

THE EFFECTS OF VITAMIN E ON BODYWEIGHT AND FAT MASS IN INTACT AND OVARIECTOMIZED FEMALE RATS

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SUMMARY: The aim of this study was to determine the effect of vitamin E deficiency and supplementation on body weight and body composition in intact and ovariectomized growing female rats. One hundred and twenty female Wistar rats aged 3 months were ovariectomized (OVX) or left intact (sham-operated). The intact and OVX rats were divided into 6 groups and given different dietary treatments, i.e. vitamin E deficient diets (VED, 75%VED, 50%VED, 25%VED), normal rat chow diet (RC) and rat chow with oral supplementation of 30mg/kg body weight of α -tocopherol (RC+ATF). Body weight of intact and OVX rats in the RC and the RC+ATF groups showed increased significantly after 15 weeks of dietary treatment. Intact and ovariectomized rats fed with VED, 75%VED, 50%VED and 25%VED did not gain weight after 15 weeks. OVX rats had significantly higher body weight than intact rats in the 50%VED, 25%VED, RC and RC+ATF groups. Fat mass of intact rats was increased only in the RC and RC+ATF groups. For OVX rats, fat mass was increased in the VED, 50%VED, RC and RC+ATF groups. OVX groups had significantly higher fat mass when compared with intact groups, however, the significance was greater for the RC and RC+ATF groups. Other parameters of body composition were not significantly affected. In conclusion, vitamin E played an important role in the weight gain of female rats and the gain was primarily due to the increase in fat mass, irrespective of the effect of ovariectomy. Alpha-Tocopherol supplementation conferred little benefit compared to giving RC diet alone in both the intact and ovariectomized female rats. The results also indicate that excessive vitamin E intake might contribute towards obesity in female rats.

Key Words: Vitamin E, body weight, fat mass, ovariectomy.

INTRODUCTION

Ovariectomy results in a hypoestrogenic state and loss of estrous cycle, while pituitary gonadotropins become markedly elevated due to lack of negative feedback. Ovariectomy was shown to induce adipocyte hypertrophy and also increased the level of epidermal growth factor (EGF). These factors are involved in the induction of obesity in the ovariectomized 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). Studies have shown that there was an increase in body weight and food intake in ovariectomized female rats as compared to intact female rats (3). The increase in body weight was paralleled by higher adiposity and greater retroperitoneal adipose tissue mass (4). Ovariectomy also increased the level of *in vivo* oxidative stress in rodents (5). This is because estrogen is known to have antioxidant properties (6).

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Body composition parameters such as fat mass, lean mass (fat-free mass) and bone mineral density can be measured using the dual-energy X-ray absorptiometer (DEXA) machine. The advantages of using the DEXA technique for body composition studies are that it required only a short duration of time, involved a minimal radiation dose, and gave regional values as well as total body values (7). We also selected this method because of the non-invasive nature, thus the same groups of animals can be followed-up on a long-term basis, and changes in body composition parameters before and after the study period can be observed. Measurement of fat mass, lean mass and bone parameters of rodents using the DEXA technique was shown to be accurate and comparable to the carcass chemical extraction method, which is considered to be the 'gold standard' in body composition measurement (8).

Vitamin E has been proven to have antioxidant properties and has an important role in protecting biological systems (9). Rats given vitamin E deficient diet was found to develop pathological abnormalities. Machlin *et al.* (10) showed that weanling rats fed with vitamin E deficient diet chronically exhibited growth retardation and necrotizing myopathy when they were older. Vitamin E was also shown to have some effects on bone growth. Vitamin E was able to stimulate the growth of trabecular bone (11). Alpha-tocopherol and vitamin E derived from palm oil was able to reverse the negative effects of estrogen deficiency on bone mineral density (12). Optimum vitamin E levels are needed to maintain bone calcification and bone mineral density (13). In terms of bodily growth, vitamin E was shown to improve growth retardation in glucocorticoid-treated rats (14). Another study did not find any significant difference in body weight of growing male rats supplemented with two different doses of palm vitamin E-rich extract (15). A more recent study (16) showed that intact and ovariectomized rats given long-term vitamin E deficient diet failed to increase their body weight as compared to rats given adequate dietary vitamin E. However, the corresponding changes in body composition parameters in those rats were not studied. There is no available literature on the *in vivo* effect of vitamin E on fat mass in humans or rats. Also, no studies have yet been done to

compare the effects of different degrees of severity of vitamin E deficiency on fat mass, lean mass and bone mineral density in female rats as they age, using the DEXA technique. Therefore, this study was done in order to determine the effects of different degrees of severity of vitamin E deficiency on body weight, as well as on components of body composition such as fat mass, lean mass and bone mineral density, in intact and ovariectomized growing female rats. The effects of vitamin E (alpha-tocopherol) supplementation on these animals were also studied.

MATERIALS AND METHODS

Animals and treatment

One hundred and twenty 3-month-old female Wistar rats were obtained from the University Breeding Center. Half of the rats were ovariectomized and the rest were sham-operated (both ovaries left intact). Both ovariectomized and intact rats were divided into 6 groups, 10 rats for each group, and given 6 types of diet; total vitamin E deficient diet (VED), 75% vitamin E deficient diet (75%VED), 50% vitamin E deficient diet (50% VED), 25% vitamin E deficient diet (25% VED), normal rat chow diet (RC) and normal rat chow diet plus 30mg/kg vitamin E (alpha-tocopherol) supplementation (RC+ATF). The rats were kept 4 per cage under 12 hour natural light/dark cycles and given tap water *ad libitum*.

Ovariectomy

The rats were anaesthetized with Ketapex and Xylazil, 1:1 (Troy Laboratories, Australia). The lower abdomen of the rats were shaved and incised. Fallopian tubes and ovaries were identified and absorbable catgut suture was used to tie the fallopian tubes below the ovaries. The ovaries were then removed. The rats were given normal diet for 2 weeks for the wound to recover before dietary manipulation was started.

