EFFECTS OF VITAMIN E ON FOOD INTAKE AND BODY WEIGHT IN RATS EXPOSED TO RESTRAINT STRESS

NUR A. M. FAHAMI*
NAFEEZA M. ISMAIL*
KHALID B. A. KHALID**

SUMMARY : Many investigators have shown that stress suppresses food intake and reduces body weight in rats. The effect of vitamin E on body weight and food intake in rats exposed to stress were studied. Male Sprague Dawley rats (n = 80) were fed with either normal chow, vitamin E deficient diet or vitamin E with or without oral supplementation of either tocopherol, tocotrienol or Tocomin at 60mg/kg body weight. The rats were treated with these different diet regimes for 28 days prior to exposure to stress. Repetitive stress was applied, whereby the rats were restrained 2 hours daily for 4 consecutive days. The body weight and food intake was measured, weekly and after exposure to stress. The findings showed that vitamin E deficiency induced by feeding rats with vitamin E deficient diet for 28 days resulted in significantly lower body weight and food intake compared to the control animals given (normal chow). Supplementation with vitamin E in different forms, tocopherol, tocotrienol or Tocomin to the deficient diet was unable to increase the body weight or food intake, where both parameters were comparable with rats fed with deficient diet alone. It is possible that the dose of the vitamin E given was inadequate to maintain the food consumption and prevent the drop in body weight. Exposure to stress caused a further reduction in both the food intake and body weight in all groups and this suggests that the normal level of vitamin E in normal chow nor the supplementation with tocopherol and tocotrienol at the dose 60mg/kg was able to protect against changes in the body weight and food intake due to stress. We therefore conclude that vitamin E deficiency and stress can reduce food intake and cause a reduction in body weight. Stress can worsen the status of the two parameters and in vitamin E supplementation at 60 mg/kg body weight is not sufficient to prevent the changes in the body weight or food intake due to vitamin E deficiency or stress.

Key Words: Stress, vitamin E, food intake, body weight.

INTRODUCTION

Shress has variable effects on body physiology, neurochemical responses and behavior of both human and animal. Food intake and body weight are some of the variables sensitive to stress (1,2). These particular variables are interesting titles of stress research not only because of the impact of food on growth and health but also because it can be measured with minimal disturbances to the animal (2). Restraint stress had been proposed as an animal model for psychological stress such as depression and anorexia nervosa, generally due to its ability to cause behavioral and physiological changes in rats (3,4).

Stress is known to stimulate corticotropin-releasing factor (CRF), which activates the hypothalamic-pituitary-adrenal (HPA) axis, seratonergic, catecholaminergic, sym-

*From Department of Pharmacology,
**From Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

pathetic nervous and immune systems (5-7). These systems are known to have the potential to inhibit food intake and reduce body weight (8-9). It is also established that CRF influence feeding behavior and mediate behavioral and physiological responses to stress (3,4,10).

The activity of the HPA axis and the resulting changes in corticosterone metabolism may regulate energy balance, mainly food intake and nutrient utilization which can be disrupted by stress-related activation of the HPA axis (11). Ainsah *et al.* (12) reported that rats given supplementation of vitamin E had a significant reduction of plasma corticosterone levels when exposed to repeated stress compared to the unsupplemented controls. These findings suggest that vitamin E supplementation may block changes in the corticosterone level in stress, which in turn may render protection against changes in the food intake and nutrient utilization that is known to occur during stress. The current study investigates the effects of vitamin E deficiency and supplementation with vitamin E either tocopherol or tocotrienol on stress-induced changes in food-intake and body weight in rats.

MATERIALS AND METHODS

Male *Sprague-Dawley* rats (n = 50) weighing initially approximately 200 - 220g were purchased from the University Breeding Center (UKM, Kuala Lumpur). The rats were divided into five equally sized groups. The control group was fed with normal rat chow (RC) while the treatment group was either given a vitamin E deficient (VED) diet or a vitamin E deficient diet plus one of the oral supplements α-tocopherol (TF), tocotrienol (TT) or Tocomin (TC) 60mg/kg body weight for 28 days. At the end of this treatment period, rats from each group were exposed to restraint-stress. All rats were kept on a regular night/day cycle, with natural light for a period of 10 hours (0700 to 1700 h). Throughout the feeding period all rats were habituated to handling to reduce their stress-related disturbances. The rats were housed two per cages in large cages with wide wire-mash bottoms to prevent coprophagy and was given free access to water throughout the experiment. All the experiment protocols were approved by the Animal Care and Use Committee of the Faculty of Medicine, National University of Malaysia (approval number: FAR/2000/NAFEEZA/30-NOVEMBER/031).

