

ORIGINAL ARTICLE

EFFECTS OF ASCORBIC ACID AND ALPHA TOCOPHEROL SUPPLEMENTATION ON BASAL TESTOSTERONE CORTISOL RATIO IN MALE SPRAGUE DAWLEY RATS

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Background: Basal testosterone cortisol ratio is considered very important to maintain homeostasis. Increase in this ratio has various beneficial effects on body. In this study we determined the effects of ascorbic acid and alpha tocopherol supplementation on basal testosterone cortisol ratio in male Sprague Dawley rats. **Methods:** It was quasi experimental study carried out in department of Physiology, Army Medical College Rawalpindi in collaboration with National Institute of Health, Islamabad during October 2006 to September 2007. Forty male Sprague Dawley rats were divided into four groups with ten rats in each group and above mentioned antioxidants supplementation were given along with standard diet for one month. After this, blood samples were taken and analyzed for serum testosterone and cortisol by ELISA and malondialdehyde levels colorimetrically. Data were analysed on SPSS version 13 and $p < 0.05$ was considered significant. **Results:** There was no significant rise in testosterone cortisol ratio in rats supplemented with single antioxidant; however rats supplemented with combination of ascorbic acid and alpha tocopherol revealed significant rise in testosterone cortisol ratio with a fall in malondialdehyde levels. **Conclusions:** Synergistic effects of ascorbic acid and alpha tocopherol resulted in a decline in reactive oxygen species induced lipid peroxidation and rise of testosterone cortisol ratio.

Keywords: Testosterone cortisol ratio, Ascorbic acid, Alpha tocopherol, Malondialdehyde

INTRODUCTION

Testosterone is the principal androgen in males. Leydig's cells are responsible for testosterone production which depends upon stimulation of these cells by Luteinizing Hormone (LH). Cortisol is one of the corticosteroids secreted from adrenal cortex. Glucocorticoids exert various deleterious effects on the interstitial Leydig cells of the testis that may range from direct inhibition of testosterone synthesis at Leydig cell level to suppression of LH receptor expression and induction of Leydig cell apoptosis.^{1,2}

The relationship between plasma testosterone and cortisol has been discussed more than 20 years ago when experiments were performed to prove that elevation of plasma cortisol concentrations resulting from insulin-induced hypoglycaemia or after the administration of cortisol was followed by decline in plasma levels of testosterone. This was not related with changes in pituitary gland, and plasma LH levels remained unchanged.³ This ratio is considered very important to maintain homeostasis and increase in ratio has various important effects on body.

Increased cortisol, decreased testosterone, and the fall of ratio between testosterone and cortisol levels is thought to happen in stress.⁴ Cortisol is a catabolic hormone while testosterone is considered as an anabolic hormone and their ratio may reveal body's catabolic/anabolic balance which is disturbed

in various conditions and leads to generalised weakness and muscle wasting.⁵ If there is an increase in glucocorticoids and a decline in testosterone levels, it shows the catabolic tendency.⁶

Our body is exposed to oxidants even without active stress. These oxidants may cause lipid peroxidation with generation of reactive oxygen species. Lipid peroxidation can be assessed by malondialdehyde (MDA) levels. Antioxidants, both water soluble and lipid soluble comprise an important aspect of the antioxidant defence system. An antioxidant has been defined as 'any substance that, when present at low concentrations compared to those of an oxidisable substrate (e.g., proteins, lipids, carbohydrates and nucleic acids) significantly delays or prevents oxidation of that substrate'.⁷

Dietary intake or supplementation with antioxidant vitamins is associated with a reduction in the incidence of chronic disease morbidity and mortality.⁸⁻¹⁰ It is proposed that antioxidants, ascorbic acid and alpha tocopherol increase testosterone cortisol ratio by decreasing lipid peroxidation at basal stress levels. No study was found in literature showing effects of above mentioned antioxidants on testosterone cortisol ratio in basal stress conditions. Therefore, this study was planned to investigate the effects of antioxidants supplementation on levels of testosterone, cortisol and testosterone cortisol ratio, in relation to Malondialdehyde.

MATERIAL AND METHODS

This study was conducted in the Department of Physiology, Army Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad. Forty male, healthy, Sprague Dawley rats, at least 60 days old were included in study. Duration of study was one year. Rats were divided into four groups. Group I served as control and took standard diet without any supplementation. Group II received ascorbic acid at a dose of 500 mg/L drinking water¹¹ (10 rats consumed one litre water in 3–4 days) and group III was given alpha tocopherol supplementation 300 mg/Kg chow +2% soya bean oil for one month.¹¹ Group IV received both ascorbic acid and alpha tocopherol supplementation for one month.