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 (17). Vitamin E deficient diet was purchased from ICN Biomedicals, USA, and did not contain any vitamin E at all. 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 repelleted, and dried in the oven at 70°C for 24 hours. Likewise, the 75% and 25% vitamin E deficient diets were made by using portions of ground vitamin E deficient diet and ground normal rat chow at a mixture of (3:1) and (1:3) respectively. Dietary treatment was carried out for 15 weeks.

Vitamin E supplementation

Alpha-tocopherol supplementation of 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 mixture per 100 g body weight was given orally 6 days a week for 15 weeks. Rats given vitamin E supplementation were fed normal rat chow *ad libitum*.

Body weight

Body weight was measured weekly using electronic weighing scales (Denver Instrument Co., USA).

Body composition measurements

Body composition measurements were obtained using the Dual-Energy X-ray Absorptiometer (DEXA XR-36) (Norland, USA), which has less than 1% coefficient of variation. The rats were anaesthetized with Ketapex and Xylazil (1:1), and placed prone for measurements. The measurements were taken before the rats were treated, and once again after the rats had been treated for 15 weeks.

Analyses of data

The data were first tested for normality. Normally distributed data were analyzed using parametric methods while data which were not normally distributed were analyzed using non-parametric methods. Data for body weight, lean mass and bone mineral density were analyzed using parametric methods. Difference between treatment groups was analyzed using the one-way analysis of variance with the Tukey's honestly significant difference as the post-hoc test. Comparison of data between the same group before and after the study period was done using the paired Student's t-test, and fat mass data were analyzed by non-parametric methods. The difference between means was determined using the Kruskal-Wallis test, comparison of data within the same group before and after treatment were done using the Wilcoxon Signed Rank test, and comparison of data

between different groups were done using the Mann-Whitney U test. All the statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS) version 10 software. A value of $p < 0.05$ was considered significant.

This study was approved by the Universiti Kebangsaan Malaysia Research and Animal Ethics Committees (UKMAEC).

