sorbitol dehydrogenase (SDH),AST and ALT that may be important in spermatogenesis by improving the lipid and energy metabolism to increase the spermatogenic cells division and acted as antioxidant in testis which protect the testis from oxidative stress to produced testosterone and estrogen ,and from other side , may be permit to LH and FSH to regulate the increased in testosterone levels .The results came in agreement with 29;30;31**.**

The bad effect of hypercholesterolemia on the sperm quality may be due to produced of reactive oxygen species (ROS) which led to oxidative stress(OS) ,since both hypercholesterolemia and hypertriglyceridemia caused an increase of oxygen radicals production and lipid peroxidation level associated with decreased antioxidative effect of glutathione(32) .Oxidative stress arises as a consequence of excessive production of reactive oxygen species and impaired antioxidant defense mechanism(33) .The generation of ROS had a toxic effect on the sperm quality as a resulte of damage the plasma membrane which contain large quantities of fatty acids and that caused defects in sperm morphology due to induce lipid peroxidation , that led to morphological changes in sperms (34).According to Agarwal *et al*, (35) they reported a significant reduction in rabbits sperm concentration and percentage of motile spermatozoa with hypercholesterolemia. They attributed the reduction of sperm viability to defects in the secretory function of the Sertoli and Leydig cells in which resulting in impaired spermatogenesis and epididymal sperm maturation process and decreased sperm motility and increased sperm abnormalities .Depending on the physiological role of taurine in acting as antioxidant agent and inhibit lipid peroxidation in spermatozoa

and protect against loss of motility , taurine could protect the sperm from ROS and free radicals and kept the motile of spermatozoa , in addition ,taurine acted as membrane stabilizing factor by inhibited sperm Na,K-ATPase activity to protect the sperm plasma membrane from the free radicals and oxidation specially when know that taurine as the major amino acid of sperm cell and seminal fluid (35;36;37; and31).The present results indicated the important role of taurine to improve the semen quality by its effect on stimulation of testosterone secretion and promoting of testis homeostasis as mentioned by Yang *et al,*(31) by increased the biochemical indicators levels ACP,LDH,SDH,AST, and ALT that have important role in spermatogenesis and improve the ability of antinociception and anti-stress in testis cells including spermatogenic cells, and protect spermatozoa due to the antioxidative effect of taurine .

تاثير التاورين على الكفاءة التناسلية لذكور الجرذان المختبرية المغذاة على جرعة عالية من الكوليسترول

نمير عبد الكريم الزبيدي محمد علي محمد الديوان

فرع الفسلجة، كلية الطب البيطري ، جامعة البصرة، البصرة ، العراق

الخلاصة

اشتملت هذه الدراسة على اعطاء جرعة عالية من الكوليسترول في الغذاء لدى ذكور الجرذان المختبرية (*Rattus norvegicus*) لاستبيان دور التاورين بجرع مختلفة على الكفاءة التناسلية خلال مدة ٤ اسابيع من العلاج . استعمل ٣٦ من ذكور الجرذان البالغة والتي قسمت عشوائيا إلى ست مجاميع متساوية (ستة حيوانات /مجموعة) . قدمت للمجموعة الاولى عليقة متوازنة واعتبرت مجموعة سيطرة ، واضيف ١.٥% تاورين للعليقة المتوازنة في المجموعة الثانية ،واضيف الكوليسترول ١.٥% للعليقة المتوازنة لحيوانات المجموعة الثالثة ، بينما اضيف ١.٥% كوليستيرول الى العليقة المتوازنة للمجاميع المتبقية مع التاورين بجرع متزايدة٤,٣,٢ % تاورين /كغم غذاء. وفي نهاية التجربة اخذت عينات مصل الدم ، بالاضافة الى تقييم مستويات تراكيز الهرمونات التناسلية (FSH,LH,Testesterone,Estrogen) كما سجلت اوزان الخصى والبربخ وحسبت اعداد النطف وحيويتها . اظهرت النتائج الدور الايجابي للتايورين في كفاءة التكاثر في الجرذان المختبرية نتيجة تاثيرفرط الكوليسترول . اذ عمل التاورين على زيادة تراكيز هرمونات التكاثر FSH,LH بالاضافة الى Testesterone بعد ان انخفضت قيم الهرمونات اعلاه نتيجة تعرضها الى جرعة عالية من الكوليسترول في الغذاء . وكنتيجة الى التحسن في قيم الهرمونات التناسلية فقد انعكس هذا على حيوية

النطف وعددها وشهدت الدراسة انخفاضا في اعداد النطف الميتة غير السوية مقارنة مع المجموعة التي تناولت الكوليسترول بجرعة عالية .

