Effects of Ovariectomy on Body Weight and Activity of 11-Beta Hydroxysteroid Dehydrogenase Type I In the Liver and Adipose Tissue of Rats

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Abstract:

The use of ovariectomized rat model in postmenopausal researches is widely used due to the similarities between the complications caused by estrogen deficiency either in ovariectomy or in natural menopause. The aim of the study was to investigate the effect of ovariectomy on the activity of the enzyme 11β-hydroxysteroid dehydrogenase type I (11β-HSDI) and consequently on body weigh. Thirty two Adult female (*Sprague Dawley*) rats, 6 months old were used in the experiment. Rats were divided into normal and ovariectomized groups, on long and short-term studies. Bilateral ovariectomy was performed under anesthesia using the ventral approach and rat's body weight was checked monthly. At the end of the study period, rats were sacrificed and tissue samples from liver and adipose tissue were collected for bioactivity assay. Ovariectomy induced significant increase *(P<O.05)* in body weight as well as in hepatic enzyme activity. In adipose tissue there was no significant difference recorded. These results suggest that ovariectomy has an effect on body weight and in modulating 11β-HSDI activity in the liver and probably in adipose tissue.

Introduction :

An animal model to study the postmenopausal symptoms and complications has long been defined in ovariectomized (OVX) rats. OVX-rats are deficient from ovarian hormones and share similar characteristics with naturally occurring menopause. Thus, the OVX animal model was used to study a list of menopausal complications such as obesity (Saruhan & Ozdemir, 2005), postmenopausal bone loss (Kalu, 1991), fracture

healing (Qiao *et al*., 2005), osteoarthritis and cartilage abnormalities (Cake *et aI*., 2005) fat and lipid metabolism (Kamei *et al*., 2005), 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in adipose tissue, liver, kidney and brain (Low *et al*., 1993) and the relationship between the oral discomfort (Seko *et al*., 2005).

Postmenopausal women are at high risk of weight gain, especially those already characterized by increased weight and fat mass (Dubnov *et al*., 2003), coronary artery diseases and stroke, as well as of osteoporosis and fractures (Sowers & La Pietra, 1995). Weight gain at menopause increases the risk of high blood pressure, high blood lipid levels, and insulin resistance. Alterations of lipid and carbohydrate metabolism are deeply related to increased risk of cardiovascular mortality and morbidity (Gaspard *et al*., 1995).

The link between obesity and the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDI) has been investigated by different studies. *in vivo* studies have shown that adipose regeneration of cortisol is enhanced in human obesity (Sandeep *et al*., 2003). Rask *et al.*, (2001) reported that adipose tissue from obese humans has increased 11βHSD-I activity. In another study it was also shown that omental adipose tissue contains significantly more 11βHSD-I activity than subcutaneous adipose tissue into adipocytes by cortisone compared to those from the subcutaneous adipose tissue (Berger *et al*., 13).

11β-HSD1 plays an important role in determining intracellular glucocorticoids concentration by the interconversion of cortisol (F) and cortisone (E) in man and corticosterone (B) and 11-dehydrocorticosterone (A) in rodents. It is one of two identified isozymes of the enzyme 11β-HSD that interconvert hormonally active cortisol and inactive cortisone (Bujalska *et al*. 2002). 11β-HSD1 was first isolated from the liver but it has a widespread central nervous system and peripheral tissue distribution and functions as NADP dependent. Type 2 (11β-HSD2) functions as NAD+ dependent dehydrogenase of adrenal glucocorticoids (Hult *et al*., 1998). Glucocorticoids are 21-carbon steroid molecules with a variety of physiologic and metabolic effects (Schleimer *et al*. 2003) and cortisol (hydrocortisone) is the principal circulating glucocorticoid in humans.

Glucocorticoids are a group of corticosteroids that affect carbohydrate metabolism by inducing liver gluconeogenesis, glycogenolysis and thus elevation of blood sugar (Allary & Annane, 2005). They also play a role in fat and protein metabolism, maintenance of arterial blood pressure and alteration of the connective tissue response to injury. Glucocorticoids have been shown to potentiate the adipogenic process and anabolic lipid metabolism in adipocytes (Berger *et al.*, 2001).

This study aimed to investigate the effect of OVX on body weight and on the activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in the liver and in adipose tissue.