After one month, intracardiac blood samples were taken in morning between 8 and 9 AM. To avoid bias due to different values among cortisol and testosterone because of diurnal variations, all samples were taken at same time. After clotting, samples were first centrifuged at 4,000 rpm at 4 °C in the cold centrifuge. Then serum was pipetted out and stored in Eppendorf storage tubes at -70 °C till analysis. Serum testosterone and cortisol were determined by ELISA and malondialdehyde levels were estimated colorimetrically with commercially available kits.

Data were analysed on SPSS-13. The arithmetic mean and standard deviation of all samples were calculated. Difference in mean among control and treated groups was calculated by 'Independent sample *t*-test'. The difference was considered significant if *p* value was found to be less than 0.05.

RESULTS

The animals in this study remained healthy and active throughout study period and took their feed properly.

Ascorbic acid supplementation for one month showed insignificant change in testosterone, cortisol and their ratio as narrated in our previously published data.¹² Similarly, one month supplementation with alpha tocopherol showed insignificant changes in study parameters as shown in the Table-1. However, combination of ascorbic acid and alpha tocopherol resulted in significant rise in testosterone levels ($p < 0.05$) and fall in cortisol ($p < 0.05$) levels. It was associated with fall in malondialdehyde levels ($p < 0.05$) as shown in Table-2. The calculated testosterone/cortisol ratio was also significantly raised in the group supplemented with combination of ascorbic acid and alpha tocopherol ($p < 0.05$) as shown in Figure-1.

Table-1: Effects of alpha tocopherol supplementation on serum testosterone, cortisol, malondialdehyde levels and testosterone/cortisol ratio in male Sprague Dawley rats

Parameter	Control Group-I (n=10)	Experimental Group-III (n=10)	<i>p</i> *
Serum Testosterone (ng/ml)	3.06±1.29	3.51±1.50	>0.05
Serum Cortisol (ng/ml)	21.00±1.41	20.20±1.87	>0.05
Serum Malondialdehyde (M)	5.25±1.29	4.65±1.59	>0.05
Testosterone Cortisol ratio	0.14±0.07	0.18±0.08	>0.05

* $p > 0.05$ is taken as insignificant

Table-2: Effects of alpha tocopherol and ascorbic acid supplementation on serum testosterone, cortisol, malondialdehyde levels and testosterone/cortisol ratio in male Sprague Dawley rats

Parameter	Control Group-I (n=10)	Experimental Group-III (n=10)	<i>p</i> *
Serum Testosterone (ng/ml)	3.06±1.29	5.76±2.79	<0.05
Serum Cortisol (ng/ml)	21.00±1.41	19.50±1.08	<0.05
Serum Malondialdehyde (M)	5.25±1.29	3.30±1.67	<0.05
Testosterone Cortisol ratio	0.14±0.07	0.29±0.15	<0.05

* $p < 0.05$ is taken as significant

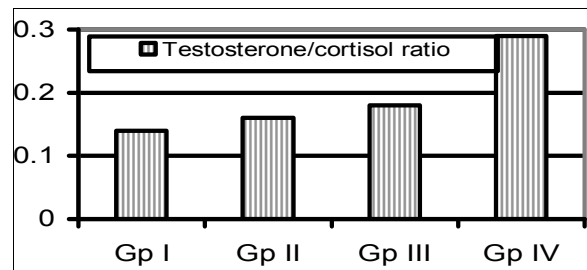


Figure-1: Effects of antioxidants supplementation on basal testosterone cortisol ratio in all groups

DISCUSSION

Decline in testosterone cortisol ratio as mentioned before is indicative of catabolic tendency in body and increasing this ratio is useful to body in various ways. Therefore this study was planned on male Sprague Dawley rats to see the individual as well as combined effects of antioxidants, ascorbic acid and alpha tocopherol on basal testosterone cortisol ratio in relation to lipid peroxidation.

In our study, the rats supplemented with ascorbic acid, show no significant change in basal testosterone levels. The literature shows that the deficiency of ascorbic acid may lead to diminished testosterone production with consequent impaired fertility.^{13,14} In our case there is no significant difference between testosterone levels among the control and ascorbic acid supplemented groups which may be due to the reason that control group was not given ascorbic acid deficient diet and therefore essential amount of ascorbic acid required to maintain normal steroidogenesis was available to controls as well, which could be responsible for such results. However high

doses of ascorbic acid are claimed to increase basal testosterone levels.¹⁵

In this group, cortisol levels were insignificantly changed. Similar results are reported by Xianqing Zhou and their colleagues have supplemented the diet of turtles for four weeks with very high amount of ascorbic acid up to 10,000 mg/Kg diet and their results show that turtles fed on high Vitamin C have no effect on basal cortisol levels.¹⁶ Lipid peroxidation was also not decreased in this ascorbic acid supplemented group as is evident by unchanged malondialdehyde levels.

