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Original article

Estrogen deprivation induces lipid profile impairment but not cardiac dysfunction in ovariectomized dogs

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Abstract

Studies in human medicine have shown that in addition to affecting the reproductive system, the hormone estrogen also has cardioprotective effects. The present study hypothesized that ovariectomized (OVH) dogs would have a higher incidence of cardiac dysfunction and impairment of lipid profiles compared to intact female dogs. Thirty healthy female dogs were divided into two groups, 15 intact female dogs and 15 OVH dogs. All the dogs underwent a physical examination, including investigation of physical parameters, blood collection for lipid profile measurement, thoracic radiography, electrocardiography and echocardiography. Physical examination parameters, electrocardiographic parameters, heart size and cardiac function in OVH dogs were not different when compared to intact female dogs. However, in the OVH dogs, triglyceride and very-low-density lipoprotein levels were increased, while high-density lipoprotein was significantly decreased compared to the intact female dogs ($P < 0.05$). Differences between the groups in total cholesterol and low-density lipoprotein did not reach statistical significance. We concluded that estrogen deprivation in dogs can induce lipid profile impairment but not cardiac performance impairment 1 year after an ovariectomy.

Key words: dog, estrogen, ovariectomy, cardiac function, lipid profiles

Introduction

Estrogens, the primary female sex hormones, include a group of steroid hormones consisting of estrone (E1), estradiol (E2) and estriol (E3). Estrogens play a vital role in reproduction and secondary female characteristics (Nelson and Bulun 2001). Estrogen acts on estrogen receptors (ERs), which are located on various cell membranes throughout the body including the reproductive system, liver, bone, brain and cardiovascular system, play a role in various functions (Mendelsohn 2002). There are two type of ERs: estrogen receptor type alpha ($ER\alpha$), found in approximately equal numbers in both male and female cardiomyocytes, and estrogen receptor type beta ($ER\beta$) which is more prevalent in cardiomyocytes in males than in females. ERs and estrogen are responsible for the cardioprotective mechanism observed in females (Prabhavathi et al. 2014).

Previous studies have found an increased risk for cardiovascular diseases, coronary heart disease and heart failure (HF) in postmenopausal women (Bui et al. 2011, Lobo et al. 2014, Bhatnagar et al. 2016). Moreover, an increase in total cholesterol, LDL and total cholesterol/high-density lipoprotein (HDL) ratio has been shown to be related to the period of menopause (Zago et al. 2004, Derby et al. 2009). Estrogen plays a role in the synthesis of HDL which is a cardioprotective substance. HDL can also help prevent coronary heart disease by increasing the transport of excess cholesterol from the arterial wall to the liver and inhibiting monocyte adhesion. It also acts as an anti-oxidant in the prevention of oxidation of low-density lipoprotein (LDL) cholesterol which is the cause of coronary heart disease and arteriosclerosis (Kilim et al. 2013). In general, levels of HDL in women are higher than in men. Estrogen also prevents the conversion of angiotensin I into angiotensin II by reducing angiotensin-converting enzyme (ACE) activity in the renin-angiotensin-aldosterone cascade, resulting in a reduction of vasoconstriction and leading to a lowering of blood pressure.

Ovariectomy (OVH) is the surgical removal of the uterine horns, the uterine body and the ovaries which are an important source of estrogen production. This procedure is a commonly used method to control dog populations. A study in an animal model reported potential cardioprotective effects of estrogen. In that study, ovariectomized rodents exhibited left ventricular hypertrophy, ventricular arrhythmia and an increase in the QT range in a prolongation of the QT interval which is associated with arrhythmia (Prabhavathi et al. 2014). This suggests the decrease in estrogen levels in OVH dogs might exhibit a similar effect on the cardiovascular

system in humans as well as in rodents. The effect of estrogen deprivation on lipid profiles and cardiac function, however, has only rarely been explored. This study aimed to investigate the effect of estrogen deprivation on lipid profiles and cardiac function by using ECG and echocardiography in ovariohysterectomized female dogs compared to intact female dogs to test the hypothesis that estrogen-deprived dogs demonstrate greater lipid profile impairment and cardiac dysfunction when compared to intact female dogs.

Materials and Methods

Animal preparation

All procedures involving animals were approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Chiang Mai University (Permit: No. S11/2559). Dog owners were informed about the study protocol, and written consent was obtained from them before starting the study.