Materials and Methods:

In this set of experiments two main things were investigated; first, the effect of ovariectomy (OVX) on body weight and second, the effect of OVX on the activity of the enzyme l1β-hydroxysteroid dehydrogenase type 1 (11β-HSDl) in the liver and adipose tissue.

Animals: Thirty Adult female (Sprague Dawley) rats, 6 months old were used in the experiment. The rats were housed at normal room temperature with adequate ventilation and normal 12-h light- dark cycle with free access to food (commercial laboratory rat's food) and water. Based on the allocated timing for sacrificing, the study was divided into short-term and long-term. In the short-term study two groups were included, the normal NOR1M (n = 8) and the ovariectomized OVX1M (n = 8) which were sacrificed one month (1 M) after the beginning of the study. In the long-term study, the normal NOR4M (n = 8) and OVX4M (n = 8) which were included were sacrificed four months (4M) after the beginning of the study. The rat's body weight was monitored and recorded monthly. This work has the approval of the Animal Ethics Committee at the Institute for Medical Research - Malaysia.

Ovariectomy: For ovariectomy (OVX), sixteen rats were anesthetized with intramuscular injection (IM) of Zoletil 50 O.lml (Virbac Laboratories, France), Ketamav O.lml (MA VLAB, Australia) and Xylazil 0.03 ml (Troy Laboratories, Australia). OVX was performed using the ventral approach. Upon recovery from anesthesia, animals were randomly

divided into OVX1M and OVX4M. To avoid the risk of infection post surgery, rats were housed in soft tissue bedded cages and the wounds were sprayed daily with a surgical disinfectant for a period of one week.

Tissue harvesting and preparation: Rats were sacrificed by cervical dislocation either one month or four months after OVX as allocated. Immediately upon sacrificing, tissue samples from liver and adipose tissue were collected and dissected on ice to minimize disturbance to the enzyme's activity within the tissues. Tissues were then stored at into -70°C environment for bioactivity assay.

11β-HSDl Activity in the Liver and Adipose Tissue: Tissues samples were thawed and homogenized in 2 ml of Krebs-Ringer buffer + 0.2% glucose at pH 7.4 in the same day of the assay. Homogenates were assayed for protein content calorimetrically (Bio-Rad, Hercules, DA, USA). Protein homogenate 80µg/ml protein for liver sample, (Moisan *et al*., 1990) and 750µg/ml protein for adipose tissue sample, (Lindsay *et al.*, 2003) was added to 2.5µl [^{3}H] corticosterone with 20 mM NADP. The total assay volume was made up to a total volume of 250 µl by adding Krebs-Ringer buffer. The final volume was than incubated at 37°C either 30 minutes (liver) or overnight for adipose tissue. Assays were in the dehydrogenase direction (corticosterone (B) to 11-dehydrocorticosterone (A), which is more stable in tissue homogenates. The reaction was terminated by addition of ethyl acetate. Steroids was separated by TLC using chloroform:ethanol (95:5, vol:vol), visualized under UV light and quantification by scintillation counting using β counter.

Statistical Analysis: Data were analyzed by T-test and one-way ANOV A. When the main effect was significant, a post-hoc test (Tukey) was applied to determine individual differences between means. A value of *(P<0.05)* was considered significant.

Results:

The Effect of Ovariectomy on Body Weight: In the short-term study, (one month after ovariectomy), the statistical T-test of these two groups showed no significant difference *(P=0.05)* in mean body weight (MBW) of the NOR1M one month after (from 244.7 ± 24g to 243.1 ± *20Ag).* On

the other hand, the OVX1M; ovariectomy induced a significant increase *(P<0.05)* in mean body weigh one month after ovariectomy. The mean body weight of those rats increased from 253.8 ± 25.6g to 282.3 ± 25.3g. Figure 1 illustrates the mean value of body weight at base line and one month after ± standard deviations (SD) for both groups.

In the long term study, (four months after ovariectomy), the statistical ANOVA of these groups showed significant increase *(P<0.05)* in MBW of the OVX4M between base line (259.32 ± 27.30g) and four months after OVX (324.91 ± 47.37g), whereas no significant difference recorded for the NOR4M. MBW of this group increased from 226.90 ± 30.77g to 255.73 ± 35.82g.