REFERENCES

1-Oda,Hiroaki. (2006) . Functions of sulure-containing amino acids in lipid metabolism .*j.of .Nutri*.136.1666S-1669S,2006.

2-Ohara,Y.;Peterson,T.E.and Harrison,D.G.(1993). Hypercholesterolemia increases endothelial superoxide anion production. *J.Clin.Invest*.,91:2546-2551.

3-Sanchez,E.E.T.;Marquette,M.L.;Brown,D.B. and Ansari,N.H.(2006). The effect of oxidative stress on human sperm morphology. *Fertil,Steril, 86(suppl.1).S444.In Am.Soc.Repro.Med.62nd* Annual Meeting .

4-Chetrok,V.M.;Botvich,T.A.;Khasina,M.A. and Artyukova,O.A.(2001). Effect of Pollack liver oil on lipid metabolism in rats testes .*Bullet Experim. Biol. Med*., 131: 116-118.

5-Zhu,Z.P.;Huang,Y.F.;Pan,I.J. and Xia ,X.Y.(2005). The effect of diabetic hyperlipidemia on the development of testes and penis in mole New Zealand rabbits. *Zhonghua Nan Ke Xue*,11:904-907.

6-Rao,K.;Du,G.H.; and Yang,W.M.(2006). Hyperlipidemia and erectile dysfunction .*Zhonghua Nan Ke Xue* ,12:643-646 .

7-Chesney, R. W. (1985). Taurine: Its biological role and clinical implications. *Adv.Pediatr.* 32: 1– 42 .

8- Wright, C. E.; Tallan, H. H.; Lin, Y. Y. and Gaull, G. E. (1986). Taurine: Biological update. *Ann. Rev. Biochem.* 55: 427– 453.

9- Danielsson, H. (1963). Present states of research on catabolism and excretion of cholesterol. *Adv. Lipid Res.* 1: 335–385.

10- Bernardi, N. (1985). On the role of taurine in the cerebellar cortex: A reappraisal. *Acta. Physiol. Pharmacol. Ther. Latinoam*. 35: 153–164.

11-Kuriyama, K. (1980). Taurine as a neuromodulator. *Fed. Proc*. 39: 2680 –2684.

12-Sturman, J. A. (1986). Nutritional taurine and central nervous system development. *Ann. NY Acad. Sci*.; 477: 196 –213

13- Pasantes- Morales H.;Martin, D.L.;Shain, W.;Martin, del and Rio, R.(1990). Taurine :functional neurochemistry ,physiology and cardiology . New York:Wiely-liss,Inc .

14- **Huxtable, R. J. (1992).** Physiological actions of taurine. *Physiol. Rev*. 72:101–163.

15- Nakamura, T.; Ogasawara, M.; Nemoto, M. and Yoshida, T. (1993). The protective effect of taurine on the biomembrane against damage produced by oxygen radicals. *Biol. Pharm. Bull*. 16: 970 –972.

16- Schaffer,S.;Takahashi,K.; and Azuma,J.(2000). Role of osmoregulation in the action of taurine .*J.Amino acid.*19:527-546.

17- Yokogoshi, H.; Mochizuki, H.; Nanami, K.; Hida, Y.;Miyachi,F. and Oda , H. (1999). Dietary taurine enhances cholesterol degradation and reduces serum and liver cholesterol concentrations in rats fed a high-cholesterol diet. *J. Nutr.* 129: 1705-1712

18- Li, J.H.; Ling, Y.Q.; Fan, J.J.; Zhang, X.P.;and Cui, S.(2006). Expression of cysteine sulfinate decarboxylase (CSD) in male reproductive organs of mice. *Histochem and Cell Biology 2006*, 125:607-613.

