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Sex differences in atherosclerosis in mice with elevated phospholipid transfer protein activity are related to decreased plasma high density lipoproteins and not to increased production of triglycerides

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Abstract

Plasma phospholipid transfer protein (PLTP) has atherogenic properties in genetically modified mice. PLTP stimulates hepatic triglyceride secretion and reduces plasma levels of high density lipoproteins (HDL). The present study was performed to relate the increased atherosclerosis in PLTP transgenic mice to one of these atherogenic effects. A humanized mouse model was used which had decreased LDL receptor expression and was transgenic for human cholesterylester transfer protein (CETP) in order to obtain a better resemblance to the plasma lipoprotein profile present in humans. It is well known that female mice are more susceptible to atherosclerosis than male mice. Therefore, we compared male and female mice expressing human PLTP. The animals were fed an atherogenic diet and the effects on plasma lipids and lipoproteins, triglyceride secretion and the development of atherosclerosis were measured. The development of atherosclerosis was sex-dependent. This effect was stronger in PLTP transgenic mice, while PLTP activity levels were virtually identical. Also, the rates of hepatic secretion of triglycerides were similar. In contrast, plasma levels of HDL were about 2-fold lower in female mice than in male mice after feeding an atherogenic diet. We conclude that increased atherosclerosis caused by overexpression of PLTP is related to a decrease in HDL, rather than to elevated hepatic secretion of triglycerides.

Keywords: Atherosclerosis; Lipid metabolism; Lipoprotein; Transgenic animal model

Atherosclerosis is the leading cause of death and morbidity in industrialized countries [1–3]. It is generally accepted that an elevated level of plasma cholesterol is an important risk factor for atherosclerosis. While the level of cholesterol in low density lipoproteins (LDL) correlates positively with the incidence of atherosclerotic disease, cholesterol in high density lipoproteins (HDL) appears to protect against the development of atherosclerosis [1,2,4]. HDL is thought to have several anti-atherogenic properties. First, HDL is involved in the reverse cholesterol transport pathway, by which excess cholesterol is transported back to the liver for excretion [5–7]. In addition, HDL has both anti-inflammatory and anti-oxidant properties [4,8–10]. One of the proteins involved in HDL metabolism is plasma phospholipid transfer protein (PLTP) [11]. We previously demonstrated that transgenic mice overexpressing human PLTP to various activity levels are more prone to the development of diet-induced atherosclerosis in a PLTP-dose dependent way [12]. Concomitantly, the plasma level of HDL was decreased. We tentatively concluded that the increase in atherosclerosis is probably caused by this decrease in plasma HDL. However,

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in addition to an effect on HDL, PLTP has an effect on the production of very low density lipoproteins (VLDL), which was first described in PLTP deficient mice [13]. The decreased atherosclerosis found in these mice was explained by decreased synthesis of apoB containing lipoproteins by the liver. We examined whether elevated PLTP activity levels would affect hepatic VLDL secretion. Indeed, PLTP over-expressing mice were found to have an increase in the synthesis of apoB containing lipoproteins [12,14], which could be an alternative explanation for the increased susceptibility to atherosclerosis development found in these mice. Thus, elevated PLTP has two atherogenic effects on lipoprotein metabolism, i.e., increasing hepatic VLDL secretion and decreasing plasma levels of HDL. In order to find out which of these mechanisms is the most important one for the development of atherosclerosis, we first determined whether male and female mice with similar levels of overexpression of PLTP differ in their susceptibility to atherosclerosis. Sex differences have been found in mouse models, including the commonly used low density lipoprotein receptor (LDLR) deficient mice and apolipoprotein (apo) E deficient mice [15–17]. In female mice, the surface area of atherosclerotic lesions is larger than in male mice. The reason for this difference is unclear. The present data show that the susceptibility to diet-induced atherosclerosis is also sex-dependent in PLTP overexpressing mice, enabling us to relate these differences with the effects on apoB containing lipoproteins versus the effects on HDL. In the present studies, we used humanized transgenic mouse models which have decreased levels of LDL receptors and express human cholesteryl ester transfer protein (CETP) [14,18]. CETP is normally not expressed in mice, but is involved in the same pathways of lipoprotein metabolism as PLTP and may be crucially involved in the process of atherosclerosis in man [19,20]. Moreover, the plasma lipoprotein profile of these mice have a closer resemblance to the profile found in humans than that of normal mice.