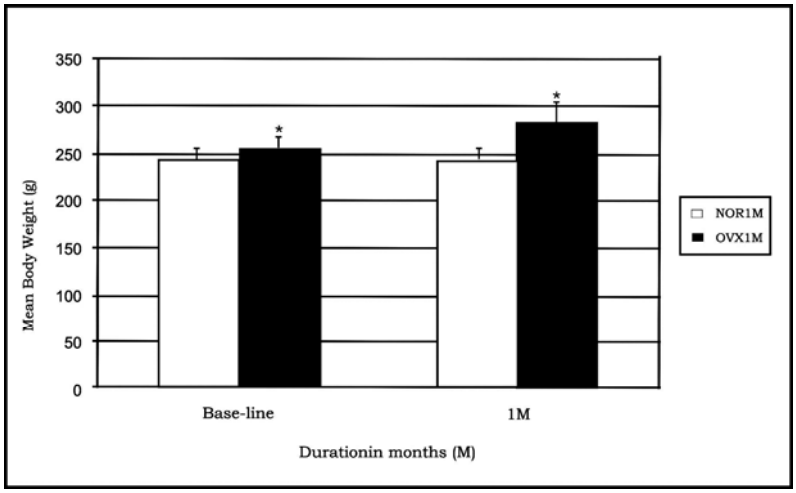

Figure (1) : Comparison of rats body weights recorded in the short-term study, (one month after ovariectomy) normal (NOR 1 M) and ovariectomized (OVX1M) rats. Ovariectomy resulted in significant increase (*, P<O.05) in mean body weight of the OVX1M group one month after compared to the mean body weight at base-line. On the other hand, results showed no significant difference in mean body weigh of the NOR1M group at baseline and one month after. Values are mean ± SD.

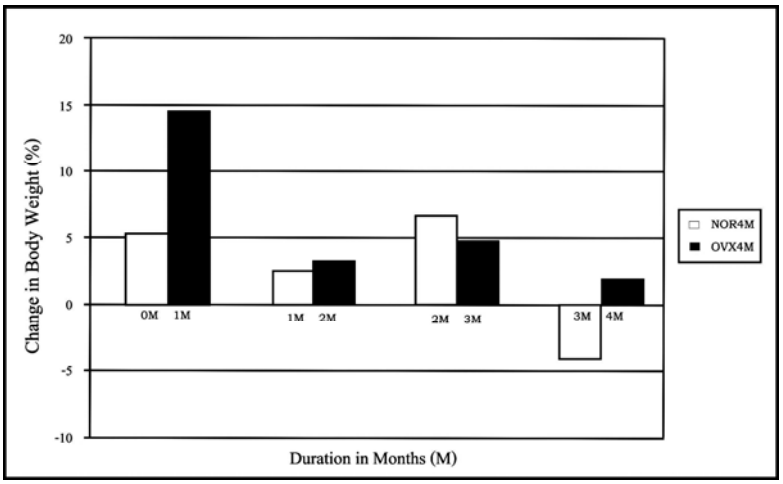

Figure (2) : Percentage changes in body weight recorded in the long-term study, (four months after ovariectomy) for the normal (NOR4M) and ovariectomized (OVX4M) groups throughout the duration of the study.

In the first month following OVX, there was an increase of 14.6% in the OVX4M group compared to 5.2% increase in the NOR4M group. In the second month, there was an increase of 2.9% in the OVX4M group compared to 2.3% in the NOR4M group. In the third month, the following changes in mean body weight were recorded: 4.6% in the OVX4M group and 6.5% in the NOR4M group. Thus, results at this stage showed less weight gain by the OVX4M group compared to the NOR4M group. In the fourth month, there was a decrease of 4.2% in the NOR4M group, compared to 1.8% increase in the OVX4M group. Percentage changes in body weight recorded for both groups are presented in Figure 2.

The physical examination of the rats: Matching the process of ovariectomyinduced weight gain, all the ovariectomized rats showed large amount of visceral/abdominal fat deposition. Figure 3 illustrates the increase in the amount of the visceral fat deposition in the OVX4M group compared to the NOR4M group as revealed by the physical examination and dissection of these rats.