19- Lobo, M.V.T.; Alonso, F.J.M. and Rio, R.M.(2000). Immunohistochemical localization of taurine in the male reproductive organs of the rat. *Journal of Histochemistry and Cytochemistry 2000*, 48(3):313-320.

20-Alvarez, J.G.and Storey, B.T.(1983). Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. *Biology of Reproduction 1983*, 29:548-555.

21- Meizel, S.; Lui, C.W.; Working, P.K.and Mrsny, R.J.(1980). Taurine and hypotaurine: their effects on motility, capacitation and the acrosome reaction of hamster sperm in vitro and their presence in sperm and reproductive tract fluids of several mammals. *Development, Growth & Differentiation 1980*,22(3):483-494.

22- Meizel, S.(1985). Molecules that initiate or help stimulate the acrosome reaction by their interaction with the mammalian sperm surface. *American Journal of Anatomy 1985*, 174(3):285-302.

23- Mrsny, R.J and Meizel, S.(1985). Inhibition of hamster sperm Na+, K+-ATPase activity by taurine and hypotaurine. *Life Science 1985*, 36(3):271-275.

24- Boatman, D.E.; Bavister, D.B.and Cruz, E.(1990). Addition of hypotaurine can reactivateimmotile golden hamster spermatozoa. *Journal of Andrology 1990*,11(1):66-72.

25- Choi,M.J.;Kim,J.H.;Chang,K. and Yung J.(2006). The effect of dietary taurine supplementation on plasma and liver lipid concentrations and free amino acid concentrations in rats fed a high cholesterol diet. Taurine 6 advances in experimental medicine and biology .Edited by S.S. Oja and P.Saransaari,Springer,New York 2006.Pg 235-242.

26 -Hoff,J. and Rlatg,L.(2000) .Methods of blood collection in the mouse ,*J. Lab . Anim.*,29:45-47 .

27- Evan , G . and Maxwell, W. M . C . (1987) . Salamona artificial insemination of sheep and goat . Butter woth , Sydney , Australia.

28-Shalaby, M.A.; El-Zorba, H.Y. and Kamel, G.M.(2004). Effect of alpha tocopherol and simvastatin on male fertility in hypercholesterolemic rats. *Pharmacol Res. 2004*; 50(2): 137-142.

29-Xiao,S.P.;Fu,W.L. and Jiang,Q.Y.(1997). Effect of taurine on development and endocrine of gonads of Yue-Huang broilers . *J.South China Agr. Univ. 1997*,18(2):94-99.

30-Yang, J.C.; Feng, Y; Sun, C.M. and Hu, J.M. (2007). Effect of taurine on the secretion of reproduction hormone of male rat. *J Anhui Agri Sci*.35 (11): 3283–3284.

31- Yang,J.C.; Wu,G.F.;Feng,Y.;Sum,C.M. and Hu,J.M.(2010).CSD mRNA expression in rats testes and the effect of taurine on testosterone secretion .*Amino acids, 2010*, 39(1):155-160.

32-De La Cruz,J.P.L.;Quintro,M.A.;Villalobos,F.S. and De La Cuesta,(2000). Lipid peroxidation and glutathione system in hyperlipidemic rabbits influence of olive oil administration .*Biochem.Biophys.Acta.,*1485:*3*6-44.

33-Sikka,S.C.(2001). Relative impact of oxidative stress on male reproductive function .*Curr.Med.Chem*.,8:851-862.

34-Sanchez,E.E.T.;Marquette,M.L.;Brown,D.B. and Ansari,N.H.(2006). The effect of oxidative stress on human sperm morphology .Fertil.Steril.,86(suppl.1) S444. *In Am. Soc.report.Med.62nd. Annual Meeting* .

35-Agarwal,S.R.;Saleh,A. and Bedaiway,M.A.(2003)..Role of reactive oxygen species in the pathology of human reproduction .*Ferti.Steril*.,79:829-843.

36-Holmes, R.P.; Goodman, H.O.; Shihabi, Z.K. and Jarrow, J.P. (1992). The taurine and hypotaurine content of human semen. *J Androl* 13:289–292

37-Hinton, B.T.(1990). The testicular and epididymal luminal amino acid microenvironment in the rat. *J. of Andrology 1990*, 11(6):498-505.