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

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ABSTRACT

Objective: To determine the effects of ascorbic acid and selenium supplementation on basal testosterone cortisol ratio in male Sprague Dawley rats.

Design: Quasi experimental study.

Place and Duration of Study: The study was carried out in the department of Physiology, Army Medical College Rawalpindi in collaboration with National Institute of Health, Islamabad during October 2006 to September 2007.

Materials and Methods: 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. Results were analyzed on SPSS version 13 and p value less than 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 selenium revealed significant rise in testosterone cortisol ratio with a fall in malondialdehyde levels.

Conclusions: Synergistic effects of ascorbic acid and selenium may have resulted in a decline in reactive oxygen species induced lipid peroxidation and rise of testosterone cortisol ratio.

Keywords: Testosterone Cortisol Ratio, Malondialdehyde, Ascorbic Acid, Selenium.

INTRODUCTION

Testosterone and cortisol are at different concentrations, maintaining a ratio. Testosterone and cortisol secretions are interrelated. Hypothalamo - pituitary- adrenal axis not only activates the adrenal cortex to release adrenal corticosteroids like cortisol and corticosterone but also inhibits the gonadotropin secretion thereby resulting in reduction of testosterone levels [1]. The ratio between the concentration of testosterone and cortisol has been widely used as a stress index [2]. Increased cortisol, decreased testosterone, and the fall of ratio between testosterone and cortisol levels are considered as endocrinological indicators of stress [3]. A fall in the testosterone/cortisol ratio is also regarded as an indicator of tiredness [4].

Testosterone is as an anabolic hormone while cortisol is considered as a catabolic hormone and their ratio may be indicative of body's anabolic / catabolic balance which is disturbed in various conditions and leads to generalized weakness and muscle wasting [5]. Therefore if basal testosterone cortisol ratio could be raised, it can have beneficial effects on body to combat stress improve stamina and reduce fatigue.

During our lifetime, we are continuously exposed to oxidants, which are of both endogenous and exogenous origin. Therefore, all of the cells in the body are exposed to oxidants at all times. Endogenous sources of oxidants include mitochondrial respiration, enzymes such as xanthine oxidase and lipoxygenase, and the NADPH oxidase/myeloperoxidase system of phagocytes [6]. An imbalance between free radical production and antioxidant defense leads to an oxidative stress state, which may be involved in aging process and even in some diseases [7].

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Stress usually involves lipid peroxidation which is a complex process that can occur in biological membranes made up of molecular oxygen reactant polyunsaturated fatty acids which may lead to the production of lipid hydroperoxides and their metabolites. Most cases involving lipid peroxidation start from a chain reaction mediated by the presence of free radicals. Lipid hydro- peroxides accumulate in the membrane thereby inactivating its receptors and enzymes, affecting its functions, causing instability and making it permeable to ions. Malondialdehyde (MDA) is the product of reactive oxygen species -mediated damage to the polyunsaturated fatty acids and is considered as an indicator of lipid peroxidation caused by free radicals [8].

Defenses against oxidative stress are antioxidants synthesized in our body and the antioxidant vitamins that we take in diets6. Many epidemiologic and some clinical trials have indicated that dietary intake or supplementation with antioxidant vitamins is associated with a reduction in the incidence of chronic disease morbidity and mortality [9-11]. Among dietary antioxidants, ascorbic acid and selenium are two important antioxidants [9-12].

To our knowledge, there is no information regarding the effects of above-mentioned antioxidants on basal testosterone cortisol ratio. It is therefore proposed that antioxidants ascorbic acid and selenium increase testosterone cortisol ratio by decreasing lipid peroxidation at basal stress levels. The effects of antioxidants supplementation on levels of testosterone, cortisol and testosterone cortisol ratio, in relation to Malondialdehyde are investigated in this study.

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, atleast 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 water [13] (10 rats consumed one liter water in 3-4 days) and group III was given Selenium supplementation 1.5 mg/kg chow for one month [14]. However, group IV received both ascorbic acid and selenium supplementation for one month.

