ApoA-I deficiency causes both hypertriglyceridemia and increased atherosclerosis in human apoB transgenic mice

Emanuel Voyiaziakis,* Ira J. Goldberg,* Andrew S. Plump,† Edward M. Rubin,§ Jan L. Breslow,† and Li-Shin Huang[1,*]

Department of Medicine,* Columbia University, College of Physicians & Surgeons, 630 W. 168th St., New York, NY 10032; Rockefeller University,† New York, NY 10021; and Lawrence Berkeley Laboratory,§ University of California, Berkeley, CA 94720

Abstract To study the role of low levels of high density lipoprotein (HDL) and apolipoprotein (apo) A-I in atherosclerosis risk, human apoB transgenic mice (HuBTg) were crossed with apoA-I-deficient (apoA-I$^{-/-}$) mice. After a high fat challenge, total cholesterol levels increased drastically due to an increase in the non-HDL cholesterol as confirmed by FPLC analysis. In addition, total cholesterol levels in A-I$^{-/-}$ HuBTg mice were lower than the control HuBTg mice, due mainly to decreased HDL-C in A-I$^{-/-}$ HuBTg mice. Analysis of atherosclerosis in the proximal aorta in mice fed a high-fat Western-type diet for 27 weeks revealed a 200% greater lesion area in female apoA-I$^{-/-}$ HuBTg mice (49740 ± 9751 μm^2) compared to control HuBTg mice (23320 ± 4981 μm^2, $P = 0.03$). Lesion size (12380 ± 3281 μm^2) in male A-I$^{-/-}$ HuBTg mice was also about 200% greater than that in the control HuBTg mice (5849 ± 1543 μm^2), although not statistically significant. Very few and small lesions were observed in both apoA-I$^{-/-}$ HuBTg and control HuBTg animals fed a chow diet. Therefore, the adverse effect of low HDL on atherosclerosis in mice was only evident when LDL-cholesterol was markedly elevated by high-fat challenge. Male apoA-I$^{-/-}$ HuBTg mice exhibited hypertriglyceridemia when challenged with a high-fat diet. This correlated with both a reduction in lipoprotein lipase activity and a decrease in lipoprotein lipase activation by HDL. In summary, low high density lipoprotein levels due to apolipoprotein A-I deficiency exacerbated the development of atherosclerotic lesions in mice with elevated atherogenic lipoproteins. This mouse model mimics human conditions associated with low HDL levels and provides additional evidence for the anti-atherogenic role of apoA-I.**—Voyiaziakis, E., I. J. Goldberg, A. S. Plump, E. M. Rubin, J. L. Breslow, and L-S. Huang.** ApoA-I deficiency causes both hypertriglyceridemia and increased atherosclerosis in human apoB transgenic mice. *J. Lipid Res.* 1998. **39:** 313–321.

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Apolipoprotein A-I (apoA-I) is the major protein component of high density lipoprotein (HDL) particles (1). In humans, an inverse correlation of both apoA-I and HDL levels with the risk for coronary heart disease (CHD) has been documented (1–3). Premature atherosclerosis occurs in patients with low HDL levels due to apoA-I deficiency and lecithin:cholesterol acyltransferase (LCAT) deficiency (1). However, not all patients with low HDL have increased risk for CHD. Patients with the apoA-I$_{Milano}$ structural variant (4) and apoC-II deficiency also have low HDL levels but do not have increased risk for CHD (1). In the cases of Tangier disease (1, 5) and lipoprotein lipase (LPL) deficiency (6, 7), reduced HDL levels are associated with increased risk for CHD in some but not all of the patients reported. Therefore, it is possible that factors associated with low HDL levels, but not low HDL alone, contribute to atherogenesis.

Studies in animal models have confirmed the protective role of apoA-I against the development of atherosclerosis. Injection of HDL and apoA-I into rabbits fed a high-cholesterol diet inhibits the progression of atherosclerotic plaques (8, 9). Studies in inbred strains of mice have shown an inverse correlation of HDL levels and atherosclerotic lesion formation (10). Human apoA-I transgenic mice with increased HDL levels do not develop atherosclerotic lesions when fed an atherogenic diet (11). Similarly, the introduction of human apoA-I into mice decreased the development of athero-

Abbreviations: apoA-I, apolipoprotein A-I; HDL, high density lipoprotein; HTGL, hepatic triglyceride lipase; PHLA, post-heparin lipolytic activities; LDL, low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; PCR, polymerase chain reaction; TG, triglycerides; WTD, Western-type diet.