Experimental design and subject collection

Thirty dogs aged 2 to 6 years with a body weight less than 20 kg were enrolled. The dogs were divided into two groups using age-matched sampling: intact female dogs ($n=15$) and OVH dogs ($n=15$). The sample size was statistically calculated using the G*Power 3.1.92 program. The OVH dogs had undergone the ovariohysterectomy procedure at least one year prior to the study. Dogs with any systemic diseases, cardiovascular diseases, infectious diseases or heartworm infestation and those which had ever been administered contraceptive drugs were excluded from the study. All the dogs underwent a standard physical examination including blood pressure followed by examination using electrocardiography, thoracic radiography, and echocardiography.

Body condition score (BCS) and non-invasive blood pressure measurement (NIBP)

All the dogs underwent physical examination and their BCS was determined using a 9-point scoring system (Baldwin et al. 2010). Blood pressure was measured using a Doppler BF2 flow meter (Parks Medical Electronics Company, USA). During the procedure, each dog was placed in the recumbent position and a cuff equal to 40% of the width of the front limb circumference was attached. A Doppler probe was placed on the digital artery area of the front paw between the carpal and metacarpal pads. Blood pressure was measured

five times; the first measurement was discarded and the mean of the next four measurements was calculated.

Blood collection and lipid profile measurement

Five milliliter samples of 12 hour fasting blood from both groups were collected by venipuncture from the cephalic or the saphenous vein with a 21-22-gauge needle. Blood samples were placed in heparin tubes and plasma was separated for lipid profile tests including total cholesterol and triglycerides, as well as the levels of high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very-low-density lipoproteins (vLDL). Blood samples were collected between 9.00 and 12.00 a.m. to reduce the risk of factors related to circadian rhythms that might differentially change estrogen levels during the day.

Total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay using a commercially available kit (Giese Diagnostics SNC, Italy). Fasting plasma HDL was determined by sensitive colorimetric and fluorometric assays using a commercially available kit (Giese Diagnostics SNC, Italy). LDL and vLDL levels were calculated using Friedewald's equation (Friedewald et al. 1972).

Thoracic radiography

Right lateral thoracic radiography was performed on all dogs and the vertebral heart scale was determined as described by Buchanan et al. (1995).

Electrocardiography

Electrocardiography was performed using standard electrocardiogram equipment (EDAN® VE-300, China). Dogs were positioned in a right lateral recumbent position without sedation on an electrical insulator cushion. Six limb leads were recorded using alligator clips. The ECG tracings were made on paper at a speed of 25 mm/sec and a sensitivity of 10 mm/mV. The mean electrical axis (MEA) was calculated using the sum of the QRS wave in lead I and the aVF lead (Edwards 1987).

Echocardiography

Echocardiography was performed using standard echocardiographic equipment with 3.8 MHz to 6.0 MHz transducers (ALOKA® SSD-3500SX, USA). All dogs were unsedated and positioned in right lateral recumbency. Standard echocardiographic views were obtained. Parameters measured using the 2D-guided M-mode included left ventricular posterior wall thickness in diastole and systole (LVPWd and LVPWs), left ven-

tricular diameter in diastole and systole (LVIDd and LVIDs), and interventricular septum thickness in diastole and systole (IVSd and IVSs). Left atrial size (LA) and aortic root size (Ao) were obtained in 2D-mode at the left atrial/aortic root level in the right parasternal cross-sectional view. Left atrium/aortic root size ratio (LA/AO), end diastolic volume (EDV), end systolic volume (ESV), and stroke volume (SV) were automatically calculated. The fractional shortening percentage (%FS), and ejection fraction percentage (%EF) were calculated automatically using the Teicholz equation.

Statistical analysis

All data are presented as mean and standard deviation (mean \pm SD). Comparison of the OVH dog group and intact female dog group was done using Student's t-test for two independent populations and R statistical software version 3.6.3 (R Core Team, Austria). Assumptions for the Student's t-test, including normality and homogeneity of variance, were tested using the Shapiro-Wilk test and Levene's test, respectively. P-values < 0.05 were considered statistically significant in all statistical analyses.

Results

The body condition scores (BCS), blood pressure (NIBP), and vertebral heart scale (VHS) did not differ between OVH dogs and intact female dogs

The mean ages of the dogs in the two groups were significantly different ($p < 0.05$), with the OVH dogs older than the intact female dogs (Table 1). However, there were no significant differences in mean body weight, BCS, NIBP or VHS between the OVH and the intact female dogs.

