VITAMIN E DEFICIENCY IMPAIRS WEIGHT GAIN IN NORMAL AND OVARIECTOMISED GROWING FEMALE RATS

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SUMMARY: Estrogen plays a role in maintaining body weight, since ovariectomy in rats, resulting in hypoestrogenemia, also results in significant weight gain. Vitamin E, an antioxidant found in many natural food sources, has been found to be beneficial in certain disease conditions. The effects of vitamin E deficiency and supplementation on body weight in intact and ovariectomised female rats were studied. 30 and 60 mg/kg body weights of palm oil-derived vitamin E, as well as 30 mg/kg body weight of pure α -tocopherol, were used. To induce vitamin E deficiency, the rats were fed vitamin E deficient and 50% vitamin E deficient diets. The control diet was normal rat chow. For the intact animals, body weights in the three vitamin E supplemented groups respectively did not differ significantly from the normal rat chow group throughout the ten-month study period. However, animals on the total and partial vitamin E deficient diets had significantly lower body weights, evident from the first month and persisting throughout the study period. Ovariectomy significantly increased body weights of the rats on the normal rat chow diet and the three vitamin E supplemented diets. However, ovariectomy failed to increase body weights of the total and partial vitamin E deficient diet groups, which did not differ significantly from their intact controls. Thus, vitamin E deficiency impaired weight gain in both estrogen-repleted and estrogen-depleted states. Further studies are needed to elucidate the mechanisms involved.

Key Words: Body weight, vitamin E, ovariectomy.

INTRODUCTION

Ovariectomy results in a hypoestrogenic state and loss of estrous cycles, whilst pituitary gonadotrophins become markedly elevated due to lack of negative feedback. Ovariectomy was shown to induce adipocyte hypertrophy and increased levels of epidermal growth factor. These factors may play a role in the induction of obesity in the ovariectomised rat (1). Estrogen receptor mRNA and estrogen receptor binding sites in rat adipose tissue were increased after ovariectomy. Estrogen replacement reduced both these alterations (2).

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Leptin, a protein secreted by white adipocytes, plays an important role in regulation of body weight. High serum levels of leptin act on the hypothalamus to decrease food intake (3). Estrogen may play a role in the regulation of circulating leptin levels. Serum leptin concentration was decreased by ovariectomy in rats and estradiol supplementation reversed this effect (4). Serum leptin levels are higher in females compared to males (5,6). Thus, ovariectomy may result in decreased serum leptins and consequent increase in food intake and body weight.

Vitamin E is an antioxidant found in many natural food sources. It has been shown to be beneficial in certain diseases, such as atherosclerosis and cancer (7-9). Vitamin E also has some effects on body growth. It improved growth retardation in glucocorticoid-treated rats (12). Deficiency in vitamin E can cause cardiac problems and neurological abnormalities (10,11).

Palm oil is a rich source of vitamin E, containing 196 ppm α -tocopherol, 201 ppm α -tocotrienol, 372 ppm β -tocotrienol and 96 ppm γ -tocotrienol (13). It was not found any significant difference in body weights of very young, growing male rats supplemented with two doses of palm vitamin E-rich extract in earlier studies (14). In this study, the effect of vitamin E deficiency on body growth and weight gain of female rats in the presence and absence of estrogen and the effects of palm vitamin E supplementation on these animals were studied.

MATERIALS AND METHODS

Animals

One hundred and twenty female Sprague-Dawley rats 3 months age were obtained from our University Breeding Center. They were divided into six groups of 20 rats each. For each group, half of the rats were ovariectomised and the rest were left intact. For ovariectomy, the rats were anaesthetized with Ketapex and Xylazil (1:1). The lower abdomen of the rats was shaved and incised. Fallopian tubes and ovaries were identified, and then absorbable catgut suture was used to tie the fallopian tubes below the ovaries. Both ovaries were then removed. The rats were given normal rat chow diet for two weeks to allow the wound to recover before dietary manipulation or vitamin E supplementation was started. All the rats were kept 5 per cage under 12 hours natural light/dark cycles and given deionized water *ad libitum*.