[1]To whom correspondence should be addressed.

sclerosis in apo[a] transgenic mice (12). Furthermore, presence of the human apoA-I transgene significantly reduced atherosclerotic lesion development in apoE-deficient mice that spontaneously develop large and advanced lesions (13, 14). Although increased HDL levels prevent the development of atherosclerotic lesions in various transgenic mouse models, low HDL levels associated with an abolition of the apoA-I gene alone do not cause atherosclerosis in mice (15). Unlike humans, mice normally have low LDL and high HDL levels. The anti-atherogenic role of apoA-I in mice may, therefore, require the presence of high levels of atherogenic lipoproteins such as LDL.

To further investigate the role of apoA-I and HDL in the development of atherosclerosis, we crossed apoA-I-deficient mice (16, 17) with human apoB transgenic mice (18) to generate animals mimicking humans with apoA-I deficiency. We examined the lipoprotein profiles and development of atherosclerotic lesions in these animals. Lack of apoA-I was associated with a greater than 2-fold increase in atherosclerotic lesion area. In addition, we observed that these animals were hypertriglyceridemic, a lipoprotein phenotype frequently associated with low HDL levels in humans (1, 19).

MATERIALS AND METHODS

Mice

ApoA-I-deficient mice (apoA-I$^{-/-}$) were created by gene targeting techniques in ES cells as described (16, 17). These mice had mixed genetic background of predominantly C57BL/6J (>75%) and SV129 strains. Human apoB transgenic (HuBTg) mice were generated as described (18). The founder also had mixed genetic background of predominantly C57BL/6J (>75%) and also FVB and SJL strains. HuBTg mice were crossed with apoA-I$^{-/-}$ mice to generate A-I$^{+/-}$ HuBTg mice. A-I$^{+/-}$ HuBTg mice were crossed with A-I$^{+/-}$ mice to generate A-I$^{-/-}$, A-I$^{-/-}$ HuBTg, HuBTg, and wild type animals. A-I$^{-/-}$ and A-I$^{-/-}$ HuBTg were intercrossed to generate A-I$^{-/-}$ HuBTg for experiments. HuBTg and wild types littermates from the previous cross were intercrossed to generate HuBTg mice for experiments. For each cross, multiple breeding pairs were used. Therefore, all animals used were of similar mixed genetic background. For each experiment, both groups of animals were matched for age and gender. Presence of the human apoB transgene was determined by polymerase chain reaction (PCR) as described (18). Genotypes for apoA-I-deficient mice were determined by PCR as described below. Mouse apoA-I-specific primers used were as follows: 5′-TCCCAGTTGATGCTCCACT GTC (sense), 5′-TACCCAACAGCCAGATGAATCC (antisense). Additionally, a neo-specific antisense primer was used: 5′-TGCGAGGCCAGAGGCCACTTGTGTAGC. PCR was carried out in a DNA Thermal Cycler (GeneAmp PCR- 9600 system, Perkin-Elmer). For apoA-I genotyping, PCR conditions began with a hot start at 99°C for 7 min. Taq DNA polymerase was added when the block cooled to 78°C. This was followed by 30 cycles of 94°C for 45 sec, 55°C for 30 sec, and 72°C for 1 min.

Mice were fed either rodent chow (PicoLab Rodent Chow 20, No. 5053; Purina Lab Chows, St. Louis, MO) or Western-type diet (WTD) (No. 88137; Teklad Premier Laboratory Diets, Madison, WI). Rodent chow consisted of 4.5% (wt/wt) fat, 0.02% (wt/wt) cholesterol and was free of casein and sodium cholate; the WTD consisted of 21% (wt/wt) fat (polyunsaturated/saturated = 0.07), 0.15% (wt/wt) cholesterol, 19.5% casein similarly free of sodium cholate (20). For quantitative atherosclerosis studies, mice were fed with WTD at 4 weeks of age and killed after 27 weeks of high-fat feeding.

Lipid and lipoprotein analysis

All lipid and lipoprotein analyses were performed on individual mice after an 8-h fast unless otherwise indicated. Food was removed at 8:00 am and blood was drawn from the retroorbital venous plexus into tubes containing EDTA at 4:00 pm the same day. Animals used for atherosclerosis studies were bled 1 week prior to killing at the age of approximately 31 weeks. Cholesterol and triglyceride (TG) analysis was performed using enzymatic assay kits as described previously (20).

For FPLC size fractionation, 200 μl of pooled and filtered mouse plasma was injected onto two Superose-6 columns in tandem as described previously (20). Eighty fractions of 0.5 ml were collected and assayed for cholesterol and TG as described above.

Quantitative atherosclerosis analysis

Mice were killed at 31 weeks of age; hearts were perfused with 0.9% saline and fixed in 10% phosphate-buffered formalin for 1 week as described (21). Fixed hearts were gelatin embedded and sectioned at 12 μm thickness in a cryostat. Sections were stained with Oil Red O and hematoxylin and counterstained with light green. The mean lesion per section of proximal aorta was measured as described (21).