After one month, intracardiac blood samples were taken. 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 [5]. After clotting, samples were first centrifuged at 4000 rpm at 4^{o}C in the cold centrifuge. Then serum was pipetted out and stored in eppendorf storage tubes at - 70^{o}C till analysis.

Serum testosterone and cortisol were determined by ELISA and malondialdehyde levels were estimated colorimetrically with commercially available kits. Data was analyzed on SPSS version 13. The arithmetic mean and standard deviation were calculated to describe the data. Difference in mean among control and treated groups was checked using 'student's 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 through out study period and took their feed properly. In the control group, rats were fed on a standard diet without any supplementation. Mean±SD serum testosterone level of the individual animals of control group was 3.06±1.29 ng/ml. Serum cortisol level was 21±1.41 ng/ml. Serum malondialdehyde level was 5.25±1.29 μM. Mean testosterone cortisol ratio in the control group was calculated to be 0.14 ± 0.07. The above-mentioned values were taken as standard and were compared with the respective values obtained from antioxidants supplemented groups for the determination of the p value.

The rats of group II which were given vitamin C supplementation showed serum testosterone levels with a mean ± SD value of 3.37 ± 1.32 ng/ml, serum cortisol level was 20.70 ± 1.15 ng/ml, Serum malondialdehyde levels value was 5.90 ± 1.58 µM. Mean Testosterone Cortisol ratio in this vitamin C supplemented group was calculated to be 0.16 ± 0.06. No significant difference was found among the values of serum testosterone, serum cortisol and serum malondialdehyde between the control and this experimental group as shown in table 1. Also statistical analysis revealed insignificant difference ($p > 0.05$) in testosterone cortisol ratio in this group as compared to the control, although it was higher in experimental group.

Mean ± SD of Serum testosterone level of group III rats given selenium supplementation was 3.65 ± 1.35 ng/ml. Serum cortisol level was found to be 20.40 ± 1.51 ng/ml, while serum malondialdehyde level was 5.81 ± 1.92 µM. Mean testosterone cortisol ratio in selenium supplemented group was calculated to be 0.18 ± 0.07 as shown in table 2. No significant difference ($p > 0.05$) was found among the values of serum testosterone, serum cortisol and serum malondialdehyde between the control and selenium alone supplemented experimental group. The statistical analysis revealed insignificant difference in testosterone cortisol ratio in this group as compared to the control.

Group IV that was given vitamin C and selenium supplementation have shown a mean ± SD value of 6.00 ± 1.87ng/ml for testosterone, Serum cortisol level was 18.80±1.62 ng/ml, while serum malondialdehyde level was 2.41 ± 1.20 µM. Mean testosterone cortisol ratio in vitamin C and selenium supplemented group was calculated to be 0.32±0.12 (table-3, fig.1)

Significant difference was found among the values of serum testosterone, serum cortisol and serum malondialdehyde between the control and this experimental group with a 'p value' less than 0.01. The statistical analysis also revealed a significant rise ($p < 0.01$) in testosterone cortisol ratio in this group as compared to the control (fig. 2 shows the testosterone cortisol ratio in all groups).

DISCUSSION

All cells of the body are exposed chronically to oxidative stress. These oxidants

Table-1: Effects of Ascorbic acid Supplementation on Serum Testosterone, Serum Cortisol, Serum Malondialdehyde levels and Testosterone Cortisol Ratio in Male Rats

Parameter Mean ± SD	Control Group-I (n=10)	Experimental Group-II (n=10)	p value*
Serum Testosterone ng/ml	3.06±1.29	3.37±1.32	> 0.05
Serum Cortisol ng/ml	21.00±1.41	20.70±1.15	> 0.05
Serum Malondialdehyde µM	5.25 ± 1.29	5.90 ± 1.58	> 0.05
Testosterone Cortisol ratio	0.14 ± 0.07	0.16 ± 0.06	> 0.05

*P-value more than 0.05 is taken as insignificant

Table-2: Effects of Selenium Supplementation on Serum Testosterone, Serum Cortisol, Serum Malondialdehyde levels and Testosterone Cortisol Ratio in Male Rats.