Treatment

The intact rats were given dietary treatment or vitamin E supplementation as follows: Vitamin E deficient diet, 50% vitamin E deficient diet, normal rat chow, palm vitamin E 30 mg/kg, palm vitamin E 60 mg/kg or α -tocopherol 30 mg/kg. The ovariectomised rats were also treated in the same way.

Diets

Normal rat chow was obtained 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 mg/kg δ-tocotrienol and 0.87 mg/kg γ-tocopherol (15). Vitamin E deficient diet was purchased from ICN Biomedicals, USA. The 50% vitamin E deficient diet was prepared by mixing equal portions of ground vitamin E deficient diet and ground normal rat chow (1:1). Water was added, the mixture was formed into small balls and dried in the oven at 70°C for 24 hours. Dietary manipulation was carried out for 10 months.

Vitamin E supplementation

Vitamin E-rich extract from palm oil (palm vitamin E) was prepared by the Palm Oil Research Institute of Malaysia (PORIM) and had the following composition 24.82% α-tocopherol, 20.73% α-tocotrienol, 26.68% γ-tocotrienol and 13.32% δ-tocotrienol. Palm vitamin E 30 mg/kg was prepared by mixing 1.5 g palm vitamin E with 50 g olive oil. For palm vitamin E 60 mg/kg, 3 g palm vitamin E was mixed with 50 g olive oil while α-tocopherol 30 mg/kg was prepared by mixing 1.5 g α-tocopherol acetate (Sigma, USA) with 50 g olive oil. 0.1 ml of the respective oils per 100 g body weight was given orally 6 days a week for 10 months. Rats given vitamin E supplementation were fed normal rat chow *ad libitum*.

Measurement of body weight

Body weights of the rats were measured by using an electronic balance (Denver Instrument Company). Each rat was weighed monthly throughout the treatment period.

Measurement of food intake

Each cage was supplied with 200 g of the specific diets. After two days, the diets still left uneaten in the cage were weighed. The difference obtained between the two values was the amount of diet consumed by the rats for two days. Fresh supply of diets were then added to the cages to maintain the total amount of 200 g. The above procedures were followed until 6 sets of data were obtained from which the average amount of diet consumed by each rat per day could be calculated.

Table 1: Diet consumption (g/day/rat) of the intact and ovariectomised female rats.

Diets	VED	50 % VED	RC	PVE 30	PVE 60	ATF
Intact	11.8 ± 0.6	17.7 ± 0.7#b	12.1 ± 0.3**	11.4 ± 0.3	13.0 ± 0.3	12.9 ± 0.4
Ovx	10.8 ± 0.2a	16.2 ± 1.0@	13.7 ± 0.5 a,b	11.9 ± 0.3	12.4 ± 0.2	12.2 ± 0.2

# indicates significant difference compared to other diet groups of intact rats ($p<0.05$).
@ indicates significant difference compared to other diet groups of ovariectomised rats ($p<0.05$).
** indicates significant difference compared to ovariectomised rats of the same diet ($p<0.01$).
Groups which share the common alphabet indicate significant difference ($p<0.05$).

Ovx : Ovariectomised
VED : Vitamin E deficient
50% VED : 50% vitamin E deficient
PVE30 : Palm vitamin E 30 mg/kg
PVE 60 : Palm vitamin E 60 mg/kg
ATF : α-tocopherol 30 mg/kg

Analyses of data

The statistical test was the one-way analysis of variance test using the Statistical Package for Social Sciences software. The Tukey's honestly significant difference test was selected as the post-hoc test. To compare data from intact group and ovariectomised groups, the unpaired T-test was used (Microsoft Excel). The results were presented as mean ± standard error of the mean (s.e.m).

RESULTS

Body weights of intact female rats

Body weights of the group given normal rat chow showed a steady increase throughout the treatment period. Body weights of the three vitamin E supplemented groups, i.e. palm vitamin E 30 mg/kg, palm vitamin E 60 mg/kg and α-tocopherol 30 mg/kg did not differ significantly from the body weights of the normal rat chow group. However, the group fed the vitamin E-deficient diet revealed a decrease of the body weight, which was continuous and worse after the eighth month. The body weights of the group fed 50% vitamin E-deficient diet initially declined, but appeared to stabilize after the fifth month. Both the vitamin E-deficient and 50% vitamin E-deficient diet groups had significantly lower body weights compared to the normal rat chow group and all the three vitamin E-supplemented groups from the first month of the study onwards (Figure 1).

