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Increased Risk of Atherosclerosis by Elevated Plasma Levels of Phospholipid Transfer Protein*

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Plasma phospholipid transfer protein (PLTP) is thought to be involved in the remodeling of high density lipoproteins (HDL), which are atheroprotective. It is also involved in the metabolism of very low density lipoproteins (VLDL). Hence, PLTP is thought to be an important factor in lipoprotein metabolism and the development of atherosclerosis. We have overexpressed PLTP in mice heterozygous for the low density lipoprotein (LDL) receptor, a model for atherosclerosis. We show that increased PLTP activity results in a dose-dependent decrease in HDL, and a moderate stimulation of VLDL secretion (≤1.5-fold). The mice were given a high fat, high cholesterol diet, which resulted in hypercholesterolemia in all animals. HDL concentrations were dramatically reduced in PLTP-overexpressing animals when compared with LDL receptor controls, whereas VLDL + LDL cholesterol levels were identical. Susceptibility to atherosclerosis was increased in a PLTP dose-responsive manner. We conclude that PLTP increases susceptibility to atherosclerosis by lowering HDL concentrations, and therefore we suggest that an increase in PLTP is a novel, long term risk factor for atherosclerosis in humans.

High density lipoproteins (HDL)[1] are believed to be protective against the development of atherosclerosis because they mediate reverse cholesterol transport, *i.e.* the transfer of cholesterol from peripheral tissues to the liver (1, 2). However, our understanding of the molecular details and key regulatory proteins involved is incomplete (3–5). One effector of this process is the ATP-binding cassette ABCA1, which is functionally deficient in Tangier disease (reviewed in Ref. 6). The high incidence of coronary artery disease in Tangier patients suggests an essential, protective role of HDL-mediated reverse cholesterol transport in the development of atherosclerosis (7). However, recent results suggest that the contribution of ABCA1 to overall reverse cholesterol transport and its role in atherogenesis are primarily related to its function in macrophages (8–10). Therefore, other potential key proteins in reverse cholesterol transport are of interest, including PLTP (11–13).

Previously, we generated transgenic mice overexpressing human PLTP and showed that plasma from these animals is more effective in preventing accumulation of cholesterol by cultured macrophages (14). We and others therefore suggested an anti-atherogenic effect for PLTP (14–16). On the other hand, overexpression of PLTP results in a decrease of plasma HDL levels (14, 17, 18), which could be an atherogenic effect. Thus, it is unclear whether the net effect of high PLTP activity levels would be atherogenic or anti-atherogenic. The complete absence of PLTP activity in PLTP knockout mice inhibits HDL maturation, which results in reduced levels of plasma HDL caused by accelerated decay (19, 20). Still, PLTP knockout mice showed decreased atherosclerosis (21). This could be partly explained by the discovery of a novel, intracellular function of PLTP in hepatocytes; it was found that PLTP deficiency caused a decrease in apolipoprotein (apo) B-containing lipoproteins in most but not all mouse models used (21). From these results, it was concluded that PLTP is involved in VLDL secretion (21).

To clearly establish the relationship between variability in PLTP activity and the development of atherosclerosis, we created a series of transgenic mice with various levels of PLTP activities. The susceptibility to diet-induced atherosclerosis was studied in these mice and related to changes in plasma lipoproteins.

EXPERIMENTAL PROCEDURES

Transgene Design and Development—The isolation of a cosmid clone with the complete human PLTP gene including its natural flanking sequences was described previously (14). A DNA construct for the generation of mice with liver-specific expression of PLTP was generated from this cosmid using mouse albumin promoter and enhancer sequences (equivalent to construct NB (22), kindly donated by Dr. R. D. Palmiter, Seattle, WA) and α-fetoprotein enhancer sequences (kindly donated by Dr. S. M. Tilghman, Princeton, NJ). The PLTP sequences upstream from exon 1 were removed, and a 2.3-kb *Not*I-*Bam*HI fragment, including the albumin promoter and enhancer sequences, was cloned into the *Bam*HI site in exon 1 of the PLTP gene. A 6.5-kb genomic fragment containing the three α-fetoprotein enhancer elements (shown to interact with the albumin promoter to result in high liver expression) (23) was cloned into the *Eco*RV site 1.5 kb downstream of the last exon of the PLTP gene (exon 16, containing the STOP codon). The cloning strategy necessitated the replacement of the genomic sequence between the *Bam*HI sites in exons 5 and 8 by the equivalent cDNA sequence (264 bp).

Mice—Transgenic mice were obtained by microinjection of fertilized oocytes as described before (14). The P1 transgenic line was originally described in Ref. 14. All of the mice used in this study were >96% C57BL/6J (N5) male mice. LDLR$^{-/-}$ mice in C57BL/6J were purchased

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[1] The abbreviations used are: HDL, high density lipoprotein; PLTP, phospholipid transfer protein; apo, apolipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; LDLR, LDL receptor.