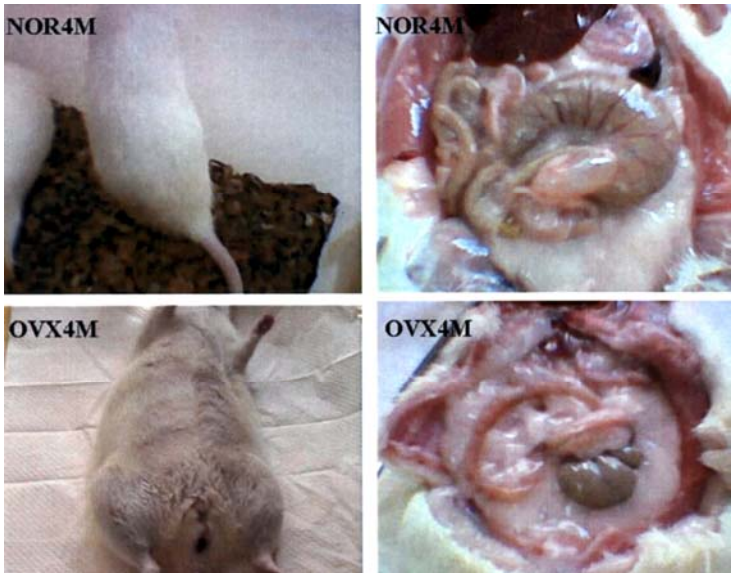

Figure (3) : Illustration of the increased amount of the visceral fat observed in the log-term study in the ovariectomized rats (OVX4M) compared to the normal rats (NOR4M) group. Ovariectomy induced the rate of adiposity that resulted in excessive visceral fat deposition and in turn body weight. The main body weight (±SD) for the NOR4M group was 255.73 ± 35.82 compared to 324.91 ± 47.37 for the OVX4M group.

The Effect of Ovariectomy on 11β-HSD1 Activity: In the both the short and long-term studies; OVX induced a significant increase *(P<0.05)* in the rate of conversion of corticosterone (B) to ll-dehydrocorticosterone (A) in the liver. The mean enzyme activity recorded for OVX1M (34.95 ± 21.67%) is significantly higher than the activity recorded for the NORlM (12.8 ± 8.04%) group. The mean enzyme activity recorded for OVX4M (43.20 ± 21.62%) is significantly higher than the activity recorded for the NOR4M (7.97 ± 4.40%) group.

In the adipose tissue the test showed no significant differences in both the short and long-term studies although the OVX4M showed a slight increase of 3.8% when compared to the NOR4M group. The following values were recorded for each group: 19.36 ± 3.73% for the NOR1M compared to

18.83 ± 6.41 % for the OVXlM and 18.59 ± 7.09% for the NOR4M compared to 22.39 ± 12.57% for the OVX4M. Generally; hepatic 11β-HSDl activity was lower than the activity in adipose tissue in both the normal groups (NORlM & NOR4M), whereas the opposite is true for the OVX groups (both OVXlM & OVX4M). Figure 4, shows enzyme activity ± standard deviation (SD) for the four groups.

Body weight and 11β-HSD-l Activity: The increase in body weight one month after OVX was associated with a significant increase *(P<0.05)* in enzyme activity when comparing NOR1M and OVX1M on one hand and NOR4M and OVX4M in the other hand. The results suggest direct relationship between body weight and enzyme activity in the liver. Figure 5 gives illustration of the relationship between body weight and enzyme activity in the liver.

Discussion :

This study investigated the effect of ovariectomy (OVX) on body weight and on the activity of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in the liver and adipose tissue. The physical examination of the ovariectomized rats revealed increased abdominal/visceral fat deposition four months after OVX, which was apparent in the OVX4M group. This observation is consistent with the finding reported by Heymsfield *et al*., (1994) and Poehlman *et al*., (1995), which have also, suggest that menopause increases central adiposity. It is also consistent with the view that menopause is associated with changes in fat distribution and that is estrogen deficiency is believed to play a role in postmenopausal women obtaining the gynoid (metabolic syndrome of overweight or obese men) fat deposition, (Reubinoff *et al*. 1995).