Body weights of ovariectomised female rats

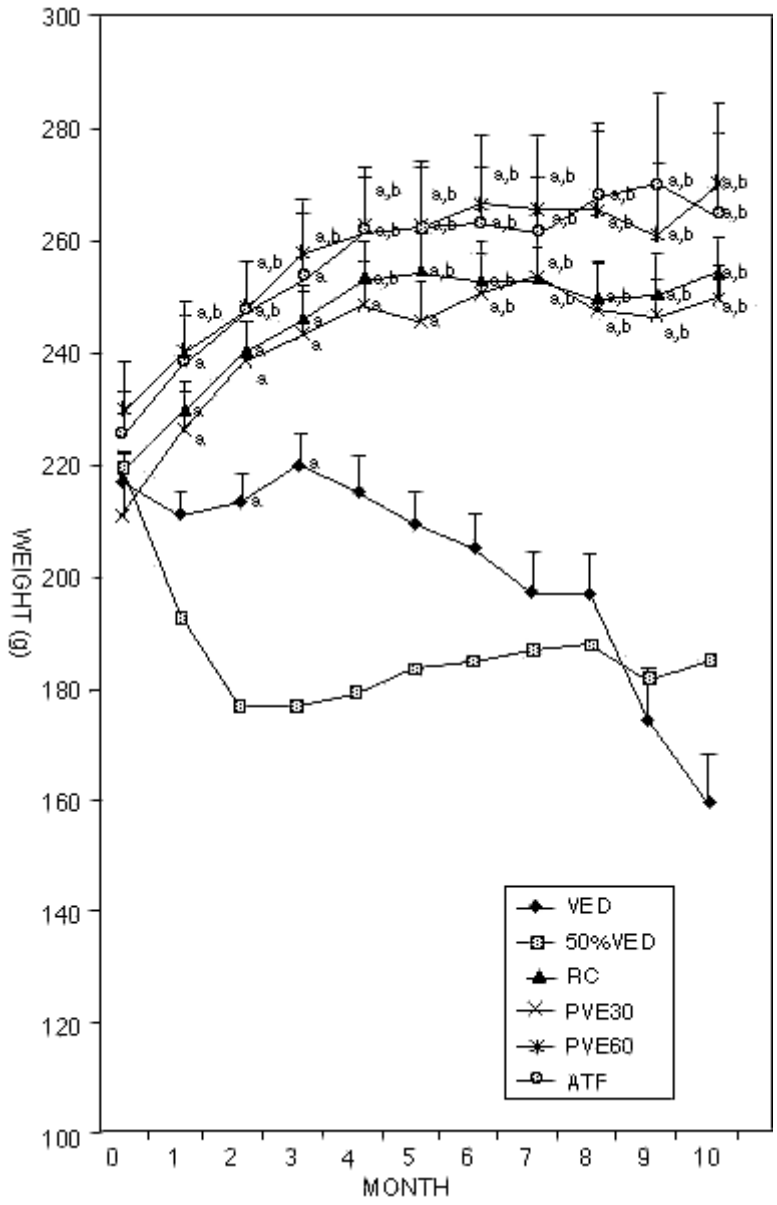
Body weights of the ovariectomised rats fed normal rat chow diet were significantly higher than its intact controls. The body weights of the ovariectomised rats supplemented with palm vitamin E 30 and 60 mg/kg and α-tocopherol 30 mg/kg did not differ significantly from the ovariectomised normal rat chow diet group. However, the vitamin E-deficient diet group had a decline in body weight, which was continuous and worse after the eighth month. The body weights of the 50% vitamin E-deficient diet group initially declined but appeared to stabilize after the fifth month. This decline in body weights was seen as early as the first month of study (Figure 2). The pattern of decline was similar and did not differ significantly from observing in the intact rats fed vitamin E-deficient and 50% vitamin E-deficient diets (Figure 1).