Rats supplemented with alpha tocopherol have shown no significant elevation of testosterone after one month supplementation. This is in accordance with the study done by Murugesan *et al*, on Leydig cells and they have also shown that alpha tocopherol given in more than normal required levels has no effect on basal testosterone release.¹⁷

Also in our study alpha tocopherol supplementation has shown no effects on serum cortisol levels. Some studies have similar results as is done by Bonnette *et al*, that when given alpha tocopherol supplementation to pigs, no effect on the basal cortisol levels was observed.¹⁸ However, various other studies point towards the fact, that alpha tocopherol protects the rats against various stressors like homocystine induced oxidative stress,¹⁵ Cadmium induced toxicity,¹⁹ nicotine induced damaging effects.²⁰

Regarding MDA levels, they were not affected by alpha tocopherol supplementation. These findings are similar to the study by Jacob *et al* in which they concluded that antioxidants supplementation has no effect on markers of oxidative damage in healthy people.²¹ Various investigators have worked on it, e.g., formaldehyde induced testicular injury can be prevented by alpha tocopherol,²² and ethane dimethane sulfonate induced testicular insult can be prevented by alpha tocopherol.²³ Therefore it can be inferred that alpha tocopherol may lower the raised MDA, but have no effect on basal MDA levels.

In our study group, supplemented with alpha tocopherol and ascorbic acid, testosterone levels were increased while cortisol levels were decreased and thus testosterone cortisol ratio was significantly increased. These results are consistent with the study of Schroder *et al*, in which they have seen that in athletes, combination of antioxidants results in statistically significant increase in testosterone cortisol ratio.²⁴ In this group, serum MDA levels were decreased. These results are similar to that of a recent study in which rats were exposed to fluoride toxicity and supplementation with alpha tocopherol and ascorbic acid reduced the levels of MDA.²⁵

Therefore it is proposed that combination of ascorbic acid and alpha tocopherol has favourable effect

on testosterone cortisol ratio. Ascorbic acid is hydrophilic and is a very important free-radical scavenger in extracellular fluids. It traps the radicals in the aqueous phase and thereby confers protection of biomembranes from peroxidative damage.²⁶ Similarly, alpha tocopherol, which is a component of plasma membrane, is an effective antioxidant and if its sufficient quantity is available at site of oxidative stress where there is free radical generation, it may neutralise the toxic effects of reactive oxygen species.²⁷

Antioxidant supplementation may protect the protein structures, prevent the reactive oxygen species induced enzyme inactivation, stabilise cell membranes, which may be responsible for this protective and favourable effect of ascorbic acid and alpha tocopherol.^{28,29}

CONCLUSION

Synergistic effects of ascorbic acid and alpha tocopherol resulted in a decline in reactive oxygen species induced lipid peroxidation and rise of testosterone cortisol ratio. The mechanism of action of these antioxidants at adrenal and Leydig cell level needs to be elucidated, which may form the basis for future research.

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REFERENCES

1. Monder C, Miroff Y, Marandici A, Hardy MP. 11 beta-hydroxysteroid dehydrogenase alleviates glucocorticoid-mediated inhibition of steroidogenesis in rat Leydig cells. *Endocrinology* 1994;134:1199–220.
2. Gao HB, Tong MH, Hu YQ, Guo QS, Ge RS, Hardy MP. Glucocorticoid induces apoptosis in rat Leydig cells. *Endocrinology* 2002;143:130–8.
3. Cumming DC, Quigley ME, Yen SSC. Acute suppression of circulating testosterone levels by cortisol in men. *J Clin Endo Metabol* 1983;57:671–3.
4. Filaire E, Bernain X, Sagnol, Lac G. Preliminary results on mood state, salivary testosterone: cortisol ratio and team performance in a professional soccer team. *Eur J Appl Physiol* 2001;86:179–84.
5. Debigare R, Marquis K, Cote CH, Tremblay RR, Michaud A, LeBlanc P, *et al*. Catabolic/Anabolic Balance and Muscle Wasting in Patients with COPD. *Chest* 2003;124:83–9.
6. Lac G, Berthon P. Changes in cortisol and testosterone levels and T/C ratio during an endurance competition and recovery. *J Sports Med Phys Fitness* 2000;40(2):139–44.
7. Halliwell B. Vitamin C: antioxidant or pro-oxidant in vivo. *Free Radic Res* 1996;25:439–54.
8. Weber P, Bendich A, Schalch W. Vitamin C and human health—a review of recent data relevant to human requirements. *Int J Vitam Nutr Res* 1996;66:19–30.
9. Enstrom JE. Vitamin C in prospective epidemiological studies. In: Packer L, Fuchs J, eds. *Vitamin C in health and disease*. New York: Marcel Dekker Inc 1997; p.381–98.
10. Gey KF. Vitamins E plus C and interacting co nutrients required for optimal health. *Biofactors* 1998;7:113–74.