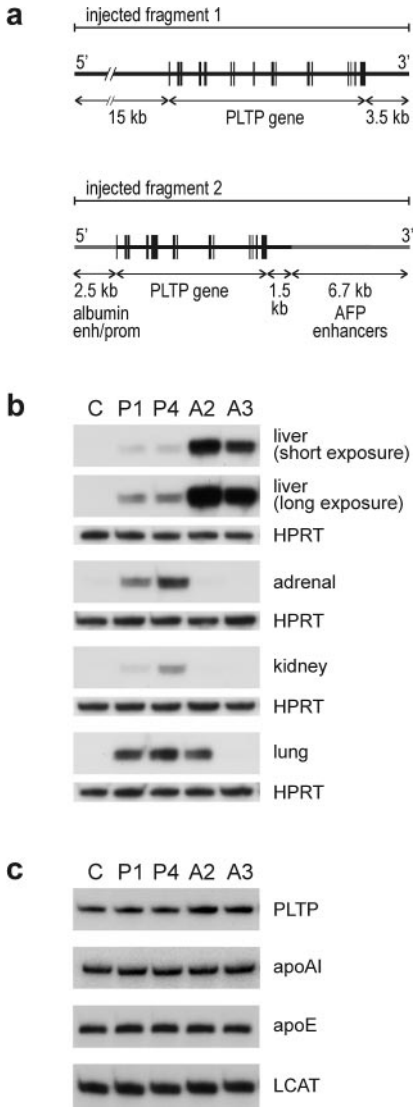

FIG. 1. **Generation of PLTP transgenic mice.** *a*, lines P1 and P4 have been generated with fragment 1, comprising the unmodified human PLTP gene, 15 kb 5′ and 3.5 kb 3′ of the natural flanking regions. Lines A2 and A3 have been generated with fragment 2, in which the mouse albumin promoter and enhancer is located 5′ to the PLTP gene, and α-fetoprotein enhancer sequences have been added at the 3′ end of the construct. *b*, mRNA analyses of the human PLTP transgene. *Lane C*, wild type littermates. *c*, mRNA expression of various mouse genes in liver. *Lane C*, wild type littermates.

from Jackson Laboratory. Controls were littermates. The mice were 12 weeks old at the beginning of the diet studies.

The mice were fed either a chow diet or an atherogenic high fat, high cholesterol diet containing 40% (w/w) sucrose, 15% (w/w) cocoa butter, 1% (w/w) cholesterol, and 0.5% (w/w) cholate (diet N; Hope Farms, Woerden, The Netherlands) for 12 weeks, preceeded by 2 weeks of diet containing 50% (w/w) sucrose (diet J; Hope Farms). Plasma samples were collected by orbital bleedings after fasting the animals overnight. All of the procedures in this study were in accordance with national and institutional guidelines.

Gene Expression Analysis by Reverse Transcription-PCR—For RNA isolation studies, the mice were killed by cervical dislocation, and the organs were quickly removed and frozen in liquid nitrogen. The tissue was either stored at −80 °C or processed immediately. Total RNA was isolated using the Ultraspec RNA isolation reagent from Biotecx Laboratories Inc. (Houston, TX) and used as a template for reverse transcription primed by oligo(dT). RNA expression was analyzed by reverse transcription-PCR (14). PCR products were run on polyacrylamide gels and visualized by autoradiography on Kodak BioMax MR-1 films or by using a PhosphorImager (Amersham Biosciences). Details on the primer sets used, and PCR conditions are available upon request via E-mail.

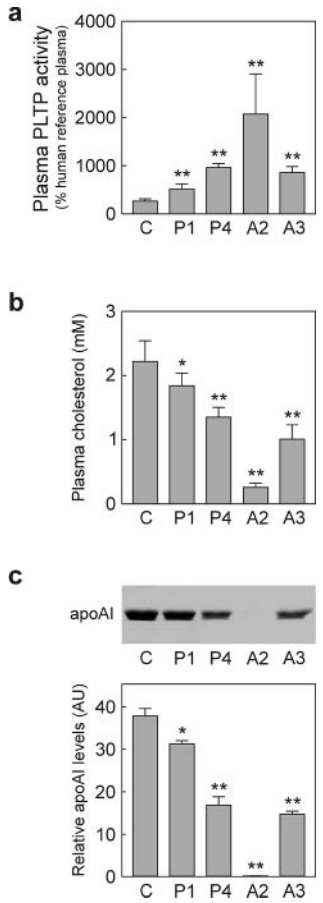

FIG. 2. **Plasma levels of PLTP activity, cholesterol and apoAI in PLTP transgenic mice.** PLTP activity (*a*) and cholesterol concentration (*b*) were measured in plasma samples from 7–12 PLTP transgenic mice or wild type littermates (*lane C*). *, $p < 0.005$; **, $p < 0.001$ *versus* control. *c*, *upper panel*, apoAI detected by staining of SDS-polyacrylamide gel with Coomassie Brilliant Blue. Plasma samples from six mice were pooled, and HDL was isolated by gradient ultracentrifugation. The samples corresponding to 12 μl of plasma were applied to the gel. *Lower panel*, three gels were scanned and quantified using ImageQuant software (Molecular Dynamics). *, $p < 0.001$ *versus* wild type littermates. All of the values are expressed as the means ± S.D.

