PRELIMINARY REPORT

Lipoproteins During the Estrous Cycle in Swine

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The purpose of this study was to examine lipoprotein values at high versus low 17β-estradiol (E2) concentrations in Yucatan miniature swine. Estrous cycles were measured by heat checking the female on a daily basis using a boar. All swine were fed a 1,050-g low-fat, standard chow diet (8% kcal from fat) once per day. Fasted (24 hours) blood samples were collected during low (early luteal, day 5) and high (late follicular, day 18) E2 concentrations to determine differences in concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL-C), high-density lipoprotein cholesterol (HDL-C), and subfractions. Concentrations of E2 differed significantly from day 5 (3.5 ± 0.7 pg/mL) to day 18 (14.2 ± 1.8 pg/mL) of the estrous cycle. Except for HDL_3-C, all lipoprotein parameters examined were significantly elevated during high E2 versus low E2. TC/HDL-C and LDL-C/HDL-C ratios were significantly lower during the high E2 phase. These results suggest that lipoprotein concentrations fluctuate during the estrous cycle of swine, with high E2 concentrations associated with elevated lipoprotein concentrations.

THE DEVELOPMENT of coronary heart disease appears to be reduced in the presence of female sex hormones. The similarities between swine and human physiology make miniature swine an attractive model for the study of cardiovascular disease and diabetes.[1-2] While data exist on lipid changes during elevated 17β-estradiol (E2) in the female menstrual cycle,[3-5] little information is available regarding the lipid fluctuations during the swine estrous cycle. It is important to characterize the lipid response during the estrous cycle in swine in order to further elucidate the appropriateness of the swine as a model in human diseases, especially those relevant to females. If found, these fluctuations must be accounted for in research designs using the swine model. Thus, the purpose of this study was to determine lipoprotein concentrations at high and low E2 concentrations in miniature swine.

MATERIALS AND METHODS

Ten female Yucatan miniature swine (Sinclair Research Center, Columbia, MO) (0.5 years and 33.0 ± 1.2 kg) had estrous cycles measured by heat checking with boar exposure as previously described by Bearden and Fuquay[6] (ovulation = day 0), a procedure previously used by our group.[1]

The pigs were housed in stainless steel cages, quartered in pairs, and separately fed approximately 1,050 g/d (~3,000 kcal) of low-fat pig chow (PMI Nutrition International, Brentwood, MO), containing 22% of kcal from protein, 8% from fat, and 70% from carbohydrate.

Blood samples were collected via vascular access port at every day 5 (early luteal, low E2) and day 18 (late follicular, high E2) for each consecutive cycle between 8 and 9 AM after a 24-hour fast. Plasma total cholesterol (TC) and triglyceride (TG) concentrations were analyzed enzymatically using diagnostic kits (Sigma, St Louis, MO). Plasma concentrations of total high-density lipoprotein cholesterol (HDL-C), HDL_2-C, and HDL_3-C were analyzed using a modified heparin-$MnCl_2$–dextran sulfate method.[7] Plasma concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation.[8] In our hands, estimated LDL-C calculated from the Freidewald equation and measured LDL-C separated by ultracentrifugation were highly correlated at $r = .94$ (n = 62 swine) (unpublished observations). Plasma concentrations of E2 were analyzed by radioimmunoassay with an antibody specific for 17β-estradiol.[1]

Average concentrations of E2 for 2 consecutive cycles and lipoproteins for 3 consecutive cycles were used in statistical analyses. Paired *t* tests were performed using Sigma Statistics version 2.0 (SPSS Inc, Chicago, IL). Significance was set at $P < .05$. Values are reported in means ± SE.

RESULTS AND DISCUSSION

The lipoprotein profile did not differ for each phase across three separate cycles (data not shown). E2 concentrations differed significantly from early luteal (day 5) to late follicular (day 18) in the cycle (3.5 ± 0.7 pg/mL *v* 14.2 ± 1.8, respectively). All of the lipoprotein parameters, except HDL_3-C, were significantly elevated during high E2 versus low E2 (Fig 1). Two thirds of the elevation in TC was accounted for by increases in HDL-C, resulting in lower TC/HDL-C and LDL-C/HDL-C ratios during the high E2 measurement (Fig 1).

In addition to increased HDL-C concentrations, LDL-C concentrations also were significantly elevated during the high E2 phase. Elevated HDL-C during high E2 agrees with the majority of human studies.[4,5] However, results on TC and LDL-C in human studies are more equivocal, with these lipids increased,[5] decreased,[3] or unchanged[4] during phases with high E2.

In contrast to our results, data from estrogen therapy studies indicate that TC and LDL-C are consistently decreased with estrogen intake.[9] However, the plasma concentrations and

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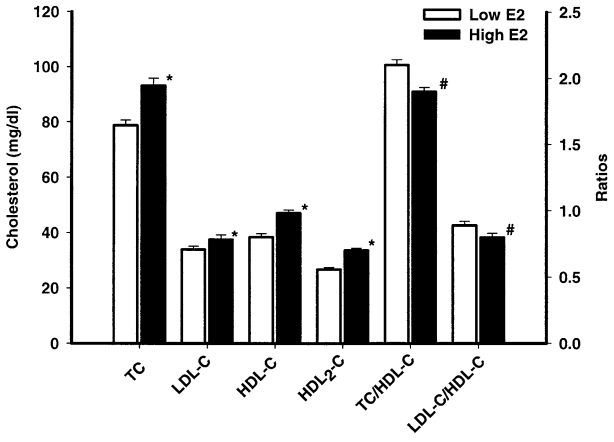

Fig 1. Lipoprotein concentrations and ratios during low E2 and high E2 phases of the estrous cycle. Values are means ± SE. *Significantly elevated during high E2 phase, $P < .05$. #Significantly lower during high E2 phase, $P < .05$.

chemical structure of synthetic estrogen used in estrogen therapy studies differ from endogenous estrogen, which makes these results difficult to compare to the present data.[9] It also is likely that lipoproteins are affected by fluctuations in other hormones, such as progesterone,[4,5] and these variables were not measured in the present study.

The relatively large increase in HDL-C during high E2 suggests a cardioprotective effect during the late follicular phase of the cycle.[10] However, the significant increase in LDL-C may counter this protection. In humans, small reductions in the TC/HDL-C ratio are associated with a substantial decrease in the risk for coronary heart disease, especially at lower ratios.[10] However, it is unknown if the modest decrease in the TC/HDL-C ratio in the present study would significantly lower the risk in these swine.

The results of this study are important because they further describe the physiological characteristics of a widely used swine model. In conclusion, these results suggest that lipoprotein concentrations fluctuate during the estrous cycle of swine, with high E2 concentrations associated with elevated lipoprotein concentrations, and these fluctuations should be considered in designing research protocols using the miniature swine model.

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