In vivo triglyceride production rates

TG production rates in WTD-fed animals were performed as described (22). Age-matched (14–16 week old) male apoA-I$^{-/-}$ HuBTg and HuBTg mice were fed WTD for 4 weeks (first set) or 8 weeks (second set).

Briefly, mice were bled to measure baseline TG levels. Mice were then injected with 15% Triton WR-1339 (Cat No: T8761, Sigma, St. Louis, MO) in 0.9% NaCl solution (0.5g/kg body weight) as described (23). Blood was collected at 90 min after injection. Plasma TG levels were assayed. Increases in TG levels above baseline at 90 min were compared between the two groups by *t* test.

Measurement of lipase enzyme activities in post-heparin plasma (PHP)

LPL and hepatic triglyceride lipase (HTGL) assays were performed using PHP from age-matched mice (14–16 weeks old) fed either chow or WTD for 2 weeks prior to assay. The animals were injected with 100 unit/kg of heparin (Elkins-Sinns, Inc., Cherry Hill, NJ) intravenously. Blood was obtained by retroorbital bleed 5 min later and plasma was frozen at −70°C within 30 min. PHP (5–10 μl) was assayed using 150 μl of a gum arabic-stabilized emulsion that contained human serum as a source of apoC-II (24). Total post-heparin lipolytic activity (PHLA) was measured in 150 mm NaCl (pH 8.2), conditions optimal for LPL activity. HTGL activity was directly measured in conditions optimal for HTGL, but inhibitory to LPL activity (1 m NaCl, pH 8.8). A monoclonal antibody specific to human LPL was used to estimate the efficiency of HTGL in human PHLA as described (25). A similar percent of mouse HTGL was then assumed to be present in the mouse PHLA assay, i.e., mouse LPL activity was estimated as total PHLA minus the estimated percent of activity due to HTGL.

The PHLA was also measured using a second glycerol-based assay that minimizes the activity of HTGL (26). The activities (μmols/free fatty acid (FFA) per ml of PHP) were obtained by comparison with a standard LPL source.

LPL activation assay

To assess the ability of plasma samples from different mice to serve as an activator of LPL, the gum arabic emulsion was prepared as above without addition of plasma (27). Variable volumes (0–40 μl) of mouse HDL sample were then added and the final volume was adjusted to 150 μl with PBS–3% BSA. Plasma samples were collected from fasted mice fed WTD for 23 weeks since the age of 4 weeks. Mouse HDL samples were obtained by precipitation method as described previously (20). Plasma samples were incubated at 56°C for 1 h prior to assay to inactivate HTGL activity found in mouse pre-heparin plasma. The emulsion was incubated at 37°C for 60 min to allow activation, presumably due to transfer of the apoC-II to the artificial emulsion. A standard source of LPL purified as described previously (28) was then added, and the assay was allowed to proceed for 60 min at 27°C. All assays were performed in triplicate and the data for the hydrolysis of TG, i.e., LPL activity, were compared.

RESULTS

Lipoprotein profiles in human apoB transgenic mice with apoA-I deficiency

ApoA-I$^{-/-}$ HuBTg and control HuBTg mice were fed either chow or WTD diet and were bled under fasting conditions. Their lipoprotein profiles are shown in **Table 1**.

TABLE 1. Lipoprotein profiles in HuBTg mice with apoA-I deficiency

		Chow[a]				WTD[b]			
Genotype	Gender	TG	TC	Non-HDL-C	HDL-C	TG	TC	Non-HDL-C	HDL-C
		mg/dl				*mg/dl*			
A-I$^{+/+}$	M	177 ± 44 (n = 23)	159 ± 35 (n = 7)	80 ± 37	78 ± 9	221 ± 66 (n = 16)	637 ± 69 (n = 6)	552 ± 64	85 ± 25
A-I$^{-/-}$	M	171 ± 44 (n = 20)	148 ± 36 (n = 6)	114 ± 39	33 ± 22	338 ± 74 (n = 11)	543 ± 52 (n = 5)	531 ± 50	12 ± 5
A-I$^{+/+}$	F	149 ± 42 (n = 10)	161 ± 11 (n = 4)	71 ± 34	90 ± 37	154 ± 28 (n = 13)	480 ± 51 (n = 4)	424 ± 55	56 ± 7
A-I$^{-/-}$	F	179 ± 42 (n = 20)	155 ± 20 (n = 8)	128 ± 34	27 ± 7	181 ± 61 (n = 11)	379 ± 70 (n = 4)	361 ± 72	18 ± 10
P value (*t* test)									
A-I$^{+/+}$ vs. A-I$^{-/-}$ (Male)		n.s.[c]	n.s.	n.s.	0.002	0.0001	0.03	n.s.	0.0004
A-I$^{+/+}$ vs. A-I$^{-/-}$ (Female)		n.s.	n.s.	0.03	0.04	n.s.	n.s.	n.s.	0.002
Male vs. female (A-I$^{+/+}$)		n.s.	n.s.	n.s.	n.s.	0.0004	0.003	0.01	0.04
Male vs. female (A-I$^{-/-}$)		n.s.	n.s.	n.s.	n.s.	0.0001	0.01	0.01	n.s.