Food intake of intact and ovariectomised female rats

Among the intact rats, the 50% vitamin E-deficient diet group had a significantly greater food intake compared to all the other groups. There were no other significant differences in food intake among the other diet groups of the intact rats (Table 1).

Food intake of the ovariectomised vitamin E deficient group was significantly less than the ovariec-

Figure 1: Body weights of the intact female rats (all treatment groups).



a, b indicate significant difference to 50% VED and VED groups respectively ($p < 0.05$)

tomised normal rat chow and 50% vitamin E-deficient diet groups but did not differ from all the three vitamin E-supplemented groups, as well as the intact normal rat chow group. However, food intake of the ovariectomised 50% vitamin E-deficient group was significantly greater than all the other ovariectomised groups, as well as the intact normal rat chow group (Table 1).

In comparing the food intake of the intact and ovariectomised rats of all the treatment groups, the ovariectomised group fed with normal rat chow showed

a higher food intake than the intact rats of the same diet. For the rest of the diet groups, the food intake of the intact and ovariectomised rats did not differ (Table 1).

DISCUSSION AND CONCLUSIONS

Vitamin E deficiency, whether total or partial (50%), induced a decline in the body weights of normal female rats, whereas vitamin E supplementation did not cause any significant changes in the body weight. This implied that metabolic rate of the rat in vitamin E deficiency increases and food consumption of the vitamin E-deficient group remained constant, a steady decline in the body weight was observed. Food consumption was significantly higher for the 50% vitamin E-deficient group compared to all the other groups and this could probably explain why, after an initial decline, the body weight started to increase after the fifth month to reach a plateau. The increased food consumption could be due to a decline in serum leptin levels. There are no reports in the literature linking serum leptins and vitamin E; therefore, further study is needed.

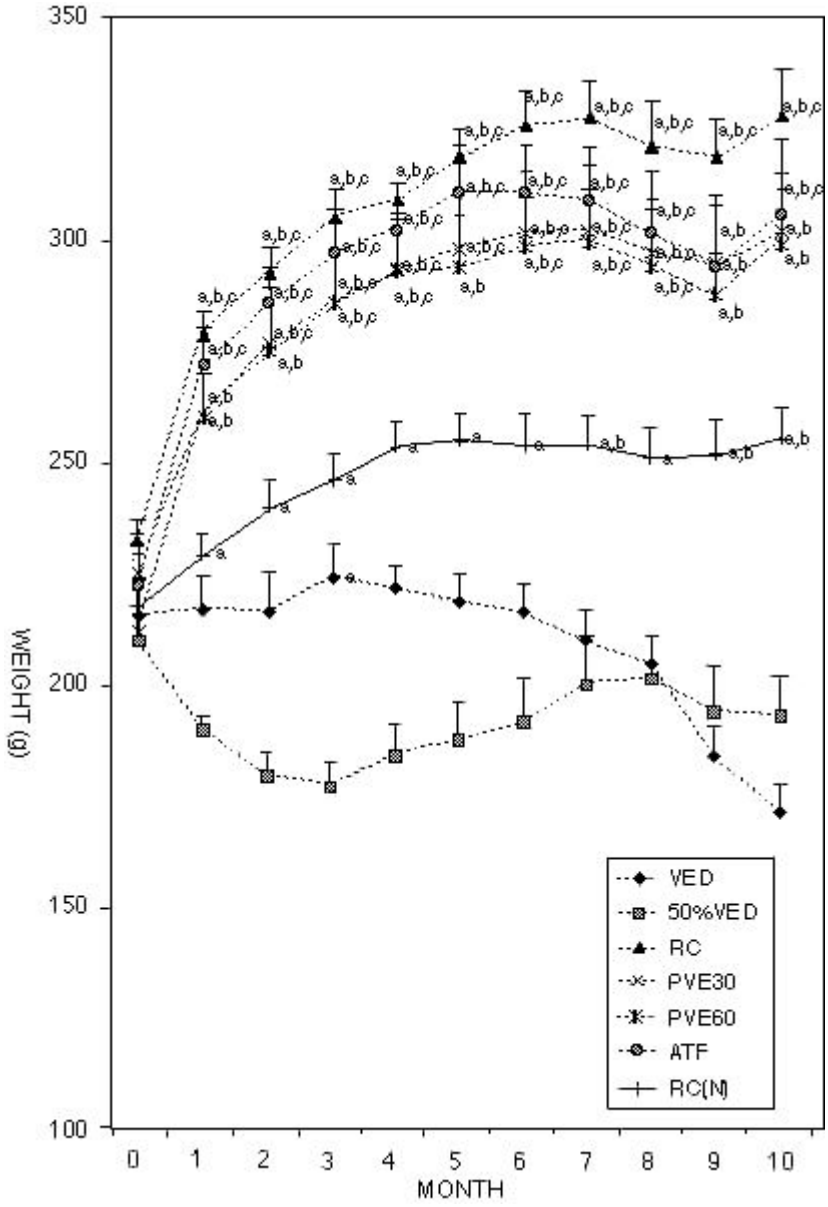
For the group of rats given normal rat chow, ovariectomy induced an increase in weight evident from the first month onwards compared to the intact rats. There was also an associated increase in food consumption by the ovariectomised rats. This is in aggreement with available literature (16,17). Estrogen was found to increase serum leptin production in both rats and human subjects (4,18). Thus, estrogen deficiency, as in ovariectomy, would cause a decline in serum leptin levels, so increasing food intake and leading to obesity. In this study, supplementation with high doses of vitamin E to the ovariectomised rats did not change the body weight with respect to that of the ovariectomised normal rat chow group. However, ovariectomy in the vitamin E deficiency groups, both total and partial failed to increase the body weight as seen in the normal rat chow group. In fact, the body weights did not differ significantly between the intact and ovariectomised rats in both the vitamin E-deficient and 50% vitamin E-deficient diet groups. Vitamin E deficiency may act to reduce weight by increasing basal metabolic rate to an extent that even ovariectomy failed to increase the body weight. Furthermore, the 50% vitamin E-deficient group was able to stabilize its body weight after 5 months by increasing its food consumption and this could be due to decreased serum leptin levels.