Restraint-Stress

Rats were restrained by placing them in individual plastic restrainer measuring approximately 12 x 5 cm for two hours daily for 4 consecutive days following model previously explained by Ainsah *et al.* (12).

Diets/ Vitamin E Supplementation

Normal rat chow diet was purchased from Gold Coin, Malaysia and contained 15.63 mg/kg α-tocopherol, 4.54 mg/kg γ-tocotrienol, 2.69 mg/kg α-tocotrienol, 1.38 δ-tocotrienol and 0.87 mg/kg γ-tocopherol (13). Vitamin E deficient diet was purchased from ICN Biomedicals, USA. Alpha-tocopherol was purchased from Sigma, USA and palm oil extracted tocotrienol and Tocomin were supplied by Carotech Sdn. Bhd., Malaysia. Tocomin, is an extract from palm oil which have a mixture of phytonutrient complex and antioxidants such as vitamin E (tocotrienols: 78% and tocopherol: 22%) and other nutrients such as phytosterol, phytocarotenoid complex and co-enzyme Q10.

Measurement of body weight

Body weight of the rats was measured using an electronic balance (Denver Instrument Company). Baseline of body weight was taken before treatment with different diet begins. At the end of the treatment period the body weight was measured again and another measurement was taken immediately after the last exposure to stress.

Measurement of food intake

Each cage was supplied 100 g of the specific diets. After three days, the remaining diet were weighed. The difference obtained between the two values was the amount of diet consumed by the rats for three days. Fresh supply of diets were then added to the cages to maintain the total amount of 100 g. The above processes were followed throughout the treatment period and continued during the experimental stress period. The average amount of diet consumed by each rat per day were then calculated.

Analyses of data

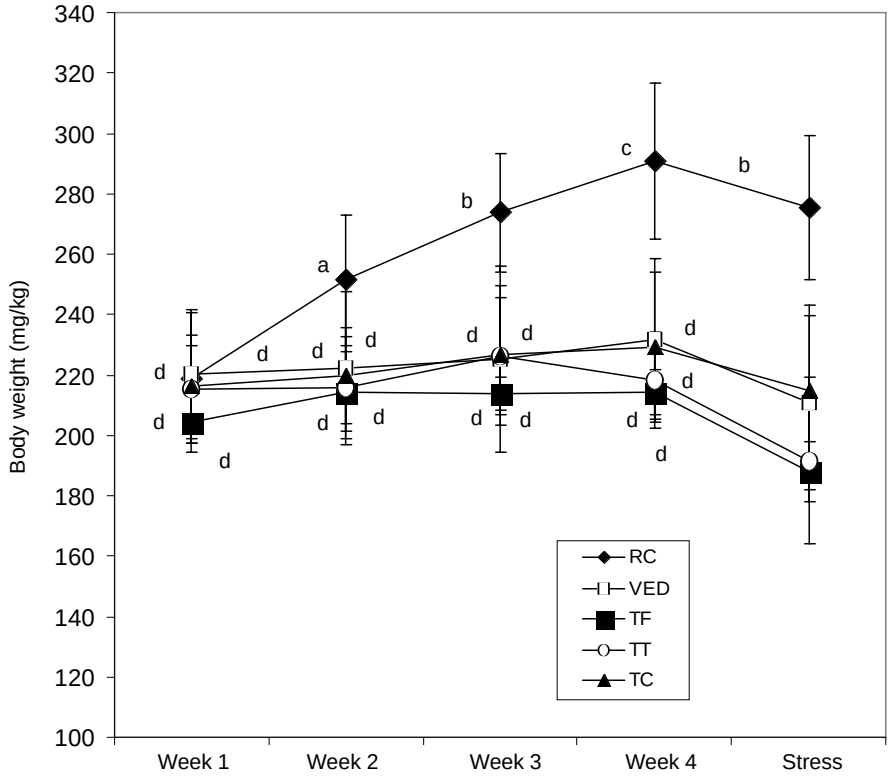
Results are expressed as means $\pm$ SEM. Statistical significance ($P<0.05$) was determined by ANOVA or student's t-test for parametric analysis and Kruskal Wallis or Wilcoxon Signed Test for non-parametric analysis where appropriate.