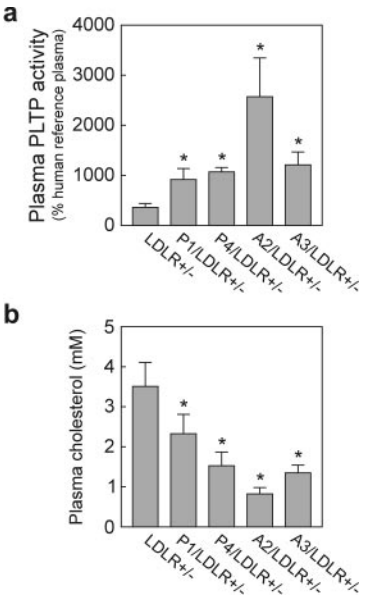

FIG. 3. **Plasma levels of PLTP activity and cholesterol in transgenic PLTP, LDLR$^{+/-}$ mice.** PLTP activity (*a*) and cholesterol concentration (*b*) were measured in plasma samples from 15–19 animals. All of the values are expressed as the means ± S.D. *, $p < 0.001$ *versus* LDLR$^{+/-}$ mice.

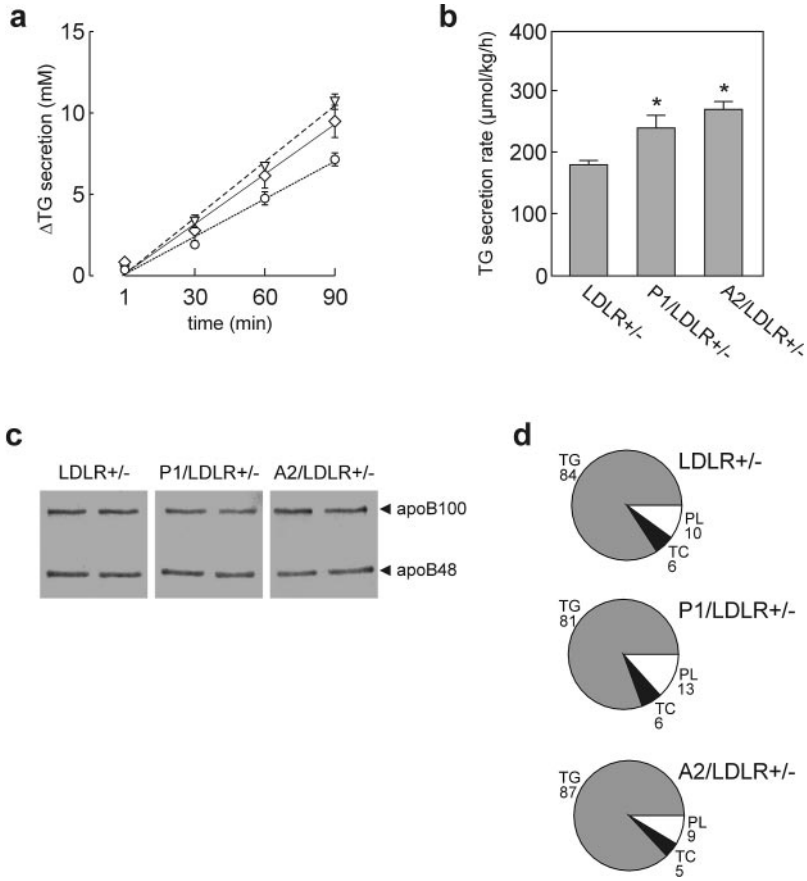

FIG. 4. **Secretion of VLDL triglycerides in transgenic PLTP, LDLR$^{+/-}$ mice.** *a*, blood was collected at 1, 30, 60, and 90 min after intravenous injection of Triton WR1339, and triglycerides were measured. ○, LDLR$^{+/-}$ mice; ◇, P1/LDLR$^{+/-}$ mice, ▽ A2/LDLR$^{+/-}$ mice. *b*, secretion rate of triglycerides/kg of body weight. *, $p < 0.001$ *versus* LDLR$^{+/-}$ mice. *c*, autoradiograms of VLDL, isolated from $t = 90$ min blood samples and applied to SDS-PAGE gels. Two representative samples from individual mice are shown. *d*, lipid composition of VLDL (w/w of total lipids).