OVH dogs demonstrated lipid profile impairment when compared to intact female dogs

In this study, we found that blood triglyceride (TG) and very-low-density lipoprotein (vLDL) levels in the OVH group were significantly higher when compared to the intact female dogs ($p < 0.05$), whereas the high-density lipoprotein (HDL) level in the OVH group was significantly lower than in the intact female dogs ($p < 0.05$). The total cholesterol per high-density lipoprotein ratio (TC/HDL ratio) was also higher in OVH dogs compared to intact female dogs. However, total cholesterol (TC) and low-density lipoprotein (LDL) were not different between the groups (Table 2).

Table 1. Age, body weight, BCS, blood pressure and VHS of intact female dogs and OVH dogs. All values are presented as mean \pm SD

Parameters	Intact female dogs (n=15)	OVH dogs (n=15)	P-value
Age (years)	3.61 \pm 1.51	4.90 \pm 1.49 *	0.026
Body weight (kg)	7.20 \pm 3.62	6.74 \pm 5.20	0.779
Body condition score (BCS)	3.50 \pm 0.63	3.6 \pm 0.66	0.674
Blood pressure (mmHg)	144.61 \pm 38.14	130.44 \pm 33.01	0.286
Vertebral heart scale (VHS)	10.0 \pm 0.91	9.7 \pm 0.75	0.413

* p<0.05 vs. intact female dogs

Table 2. Lipid profiles of intact female dogs and OVH dogs. All values are presented as mean \pm SD

Lipid	Intact female dogs (n=15)	OVH dogs (n=15)	P-value
TC	190.13 \pm 25.41	197.00 \pm 56.21	0.671
TG	56.4 \pm 23.03	99.20 \pm 70.23*	0.033
LDL	25.67 \pm 18.37	45.79 \pm 32.69	0.075
HDL	150.00 \pm 9.83	132.78 \pm 21.18*	0.027
vLDL	11.28 \pm 4.61	20.53 \pm 14.31*	0.025
TC/HDL ratio	1.19 \pm 0.11	1.31 \pm 0.17 *	0.044

* p<0.05 vs. intact female dogs. TC = total cholesterol, TG = triglyceride, LDL = low-density lipoprotein, HDL = high-density lipoprotein, vLDL = very-low-density lipoprotein, TC/HDL ratio = total cholesterol/ high-density lipoprotein ratio

OVH dogs did not show a difference in the incidence of electrocardiogram abnormalities from Lead II when compared to intact female dogs

In this study, we investigated Lead II component morphology as well as the mean electrical axis. All ECG parameters in both groups were within normal limits. In addition, we found the amplitude of the ECG components (P wave, R wave, and T wave) and the duration of the ECG components (P wave duration, PR interval, QRS duration, and QT interval) were not significantly different between the groups. Moreover, the mean electrical axis in both groups was within normal limits, i.e., 40-100 degrees, and was not different between the intact and OVH groups (Table 3).

OVH dogs did not show a difference in the incidence of cardiac dysfunction or cardiac abnormality when compared to intact female dogs

Echocardiographic study found that all echocardiographic parameters in both intact and OVH dogs were within normal limits. Specifically, the left ventricular cardiac wall thickness during systole and diastole (IVSs, LVPWs, IVSd, LVPWd) and the left ventricular diameter during systole and diastole (LVIDs and LVIDd) were not significantly different between the

groups. In addition, the left atrial size (LA) and the left atrium/aortic root ratio (LA/Ao) in both groups were within normal limits and did not differ between the groups. Furthermore, cardiac function indicated by %FS, %EF, EDV, ESV, and SV was not different in the intact and the OVH female dogs (Table 4).

Discussion

In this study, we investigated the effect of estrogen deprivation on BCS, cardiac function and lipid profiles in estrogen-deprived dogs (OVH group) and compared that to intact female dogs. The two major findings of this study are as follows. First, the estrogen-deprived dogs demonstrated blood lipid profile impairment, indicated by increased plasma triglyceride and vLDL, and decreased plasma HDL when compared to the intact female dogs. Second, cardiac function, cardiac size, and cardiac electrophysiology in the estrogen-deprived dogs were not different when compared to the intact female dogs.

In human medical studies, the estrogen deprivation which occurs after menopause has been linked to an increase in mortality rate from cardiovascular diseases in women compared to men (Rivera et al. 2009). Estrogen deprivation has also been shown to result in more severe myocardial damage and left ventricular dysfunction.