Parameter Mean ± SD	Control Group-I (n=10)	Experimental Group-III (n=10)	p value*
Serum Testosterone ng/ml	3.06±1.29	3.65 ± 1.35	> 0.05
Serum Cortisol ng/ml	21.00±1.41	20.40 ± 1.51	> 0.05
Serum Malondialdehyde µM	5.25 ± 1.29	5.81 ± 1.92	> 0.05
Testosterone Cortisol ratio	0.14 ± 0.07	0.18 ± 0.07	> 0.05

*P-value more than 0.05 is taken as insignificant

Table-3 Effects of Ascorbic Acid and Selenium Supplementation on Serum Testosterone, Serum Cortisol, Serum Malondialdehyde levels and Testosterone Cortisol Ratio in Male Rats

Parameter Mean ± SD	Control Group-I (n=10)	Experimental Group-IV (n=10)	p value*
Serum Testosterone ng/ml	3.06±1.29	6.00 ± 1.87	< 0.01
Serum Cortisol ng/ml	21.00±1.41	18.80 ± 1.62	< 0.01
Serum Malondialdehyde µM	5.25 ± 1.29	2.41 ± 1.20	< 0.01
Testosterone Cortisol ratio	0.14 ± 0.07	0.32 ± 0.12	< 0.01

*P-value less than 0.05 is taken as significant

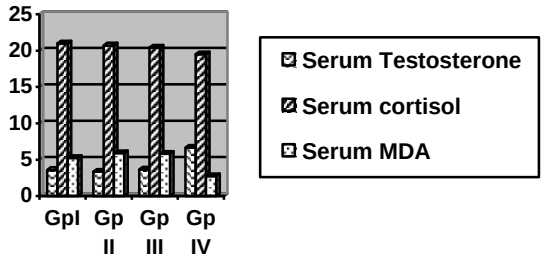

Fig.1: Effects of ascorbic acid and selenium supplementation on serum testosterone, cortisol and malondialdehyde in all groups.

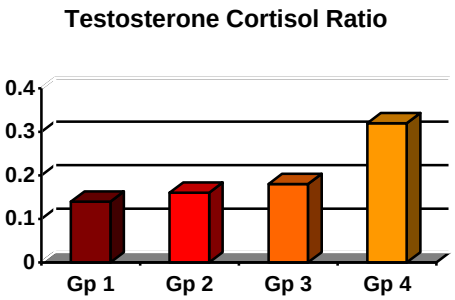

Fig.2: Effects of antioxidants supplementation on basal testosterone cortisol ratio in all groups

not only come from exogenous sources but also from endogenous sources as a result of various metabolic processes. To combat such prooxidants, antioxidant defense system plays a vital role. An important contribution to antioxidant defense system comes from nutrients which may include both water soluble and lipid soluble substances like beta carotene, alpha tocopherol, ascorbic acid and selenium [15].

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, vitamin C and selenium on basal testosterone - cortisol ratio in relation to lipid peroxidation.

In our second group of study, the rats supplemented with ascorbic acid, there was no significant change in basal testosterone levels as shown in (table-1). This is consistent with the study of Ito et al, who have shown that in rats, a species that is unable to synthesize ascorbic acid, deprivation of ascorbic acid do not effect the basal testosterone levels in adult rats although levels of leutinizing hormone may decrease [16]. It has also been reported that ascorbic acid can increase the basal testosterone levels as shown by Sonmez et al, but they used ascorbic acid supplementation at a dose of 500 mg/kg/day/rat which is a very high dose as compared to ours which was 500 mg/L drinking water that was drunk by 10 rats in 3-4 days. This could have affected the levels of testosterone in this study [17].

In this group, cortisol levels were insignificantly changed. Similar results are reported by Henrique et al in an animal study on sea bream with no effect of high dose of ascorbic acid on cortisol levels [18]. Lipid peroxidation was also not decreased in this ascorbic acid supplemented group as is evident by unchanged malondialdehyde levels. This is in accordance with the study on human males in which they have suggested that ascorbic acid has no effect on MDA concentration [19].