TABLE I
PLTP activity and plasma cholesterol in mice fed an atherogenic diet
Total cholesterol (TC) is expressed in mM; PLTP activity is expressed as percentages of human reference plasma.

	n	4 weeks		12 weeks			
		PLTP	TC	PLTP	TC	VLDL/LDL TC	HDL TC
LDLR$^{+/-}$	19	358 ± 94	13.4 ± 5.4	329 ± 130	11.0 ± 2.2	8.2 ± 1.8	1.32 ± 0.51
P1/LDLR$^{+/-}$	17	762 ± 74[a]	10.8 ± 2.9[a]	662 ± 99	9.6 ± 3.1	7.9 ± 2.9	0.55 ± 0.23[b]
P4/LDLR$^{+/-}$	19	1081 ± 256[b]	12.2 ± 3.3	1030 ± 179[c]	10.7 ± 3.1	7.8 ± 2.3	0.21 ± 0.08[b]
A2/LDLR$^{+/-}$	13	4023 ± 1328[b]	12.3 ± 2.7	4583 ± 1705[b]	9.6 ± 4.8	6.7 ± 2.9	0.16 ± 0.07[b]
A3/LDLR$^{+/-}$	17	1088 ± 223[b]	11.6 ± 4.5	956 ± 245[c]	10.1 ± 5.2	7.8 ± 3.4	0.24 ± 0.14[b]

[a] Significantly different from LDLR$^{+/-}$, $p < 0.05$.
[b] Significantly different from LDLR$^{+/-}$, $p < 0.01$.
[c] Significantly different from LDLR$^{+/-}$, $p < 0.001$.

Lipoproteins and PLTP Analysis—Lipoprotein fractions were obtained by ultracentrifugation of plasma samples in a Beckman 42.2 Ti rotor (42000 rpm, 3 h, 12 °C) at $d = 1.063$ g/ml. The tubes were sliced, and two fractions were collected: VLDL + LDL, $d < 1.063$ g/ml; and HDL, $d > 1.063$ g/ml. Alternatively, the lipoprotein fractions were isolated by gradient ultracentrifugation in a Beckman SW60 Ti rotor (24). The total cholesterol was measured with commercial kits (Roche Molecular Biochemicals). PLTP activity was measured as described before (14) and expressed as a percentage of human reference plasma (100% is equivalent to 14 μmol/ml/h).

VLDL Triglyceride Secretion Experiments—The *in vivo* production of triglycerides by the liver, secreted as VLDL, was measured in mice that were fasted overnight. 500 mg/kg body weight of Triton WR1339 (Tyloxapol; Sigma) was injected intravenously. At 1, 30, and 60 min after injection, 40 μl of blood was collected via orbital puncture. At 90 min after the injection, the mice were sacrificed, and an additional large blood sample was collected. From these samples, VLDL was isolated by gradient ultracentrifugation. Triglyceride levels in each of the blood samples as well as triglycerides, free cholesterol, cholesterol esters, and phospholipids in VLDL were determined via commercially available kits (25). The accumulation of triglycerides in plasma was linear in the time period studied. From the slope, the rate of secretion was calculated and related to the body weight of the animals ($n = 5$–9 animals/group). A small number of animals was injected intravenously with 100 μCi of [^{35}S]methionine (ICN, Zoetermeer, The Netherlands).

Quantification of Atherosclerosis—After 12 weeks of the high fat, high cholesterol diet, the animals were anesthesized with isofluran, the thorax was opened, and the animals were subjected to perfusion fixation through the left ventricle of the heart using phosphate-buffered 4% formalin. The heart and aortic arch were dissected and processed for cryosectioning. 10-μm cryosections of the valves in the aortic root were stained with Oil Red O and Mayer's hematoxilin. The sections were photographed with a Sony digital camera. The atherosclerotic area was measured in five sections at 80-μm intervals using the NIH Image based Scion Image image processing and analyzing software (available from www.scioncorp.com) according to Paigen *et al.* (26).

Statistics—All of values are expressed as the means ± S.D. Statistical analyses are from one-way analysis of variance with Bonferroni multiple comparison tests performed with Intercooled Stata 6.0 software (Stata Corp., College Station, TX).

RESULTS

Overexpression of PLTP Decreases Plasma HDL—To investigate variation in PLTP expression levels as a risk for the development of atherosclerosis, we generated several lines of transgenic mice overexpressing various levels of human PLTP. These mice were made with two genomic constructs in which