RESULTS

Body weights of different treatment groups

Body weights of the group given normal rat chow showed a steady devation while the rats given vitamin E deficient diet (VED) revealed a significant reduction of the body weight throughout the treatment period. These weight changes became obvious starting the second week. Body weight of the three vitamin E supplemented groups, i.e. tocopherol (TF), tocotrienol (TT) and Tocomin (TC) at 60 mg/kg did not increase the weight significantly and remained unchanged throughout the treatment period. By the end of the experiment the body weight of the VED

Figure 1: Effects of vitamin deficiency and supplementation of various vitamin E preparations and restraint stress on body weight in rats. Values are mean ± SEM (n=10/group). Different letters indicate significant difference ($p<0.05$).



group was 37% lower compared to rats given the normal rat chow although the mean weight at the start of the treatment was approximately similar. The supplementation with these different vitamin preparation did not improve the body weight it remained as low as the rats given the vitamin E deficient diet.

Body weights of different treatment groups after exposure to stress

Body weight of the rats exposed to stress fed with normal rat chow diet was significantly higher compared to the rats given vitamin E deficient diets. Stress causes a significant reduction in body weights which was approximately 14% ($P=0.042$) in the group given normal rat diet and 11% reduction in rats given vitamin E deficient diet compared to their mean weight at week 4 of treatment (just before exposure to stress). Supplementation with either TF, TT or TC at 60 mg/kg body weight was unable to block the reduction of body weight due to stress, where their body weights was significantly different to their body weight prior to exposure to stress.

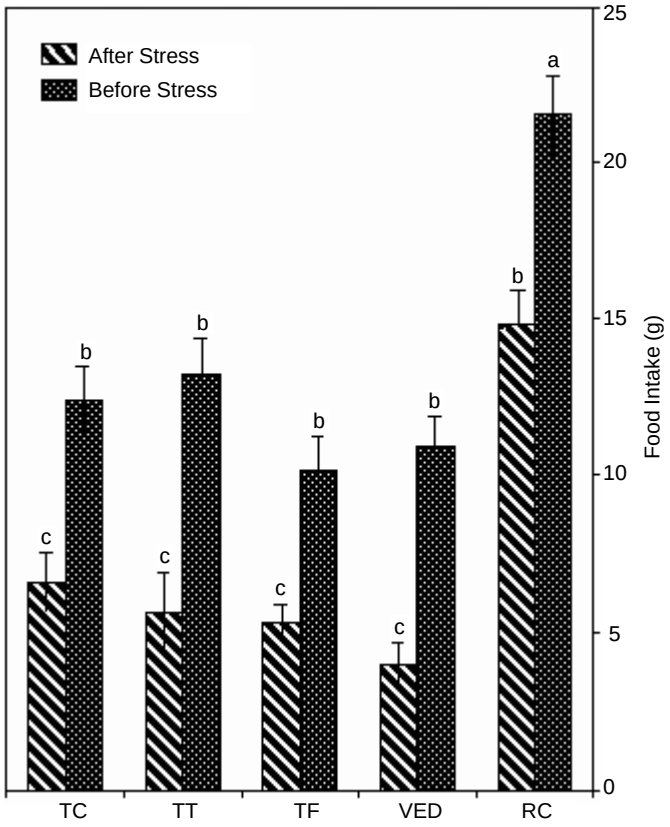
Food Intake of the rats before and after exposure to stress

Prior to exposure to stress, the rats given normal rat chow had a significantly greater food intake compared to all the other groups. There were no other differences in food intake among the other diet groups, (Figure 2). Food intake in rats exposed to stress was significantly less before exposure to stress compared to their food intake. The food intake in the group given rat chow diet after exposure to stress was similar to the food intake in rats receiving vitamin E deficient diets but not exposed to stress. Supplementation with different vitamin E did not improve food intake in the rats given vitamin E deficient diet, where the food intake was comparable with rats given vitamin E deficient diet alone.