Selenium an important constituent of dietary antioxidants, when supplemented to rats in third group of our study have shown insignificant rise in basal levels of testosterone. Prostate cancer prevention trail in which El-Bayoumy et al., have given selenium supplemented yeast to adult males of 19 to 43 years of age for 12 months has also shown that selenium has no effect on basal testosterone levels and suggested that selenium is minimally affecting the metabolism of testosterone [20].

Similarly, the basal plasma cortisol levels were unchanged in selenium supplemented group. This is in accordance with the results of Chanoine et al., who have shown in an experimental study on rats that selenium deficient diet has no effect on basal secretions of adrenal cortex [21]. The oxidative stress marker MDA in selenium alone supplemented group of our study was also not effected by selenium supplementation alone. Miller et al has in a study on fish by exposing fish to excessive water selenite, suggested similar findings that selenium has

no effect on lipid peroxidation [22]. However, the role of selenium in preventing rise of lipid peroxidation i.e., MDA levels in response to various stressors is well documented [23].

The results of the fourth group whose diet supplemented with a combination of vitamin C and selenium has shown an increase in serum testosterone and fall in cortisol levels as shown in table 3, with resultant favorable increase in testosterone cortisol ratio. The levels of MDA significantly decreased in this group of our study. There is no study found apparently in literature that shows the effect of this combination on testosterone cortisol ratio. However literature shows that in case of stress, both antioxidants can exert favorable effects on these hormones [24, 25].

Our study shows that supplementation with vitamin C and selenium in combination reduces the basal MDA levels. It means it may decrease the lipid peroxidation resulting from various intrinsic metabolic processes. Regarding the effects of this combination, it can be explained on the basis of this fact that selenium has a sparing effect on ascorbic acid. This was narrated in a study on cultured liver cell lines exposed to oxidative stress. The mechanism proposed for this increased availability is that the selenoenzyme thioredoxin reductase (TR) can recycle ascorbic acid. Selenium also enhances the ability of glutathione peroxidase which may scavenge the hydroperoxides generated by the cells. As a result of this, ascorbic acid is spared from destruction with consequent increase in ascorbic acid available at cell level. Prevention of oxidants induced cell damage result in stabilization of cell membranes [26].

Therefore it is proposed that combination of ascorbic acid and selenium has additive effect to decrease basal stress. 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 [27]. Similarly, Selenium through its various selenoproteins like GPx, mitochondrial selenoproteins GPx, selenprotein P and thioredoxin reductase confer protection against lipid peroxidation induced damages [28].

Antioxidant supplementation may protect the protein structures, prevent the enzyme reactivation by ROS, stabilize cell membranes, which may be responsible for this protective and favorable effect on anabolic catabolic ratio as shown in (fig. 2) [28, 29].

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.

CONCLUSION

Synergistic effects of ascorbic acid and selenium may have resulted in a decline in reactive oxygen species induced lipid peroxidation and rise of testosterone cortisol ratio.