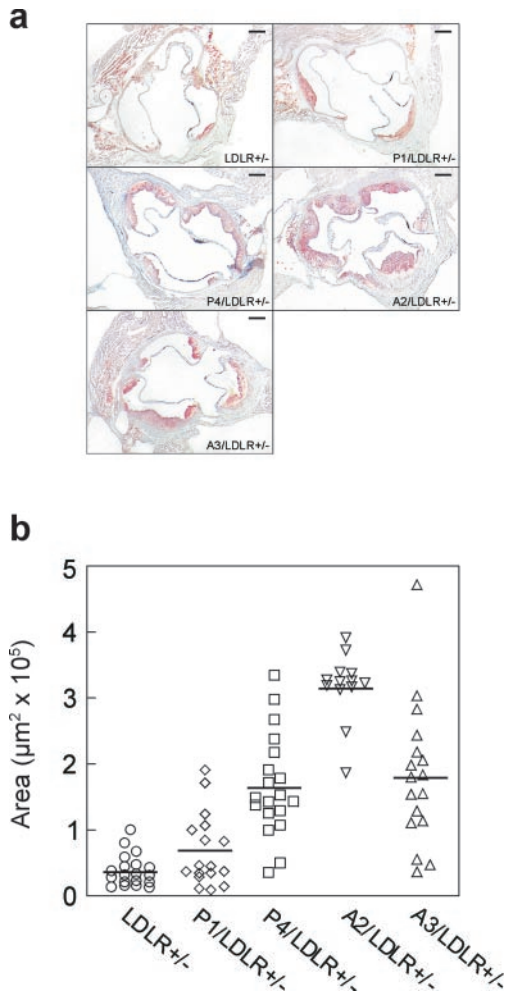

FIG. 5. **Atherosclerosis in transgenic PLTP, LDLR$^{+/-}$ mice after 12 weeks of the atherogenic diet.** *a*, representative cross-sections of the proximal aorta. The sections were stained with Oil Red O and counterstained with hematoxilin. *Scale bars*, 200 μm. *b*, lesion area for individual animals and mean of the lesion area for each group (*horizontal line*). $p < 0.001$ *versus* LDLR$^{+/-}$ mice for P4/LDLR$^{+/-}$ mice, A2/LDLR$^{+/-}$ mice, and A3/LDLR$^{+/-}$ mice.

PLTP expression is either under the control of its native promoter, resulting in the normal, multi-organ expression pattern of PLTP (14, 16, 27, 28), or under the control of the albumin promoter together with the albumin enhancer and α-fetoprotein enhancers, resulting in expression in hepatocytes. The mouse lines with these constructs were arbitrarily called P1 or P4 (multi-organ expression) and A2 or A3 (expression in hepatocytes), respectively (Fig. 1*a*). The expression of human PLTP was investigated by reverse transcription-PCR, using primers specific for human PLTP. As shown in Fig. 1*b*, there is no signal from endogenous, murine PLTP in control, nontransgenic animals. Human PLTP mRNA can be detected in liver, adrenal glands, and lung, and, to a lesser extent, in kidney in P1 and P4 mice. A2 and A3 mice expressed the transgene predominantly in liver. The level of hepatic mRNA is much higher in the A2 and A3 lines when compared with the P1 and P4 lines. Human PLTP mRNA was also detected in lungs in the A2 line mice; however, the expression in liver is much more prominent. The expression in liver of murine PLTP, studied with primers specific for murine PLTP, and other genes encoding proteins involved in HDL metabolism were not altered by overexpression of human PLTP (Fig. 1*c*).

Plasma PLTP activity levels in the different lines of transgenic mice ranged from 2-fold (P1 mice) to 9-fold (A2 mice) higher than the activity in wild type littermates (Fig. 2*a*). Cholesterol concentration decreases with increasing PLTP activity in plasma from these transgenic mouse lines to less than 10% of the normal levels in the A2 mice (Fig. 2*b*). Because plasma cholesterol in mice is mainly in HDL, the decrease in cholesterol should be reflected by a decrease of this class of lipoproteins. Fig. 2*c* confirms that the most prominent protein in HDL, apoAI, decreases with increasing PLTP activity to levels below detection in the A2 mice (Fig. 2*c*).

In contrast to humans, mice lack substantial levels of plasma LDL and hardly develop atherosclerosis. Therefore, we crossbred human PLTP transgenic mice into an LDLR$^{+/-}$ background, which has been shown to be a good model for moderate atherosclerosis development (29). This background results in an increase in PLTP activity: 360 ± 80% (n = 19) in LDLR$^{+/-}$ mice *versus* 266 ± 47% (n = 12) in wild type mice ($p < 0.02$; compare Fig. 3*a* with Fig. 2*a*). Also in mice with LDLR$^{+/-}$ background, there is an inverse correlation between PLTP activity and cholesterol levels in plasma (Fig. 3). As expected, the plasma cholesterol levels were higher in LDLR$^{+/-}$ mice: 3.5 ± 0.6 mM (n = 19) *versus* 2.2 ± 0.3 mM (n = 12) in C57BL/6J wild type mice ($p < 0.0001$). However, in the transgenic lines with substantially elevated PLTP levels (P4/LDLR$^{+/-}$, A3/LDLR$^{+/-}$, and A2/LDLR$^{+/-}$ mice), plasma cholesterol was decreased to levels below those found in the wild type control animals (LDLR$^{+/+}$; compare Fig. 3*b* with Fig. 2*b*).

Overexpression of PLTP Results in a Moderate Increase in VLDL Secretion—Because PLTP deficiency impairs VLDL secretion by the liver (21), we tested whether overexpression of PLTP would result in increased VLDL triglyceride secretion. We made a comparison between control LDLR$^{+/-}$ mice and P1/LDLR$^{+/-}$, which is the transgenic line with the lowest expression of PLTP, and A2/LDLR$^{+/-}$ mice, which have the highest expression of PLTP. In addition, hepatic expression of PLTP is greatly increased in A2/LDLR$^{+/-}$ mice (Fig. 1*b*), and thus a hepatocyte-specific effect should be highly augmented in these animals. Hepatic triglyceride secretion appeared to be increased by PLTP overexpression but only to a moderate extent (Fig. 4*a*). The rate of triglyceride secretion was only 1.3- and 1.5-fold higher than in control animals in P1/LDLR$^{+/-}$ mice and A2/LDLR$^{+/-}$ mice, respectively (Fig. 4*b*). PLTP overexpression did not induce any gross changes in the ratio of apoB100/apoB48 (Fig. 4*c*). The lipid composition of secreted VLDL was similar in the three groups (Fig. 4*d*).