[a]At the time of lipid profile mice on the chow diet were approximately 20 weeks of age and mice on WTD were approximately 31 weeks of age. The same sets of animals were analyzed for TC, non-HDL-C, and HDL-C.
[b]Mice were weaned to WTD at 4 weeks for 26 weeks duration and bled under fasting conditions.
[c]Log transformed TG was used for *t* test.

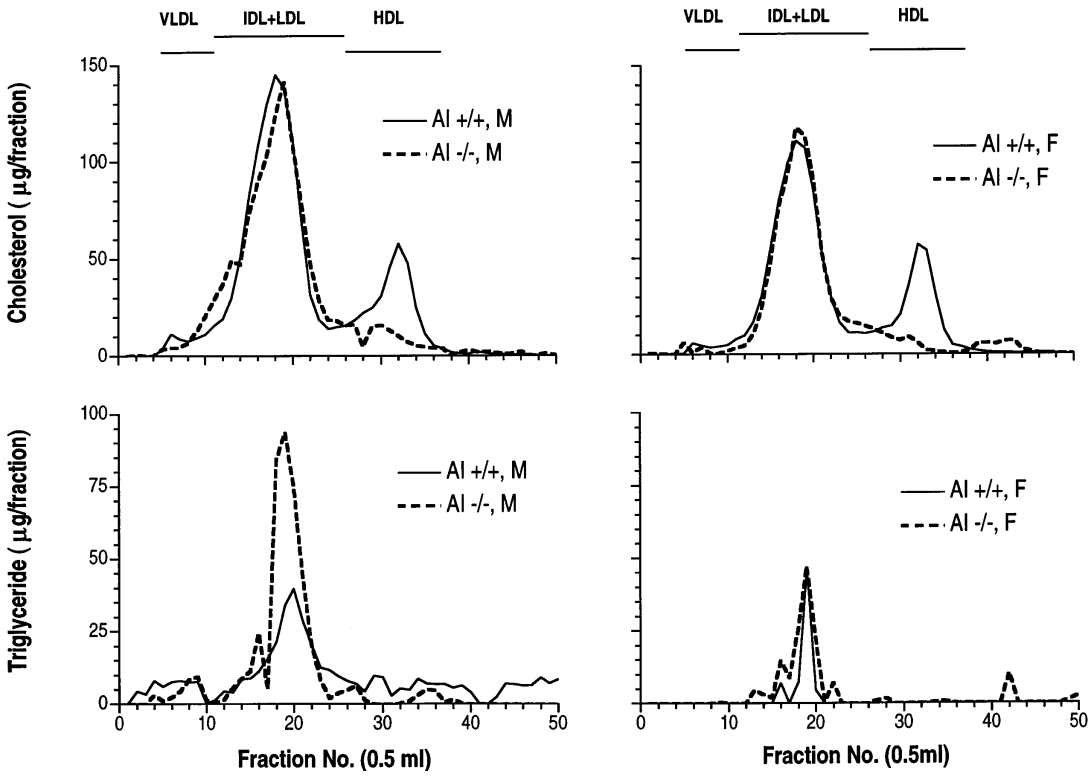

Fig. 1. FPLC profiles of WTD-fed HuBTg mice with apoA-I deficiency. Animals were fed with WTD for 26 weeks prior to bleeding. Pooled mouse plasma (200 µl) from A-I$^{-/-}$ HuBTg (dashed line) and control HuBTg (solid line) were subjected to two Superose-6 columns in series and 0.5-ml fractions were collected. The cholesterol (top) or triglyceride (bottom) in each fraction is plotted in micrograms (y-axis), against fraction number (x-axis). FPLC profiles for male mice are shown on the left, and female mice are shown on the right.

On chow diet, HDL-C was significantly lower in both male and female A-I$^{-/-}$ HuBTg compared to HuBTg controls. Although no significant differences in total cholesterol levels were observed between these groups, cholesterol levels in the non-HDL fraction were higher in female A-I$^{-/-}$ HuBTg mice compared to control female HuBTg mice. A similar but not statistically significant result was observed in male mice.