RESULTS

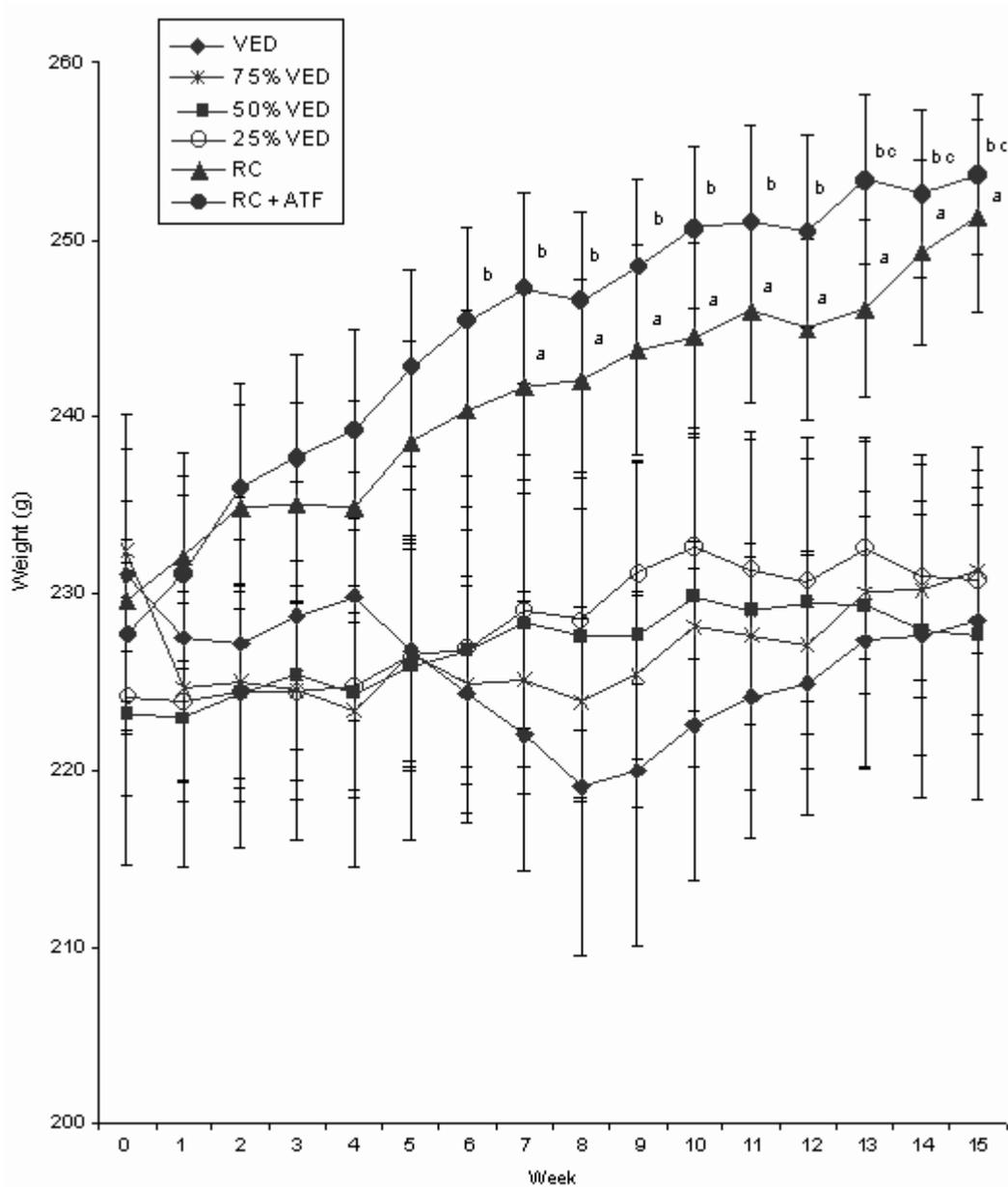
Body weight

Body weight of intact rats in RC and RC+ATF groups showed steady increase throughout the treatment period and did not differ significantly from each other (Figure 1a). However, for groups given vitamin E deficient diets, their weight remained almost the same and did not differ significantly from each other throughout the treatment period (Figure 1a). Their weight was also significantly lower than the RC and RC+ATF from the sixth week onwards (Figure 1a). The pattern of weight change for ovariectomized rats were quite similar to their intact counterparts, except that significant difference between the vitamin E deficient groups and the RC and RC+ATF groups could be noticed as early as the first week of treatment (Figure 1b). When body weight values after treatment for ovariectomized rats were compared with that of intact rats, it was found that the weight of ovariectomized rats were significantly higher than intact rats in the 50%VED ($p < 0.01$), 25%VED ($p < 0.05$), RC ($p < 0.01$) and RC+ATF ($p < 0.01$) groups (Figure 2).

Fat mass

After 15 weeks, fat mass of intact rats was significantly increased only in the RC ($p < 0.05$) and RC+ATF ($p < 0.05$) groups (Figure 3a). However, after treatment values for fat mass of those two groups did not differ significantly from each other (Figure 3a). Fat mass values after treatment for RC and RC+ATF groups of intact rats were also significantly higher than all the vitamin E deficient groups (Figure 3a). For ovariectomized rats, fat mass was significantly increased in the VED ($p < 0.05$), 50%VED ($p < 0.05$), RC ($p < 0.01$) and RC+ATF ($p < 0.01$) groups after 15 weeks (Figure 3b). After treatment values for the RC and RC+ATF groups were not significantly different (Figure 3b). Fat mass values after treatment for RC and RC+ATF

Figure 1a : Body weight of intact female rats (all treatment groups).



VED : vitamin E deficient

75%VED : 75% vitamin E deficient

50%VED : 50% vitamin E deficient

25%VED : 25% vitamin E deficient

RC : rat chow

RC+ATF : rat chow + vitamin E supplementation

a: RC significantly higher than VED, 75%VED, 50%VED, 25%VED (p<0.05)

b: RC+ATF significantly higher than VED, 75%VED, 50%VED, 25%VED (p<0.05)

c: RC+ATF significantly higher than VED (p<0.01)

groups of ovariectomized rats were also significantly higher than the four vitamin E deficient groups (Figure 3b). When fat mass values after treatment for ovariectomized rats were compared with that of intact rats, it was found

that the fat mass of ovariectomized rats were significantly higher than intact rats in the VED (p<0.05), 75%VED (p<0.05), 50%VED (p<0.05), 25%VED (p<0.05), RC (p<0.01) and RC+ATF (p<0.01) groups (Figure 3c).

Table 1: Body weights of intact and ovariectomized female rats before and after treatment.

Groups	Before treatment		After treatment	
	Intact	Ovariectomized	Intact	Ovariectomized
VED	231.1 ± 9.1	239.6 ± 4.5	228.5 ± 6.5 a,e	247.6 ± 8.5 g,k
75%VED	232.5 ± 5.7	244.7 ± 4.3	231.3 ± 4.7b,f	242.3 ± 8.1h,l
50%VED	223.1 ± 3.9	249.8 ± 5.9	227.6 ± 9.4 c	259.2 ± 5.6 # i,m
25%VED	224.2 ± 5.6	250.8 ± 8.6	230.7 ± 7.6d	261.1 ± 9.0 # j,n
RC	229.6 ± 5.7	248.3 ± 6.4	251.4 ± 5.5* a,b,c,d	291.2 ± 4.8*# g,h,i,j
RC+ATF	227.7 ± 5.4	248.3 ± 8.7	253.7 ± 4.5*e,f	296.7 ± 6.4*#k,l,m,n

Groups with the same alphabet are significantly different ($p < 0.05$).

* indicates significant difference after treatment compared to before treatment for the same treatment group ($p < 0.05$).

indicates significant difference after treatment between the intact and the ovariectomized groups given the same treatment ($p < 0.05$).

Lean soft tissue mass

For lean mass, there was significant increase after 15 weeks in all groups of intact rats - VED ($p < 0.05$); 75%VED ($p < 0.001$); 50%VED ($p < 0.001$); 25%VED ($p < 0.01$); RC ($p < 0.01$); RC+ATF ($p < 0.001$) (Figure 4a). However, no significant difference was found after treatment amongst all the intact groups (Figure 4a). For ovariectomized rats, there was also significant increase in lean mass after 15 weeks for all groups - VED ($p < 0.01$); 75%VED ($p < 0.001$); 50%VED ($p < 0.001$); 25%VED ($p < 0.01$); RC ($p < 0.001$); RC + ATF ($p < 0.00001$) (Fig. 4b). However, no significant difference was found after treatment amongst all the ovariectomized groups (Fig. 4b). After 15 weeks, lean mass values of ovariectomized rats were not significantly different from intact rats for all groups.

Bone mineral density

Bone mineral density increased significantly for all the groups of intact and ovariectomized rats after 15 weeks of treatment. There were no significant differences between the bone mineral density of the intact and ovariectomized rats at 15 weeks treatment.