Consistent with the physical appearance, ovariectomy was found to induce a significant increase in rats' body weight. The increase in body weight was a clear sign that the rats were entering the menopausal stage as weight gain is one of prime consequences of menopause. The effect of OVX persisted to four months after as the increase in body weight of the OVX-rats were statistically significant when compare to the normal group through out the duration of the study. The increase in body weight following OVX could be explained by decrease in ovarian hormone levels

namely estrogen as it is well established that OVX cause estrogen deficiency, (Eskin *et al*., 2003). Estrogen was reported to be the prime hormone responsible for the postmenopausal changes. It also has been found to influence eating behavior and cause weight gain in animals, (Heymsfield *et al*. 1994). In this study, although food intake was not calculated, the increased food intake was observed in all of the ovariectomized rats.

The effect of ovariectomy on hepatic 11βHSD-l activity in the liver and adipose issue was investigated. It was recorded that hepatic enzyme activity was significantly elevated after ovariectomy. The increase in 11-βHSDl activity in the liver is consistent with the results reported by *Low* and his group (Low *et al.*, 1993) where they reported that gonadectomy resulted in a marked increase in 11β-HSD activity in female liver. Our finding here contradicts the finding by Stewart, and his group (Stewart *et al*., 1999) where they reported hepatic 11β-HSDl is reduced in obesity which was explained to be part of the compensatory change to reduce the local intrahepatic glucocorticoid load.

The elevation in hepatic 11β-HSDl activity following ovariectomy could again be attributed to the metabolic changed cause by ovariectomy-induced estrogen deficiency. In adipose tissues, in the short-term study, OVX was not shown to alter 11β-HSDl activity. The percentages recorded for both groups were almost equal. In the long-term study, OVX was shown to induce a slight increase in 11β-HSDl activity. Although the difference was not found to be statistically significant, it is consistent with Rask *et al.*, (2001), who reported that adipose tissue from obese humans has increased 11β-HSDl activity, which again supports the menopause role in fat re deposition.

Conclusion:

These results suggest that in rats, ovariectomy-induced estrogen deficiency have induced an increase in body weight as a consequence of increasing visceral fat deposition. It also has an effect in modulating 11β-HSDl activity in the liver and in adipose tissue.

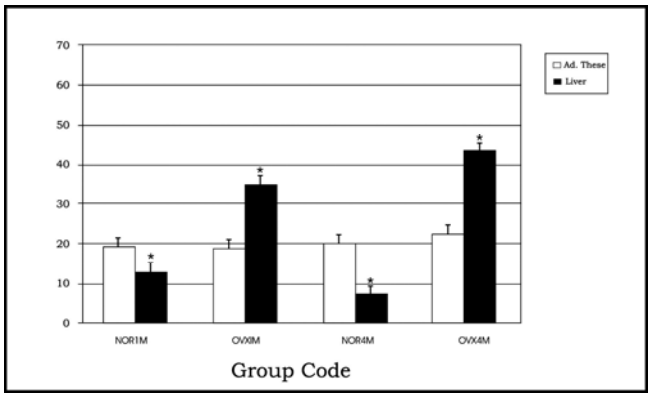

Figure (4) : Comparison between 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDI) activity in the liver and adipose tissue. In the short-term study, (one month after ovariectomy – NOR1M & OVX1M) as well as in the long-term study, (four months after ovariectomy - NOR4M & OVX4M), there was a significant increase in hepatic 11β-HSDI activity. In the adipose tissue, no significant difference detected between the groups. (*, # significant at $P = 0.05$)

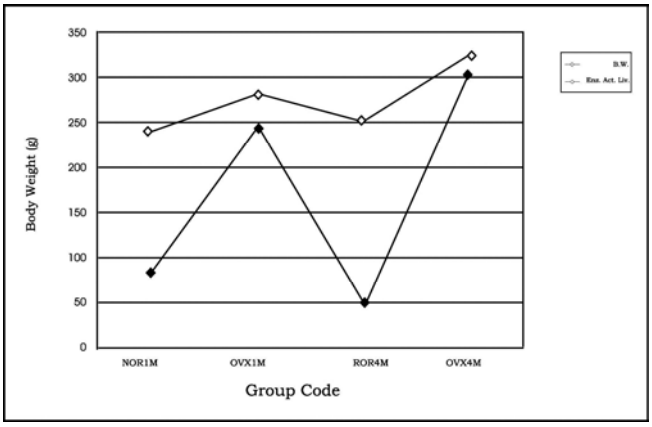

Figure (5) : The relationship between body weight and hepatic 11β-hydroxysteroid dehydrogenase type 1 (11β-HSDl. The graph shows that the significant increase in body weight was associated with a significant increase in 11β-HSDI activity. This was clear in the short-term study (one month after ovariectomy – NOR1M & OVX1M) as well as in the long-term study (four months after ovariectomy - NOR4M & OVX4M). These results suggested the existence of a direct relationship between the two parameters. In the short-term study, as well as in the long-term study,