*Estrogen deprivation induces lipid profile impairment ...*Table 3. Electrocardiographic data of intact female dogs and OVH dogs. All values are presented as mean \pm SD

ECG parameters	Intact female dogs (n = 15)	OVH dogs (n = 15)	P-value
P wave (mV)	0.24 \pm 0.09	0.57 \pm 1.23	0.307
P wave duration (sec)	0.04 \pm 0.00	0.04 \pm 0.01	0.667
PR interval (sec)	0.09 \pm 0.02	0.09 \pm 0.02	0.067
R wave (mV)	1.23 \pm 0.61	1.49 \pm 0.60	0.243
QRS duration (sec)	0.04 \pm 0.01	0.04 \pm 0.01	0.559
T wave (mV)	0.20 \pm 0.08	0.21 \pm 0.06	0.693
QT interval (sec)	0.17 \pm 0.02	0.17 \pm 0.02	0.699
Mean electrical axis (degrees)	68.40 \pm 14.34	71.93 \pm 11.46	0.462

* p<0.05 vs. intact female dogs

Table 4. Echocardiographic parameters of intact female dogs and OVH dogs. All values are presented as mean \pm SD

Parameters	Intact female dogs (n=15)	OVH dogs (n=15)	P-value
IVSd (cm)	0.61 \pm 0.20	0.70 \pm 0.12	0.152
LVIDd (cm)	2.37 \pm 0.41	2.148 \pm 0.49	0.191
LVPWd (cm)	0.65 \pm 0.16	0.67 \pm 0.19	0.852
IVSs (cm)	0.87 \pm 0.17	0.97 \pm 0.19	0.147
LVIDs (cm)	1.40 \pm 0.31	1.26 \pm 0.47	0.338
LVPWs (cm)	1.00 \pm 0.26	1.01 \pm 0.17	0.967
EDV (ml)	21.22 \pm 8.59	16.97 \pm 9.63	0.212
ESV (ml)	5.60 \pm 2.90	4.88 \pm 5.12	0.639
SV (ml)	15.78 \pm 6.18	12.57 \pm 5.82	0.154
EF (%)	74.68 \pm 7.86	75.61 \pm 11.99	0.805
FS (%)	43.22 \pm 6.00	42.65 \pm 9.09	0.847
LA/Ao	1.23 \pm 0.16	1.21 \pm 0.11	0.714
LA (cm)	1.69 \pm 0.38	1.70 \pm 0.36	0.876
Ao (cm)	1.39 \pm 0.29	1.44 \pm 0.23	0.578

* p<0.05 vs. intact female dogs. IVSd = interventricular septal end diastole, LVIDd = left ventricular internal diameter end diastole, LVPWd = left ventricular posterior wall end diastole, IVSs = interventricular septal end systole, LVIDs = left ventricular internal diameter end systole, LVPWs = left ventricular posterior wall end systole, EDV = end-diastolic volume, ESV = end-systolic volume, SV = stroke volume, %EF = % ejection fraction, %FS = % fractional shortening, LADs = left atrial diameter, Ao = aortic root diameter, LA/Ao = ratio of left atrial diameter to aortic root diameter

tion after myocardial ischemia and reperfusion (I/R) injuries and in cases of impaired mitochondrial function (Zhai et al. 2000, Sivasinprasasn et al. 2017, Sivasinprasasn et al. 2019). Interestingly, estrogen supplements can reduce the risk of cardiovascular diseases and exert a cardioprotective effect on the heart (Rivera et al. 2009).

In this study, both groups of dogs were of adult age. Their physical parameters, including blood pressure, body weight, BCS, VHS and cardiac function, were not statistically different between the groups. This lack of differentiation could be due to the duration of estrogen

depletion not having been long enough to show the full effect. Although coronary heart disease is commonly observed in humans (Muka et al. 2016), in small animal veterinary medicine coronary heart disease is rarely reported, which may indicate that cardiac function after estrogen depletion is more likely to remain normal in dogs than in humans. Alterations of lipid profiles in humans during the menopause transition have been linked to cardiovascular risk factors (Udo et al. 2014). These same alterations were also found in the estrogen-deprived dogs in this study. The body condition scores of the dogs, however, were not affected, a result which

is inconsistent with results of research in a rodent model which reported that OVH rats had increased body weight compared to intact female rats (Sivasinprasasn et al. 2017). In that rat study, animals receiving estrogen replacement after OVH had significantly reduced body weight compared to those without estrogen replacement. Other studies have demonstrated that the mouse brain has Leptin receptors and the SH2-tyrosine phosphatase Shp2, which are involved in estrogen signaling for proper appetite control and energy use (Kararigas et al. 2014). Those receptors could potentially also be involved in the reduction of body weight.