Food intake

Food intake of the 75%VED, 50%VED, 25%VED, RC and RC+ATF groups for both the intact and ovariectomized groups were all significantly higher than corresponding VED group. Food intake of the ovariectomized

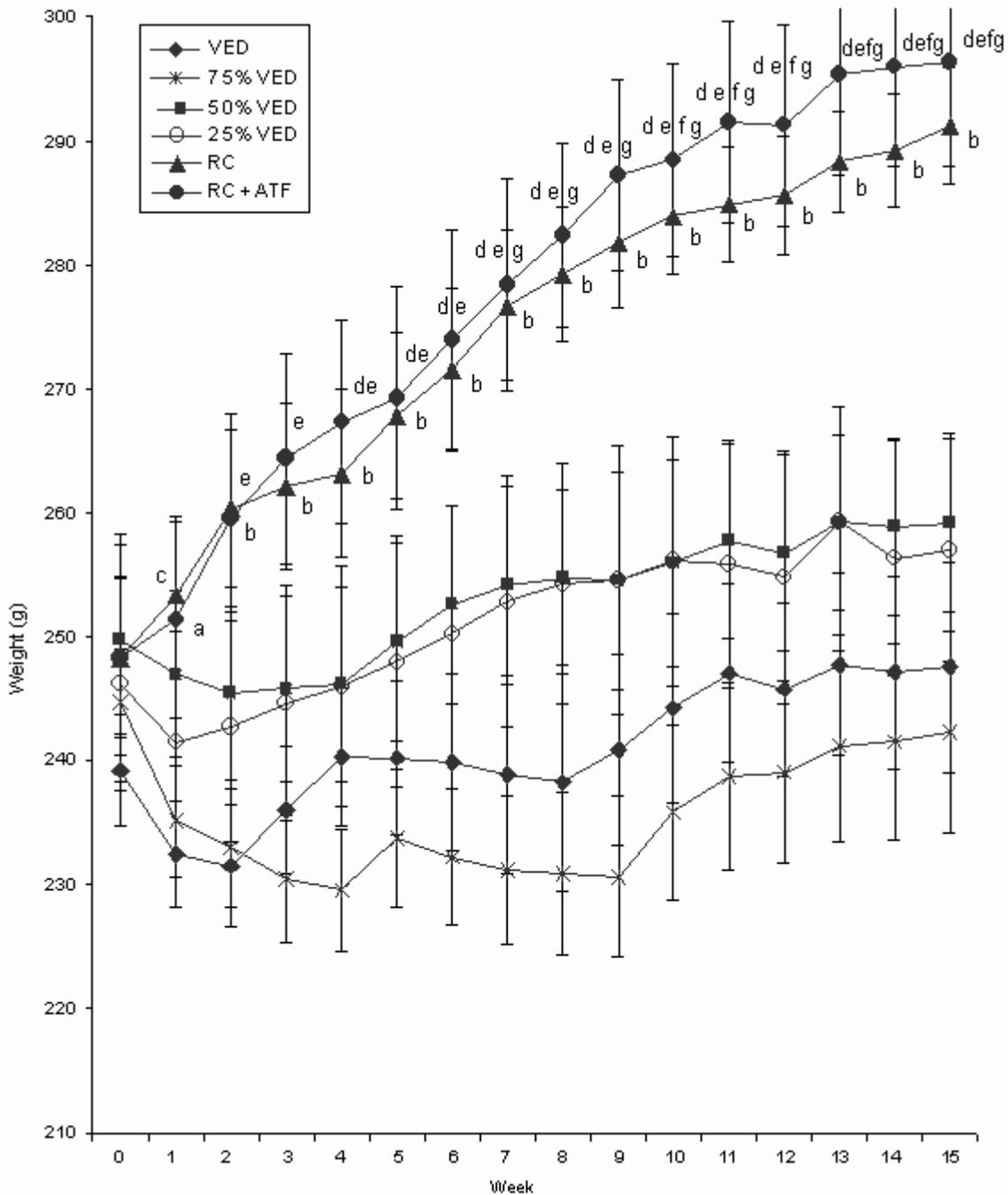
50%VED, 75%VED, RC and RC+ATF groups were higher than their intact counterparts (Figure 5).

DISCUSSION

Intact (sham-operated) rats fed with normal rat chow diet (RC group) showed progressive increase in weight throughout the study. This result confirmed the results of previous studies (18, 19). Newby *et al.* (20) found that the weight of rats continued to increase until they were two years old. They concluded that the increase in weight was mainly due to the increase in fat mass. However, Newby *et al.* (20) did not look into the effect of any vitamins on the increase of body weight and fat mass in rats. In this current study, we found that the increase in weight after a period of time was not seen in rats given vitamin E deficient diets, indicating that vitamin E was indeed needed in exhibiting this effect.

Giving vitamin E deficient diets to female rats inhibited the increase in weight as they aged. This result was similar to the one obtained by Ima-Nirwana (16). Vitamin E deficiency had also been shown to cause atrophy of skin, muscle dystrophy and thinning of bone (10), and this might also contribute towards the failure to gain weight in rats given vitamin E deficient diets in this study. It had been suggested that a decrease in the production of reactive oxygen species was able to prevent increases in the metabolic rate of animals (21). This further suggests that vitamin E (an antioxidant) could result in slowing down of body metabolism and favour fat accumulation.

Figure 1b : Body weight of ovariectomized female rats (all treatment groups).



VED : vitamin E deficient

75%VED : 75% vitamin E deficient

50%VED : 50% vitamin E deficient

25%VED : 25% vitamin E deficient

RC = rat chow

RC+ATF : rat chow + vitamin E supplementation

a: RC significantly higher than VED, 75%VED, 50%VED, 25%VED (p<0.05)

b: RC significantly higher than VED, 75%VED, 50%VED, 25%VED (p<0.01)

c: RC+ATF significantly higher than VED and 75%VED (p<0.05)