11. Hsu PC, Liu MY, Hsu CC, Chen LY, GuoYL. Effects of vitamin E and/or C on reactive oxygen species-related lead toxicity in the rat sperm. *Toxicology* 1998;128(3):169–79.
12. Lodhi GM, Butt IF, Hussain MM, Aslam M. Effects of ascorbic acid and selenium supplementation on basal testosterone cortisol ratio in male Sprague Dawley rats. *Pak Armed Forces Med J* 2009;59(1):14–9.
13. Sapra MM, Sharma PP, Kothari LK. Effect of vitamin C deficiency on testicular structure in the guinea pig. *J Postgrad Med* 1987;33:69–73.
14. Chitra KC, Latchoumycandane C, Mathur PP. Chronic effect of endosulfan on the testicular functions of rat. *Asian J Androl* 1999;1:203–6.
15. Sonmez M, Turk G, Yuci A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. *Theriogenology* 2005;63:2063–72.
16. Zhou X, Xie M, Niu C, Sun R. The effects of dietary vitamin C on growth, liver vitamin C and serum cortisol in stressed and unstressed juvenile soft-shelled turtles (*Pelodiscus sinensis*) *Comp Biochem Physiol A Mol Integr Physiol* 2003;135(2):263–70.
17. Murugesan P, Muthusamy T, Balasubramanian K, Arunakaran J. Studies on the protective role of vitamin C and E against polychlorinated biphenyl (Aroclor 1254) induced oxidative damage in Leydig cells. *Free Radic Res* 2005;39(11):1259–72.
18. Bonnette ED, Kornegay ET, Lindemann MD, Notter DR. Influence of two supplemental vitamin E levels and weaning age on performance, humoral antibody production and serum cortisol levels of pigs. *J Animal Sci* 1990;68(5):1346–53.
19. Ognjanovic BI, Pavlovic SZ, Maletic SD, Zikic RV, Stajn AS, Radojicic RM, *et al.* Protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium. *Physiol Res* 2003;52(5):563–70.
20. Hijazi MM, Janjua MZ, Islam ZU, Furqan M. Protective effect of vitamin E against Nicotine induced morphological changes in rat testis. *Ann Abbassi Shaheed Hosp* 2002;7(2):374–82.
21. Jacob RA, Aiello GM, Stephensen CB, Blumberg JB, Milbury PE, Wallock LN, *et al.* Moderate Antioxidant Supplementation has no Effect on Biomarkers of Oxidant Damage in Healthy Men with Low Fruit and Vegetable Intakes. *J Nutr* 2003;133:740–3.
22. Zhou DX, Qiu SD, Zhang J, Tian H, Wang HX. The protective effect of vitamin E against oxidative damage caused by formaldehyde in testis of adult rats. *Asian J Androl* 2006;8(5):584–8.
23. Sahinturk V, Guclu C, Baycu C. Protective effects of vitamin E on ethane dimethane sulfonate-induced testicular toxicity in rats. *Asian J Androl* 2007;9(1):117–24.
24. Schroder H, Navarro E, Mora J, Galiano D, Tramullas A. Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. *Eur J Nutr* 2001;40:178–84.
25. Guney M, Oral B, Take G, GiraySG, Mungana T. Effect of fluoride intoxication on endometrial apoptosis and lipid peroxidation in rats: Role of vitamins E and C. *Toxicology* 2007;231:215–23.
26. Harapanhalli RS, Yaghmai V, Giuliani D, Howell RW, Rao DV. Antioxidant effects of vitamin C in mice following X-irradiation. *Res Commun Mol Pathol Pharmacol* 1996;94:271–87.
27. John S, Kale M, Rathore N, Bhatnagar D. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem* 2001;12:500–4.
28. Rayman MP. The importance of selenium to human health. *Lancet* 2000;356:233–41.
29. Schroder H, Navarro E, Mora J, Galiano D, Tramullas A. Effects of alpha-tocopherol, beta-carotene and ascorbic acid on oxidative, hormonal and enzymatic exercise stress markers in habitual training activity of professional basketball players. *Eur J Nutr* 2001;40:178–84.

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