In addition, our results demonstrated that in OVH dogs TG and vLDL levels were significantly increased, whereas HDL levels were significantly decreased when compared to intact female dogs ($p < 0.05$). Moreover, the total cholesterol per high-density lipoprotein ratio (TC/HDL ratio) was higher in the OVH dogs compared to the intact female dogs. A previous study reported that changes in lipid profiles and other cardiovascular risk factors were associated with the onset of menopause. Additionally, total cholesterol (TC), low-density lipoprotein cholesterol (LDL), apolipoprotein A1 and apolipoprotein B triglycerides were significantly higher in post-menopausal women than premenopausal women. This relates to the significantly higher systolic blood pressure and fibrinogen levels in post-menopausal women, an indication of increased cardiovascular risks (Bonithon-Kopp et al. 1990). Estrogen deprivation has been shown to be associated with an atherogenic lipid profile increases in TC, TG, VLDL, LDL levels and decreases in HDL level which can be a cause of atherosclerosis and coronary heart disease. In contrast, HDL leads to inhibition of adhesion of monocytes and also acts as an antioxidant which helps prevent peroxidation of LDL (Kilim et al. 2013). These results are in line with our study which suggests that estrogen deprivation is associated with lipid profile impairment in neutered female dogs. Moreover, previous studies in dogs have demonstrated that an age of more than 7 years can induce lipid metabolism disorder through increased plasma cholesterol and triglyceride (Kawasumi et al. 2014, Usui et al. 2014). In this study, although the OVH dogs were on average older than the intact female dogs (4.9 years vs. 3.6 years, respectively), both groups were between 2 and 6 years which is defined as a young dog (Kawasumi et al. 2014). The present study suggests that estrogen deprivation might enhance lipid metabolism disorder in young dogs as well. Further clinical studies with a larger sample of dogs of the same age need to be conducted.

Previous studies in dogs demonstrated that hypercholesterolemia and lipidemia are related to arteriosclerosis (Boynosky et al. 2014), and that they can induce

myocardial infarction in dogs (Detweiler et al. 1961, Lui et al. 1986, Falk et al. 2000, Boynosky et al. 2014). In addition, a previous study of sixty-five dogs demonstrated that 78% of the dogs had a myocardial infarction and suggested that arteriosclerosis in dogs may be an important cause of sudden death and of death during general anesthesia (Falk et al. 2000). Although in this study cardiac function did not change one year after an ovariohysterectomy, OVH dogs did exhibit lipid profile impairment which might indicate a potential increase in the severity of impairment in the future. This suggests that the lipid profiles as well as cardiac function of OVH dogs should be monitored annually. Additionally, cardiac function and blood profiles in OVH dogs should be monitored.

In regard to electrocardiographic and echocardiographic parameters, this study found that lead II ECG parameters in both intact and neutered female dogs were within normal limits and that there was no difference between the groups. This is consistent with a previous study of ovariectomy in female dogs which reported that QT interval, QTc interval, and QT dispersion were not changed one month after an ovariectomy and that the operation did not affect left ventricular repolarization (Fülöp et al. 2006). On the other hand, results of the present study are not consistent with a study of ovariohysterectomy in female rats which reported that female rats with estrogen deprivation had a prolongation of their QT interval (Prabhavathi et al. 2014) as well as with another study which reported an increase in the PQ interval in ovariectomized dogs (Fülöp et al. 2006). The present study also found that an ovariohysterectomy in female dogs did not induce cardiac dysfunction one year after the operation; however, that result is not consistent with previous rat model studies which have reported that the %FS and %EF values of sterilized female rats are slightly lower than those of intact female rats (Sivasinprasasn et al. 2016). These discrepancies might be related to the species as well as to the period of time after the OVH. The present study suggests that an ovariohysterectomy and the associated estrogen deprivation does not affect cardiac electrophysiology or cardiac function in neutered female dogs one year after the operation. Further study should be conducted using a larger sample and with the same individual dogs before and after OVH to reduce the potential influence of other factors, e.g., the dog owner and the environment.

Conclusions

Dogs which have received an ovariohysterectomy procedure have harmful increased levels of lipid pro-

files, including TG and vLDL, and reduced HDL levels, compared to dogs that have not received OVH; however, OVH does not affect cardiac function or cardiac electrophysiology.

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