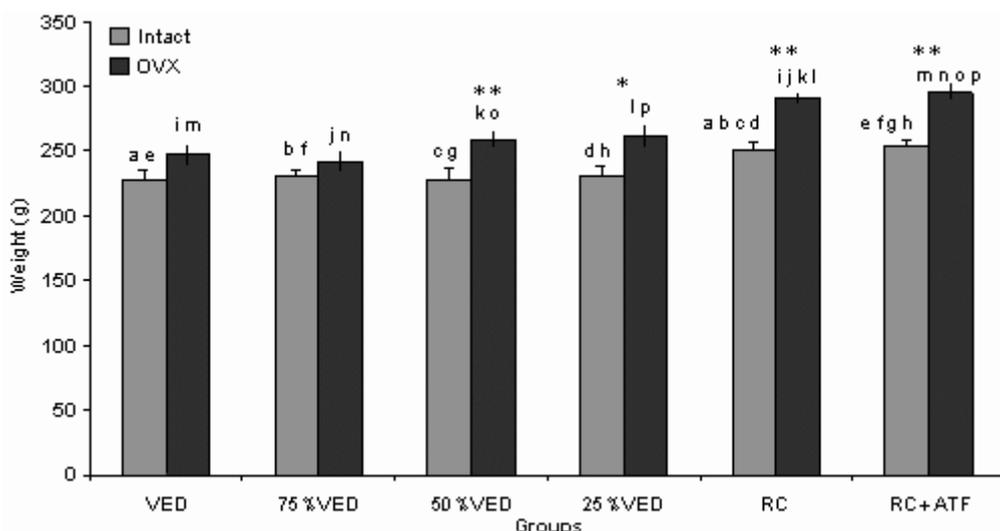
d: RC+ATF significantly higher than 50%VED and 25%VED (p<0.05)

e: RC+ATF significantly higher than VED and 75%VED (p<0.01)

f: RC+ATF significantly higher than 50%VED (p<0.01)

g: RC+ATF significantly higher than 75%VED (p<0.001)

Figure 2: Body weight of intact and ovariectomized rats after treatment.



Groups which share the common alphabet indicate significant ($p < 0.05$). *, ** indicate significant difference between intact and ovariectomized rats ($p < 0.05$, $p < 0.01$ respectively).

The increase in body weight in intact rats supplemented with alpha-tocopherol (RC+ATF group) did not differ significantly with the intact RC group. This at the same time result was similar to that obtained by Ima-Nirwana (16). This suggests that the RC diet contained sufficient vitamin E and giving more than the optimum vitamin E requirement did not have any added effect on body weight. Traber (22) reported that alpha-tocopherol absorption efficiency decreased when the amount given to animals was increased. An increase in the concentra-

tion of alpha-tocopherol in plasma might also saturate the alpha-tocopherol transfer protein (TTP) (23) responsible for transferring alpha-tocopherol from lipoprotein in circulation to effector cells.

The weight of ovariectomized rats given normal rat chow diet (OVX RC group) *ad libitum* increased steadily throughout the study period, the pattern being similar to the intact rats. However, when the weight of ovariectomized rats were compared with the weight of intact rats after 15 weeks, it was noted that the weight of ovariec-

Table 2: Body fat mass of intact and ovariectomized female rats before and after treatment.

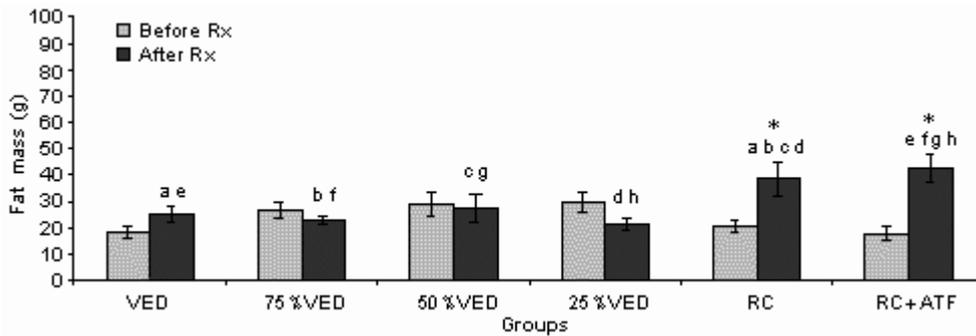
Groups	Before treatment		After treatment	
	Intact	Ovariectomized	Intact	Ovariectomized
VED	18.4 ± 7.2	20.5 ± 8.4	25.2 ± 9.5 a,e	46.1 ± 20.5* # i,m
75%VED	26.6 ± 10.3	27.6 ± 11.8	22.9 ± 5.6 b,f	35.2 ± 8.4* # j,n
50%VED	28.7 ± 14.3	29.4 ± 18.4	27.2 ± 16.2 c,g	42.6 ± 13.6 # k,o
25%VED	29.7 ± 11.3	26.13 ± 12.3	21.1 ± 7.6 d,h	30.2 ± 8.9* # l,p
RC	20.5 ± 6.6	24.8 ± 12.4	38.4 ± 21.0*a,b,c,d	59.1 ± 15.2*# i,j,k,l
RC+ATF	17.7 ± 8.8	24.4 ± 9.4	42.3 ± 16.5*e,f,g,h	75.6 ± 33.1* # m,n,o,p

Groups with the same alphabet are significantly different ($p < 0.05$).

* indicates significant difference after treatment compared to before treatment for the same treatment group ($p < 0.05$).

indicates significant difference after treatment between the intact and the ovariectomized groups given the same treatment ($p < 0.05$).

Figure 3a: Fat mass of intact rats before and after treatment.



Groups which share the common alphabet indicate significant ($p < 0.05$). Rx: treatment. * indicates significant difference between intact and ovariectomized rats ($p < 0.05$) respectively.