Overexpression of PLTP Increases the Susceptibility to Atherosclerosis—Subsequently, mice from each of the five groups tested in Fig. 3 were fed a high fat, high cholesterol diet for 12 weeks. The plasma levels of total cholesterol were highly increased after 4 weeks, to 13.4 ± 5.4 mM in LDLR$^{+/-}$ control mice (*versus* 3.5 ± 0.6 mM before the diet). The overexpression of human PLTP did not seem to affect this, because the high plasma cholesterol levels in the PLTP transgenic mice on the high fat, high cholesterol diet were essentially the same as in the control mice (Table I).

At the end of the 12-week diet period, PLTP activity, total cholesterol, VLDL + LDL cholesterol, and HDL cholesterol were measured (Table I). Total cholesterol does not increase further between 4 and 12 weeks on the high fat, high cholesterol diet. There is no difference between the various genotypes. Also VLDL + LDL cholesterol is not significantly affected in any group in comparison with control mice. In contrast, HDL cholesterol is decreased in all human PLTP expressing mice in comparison with control animals. In the transgenic line with moderate expression of the PLTP transgene, P1/LDLR$^{+/-}$, a 60% reduction of HDL cholesterol was

observed; in the other PLTP transgenic lines, this reduction was more than 80%.

Fig. 5*a* shows representative photographs of the aortic root sections with atherosclerotic lesions of the various groups after 12 weeks of the high fat, high cholesterol diet. Fig. 5*b* shows the individual and mean atherosclerotic lesion areas. In the P1/LDLR$^{+/-}$ mice, there is a tendency toward more severe atherosclerosis than in controls, but the difference was not statistically significant. However, in all other groups of PLTP transgenic mice, the atherosclerotic lesion area is significantly increased. The A2/LDLR$^{+/-}$ mice, which have the highest level of plasma PLTP activity, also have the highest atherosclerotic lesion area ($p < 0.001$; compared with all other transgenic lines).

DISCUSSION

In the present study, we found that elevation of plasma PLTP activity has different effects on lipoproteins: secretion of VLDL is increased, whereas plasma HDL-cholesterol levels are decreased. In addition, we found a PLTP dose-dependent increase of the development of atherosclerosis.

Lines A3 (hepatocyte-specific expression) and P4 (not liver-specific) have comparable plasma PLTP activity levels and show no statistically significant differences in any of the parameters studied here. Thus, the effects described in this report are likely caused by plasma PLTP, regardless of the site of synthesis.

Although there is a stimulatory effect of PLTP on VLDL secretion by the liver, this effect seems to be saturated by moderately increased PLTP expression. Therefore, the effect of PLTP on VLDL secretion is an unlikely explanation for the increase in the development of atherosclerosis that we found in PLTP transgenic mice. On the other hand, plasma HDL levels are clearly related to the activity levels of plasma PLTP. In the highest expressing line of PLTP transgenic mice (A2), both HDL cholesterol and HDL protein (apoAI) were virtually absent from plasma, a result also found in adenoviral transfected mice that express very high levels of PLTP (17, 18). This is explained by the surface activity of PLTP on phospholipids in the outer shell of the HDL particles, causing instability and increased HDL catabolism (18). In preliminary studies, we found that plasma decay of labeled HDL cholesterylether is accelerated in our PLTP transgenic mice.[2] The decrease in apoAI/HDL is probably not the result of decreased apoAI synthesis, because hepatic apoAI mRNA levels are unchanged in both of the PLTP transgenic lines (Fig. 1*c*). From the present studies it is clear that PLTP lowers plasma HDL in mice fed a high fat, high cholesterol diet, whereas VLDL/LDL levels remain unchanged by expression of human PLTP (Table I).

There is a large body of evidence showing an inverse relationship between circulating levels of HDL and the incidence of atherosclerosic disease. Low levels of plasma HDL are generally associated with an increased risk for the development of atherosclerosis. The initial finding that apoAI-deficient mice did not show atherosclerosis (30) remained a single observation, which is probably due to the fact that the mice were not backcrossed to an atherosclerosis-prone genetic background. Later it was found that apoAI deficiency does result in enhanced atherosclerosis in atherosusceptible backgrounds (31, 32). The underlying molecular mechanism of the atheroprotective effect of HDL is complex because HDL has multiple functions that contribute to this relationship. These include a key role in reverse cholesterol transport, antioxidant effects via proteins associated with HDL like paraoxonase, and anti-inflammatory properties of HDL (4, 33–36).

[2] R. van Haperen, A. van Tol, T. van Gent, L. Scheek, P. Visser, A. van der Kamp, F. Grosveld, and R. de Crom, unpublished results.