DISCUSSION

Vitamin E deficiency induced a decline or a maintenance in the body weights of normal rats, whereas vitamin E supplementation either tocopherol, tocotrienol or Tocomin did not cause any significant changes in improv-

Figure 2: Effects of vitamin deficiency and supplementation of various vitamin E preparations and restraint stress on daily food intake in rats. Values are mean ± SEM (n=10/group). Different letters between bars indicate significant difference ($p<0.05$).



ing the body weight. Other studies had reported that vitamin E deficiency inhibits normal body growth in rats (14,15). A study by Pillai *et al.* (16) found that rats with vitamin E deficiency can experience muscle necrosis which can lead to a reduction in body weight. Thus it is possible that tocopherol, tocotrienol or Tocomin supplementation at 60 mg/kg body weight used in this study was insufficient. A larger dose or a dose-dependent study is warranted to see the possible effects of vitamin E supplementation in maintaining normal body weights in rats given a vitamin E deficient diet.

Another possible explanation on the lower body weight in rats given vitamin E deficient diet would be the lower food intake in this group of rats compared to the normal rat chow group. This suggests that vitamin E deficiency may cause a reduction in appetite which leads a reduced food consumption. Supplementation with vitamin E to these rats does not improve the food intake towards normal, which is possibly because the dose given was insufficient to block the altered appetite.

Exposure to restraint stress for 2 hours a day for 4 consecutives days resulted in a significant reduction of body weight in all groups studied. A recent study (17) showed a similar finding where rats exposed to stress had a lower body weight as compared to their initial body weight. Studies had also shown that exposing rats to a repetitive restraint stress causes a more conspicuous reduction in body weight compared to a single exposure to stress (9). Acute release of CRF during repetitive stress exposure causes a temporary hypophagia with continuous suppression of body weight in rats (17). Likewise our study revealed that exposure to stress causes a significant reduction in food consumption in rats of all groups. We also observed that rats exposed to stress did not make an attempt to overeat or increase food intake after every exposure to stress, and this suggests that hyperphagia does not occur in response to a reduced body weight in stress.

Smagin *et al.* (1) found that these reductions of bodyweight can be blocked by infusion of CRF antagonist to the

third ventricle prior to exposure to stress. While other studies had shown that body weight response towards stress is similar to the observation in rats receiving lesions on the lateral hypothalamus (17) or with presence of inflammation in this area (18). Corticotropin-Releasing-Factor increased as a responce toward stress is regulated by various locations in the brain including those involved in the regulation of food intake (6,19).These findings from the referred studies suggest that physiological responses to stress involving body weight and food intake depend on central mechanisms.

Supplementing the rats with either tocopherol, tocotrienol or Tocomin was unable to block the effect of stress on the changes in both the body weight of the rats as well as the amount of food consumed. A study by Ainsah *et al.* (12) showed that supplementation of tocopherol blocks the increasing corticosterone level due to stress. Thus it is possible that the inability of either tocopherol or tocotrienol to maintain the food intake and the body weight of the rats may be because of their inability to block the effect of stress causing an acute release of CRF acting centrally.

In conclusion, vitamin E deficiency decreased body weight and food intake in normal rats. Exposure to repetitive stress for 2 hours a day for 4 consecutive days cause a reduction in both food intake and body weight in all groups studied. Supplementation with 60 mg/kg BW of tocopherol, tocotrienol or Tocomin did not affect the body weight or food intake in normal or stressed rats. Further studies are warranted to elucidate the doses of vitamin E with probable effect on body weight and food intake in both vitamin E deficiency and stress.

ACKNOWLEDGEMENTS

This study was made possible by the research grant (F/9/2000) from the Faculty of Medicine, UKM. The authors would also like to acknowledge Carotech Sdn. Bhd. for the supply of tocotrienol and tocomin for this research.

REFERENCES

1. Smagin GN, Howell LA, Redmann SJ, Ryan DH, Harris RB : Prevention of stress-induced weight loss by third ventricle CRF receptor antagonist. Am J Physiol. Regulatory Integrative Comp Physiol, 276: R1461-R1468, 1999.