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REFERENCES

1. Li XF, Michell JC, Wood S, Coen CW, Lightman SL, O'Byrne KT. The effect of oestradiol and progesterone on hypoglycaemic stress-induced suppression of pulsatile luteinizing hormone release and on corticotrophin-releasing hormone RNA expression in the rat. J. Neuroendocrinol. 2003; 15: 468–76.
2. Uchida MC, Bacurau RFP, Navarro1 F, Pontes Jr FL, Tessuti VD, Moreau RL et al. Alteration of testosterone: cortisol ratio induced by resistance training in women. Rev Bras Med Esporte. 2004; 10: 3: 169-72.
3. 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.
4. Maso F, Lac G, Filaire E, Michaux O, Robert A. Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. Br J Sports Med. 2004; 38; 3: 260-3
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. Frei B, England L, and Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma (oxidant stress/lipid peroxidation/protein thiols/a-tocopherol. Proc Nati Acad Sci. 1989; 86: 6377-81.
7. Finaud J, Lac G, Filaire E. Oxidative stress: relationship with exercise and training. Sports Med. 2006; 36: 4: 327-58.
8. Kazez A, Kucukaydin N, Kucukaydin M, Kontas O, Okur H, Dogan P. A model of nti-in-induced necrotizing enterocolitis: the role of distension. J Pediatr Surg. 1997; 32: 1466-9.
9. 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.
10. Enstrom JE. Vitamin C in prospective epidemiological studies. 1997. In: Packer L, Fuchs J, eds. Vitamin C in health and disease. New York: Marcel Dekker Inc. 381–98.
11. Gey KF. Vitamins E plus C and interacting co nutrients required for optimal health. Biofactors. 1998; 7:113–74.
12. Schwarz S. Essentiality and metabolic functions of selenium. Med Clin North Am. 1976; 60: 745-58.
13. Hsu PC, Liu MY, Hsu CC, Chen LY, Guo YL. Effects of vitamin E and/or C on reactive oxygen species-related lead toxicity in the rat sperm. Toxicology. 1998; 128; 3: 169-79.
14. Tanguy S, Boucher F, Besse S, Ducros V, Favier A, de Leiris J. Trace elements and cardioprotection: increasing endogenous glutathione peroxidase activity by oral selenium supplementation in rats limits reperfusion-induced arrhythmias. J Trace Elem Med Biol. 1998; 12; 1: 28-38.
15 Mayne ST. Antioxidant Nutrients and Chronic Disease: Use of Biomarkers of Exposure and Oxidative Stress Status in Epidemiologic Research. J. Nutr. 2003;133: 933–40.
16 Ito Y, Tsuji M, Terada N, Mori H. Testosterone production in mature scorbutic mutant rats unable to synthesize ascorbic acid. Int.J Androl. 1992; 15: 160–9
17 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.
18 Henrique MMF, Gomes EF, Gouillou-Coustans MF, Oliva-Teles A, Davies SJ. Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of sea bream, Sparus aurata. Aquaculture. 1998; 161: 415-26.
19 Bayerle-Eder M, Pleiner J, Mittermayer F, Schaller G, Roden M, Waldhaus W, et al. Effect of systemic vitamin C on free fatty acid induced lipid peroxidation. Diabetes and Metabolism. 2004; 30: 5: 433-9.
20 El-Bayoumy K, Richie Jr JP, Boyiri T, Komninou D, Prokopczyk B, Trushin N, et al. Influence of Selenium-enriched Yeast Supplementation on Biomarkers of Oxidative Damage and Hormone Status in Healthy Adult Males: A Clinical Pilot Study. Cancer Epidemiology, Biomarkers & Prevention. 2002;11: 1459-65.
21 Chanoine JP, Wong AC, Lavoie JC. Selenium deficiency impairs corticosterone and leptin responses to adrenocorticotropin in the rat. Biofactors. 2004; 20: 2: 109-18.
22 Miller WL. Molecular biology of steroid hormone synthesis. Endocr Rev. 1988; 9: 295-318.
23 El-Sharaky AS, Newairy AA, Badreldeen MM, Eweda SM, Sheweita SA. Protective role of selenium against renal toxicity induced by cadmium in rats. Toxicology. 2007; 235: 3: 185-93
24 Chitra KC, Latchoumycandane C, Mathur PP. Chronic effect of endosulfan on the testicular functions of rat. Asian J Androl. 1999; 1: 203-6.
25 Bekpinar S, Tugrul Y. Influence of selenium supplementation in non-toxic doses on testis lipid peroxide and antioxidant levels in chronic alcohol-fed rats. Alcohol. 1995; 30: 5: 645-50.
26 Li X, Hill KE, Burk RF, May JM. Selenium spares ascorbate and alpha-tocopherol in cultured liver cell lines under oxidant stress. F.E.B.S. Lett. 2001; 508; 3: 489-92
27 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.
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.