Previously, we and others suggested that overexpression of PLTP resulted in an increase in the anti-atherogenic potential of HDL, because of an increased formation of pre-β-HDL in plasma (14, 16, 18). In addition, plasma from PLTP transgenic animals was more efficient in preventing the accumulation of cholesterol by cultured macrophages (14). Still, the present study shows that atherosclerosis is increased in mice overexpressing PLTP. From these *in vivo* experiments we conclude that the decrease in total HDL apparently is the deciding factor and not a possible effect on pre-β-HDL. This conclusion is corroborated by recent findings bringing the key role of pre-β-HDL in cellular cholesterol efflux under discussion (37).

We conclude that high plasma activity levels of PLTP are atherogenic via a lowering effect on HDL cholesterol. The elevations in plasma PLTP activity in the transgenic mice we used started at 2-fold. Until now, such differences have not been found in humans. However, atherosclerosis is a long term process, taking several decades in humans. This process has to be accelerated to be able to do studies in genetically modified animals. Therefore, models are used with substantial overexpression, which have proven to be very informative for proteins involved in cholesterol metabolism, including ABCA1 (38), apoA-IV (39), cholesteryl ester transfer protein (40), and lecithin:cholesterol acyltransferase (41). Actually, PLTP transgenic mice with an elevated activity of 30% have been generated and showed no effects on plasma lipids and lipoproteins (16). Mice with only one functional PLTP allele (PLTP$^{+/-}$) do not have any abnormalities in lipoproteins when fed normal chow or a high fat, high cholesterol diet (19). Therefore, physiological PLTP levels might be considered "high." It is conceivable that moderate differences in PLTP levels found among humans might affect the life-long process of the development of atherosclerotic disease. Until now, mutations in the PLTP gene resulting in differences in PLTP activity have not been found. However, the available data show high PLTP activity levels in conditions that have been associated with an increased risk of coronary artery disease, *i.e.* diabetes type 1 and 2, and obesity (12, 42, 43). Together with mouse data described here, this implies that high PLTP activity is a potential risk factor for coronary artery disease in humans.

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REFERENCES

1. Lusis, A. J. (2000) *Nature* **407,** 233–241
2. Glass, C. K., and Witztum, J. L. (2001) *Cell* **104,** 503–516
3. Sviridov, D., and Nestel, P. (2002) *Atherosclerosis* **161,** 245–254
4. Libby, P. (2001) *Am. J. Cardiol.* **88,** 3N–8N
5. Fielding, C. J., and Fielding, P. E. (2001) *Biochim. Biophys. Acta* **1533,** 175–189
6. Oram, J. F., and Lawn, R. M. (2001) *J. Lipid Res.* **42,** 1173–1179
7. Tall, A. R., and Wang, N. (2000) *J. Clin. Invest.* **106,** 1205–1207
8. Groen, A. K., Bloks, V. W., Bandsma, R. H., Ottenhoff, R., Chimini, G., and Kuipers, F. (2001) *J. Clin. Invest.* **108,** 843–850
9. Tall, A. R., Wang, N., and Mucksavage, P. (2001) *J. Clin. Invest.* **108,** 1273–1275
10. van Eck, M., Bos, I. S., Kaminski, W. E., Orso, E., Rothe, G., Twisk, J., Bottcher, A., Van Amersfoort, E. S., Christiansen-Weber, T. A., Fung-Leung, W. P., Van Berkel, T. J., and Schmitz, G. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 6298–6303
11. Bruce, C., Chouinard, R. A., and Tall, A. R. (1998) *Annu. Rev. Nutr.* **18,** 297–330
12. Huuskonen, J., Olkkonen, V. M., Jauhiainen, M., and Ehnholm, C. (2001) *Atherosclerosis* **155,** 269–281
13. van Tol, A. (2002) *Curr. Opin. Lipidol.* **13,** 135–139
14. van Haperen, R., van Tol, A., Vermeulen, P., Jauhiainen, M., van Gent, T., van den Berg, P., Ehnholm, S., Grosveld, F., van der Kamp, A., and de Crom, R. (2000) *Arterioscler. Thromb. Vasc. Biol.* **20,** 1082–1088
15. Albers, J. J., Tu, A. Y., Paigen, B., Chen, H., Cheung, M. C., and Marcovina, S. M. (1996) *Int. J. Clin. Lab. Res.* **26,** 262–267
16. Jiang, X., Francone, O. L., Bruce, C., Milne, R., Mar, J., Walsh, A., Breslow, J. L., and Tall, A. R. (1996) *J. Clin. Invest.* **98,** 2373–2380
17. Ehnholm, S., van Dijk, K. W., van 't Hof, B., van der Zee, A., Olkkonen, V. M.,