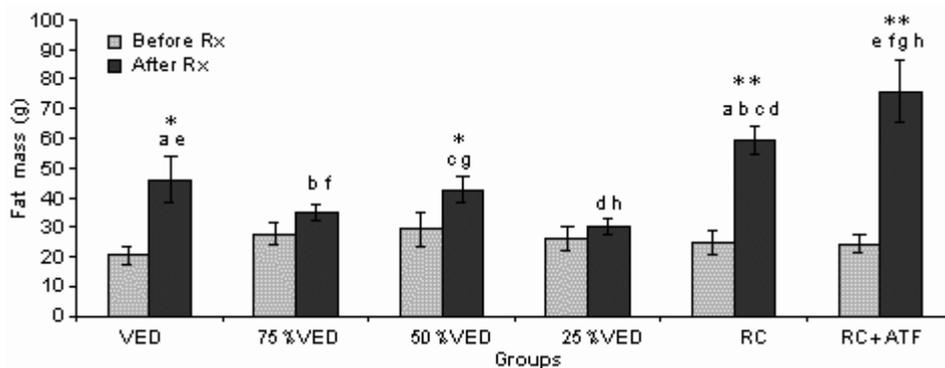
tomized rats were higher than intact rats for almost all the groups, except for those which experience very severe vitamin E deficiency (VED and 75%VED). One of the reasons for this is the higher food intake of ovariectomized rats (Figure 6), and this result is reported by previous studies (24-26). This result also supports the hypothesis that apart from estrogen deficiency, an optimum amount of vitamin E *in vivo* is also required to manifest the higher weight gain seen in ovariectomized rats after a period of time.

Estradiol, either the naturally occurring type secreted by the ovaries, or given exogenously, was able to decrease food intake and body weight of female rats (25). Ovariectomized rats injected with estradiol benzoate did not experience increase in body weight as compared to

ovariectomized rats injected with vehicle only (19). The OVX rats given vitamin E deficient/low vitamin E diet did not gain weight after 15 weeks, and the weight of OVX rats in the VED and 75%VED groups did not differ significantly from their intact counterparts, suggesting that vitamin E deficiency might also have an effect on metabolism, although further studies need to be done to confirm this observation. Apart from estrogen, vitamin E was needed to manifest the weight gain seen in OVX rats. Further studies need to be done in order to define the nature of interaction between vitamin E and estrogen in manifesting the weight gain seen in this study.

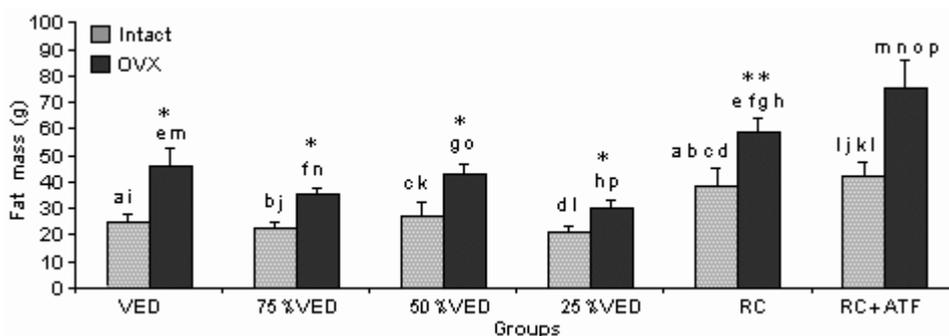
After 15 weeks, it was shown that fat mass of the intact RC group increased significantly as they aged. This increase in fat did not occur in rats given the various vita-

Figure 3b : Fat mass of ovariectomized intact rats before and after treatment.



Groups which share the common alphabet indicate significant ($p < 0.05$). Rx: treatment. *, ** indicate significant difference between intact and ovariectomized rats ($p < 0.05$, $p < 0.01$) respectively.

Figure 3c: Fat mass of intact and ovariectomized rats after treatment.



Groups which share the common alphabet indicate significant ($p < 0.05$). *,** indicate significant difference between intact and ovariectomized rats ($p < 0.05$, $p < 0.01$ respectively).

min E deficient diets indicating that an optimum amount of vitamin E was required for the increase in fat mass of rats as they grew older. Vitamin E, acting as an antioxidant, will decrease the level of oxidative stress *in vivo*. The decrease in oxidative stress level might help to stabilize the integrity of enzymes that are involved in liposynthesis. The presence of adequate level of vitamin E may also protect against lipolysis, whereby increase in lipid peroxidation (for example in conditions of low vitamin E levels) could theoretically facilitate lipolysis. The lipid peroxidation process could also possibly directly destroy adipose cells. Thus, the possible ability of vitamin E in curbing lipolysis and adipose cell destruction as well as facilitating liposynthesis, could explain why fat mass increase observed in this study. Further studies need to be done in order to confirm these proposed mechanisms.

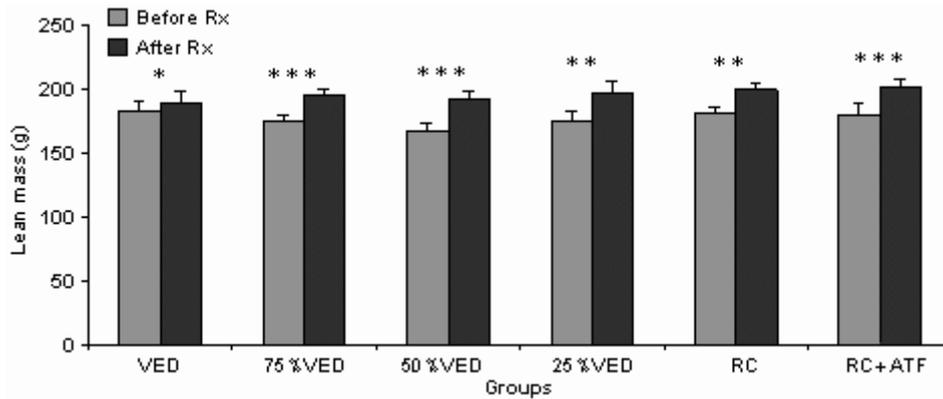
Fat mass of ovariectomized rats given normal rat chow (RC group) increased significantly after 15 weeks. This result is in compliance with other studies (3, 30). Fat mass increased significantly after 15 weeks for some of the vitamin E deficient groups of ovariectomized rats, but the significance of the increase was lower than that of the RC and RC+ATF groups. This further adds to the proof that vitamin E is essential for the gain in fat mass as the rats grew older. However, the estrogen deficiency state that occurred after ovariectomy could by itself stimulate fat mass accumulation in the body. Ovariectomy has been shown to cause an increase in food intake (24, 26), whereas estradiol hormone replacement therapy reduced food intake in ovariectomized rats (18, 19). Results from previous studies also implied that thermogenesis and basal metabolic activity of ovariectomized rats decreased

Table 3 : Lean soft tissue mass of intact and ovariectomized female rats before and after treatment.