2. Valles A, Marti O, Garcia A, Armario A : Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. Am J Physiol. Regulatory Integrative Com Physiol, 279(3): R1138-R1144. 2000

3. Kennett GA, Dourish CT, Curzon G : 5-HT1B agonist induce anorexia at a postsynaptic site. Eur J Pharmacol, 141:429-435, 1987.

4. Krahn DD, Gosnell BA, Levine AS, Morley JE : Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. Brain Res, 443:63-69, 1988.

5. Owen PC, Smith R : Opiod peptides in blood and cerebrospinal fluid during acute stress. Clin Endocrinol Metab, 1:303-308, 1987.

6. Makino S, Smith MA, Gold PW : Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hipothalamus paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor MRNA level. Endocrinology, 136: 3299-309, 1995.

7. Turnbull AV, Rivier CL : Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev 79:1-71, 1997.

8. Harris RBS, Zhou J, Youngblood BD, Rybkin II, Smagin GN, Ryan DH : Effect of repeated stress on bodyweight and body composition of rats fed low-and high fat diets. Am J Physiol. Regulatory Integrative Comp Physiol, 275:R1928-1938, 1998.

9. Rybkin II, Zhou Y, Volaufova J, Smagin GN, Ryan DH, Harris RBS : Effect of restraint stress on food intake and bodyweight is determined by time of the day. Am J Physiol. Regulatory Integrative Com Physiol, 273:R1612-1622, 1997.

10. Sutton RE, Koob GF, Le Moal M, Rivier J, Vale W : Corticotropin releasing factor produces behavioral activatio in rats. Nature, 297: 331-333, 1982.

11. Dallman MF, Akana SF, Bhatnagar S, Bell ME, Strach AE : Bottomed out: metabolic significance of the circadian trough in glucocorticoid concentration. Int J Obes Relat Metab Disord, 24: S40-S46, 2000.

12. Ainsah O, Nabishah BM, Osman CB, Khalid BAK : Naloxone and vitamin E block stress-induced reduction of locomotor activity and elevation of plasma corticosterone. J Clin Exp Pharmacol and Physio, 26:444-448, 1999.

13. Asma H, Alini M : The effect of different types of palm oil on lipid profile and erythrocyte anti-oxidant activity. Thesis Hons Universiti Kebangsaan Malaysia, 1996.

14. Azman A : Aras leptin dan TNF-a dalam tikus yang defisien dan disuplementasi vitamin E. Tesis Sarjana Universiti Kebangsaan Malaysia, Kuala Lumpur, 2002.

15. Norazlina M : The effects of vitamin E, vitamin D and calcium on experimental osteoperosis. Tesis Doktor Falsafah Universiti Kebangsaan Malaysia, Kuala Lumpur, 2001.

16. Pillai SR, Traber MG, Kayden HJ, Cox NR, Toivio-Kinnucan M, Wright JC, Braund KG, Whitley RD, Gilger BC, Steiss JE : Concomitant brainstem axonal dystrophy and necrotising myopathy in vitamin E deficient rats. J Neurol Sci, 123:64-73, 1994.

17. Harris RBS, Mitchell TD, Simpson J, Redmann Jr SM, Youngblood BD, Ryan DH : Weight loss in rats exposed to repeated acute restraint stress is independent of energy and leptin status. Am J Physiol. Regulatory Integrative Comp Physiol, 282: R77-R88, 2002.

18. LennieTA, McCarthy DO, Keesey RE : Body energy status and the metabolic responses to acute inflammation. Am J Physiol, 269:R1024-1031, 1995.

19. Shimizu N, Take S, Hori T, Oomura Y : In vivo measurement of hypothalamic seratogenin release by intracerebral microdialysi: significant enhancement by immobilization stress in rats. Brain Res Bull, 28:727-734, 1992.

Correspondence:
Nur Azlina Mohd Fahami
Department of Pharmacology,
Faculty of Medicine,
Universiti Kebangsaan Malaysia,
Kuala Lumpur, MALAYSIA.
e-mail: nazlina@medic.ukm.my