Jauhiainen, M., Hofker, M., Havekes, L., and Ehnholm, C. (1998) *J. Lipid Res.* **39,** 1248–1253
18. Föger, B., Santamarina-Fojo, S., Shamburek, R. D., Parrot, C. L., Talley, G. D., and Brewer, H. B. (1997) *J. Biol. Chem.* **272,** 27393–27400
19. Jiang, X. C., Bruce, C., Mar, J., Lin, M., Ji, Y., Francone, O. L., and Tall, A. R. (1999) *J. Clin. Invest.* **103,** 907–914
20. Qin, S., Kawano, K., Bruce, C., Lin, M., Bisgaier, C., Tall, A. R., and Jiang, X. (2000) *J. Lipid Res.* **41,** 269–276
21. Jiang, X. C., Qin, S., Qiao, C., Kawano, K., Lin, M., Skold, A., Xiao, X., and Tall, A. R. (2001) *Nat. Med.* **7,** 847–852
22. Pinkert, C. A., Ornitz, D. M., Brinster, R. L., and Palmiter, R. D. (1987) *Genes Dev.* **1,** 268–276
23. Camper, S. A., Godbout, R., and Tilghman, S. M. (1989) *Prog. Nucleic Acids Res. Mol. Biol.* **36,** 131–143
24. Redgrave, T. G., Roberts, D. C., and West, C. E. (1975) *Anal. Biochem.* **65,** 42–49
25. Lie, J., de Crom, R., Jauhiainen, M., van Gent, T., van Haperen, R., Scheek, L., Jansen, H., Ehnholm, C., and van Tol, A. (2001) *Biochem. J.* **360,** 379–385
26. Paigen, B., Morrow, A., Holmes, P. A., Mitchell, D., and Williams, R. A. (1987) *Atherosclerosis* **68,** 231–240
27. Day, J. R., Albers, J. J., Lofton-Day, C. E., Gilbert, T. L., Ching, A. F., Grant, F. J., O'Hara, P. J., Marcovina, S. M., and Adolphson, J. L. (1994) *J. Biol. Chem.* **269,** 9388–9391
28. Jiang, X. C., and Bruce, C. (1995) *J. Biol. Chem.* **270,** 17133–17138
29. Arai, T., Wang, N., Bezouevski, M., Welch, C., and Tall, A. R. (1999) *J. Biol. Chem.* **274,** 2366–2371
30. Li, H., Reddick, R. L., and Maeda, N. (1993) *Arterioscler. Thromb.* **13,** 1814–1821
31. Voyiaziakis, E., Goldberg, I. J., Plump, A. S., Rubin, E. M., Breslow, J. L., and Huang, L. S. (1998) *J. Lipid Res.* **39,** 313–321
32. Hughes, S. D., Verstuyft, J., and Rubin, E. M. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17,** 1725–1729
33. Tall, A. R., Jiang, X., Luo, Y., and Silver, D. (2000) *Arterioscler. Thromb. Vasc. Biol.* **20,** 1185–1188
34. Durrington, P. N., Mackness, B., and Mackness, M. I. (2001) *Arterioscler. Thromb. Vasc. Biol.* **21,** 473–480
35. Navab, M., Berliner, J. A., Subbanagounder, G., Hama, S., Lusis, A. J., Castellani, L. W., Reddy, S., Shih, D., Shi, W., Watson, A. D., Van Lenten, B. J., Vora, D., and Fogelman, A. M. (2001) *Arterioscler. Thromb. Vasc. Biol.* **21,** 481–488
36. Shah, P. K., Kaul, S., Nilsson, J., and Cercek, B. (2001) *Circulation* **104,** 2376–2383
37. Sviridov, D., Miyazaki, O., Theodore, K., Hoang, A., Fukamachi, I., and Nestel, P. (2002) *Arterioscler. Thromb. Vasc. Biol.* **22,** 1482–1488
38. Joyce, C. W., Amar, M. J., Lambert, G., Vaisman, B. L., Paigen, B., Najib-Fruchart, J., Hoyt, R. F., Jr., Neufeld, E. D., Remaley, A. T., Fredrickson, D. S., Brewer, H. B., Jr., and Santamarina-Fojo, S. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 407–412
39. Duverger, N., Tremp, G., Caillaud, J. M., Emmanuel, F., Castro, G., Fruchart, J. C., Steinmetz, A., and Denefle, P. (1996) *Science* **273,** 966–968
40. Herrera, V. L., Makrides, S. C., Xie, H. X., Adari, H., Krauss, R. M., Ryan, U. S., and Ruiz-Opazo, N. (1999) *Nat. Med.* **5,** 1383–1389
41. Hoeg, J. M., Santamarina-Fojo, S., Berard, A. M., Cornhill, J. F., Herderick, E. E., Feldman, S. H., Haudenschild, C. C., Vaisman, B. L., Hoyt, R. F., Jr., Demosky, S. J., Jr., Kauffman, R. D., Hazel, C. M., Marcovina, S. M., and Brewer, H. B., Jr. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 11448–11453
42. Riemens, S., van Tol, A., Sluiter, W., and Dullaart, R. (1998) *Atherosclerosis* **140,** 71–79
43. Colhoun, H. M., Scheek, L. M., Rubens, M. B., Van Gent, T., Underwood, S. R., Fuller, J. H., and Van Tol, A. (2001) *Diabetes* **50,** 652–659