When animals were fed a WTD for 26 weeks, cholesterol levels in all fractions increased compared to chow diet. In addition, significant gender differences in the cholesterol levels in these fractions were observed. However, the major difference between control HuBTg and A-I$^{-/-}$ HuBTg mice was in the HDL-C fraction. In male control HuBTg mice, total cholesterol increased from 159 ± 35 (chow diet) to 637 ± 67 mg/dl (WTD). A comparable increase was observed in male A-I$^{-/-}$ HuBTg mice in which total cholesterol increased from 148 ± 36 (chow diet) to 543 ± 93 mg/dl (WTD). No significant difference was observed in the non-HDL fractions, whereas HDL-C was significantly lower in A-I$^{-/-}$ HuBTg mice (12 ± 5 mg/dl) compared to control HuBTg mice (85 ± 25 mg/dl). Similar results were observed in female mice. In addition in both female control and A-I$^{-/-}$ HuBTg mice, both total cholesterol levels (480 ± 51 and 379 ± 70 mg/dl, respectively) and non-HDL cholesterol levels (424 ± 55 and 361 ± 72 mg/dl, respectively) were significantly lower than those in the male mice described above. Although there was a significant gender difference in HDL-C in control HuBTg mice, no difference was observed in A-I$^{-/-}$ HuBTg mice. These data showed that upon WTD feeding, lower HDL cholesterol levels were the major difference between control HuBTg and A-I$^{-/-}$ HuBTg mice.

Differences in the lipoprotein fractions in animals fed on WTD were confirmed by the FPLC profiles of pooled mouse plasma from these animals. As shown in the top-left panel of **Fig. 1**, the area under the curve corresponding to the IDL and LDL fractions was similar between male control and A-I$^{-/-}$ HuBTg mice, whereas there was a diminished area in the HDL fractions in male A-I$^{-/-}$ HuBTg mice. Similar FPLC profiles were observed in female mice (Fig. 1, top-right panel). The only difference between the WTD-fed male and female mice was in the non-HDL fraction as shown

in Table 1. These data showed that the A-I$^{-/-}$ HuBTg mouse has a low HDL and high LDL phenotype.

TG levels in these animals were also assessed under fasting conditions. In chow-fed animals, no significant difference in TG levels was observed between control and A-I$^{-/-}$ HuBTg mice of either gender as shown in Table 1. However, upon long duration of WTD feeding (26 weeks), total TG levels were significantly higher in male A-I$^{-/-}$ HuBTg mice (338 ± 74 mg/dl) compared to male control HuBTg mice (221 ± 66 mg/dl). FPLC analysis of plasma from WTD-fed male mice showed that the increase of TG in A-I$^{-/-}$ HuBTg was mainly in the LDL-sized particles (Fig. 1, bottom-left panel). In female mice, the TG was also mostly in the LDL-sized particle (Fig. 1, bottom-right panel). Although TG levels were slightly higher in A-I$^{-/-}$ HuBTg mice (181 ± 61 mg/dl) than those in control HuBTg mice (154 ± 20 mg/dl) (Table 1), this difference was not statistically significant. In addition, TG levels in WTD-fed females (both A-I$^{-/-}$ and control HuBTg mice) were not significantly changed compared to chow-fed female mice.

Atherosclerotic lesions in the proximal aorta

To determine the effect of low HDL levels on the development of atherosclerotic lesions, the proximal aorta from A-I$^{-/-}$ HuBTg and control mice was assessed for the extent of lesion development and area. We examined proximal aortas from chow-fed mice approximately 31 weeks old and found no or few and small lesions in control HuBTg and also in A-I$^{-/-}$ HuBTg mice (**Table 2**). To better assess the lesion development, we challenged these animals with WTD at the age of 4 weeks for a duration of 27 weeks. As shown in Table 2, the mean lesion area in female A-I$^{-/-}$ HuBTg mice (49740 ± 9751 μm^2) was significantly larger (2.1-fold, $P = 0.03$) than that in female control HuBTg mice (23320 ± 4981 μm^2). The mean lesion area in male A-I$^{-/-}$ HuBTg mice (12380 ± 3281 μm^2) was also 2-fold larger, although not statistically significant, than that in male control mice (5849 ± 1543 μm^2). As shown in Table 2, female mice developed much more extensive lesions; approximately 4-fold greater lesion area was found between comparable genotypes. These results demonstrated that low HDL levels due to apoA-I deficiency exacerbated the development of atherosclerotic lesions in mice containing elevated LDL levels due to overexpression of the human apoB transgene.