Groups	Before treatment		After treatment	
	Intact	Ovariectomized	Intact	Ovariectomized
VED	182.6 ± 8.4	174.1 ± 6.7*	189.3 ± 8.7	190.6 ± 6.7*
75%VED	174.9 ± 4.6	174.6 ± 5.5*	195.5 ± 3.7	198.2 ± 5.8*
50%VED	167.5 ± 5.7	172.0 ± 5.8*	191.3 ± 5.8	212.2 ± 6.8*
25%VED	174.1 ± 8.5	171.2 ± 10.0*	196.7 ± 8.6	213.7 ± 9.0*
RC	180.2 ± 5.5	173.3 ± 3.9*	199.2 ± 5.1	212.9 ± 5.2*
RC+ATF	179.4 ± 10.1	171.3 ± 5.1*	201.8 ± 5.6	208.9 ± 7.2*

* indicates significant difference after treatment compared to before treatment for the same treatment group ($p < 0.05$).

Figure 4a: Lean mass of intact rats before and after treatment.



Groups which share the common alphabet indicate significant ($p < 0.05$). Rx: treatment. *, **, *** indicate significant difference between intact and ovariectomized rats ($p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively).

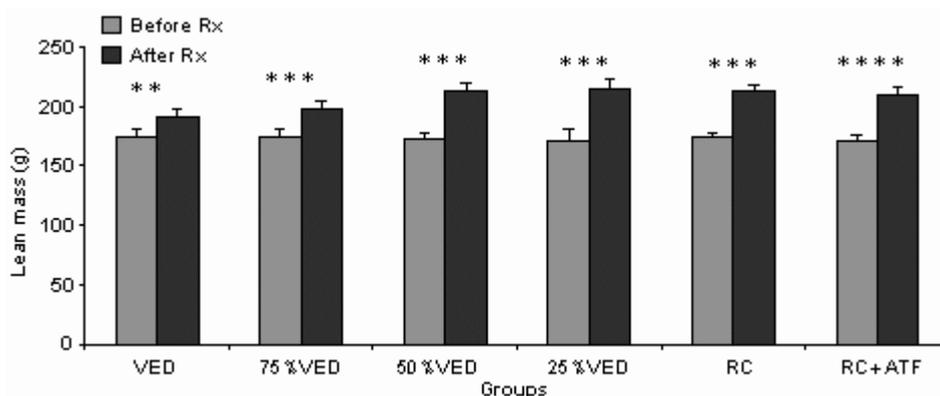
as compared to intact rats (31, 32) and estradiol hormone replacement had been shown to increase thermogenic activity of ovariectomized rats (33).

After 15 weeks, we also noticed that fat mass of ovariectomized rats is bigger than that of intact rats for all groups. This result is in agreement with other studies (1, 3, 4, 30). However, the increase in fat mass when ovariectomized rats was compared to intact rats. This was less significant in the vitamin E deficient groups which indicates that vitamin E deficiency inhibited the bigger increase in fat mass of ovariectomized and intact rats seen in groups given adequate vitamin E in their diet (RC and RC+ATF groups). Vitamin E might act synergistically with estrogen

deficiency to further increase fat mass content, as compared to the effect of estrogen deficiency alone.

Since the pattern of changes in fat mass of intact and ovariectomized rats in our study were quite similar to that of body weight changes, and both parameters seemed to be influenced by vitamin E, it seemed very likely that the body weight changes in this study is determined by fat mass, and vitamin E might be involved in influencing this relationship. Therefore, vitamin E affects bodyweight through its effect on fat mass in female rats. There is also strong evidence from our study that consuming more than the required amount of vitamin E seemed to promote fat accumulation in female rats, which could lead to obesity as they grew older.

Figure 4b: Lean mass of ovariectomized rats before and after treatment.



Groups which share the common alphabet indicate significant ($p < 0.05$). Rx: treatment. **, ***, **** indicate significant difference between intact and ovariectomized rats ($p < 0.01$, $p < 0.001$ and $p < 0.00001$ respectively).

Table 4: Bone mineral density of intact and ovariectomized female rats before and after treatment.

Groups	Before treatment		After treatment	
	Intact	Ovariectomized	Intact	Ovariectomized
VED	0.116 ± 0.003	0.118 ± 0.003*	0.118 ± 0.002	0.121 ± 0.002*
75%VED	0.115 ± 0.002	0.116 ± 0.002*	0.122 ± 0.002	0.122 ± 0.003*
50%VED	0.112 ± 0.003	0.111 ± 0.002*	0.121 ± 0.002	0.122 ± 0.002*
25%VED	0.116 ± 0.003	0.115 ± 0.002*	0.122 ± 0.002	0.123 ± 0.002*
RC	0.116 ± 0.003	0.113 ± 0.002*	0.126 ± 0.002	0.127 ± 0.002*
RC+ATF	0.114 ± 0.002	0.116 ± 0.002*	0.124 ± 0.001	0.129 ± 0.002*#

* indicates significant difference after treatment compared to before treatment for the same treatment group (p<0.05).

