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Translational

The Effects of Estrogen on Serum Level and Hepatocyte Expression of PCSK9

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ABSTRACT

Objective. We previously reported that serum PCSK9 levels are higher in postmenopausal women than in premenopausal women in a Han Chinese population. Whether this difference is related to estrogen has not been well-characterized. This study aims to examine if the alteration in estrogen level is responsible for the changes of serum PCSK9 concentration.

Materials/Methods. A sandwich ELISA assay was used to measure serum PCSK9 levels in 727 healthy women aged from 26 to 85 years old. Anthropometric and biochemical examination of parameters such as estrogen and serum lipids was also performed for these individuals. Next, we measured serum PCSK9 and estrogen levels of 30 healthy fertile women (24–26 years old) in their menstrual cycles and analyzed the correlation between serum PCSK9 level and estrogen concentration. Moreover, cell culture studies were carried out to examine if estrogen at physiological and non-physiological concentrations regulates hepatocyte PCSK9 expression.

Results. Serum PCSK9 concentrations were significantly increased with aging. Aged group had higher serum PCSK9 levels than the middle aged group and the young group (60.29 ± 28.47 vs 71.38 ± 34.22 vs 83.81 ± 33.50 ng/ml). Serum PCSK9 levels were positively correlated with age, BMI, serum total cholesterol and LDL-cholesterol ($P < 0.01$), but not correlated with estrogen levels. There was no significantly difference of PCSK9 levels between the lower and the upper estradiol (E2) tertiles in the 727 women. There was either no significant difference in PCSK9 levels during the menstrual, ovulatory, luteal phases in the 30 healthy fertile women. Cell culture studies showed that 17β-estradiol at physiological concentrations did not significantly alter PCSK9 expression in human hepatocytes.

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; WHR, waist circumference/hip circumference; E2, estradiol.

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Conclusion. The serum PCSK9 levels were higher in postmenopausal women than those in pre-menopausal women. However, the difference in serum PCSK9 levels between postmenopausal and premenopausal woman appeared to be independent of estrogen status, and estrogen at physiological concentrations does not affect human hepatocyte PCSK9 expression.

1. Introduction

Elevated plasma LDL-cholesterol (LDL-C) level is a major risk factor for cardiovascular diseases [1]. LDL-C increases with age, and postmenopausal women have higher LDL-C than premenopausal women or men of the same age [2], conferring increased risk of cardiovascular diseases. Estrogen has beneficial effects on glucose homeostasis and lipid metabolism in liver and peripheral tissues. Estrogen treatment reduces LDL-C, apolipoprotein B, and lipoprotein (a) levels, while increasing HDL-C and ApoAI levels [3]. As several studies indicated, high dose estrogen treatment is the most potent way to induce hepatic gene expression of LDL receptors in rats [4] and men [5].

Proprotein convertase subtilisin/kexin type 9 (PCSK9), a member of the proprotein convertase family of zymogens, plays a key role in cholesterol homeostasis and atherosclerosis [6]. PCSK9 mediates degradation of LDLR on the surface of hepatocytes and thus decreases the uptake of LDL by the liver, leading to increased plasma LDL-C levels [7]. Gain-of-function mutations in PCSK9 cause a rare autosomal dominant form of severe hypercholesterolemia and lead to premature coronary heart disease [8], whereas loss of function mutations is more common and is associated with reduced plasma levels of LDL-C and the risk of coronary heart disease [9].

Large population based studies carried out by ours [10] and others [11] showed that PCSK9 levels were significantly lower in premenopausal women than in postmenopausal women. Whether this difference was related to estrogen remains unknown. In an attempt to investigate the effects of endogenous estrogen levels on serum PCSK9 concentrations and cholesterol metabolism, estrogen and PCSK9 levels were measured in 727 healthy Chinese women aged from 26 to 85 years old and the relationship between them was examined. In order to further test if physiological estrogen variation has effects on PCSK9 during menstrual cycle, we also measured serum PCSK9 and estrogen levels in 30 healthy fertile women (24.85 ± 0.83 years old) in their menstrual, ovulatory and luteal phase respectively. Lastly, we carried out cell culture studies to test if 17β-estradiol at physiological concentrations affects hepatocyte PCSK9 expression.

2. Methods

2.1. Study Subjects

A total of 727 females were randomly selected from PPID Nanjing Study (Study of Prediction, Prevention and Intervention for type 2 diabetes in Nanjing) in China. In this study, the premenopausal group (26–40 years old of age) contained only women with regular menstrual cycles within the 12 months preceding the study. The postmenopausal group (61–85 years old of age) included women with an intact uterus and ovaries, who had amenorrhea for at least 12 months before the study entry. Exclusion criteria for all participants were: type 2 diabetes, hyperthyroidism, liver, kidney and other diseases associated with lipid metabolism disorders, uterine fibroids, endometriosis, ovarian cancer and other diseases related to the disorder of the hormone levels, statins as lipid-lowering drugs and hormone therapy history. To measure the alterations of serum PCSK9 and estrogen levels during menstrual cycle, 30 healthy fertile women aged from 24 to 26 years old were enrolled.

The study protocol was approved by the Human Research Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (no. 2011-SR-128). Written informed consent was obtained from all subjects.

2.2. Blood Sample Collection and Storage

Venous blood (3 ml) was obtained from each of the 727 participants after being fasted for 12 h. Venous blood (3 ml) was collected in the menstrual period (on the 3rd day of the menstrual cycle), ovulatory period (on the 14th day before the next menstrual cycle) and luteal period (on the 7th day before the next menstrual cycle) from 30 healthy fertile women who were fasted overnight. Blood samples were centrifuged at 3000 rpm for 15 min at 4 °C, and serum samples were separated and stored at −80 °C.

2.3. Physical Examination and Biochemical Tests

Body weight, height, waist circumference, and hip circumference were measured in accordance with the international standards. Fasting blood glucose, total serum cholesterol, triglyceride, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations were measured by enzymatic methods (Chemistry Analyzer Au2700, Olympus Medical Engineering Company, Japan).

2.4. Serum Concentrations of Estradiol (E2) Determination

Serum concentrations of estradiol (E2) were measured by a radioimmunoassay with commercially available kits (Bnibt, Beijing, China) following the manufacturer's instructions.

2.5. PCSK9 ELISA

PCSK9 was measured by a sandwich ELISA assay as previously described [10].