Triglyceride levels and production rates

To determine whether increased TG production or decreased clearance of TG was responsible for the increase in plasma TG levels in WTD-fed male A-I$^{-/-}$ HuBTg mice, in vivo TG production rates were determined by intravenous injection of Triton WR-1339 which inhibits lipolysis. TG accumulating in the plasma 90 min after injection were compared between male A-I$^{-/-}$ HuBTg and HuBTg controls. Because animals from the two experiments were on WTD for different duration (4 weeks vs. 8 weeks), results from these two experiments are shown separately. As shown in **Fig. 2**, both experiments showed no significant differences in the accumulation of TG at the 90-min time point (first set: 310 ± 52 mg/dl for HuBTg vs. 346 ± 132 mg/dl for A-I$^{-/-}$ HuBTg; second set: 277 ± 41 mg/dl for HuBTg vs. 301 ± 88 mg/dl for A-I$^{-/-}$ HuBTg). These results indicated that hypertriglyceridemia in male A-I$^{-/-}$ HuBTg mice was not due to an increase in TG production rates in these animals.

TABLE 2. Atherosclerotic lesions in HuBTg mice with apoA-I deficiency

Genotype	Diet[a]	Gender[b]	N	Lesion Area per Section	*P* value (*t* test)
				mean μm^2 ± SEM	
A-I$^{+/+}$	Chow	M and F	15	307 ± 221	
A-I$^{-/-}$	Chow	M and F	13	105 ± 42	n.s.
A-I$^{+/+}$	WTD	F	9	23320 ± 4981	
A-I$^{-/-}$	WTD	F	10	49740 ± 9751	0.03[c]
A-I$^{+/+}$	WTD	M	11	5849 ± 1543	
A-I$^{-/-}$	WTD	M	11	12380 ± 3281	n.s. (0.09)

[a]Mice were weaned to either chow or WTD at 4 weeks for 27 weeks duration.

[b]Significant differences in lesion sizes between WTD-fed male A-I$^{+/+}$ and female A-I$^{+/+}$ mice ($P = 0.002$) and between WTD-fed male A-I$^{-/-}$ and female A-I$^{-/-}$ mice ($P = 0.001$).

[c]Significant difference in the mean lesion area between A-I$^{+/+}$ and A-I$^{-/-}$ mice.

LPL Activity

As there was no difference in the TG production rates by the male A-I$^{-/-}$ HuBTg and the male control HuBTg mice, we assessed whether the increase in TG levels was associated with differences in LPL actions. We first assessed the post-heparin lipolytic activities of LPL and HTGL in these animals. As shown in **Table 3**, both PHLA and HTGL activities were significantly lower in the male A-I$^{-/-}$ HuBTg mice compared to controls either on WTD or chow diet. However, the percent decrease of the enzyme activities in the WTD-fed A-I$^{-/-}$ HuBTg animals (51–55%) was much higher than that in control HuBTg mice (18–28%). A similar trend persisted for the estimated LPL activities in WTD-fed male mice using either gum arabic or a glycerol-based emulsion (Table 3).

In a separate experiment, post-heparin lipolytic activities of LPL and HTGL from WTD-fed female mice were also assessed. Table 3 shows that the activities of both enzymes were also lower in female A-I$^{-/-}$ HuBTg mice than female controls. However, the percent de-

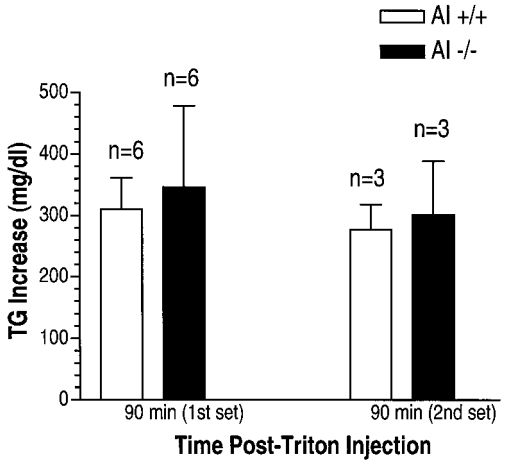

Fig. 2. In vivo TG production. Male mice were fed with WTD for either 4 weeks (1st set: shown on the left) or 8 weeks (2nd set: shown on the right) prior to experiments. Animals were fasted for 8 h and injected with Triton WR-1339. Mice were bled immediately prior to injection and 90 min after injection. TG levels were measured and the increase of TG levels over the baseline is shown in histograms. Increase of TG (mg/dl) over the baseline is shown on the y-axis. The first set of animals was on WTD for 4 weeks (shown on the left). Male A-I$^{-/-}$ HuBTg mice are shown in solid bars and male controls with open bars. No statistical differences were observed between the two groups of animals when compared by *t* test.