One study showed that the presence of corticosterone was required for ovariectomy to exert its action, such as body weight gain, total energy and fat gains (19). This finding could also be considered in relating vitamin E deficiency and impaired weight gain in ovariectomised animals although there are no reports in the literature relating vitamin E and corticosterone. Similarly, no previous studies have been reported on vitamin E deficiency, estrogen and body weight. However, reports on relationship of vitamin E and estrogen on lipid profiles showed that hormone replacement and vitamin E combined therapy is effective in prevention of cardiovascular diseases (20).

In this study, the weight loss cannot be attributed to a lower calorie content of the vitamin E deficient diet, since if that was so, then body weights of the rats fed the 50% vitamin E deficient diet should be midway between the body weights of the normal rat chow and vitamin E deficient diet groups. This was not so, in fact body weights of the rats in the 50% vitamin E deficient diet group was even lower than that of the vitamin E deficient group in the first 4 months of the study despite higher food intake by the 50% vitamin E deficient group of rats. Furthermore, the ovariectomised vitamin E deficient and 50% vitamin E deficient rats did not exhibit the weight increase, which should occur if the weight loss was attributed solely to lack of calories.

In conclusion, vitamin E deficiency, both total and partial decreased body weights in normal and estrogen deficient female rats. Vitamin E deficiency appeared to inhibit the post-ovariectomy weight gain that was seen in the ovariectomised rats given normal rat chow or vitamin E supplemented diets. Supplementation with palm vitamin E 30 mg/kg and 60 mg/kg or with pure α-tocopherol 30 mg/kg did not affect body weights in the normal and estrogen deficient female rats. Further

Figure 2: Body weights of the ovariectomised female rats (all treatment groups) and intact rats (normal rat chow).



a,b,c indicate significant difference to 50% VED, VED and RC(N) groups respectively ($p<0.05$)

studies are needed to elucidate the mechanisms involved.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Science, Technology and Environment for funding this project under grant IRPA/UKM 06-02-02-0053. We would also like to thank Ms. Kiftiah Ahmed and Ms. Sariza Taib for their technical assistance.