References:

1. Allary, J. and Annane, D. 2005. Glucocorticoids and sepsis. Minerva Anestesiol 71:759-68.
2. Berger, J., Tanen, M., Elbrecht, A., Hermanowski-Vosatka, A., Moller, D.E., Wright, S.D. & Thieringer, R. 2001. Peroxisome Poliferator-activated Receptor γ Ligand Inhibited Adipocytes 11β-Hydroxysteroid Dehydrogenase Type 1 Expression and Activity. The Journal of Biological Chemistry & Molecular Biology. 276 (16): 12629-12635.
3. Bujalska, I.J., Walker, E.A, Hewison, M. & Stewart, P.M. 2002. A Switch in Dehydorgenase Activity of 11β-Hydroxysteroid Dehydrogenase Type 1 upon Differentiation of Human Omental Adipose Stromal Cells. The Journal of Clinical Endocrinology & Metabolism 87 (3):1205-1210.
4. Cake, M.A., Appleyard, R.C., Read, R.A., Smith, M.M., Murrell, G.A. & Ghosh, P. 2005. Ovariectomy alters the structural and biomechanical properties of ovine femoro-tibial articular cartilage and increases cartilage iN OS. Osteoarthritis Cartilage. 13 (12):1066-75.
5. Dubnov, G., Brzezinski, A. & Berry, E.M. 2003. Weight control and the management of obesity after menopause: the role of physical activity. Maturitas 44 (2):89-101.
6. Eskin, B.A., Synder, D.L., Roberts, J. & Aloyo, V.J. 2003. Cardiac Norepinephrine Release: Modulation by Ovariectomy And Estrogen. Society for Experimental Biology and Medicine. 228: 194-199.
7. Gaspard, U.J., Gottal, J.M., Frederic, A. & van den Brule, 1995. Postmenopausal changes of lipid and glucose metabolism: a review of their main aspects. Maturitas. 21 (3):171-178.
8. Heymsfield, S.B. Gallagher, D. & Poehlman, E.T. 1994. Menopausal changes in body composition and energy expenditure. Exp Gerontol 29 (3-4): 377-89).
9. Hult, M., Jomvall, H. & Oppermann, C.T. 1998. Selective Inhibition of Human Type 1 11β-Hydroxysteroid Dehydrogenase by Synthetic Steroids and
1. Xenobiotics. FEBS Letters. 441:25-28.
10. Kalu, D.N. 1991. The ovariectomized rat model of postmenopausal bone loss. Bone and Mineral. 15 (3): 175-191.