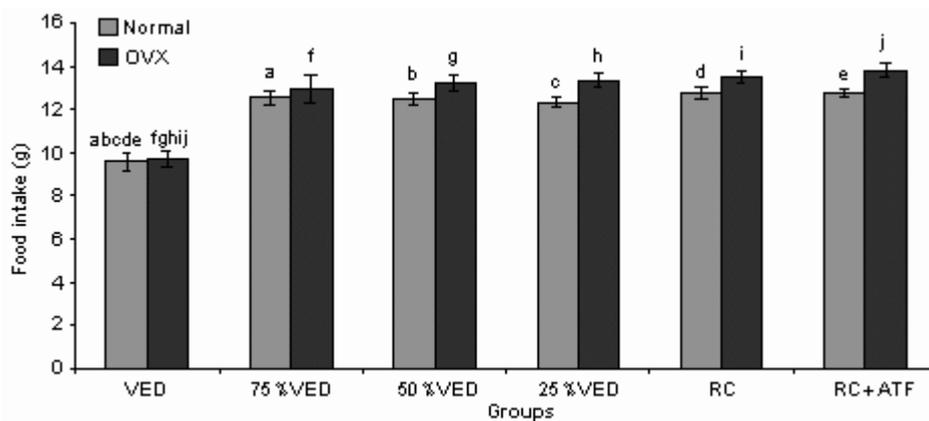
indicates significant difference after treatment between the intact and the ovariectomized groups given the same treatment (p<0.05).

The pattern of food intake was different from the pattern of body weight increase as well as the pattern of body fat mass changes for both the intact and ovariectomized groups. This indicates the changes in body weight and fat mass cannot be solely explained by changes in food intake. However, the results showed that severe vitamin E deficiency, as in the VED group, significantly suppressed appetite of the animals compared to the other groups.

Lean mass (fat-free mass) value is an indication of skeletal muscle content (7, 34). There was a significant increase in lean mass after treatment (as compared to before treatment values) in all groups of intact and ovariectomized rats. However, there was no significant

difference in the lean mass values after treatment between the groups of intact rats and ovariectomized rats. These results indicate that vitamin E had only a minimal effect on the growth of lean mass of these young growing female rats. Supplementation of vitamin E did not seem to have any added effect on lean mass development as compared to giving normal rat chow alone suggesting that vitamin E requirement is not essential needed for growth of lean mass. Simard and Srivastava (36) found that rabbits given vitamin E deficient diet chronically eventually developed nutritional muscular dystrophy. They suggested that severe vitamin E deficiency could disrupt the normal pattern of protein synthesis. In this study, the rats

Figure 5: Mean food intake per day for all groups.



Groups which share the common alphabet indicate significant difference (p<0.05).

Table 5: Mean food intake per day of ovariectomized and intact female rats.

Groups	Intact	Ovariectomized
VED	9.6 ± 0.4 a,b,c,d,e	9.7 ± 0.4 f,g,h,i,j
75%VED	12.6 ± 0.3 a	13.0 ± 0.6 f
50%VED	12.5 ± 0.3 b	13.2 ± 0.3 g
25%VED	12.4 ± 0.2 c	13.4 ± 0.4 h
RC	12.8 ± 0.3 d	13.5 ± 0.3 i
RC+ATF	12.8 ± 0.2 e	13.8 ± 0.3 j

Groups with the same alphabet are significantly different ($p < 0.05$).

were at first given normal rat chow diet until they were 3 months old, and only after that dietary manipulation was initiated. Since vitamin E is a fat-soluble vitamin, most probably it was stored in various tissues and were then be utilized by the rats during the 15 week treatment period. This may be the reason why in rats given total vitamin E deficient diet in this study, lean mass growth still occurred. From the results of this study, we noticed that the pattern of changes in body weight and lean mass before and after treatment were different, suggesting that the increase in weight as the rats grew older were not contributed by lean mass.

For both intact and ovariectomized rats, there was significant increase in bone mineral density values after treatment (as compared to before treatment) for all groups, except the VED group. This meant that only very severe vitamin E deficiency could prevent the increase in bone mineral density after 15 weeks, suggesting that optimal vitamin E concentration *in vivo* was essential to exhibit the increase in bone mineral density as the rats grew older. One study suggested that vitamin E was able to stimulate the growth of trabecular bone (11). On the other hand, vitamin E deficiency induced hypocalcemia, decreased the active transport process of calcium into the small intestine, as well as the saturation point of minerals in bone (38). However, in our current study, the rats were still young and growing, and factors such as growth hormone and calcium levels might play more important roles in these young, growing female rats. These factors, espe-

cially the high growth hormone level in young rats, may mask the effect of estrogen deficiency in decreasing bone mineral density as well as bone growth (39). Therefore, the increase in bone mineral density was still seen after 15 weeks of treatment, except for rats that received a diet that is totally deficient in vitamin E (the VED group). For this particular group of rats, the presence of lipid peroxidation products and free radicals, which was worsened by estrogen deficiency in the ovariectomized VED group, would probably have disrupted bone growth. This result was supported by a previous study which reported that palm vitamin E or alpha-tocopherol supplementation could bring bone mineral density values of ovariectomized rats to be similar to those of intact rats given normal rat chow (13). Even though ovariectomy was expected to decrease bone mineral density values, it was found from this study that there was no difference in the values of bone mineral density between the intact and ovariectomized rats. The treatment period of 15 weeks was probably not sufficient to decrease the bone mineral density of the ovariectomized rats. This result was supported by a previous study which found that after 3 month of ovariectomy, there was still no significant difference in bone mineral density between intact and ovariectomized rats given normal rat chow (40).

In conclusion, vitamin E is needed for the increase in body weight seen in intact and ovariectomized rats. The increase in weight was mainly due to increase in body fat mass. Vitamin E was needed for optimum growth of bone in growing rats. Growth of lean soft tissue appeared to be independent of vitamin E. Therefore, antagonism of the effect of vitamin E on body fat may be beneficial in preventing obesity, especially that associated with estrogen deficiency in menopause. Antagonism of the effects of vitamin E on appetite may also be beneficial for treatment of obesity.

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