1. Methods

1.1. Animals

The human PLTP transgenic mice (huPLTPtg) were described before (line P1; [12,21]). LDL receptor knockout mice were purchased from Jackson Laboratory. Human CETP transgenic mice (huCETPtg) were kindly provided by Dr. A.R. Tall (Columbia University, New York). All mice were in C57BL/6J background for at least 8 generations. LDLR$^{+/-}$/huCETPtg and LDLR$^{+/-}$/huCETPtg/huPLTPtg mice were created by crossbreeding. After weening, animals were kept on a chow diet (Hope Farms, The Netherlands). For the induction of atherosclerosis, mice were fed a high fat high cholesterol (HFHC) diet for 14 weeks, which contained 40% w/w sucrose 15% w/w fat, 1% w/w cholesterol and 0.5% w/w sodium cholate (Hope Farms, The Netherlands). Mice were 12 weeks old at the beginning of the diet studies. Animals were housed under standard conditions and had free access to water and food. After fasting overnight, blood samples were collected from the orbital plexus by using Vitrex™ sodium-heparinized micropipettes (80 IU) (Modulohm A/S, Copenhagen, Denmark) and immediately stored on ice. Blood was centrifuged at 2700 rpm for 15 min at 4 °C. Plasma was either used directly or stored in small aliquots at −80 °C before analysis. All experiments were performed according to national and institutional guidelines. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

1.2. Separation of plasma lipoproteins by density gradient centrifugation

Lipoprotein fractions (HDL and non-HDL) used in Table 1 were collected following ultracentrifugation at density 1.063 g/mL in a Beckman 42.2 Ti rotor (42,000 rpm, 3 h, 12 °C). The non-HDL fraction includes VLDL, IDL and LDL.

1.3. Quantification of cholesterol

Total plasma cholesterol (TC) was determined enzymatically with the Free Cholesterol C kit no. 274-47109 (WAKO, Neuss, Germany) after hydrolysis of cholesteryl esters with cholesterol esterase from *Candida cylindracea* (Boehringer, Mannheim, Germany).

1.4. Plasma activity assays

CETP and PLTP activity assays were performed as described before [18]. The activities are expressed as arbitrary units (AU); 1 AU is the activity found in human reference pool plasma. The activities are: CETP 216 nmol/mL/h; PLTP 14 μmol/mL/h.

1.5. In vivo hepatic VLDL secretion

VLDL secretion experiments were performed using the Triton WR1339 method, as described [14].

1.6. Histological assessment of atherosclerosis

After 14 weeks of HFHC diet feeding, mice were sacrificed after blood collection as described in the animal section. The hearts were dissected, stored in phosphate buffered 4% formaldehyde until processed. Atherosclerotic areas in the aortic valves were quantified by computer assisted measurements as described [12,22]. To evaluate whether PLTP has an effect on accumulation of free cholesterol in atherosclerotic lesions, the presence of cholesterol clefts in the sections was evaluated as described [23].

1.7. Statistical analysis

Data are expressed as means±SEM. Differences between two groups of mice were analyzed by two sample Wilcoxon rank-sum tests by using Intercooled Stata 6.0 software (Stata corporation, College Station, TX, USA).

Table 1
Plasma levels of lipoprotein cholesterol on chow and HFHC diet

Genotype	LDLR$^{+/-}$/huCETPtg		LDLR$^{+/-}$/huCETPtg/huPLTPtg	
Sex	Male	Female	Male	Female
Non-HDL-C, chow	0.8±0.1	1.1±0.1[a]	0.6±0.1[b]	0.8±0.1[a, b]
HDL-C, chow	1.7±0.1	1.8±0.1	1.1±0.1[b]	1.0±0.1[b]
Non-HDL-C, HFHC	9.9±1.3	8.2±0.9	7.3±0.8[c]	6.9±0.7
HDL-C, HFHC	1.4±0.1	0.6±0.1[a]	0.6±0.1[b]	0.3±0.03[a, b]

Cholesterol (C) concentrations are in mmol/L.
n=14–20 per group.
[a] $P<0.001$, male versus female of same genotype.
[b] $P<0.001$, LDLR$^{+/-}$/huCETPtg versus LDLR$^{+/-}$/huCETPtg/huPLTPtg of same sex.
[c] $P=0.002$, LDLR$^{+/-}$/huCETPtg versus LDLR$^{+/-}$/huCETPtg/huPLTPtg of same sex.