11. Kamei, Y., Suzuki, M., Miyazaki, H., Tsuboyama-Kasaoka, N., Wu, J., Ishimi, Y. & Ezaki, O. 2005. Ovariectomy in mice decreases lipid metabolism-related gene expression in adipose tissue and skeletal muscle with increased body fat. J Nutr Sci Vitaminol. 51(2):110-7.
12. Lindsay, R.S., Wake, D.J., Nair, S., Bunt, J.D., Livingstone, KW., Permana, P.A., Antonio, T.P. & Walker, B.R. 2003. Subcutaneous Adipose 1113 Hydroxysteroid Dehydrogenase Type 1 Activity and Messenger Ribonucleic Acid Levels Are Associated with Adiposity and Insulinemia in Pima Indians and Caucasians. The Journal of Clinical Endocrinology & Metabolism. 88 (6): 2738-2744.
13. Low, S.C., Assaad, S.N., Raj an, V., Chapman, K.E., Edwards, C.R. & Seckl, J.R. 1993. Regulation of 11 beta-hydroxysteroid dehydrogenase by sex steroids in vivo: further evidence for the existence of a second dehydrogenase in rat kidney. J Endocrinol. 139 (1):27-35.
14. Moisan, M.P., Seckl, J.R. & Edwards, C.R. 1990. 11Beta-hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: localization in hypothalamus, hippocampus, and cortex. Endocrinology. 127:1450-1455.
15. Poehlman, E.T., Toth, M.J. & Gardner, A.W. 1995. Changes in energy balance and body composition at menopause: a controlled longitudinal study. Ann. Intern. Med. 123(9): 673-5.
16. Qiao, L., Xu, K.H., Liu, H.W. & UU HQ. 2005. Effects of ovariectomy on fracture healing in female rats. J Sichuan University (Medical science edition). Sichuan Da Xue Xue Bao (Yi Xue Ban) 36(1):108-11.Saruhan BG, Ozdemir N. 2005. Effect of ovariectomy and of estrogen treatment on the adrenal gland and body weight in rats. Saudi Med J. 26 (11): 1705-9.
17. Rask, E., Olsson, T., Soderberg, S., Andrew, R., Livingstone, D.E.W., Johnson, O. & Walker, RR. 2001. Tissue-specific Oysregulation Of Cortisol Metabolism In Human Obesity, J Clin Endocrinol Metab. 86:1418-1421.
18. Reubinoff, RE., Wurtman, J. & Rojanski, L. 1995. Effects of hormone Replacement Therapy on Weight, Body Fat Composition, Fat Distribution and Food Intake in Early Postmenopausal Women: A Prospective Study. Fertil. Steril. 64: 963-8.
19. Schleimer, R.P., Spahn, J.D., Covar, R. & Szefler, S.J. 2003. Glucocorticoids. Elsevier health e-books. Ch. 52.

20. Sandeep, T.C., Andrew, R., Homer, N.Z.M. & Walker, B.R. 2003. Effects of the 11beta-Hydroxysteroid Dehydrogenase Inhibitor Carbenoxolone on Insulin Sensitivity in Human Obesity. Endocrine Abstract 5:81.

21. Seko, K., Kagami, H., Senga, K., Ozeki, K., Mizutani H. & Ueda, M. 2005. Effects of ovariectomy and estrogen replacement on rat oral mucosa. Maturitas 50 (1):44-51.

22. Sowers, M.R. & La Pietra, M.T. 1995. Menopause: its epidemiology and potential association with chronic diseases. Epidemiol Rev. 17(2):287-302.

23. Stewart, P.M., Boulton, A., Kumar, S., Clark, P.M.S & Shackleton, C.H.L. 1999. Cortisol metabolism in human obesity: impaired cortisone → cortisol conversion in subjects with central adiposity, J Clin Endocrinol Metab. 84: 1022-1027.

الملخص :

إن استخدام استئصال المبيض كنموذج لبحوث ما بعد سن اليأس في العالم واسعة الانتشار، وذلك لتشابه التعقيدات والمشاكل المترتبة على نقص هرمون الاسروجين بسبب أما استئصال المبيض أو بلوغ سن اليأس الطبيعي. تهدف هذه الدراسة إلى التعرف على تأثير استئصال المبيض على نشاط إنزيم 11β-hydroxysteroid dehydrogenase type I ، وبالتالي على وزن الجسم. إثنان وثلاثون من إناث الجرذان عمرها ٦ أشهر استخدمت في هذه التجربة. قسمت الحيوانات إلى مجموعتين الأولى طبيعية والثانية مستأصلة المبيض مبينة على دراسة قصيرة وأخرى طويلة المدى. تم استئصال المبيضين تحت تأثير المخدر من الناحية السفلى لبطن الحيوان. وتم تسجيل وزن الحيوانات شهريا. في نهاية التجربة تم قتل الحيوانات وأخذ عينات من الكبد وأنسجة الشحم لإخضاعها لفحص النشاط الحيوي، أظهرت النتائج حدوث زيادة حقيقية ($p<0.55$) في وزن الجسم ونشاط إنزيم الكبد. أما في أنسجة الشحم فلم تظهر النتائج فروق حقيقية. تشير هذه النتائج إلى أن استئصال المبايض تساهم في زيادة وزن الجسم ونشاط إنزيم 11β-hydroxysteroid dehydrogenase type I في الكبد مع احتمال ذلك في أنسجة الشحم.