REFERENCES

1. Kurachi H, Adachi H, Ohtsuka S, Morishige K, Amemita K, Keno Y, Shimomura I, Tokunaga K, Miyake K and Matsuzawa Y : Involvement of epidermal growth factor in inducing obesity in ovariectomised mice. Am J Physiol, 265:E323-E331, 1993.

2. Mishikagawa Y, Ikegami H, Sakata M, Mizutani T, Morishige K, Kurachi K, Hirota K, Miyake A and Tamizawa O : Ovariectomy increases the level of estrogen receptor mRNA and estrogen receptor binding sites in female rat adipose tissue. J Endocrinol Invest, 16:579-583, 1993.

3. Bray GA : Obesity and reproduction. Hum Reprod, 12: 26-32, 1997.

4. Shimizu H, Shimomura Y, Nakamishi Y, Futawatari T, Ohtani K, Sato N and Mori M : Estrogen increases in vivo leptin production in rats and human subjects. J Endocrinol, 54:285-292, 1997.

5. Ambrosius WT, Compton JA, Bowsher RR and Pratt JH : Relation of race, age and sex hormone differences to serum leptin concentrations in children and adolescents. Horm Res, 49: 240-246, 1998.

6. Hickey MS, Israel RG, Gardiner SN, Considine RV, McCammon MR, Tyndall GL, Houmard JA, Marks RH and Caro JF : Gender differences in serum leptin levels in humans. Biochem Mol Med, 59:1-6, 1996.

7. Schwenke DC and Behr SR : Vitamin E combined with selenium inhibits atherosclerosis in hypercholesterolemic rabbits independently of effects on plasma cholesterol concentrations. Circ Res, 83:366-377, 1998.

8. Xu R, Yokoyama WH, Irving D, Rein D, Walzem RL and German JB : Effect of dietary catechin and vitamin E on aortic fatty streak accumulation in hypercholesterolemic hamsters. Atherosclerosis 137:29-36, 1998.

9. Nesaretnam K, Stephen R, Dils R and Darbre P : Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. Lipids, 33:461-469, 1998.

10. Miwa K, Miyagi Y, Igawa A, Nakagawa K and Inoue H : Vitamin E deficiency in variant angina. Circulation 94:14-18, 1996.

11. Tanyel MC and Mancano LD : Neurologic findings in vitamin E deficiency. Am Fam Physician, 55:197-201, 1997.

12. Ohtsuka A, Ohtani T, Horiguchi H, Kojima H and Hayashi K : Vitamin E reduces glucocorticoid-induced growth inhibition and lipid peroxidation in rats. J Nutr Sci Vitaminol, 44:237-247, 1998.

13. Elson CE : Tropical oils: Nutritional and scientific issues. Critical Rev Food Sci Nutr, 31:79-102, 1992.

14. Yee JK and Ima-Nirwana S : Palm vitamin E protects against ferric-nitrilotriacetate-induced impairment of bone calcification. Asia Pacific J Pharmacol, 13:1-7, 1998.

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

16. Cikos S, Kuchar S and Koppel J : Body weight and skeletal growth in female rats after ovariectomy. Vet Med (Praha), 38:729-737, 1993.

17. McElroy JF and Wade GN : Short-and long-term effects of ovariectomy on food intake, body weight, carcass composition and brown adipose tissue in rats. Physiol Behav, 39:361-365, 1987.

18. Mannucci E, Ognibene A, Becorpi A, Cremasco F, Pellegrini S, Ottanelli S, Rizello SM, Massi G, Messeri G and Rotella CM : Relationship between leptin and estrogens in healthy women. Eur J Endocrinol, 139:198-201, 1998.

19. Deshaies Y, Dagnault A, Lalonde J and Richard D : Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. Am J Physiol, 273:E355-E362, 1997.

20. Inal M, Sunal E, Kanbak G and Zeytinoglu S : Effects of postmenopausal hormone replacement and alpha-tocopherol on the lipid profiles and antioxidant status. Clin Chim Acta, 268: 21-29, 1997.

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