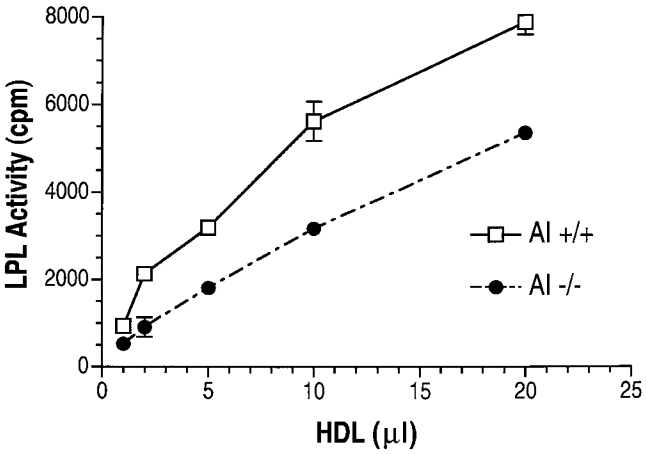

Fig. 3. In vitro LPL activation by mouse HDL. Pooled mouse HDL samples from WTD-fed male mice were used as sources of activators for purified bovine LPL in the LPL activation assays. All blood samples were collected under fasting conditions. Various volumes of mouse HDL samples were used as activators for the bovine LPL used in the LPL assays described in the Methods section. Volume of mouse HDL is shown on the x-axis. The activity of LPL is shown as cpm on the y-axis. Male A-I$^{-/-}$ HuBTg mice are shown as solid circles, and male controls as open squares. The standard error bars at each point are indicated, unless they fall within the symbol.

crease in the activities between the two groups of animals (i.e., A-I$^{-/-}$ HuBTg vs. HuBTg) was less in females (33–36%) than that in males (49–55%) (Table 3). This may contribute to the gender difference in the clearance of TG upon high-fat feeding.

We also assessed HDL from male mice as a source of apoC-II for LPL activation. As shown in **Fig. 3**, HDL from male A-I$^{-/-}$ HuBTg mice activated purified bovine LPL significantly less than did that of male control HuBTg mice. This was further confirmed by assaying HDL (10 μl) from individual male mice fed WTD; ability of HDL from male A-I$^{-/-}$ HuBTg mice (n = 6) to activate LPL activity in vitro was reduced by 46% (P = 0.002) compared to that from male control HuBTg

TABLE 3. Post-heparin plasma (PHP) lipase ativities in HuBTg mice with apoA-I deficiency

Diet	Genotype	Gender	n	PHLA[a]	HTGL	LPL	PHLA[b] (Glycerol-based)
Chow	A-I$^{+/+}$	M	6	62.6 ± 2.2	106.7 ± 4.9	43.0 ± 1.7	
	A-I$^{-/-}$	M	6	49.7 ± 6.1	77.7 ± 14.3	35.4 ± 4.2	
P value[c]				0.0003	0.0004	0.001	
% Decrease (A-I$^{-/-}$ vs. A-I$^{+/+}$)[d]				18%	28%	19%	
WTD[e]	A-I$^{+/+}$	M	8	43.5 ± 5.9	76.5 ± 14.7	32.4 ± 4.5	52.1 ± 7.9
	A-I$^{-/-}$	M	8	21.4 ± 4.3	34.3 ± 9.1	16.6 ± 4.1	19.8 ± 5.3
P value				<0.0001	<0.0001	<0.0001	<0.0001
% Decrease (A-I$^{-/-}$ vs. A-I$^{+/+}$)				51%	55%	49%	
WTD[e]	A-I$^{+/+}$	F	7	33 ± 10	36 ± 24	32 ± 10	
	A-I$^{-/-}$	F	5	21 ± 10	24 ± 5	20 ± 10	
P value				0.03	0.01	0.030	
% Decrease (A-I$^{-/-}$ vs. A-I$^{+/+}$)				36%	34%	33%	

[a]Enzyme activities for all enzymes are expressed as μmol/free fatty acid per ml of PHP ± standard deviation.
[b]PHLA (post-heparin lypolytic activity) was measured with a glycerol-based assay. Enzyme assays for chow-fed males, WTD-fed males, and WTD-fed females were performed at different times.
[c]*P* value was derived from *t* test.
[d]% Decrease of enzyme activity in A-I$^{-/-}$ plasma compared to A-I$^{+/+}$ plasma.
[e]Mice were fed with WTD for 2 weeks prior to assay.

mice (n = 8). Therefore, increased TG levels in A-I$^{-/-}$ HuBTg mice, particularly in male mice, was associated with in vitro defects in post-heparin lipase.

DISCUSSION

In this report, we demonstrated that low HDL levels due to apoA-I deficiency exacerbated the development of atherosclerotic lesions in mice with elevated LDL levels, a mouse model generated by crossing the apoA-I-deficient and human apoB transgenic mouse lines. We confirmed that a low HDL level itself is insufficient for the initiation of atherosclerosis in mice. Despite drastic reduction of HDL levels in chow-fed A-I$^{-/-}$ HuBTg mice, these animals had no or few lesions. Only when fed a Western-type diet with high-fat and high-cholesterol content did LDL levels in these animals become elevated enough to produce measurable lesions. After 6 months of WTD feeding, female A-I$^{-/-}$ HuBTg mice had a significant increase (>2-fold) in lesion sizes compared to control animals. A similar 2-fold increase, although not statistically significant, was observed in male A-I$^{-/-}$ HuBTg mice compared to male control HuBTg mice. Our data show that the protective effect of apoA-I, and hence high HDL levels, is not evident unless the level of atherogenic lipoproteins rises sufficiently to initiate atherosclerotic lesion development.

Although the basis of the gender differences is not known, larger lesion formation in female mice has been documented in both inbred strains and induced mutant mice (29, 30). We also observed this sex difference given that female A-I$^{-/-}$ HuBTg and control HuBTg mice had 4-fold larger mean lesion areas than their male counterparts. Again, increased lesion areas were found in both genders of A-I$^{-/-}$ HuBTg mice relative to controls.

In our studies a high fat diet mimicking the average diet consumption in the Western world was capable of initiating atherosclerosis in both HuBTg mice and A-I$^{-/-}$ HuBTg mice. The atherosclerotic lesion development was, however, slower than that induced by the atherogenic cholic acid-containing high-fat diet; male HuBTg mice developed few and small lesions (<500 μm^2) after 14 weeks of WTD feeding (data not shown) even though total cholesterol levels in these animals were above 400 mg/dl. Previous studies in HuBTg mice showed that feeding with a cholic acid-containing high-fat diet produced large atherosclerotic lesions within 18 weeks of feeding (31, 32). Different effects on the lipid profiles by the WTD versus the cholic acid-containing diet were also observed. The cholic acid-containing atherogenic diets increased LDL-size particles (mainly B-48-containing lipoproteins) and reduced HDL cholesterol levels in HuBTg mice (31, 32). In contrast, the WTD diet increased both LDL and HDL cholesterol levels in A-I$^{-/-}$ HuBTg and HuBTg mice (Table 1 and Fig. 1). The increase in the apoB proteins was mainly of B-100 when plasma samples from these animals were analyzed by Western blot analysis (data not shown). The atherogenesis induced by WTD and cholic acid-containing atherogenic diets may therefore be different in these animals.

The exact mechanism for the anti-atherogenic effect of HDL is not clear. One hypothesis is that HDL performs reverse cholesterol transport, a process that prevents atherosclerotic lesion formation by removing excess cholesterol from the artery (33). A number of other hypotheses for the protective actions of HDL have also been suggested (33, 34). Premature atherosclerosis occurs in humans with low HDL levels associated with apoA-I deficiency (1–3). Using a mouse model with low HDL and high LDL levels, we confirmed that apoA-I is responsible for the protective effect of HDL. Although it is unlikely to be the case, we cannot rule out the possibility that loci other than apoA-I may also have an effect on lesion development in these animals due to the mixed background. In fact, while our work was in preparation, Hughes et al. (35) also reported an increased susceptibility of apoA-I$^{-/-}$ HuBTg mice to diet-induced atherosclerosis. Their study was carried out using a cholic acid-containing high-fat diet and may differ in the atherogenesis induced when compared to the WTD as discussed above. A recent study by Zhang et al. (36) showed that ablation of apoA-I genes in apoE-deficient animals did not increase the size of the lesions formed spontaneously in the chow-fed animals. This further validated the usefulness of the A-I$^{-/-}$ HuBTg mouse model to study the effect of low HDL levels on the development of atherosclerosis.

In addition, we showed that hypertriglyceridemia was associated with apoA-I deficiency in male apoB transgenic mice due to enrichment of triglyceride in the LDL-sized particles. The increase in TG in these animals was unmasked only after high-fat consumption, possibly because TG removal was not limited under the control, chow-diet, conditions. Mechanistic studies revealed defects in TG clearance but not TG production in these animals. Because TG clearance requires LPL-mediated lipolysis followed by remnant clearance in the liver, abnormalities in this pathway may have contributed to the accumulation of TG in these animals. Two possible reasons for this are decreased LPL activation due to loss of HDL–apoC-II or a reduction in post-heparin lipase activities. The reason for this latter finding is unknown. Decreased post-heparin LPL activities

were found in some bunt not all patients with LCAT deficiency (37–39). In patients with Tangier disease, LPL activity was also decreased in some patients (40). Our data showing hypertriglyceridemia with HDL deficiency in the male A-I$^{-/-}$ HuBTg mice are consistent with human HDL defective states; patients with apoA-I deficiency, without the concomitant loss of apoC-III (41, 42), and patients with the apoA-I$_{Milano}$ structural variant have low HDL and high triglyceride levels (43).

In summary, we confirmed that apoA-I is protective against the development of atherosclerosis. The apoA-I$^{-/-}$ HuBTg mouse provides a useful model to study the effect of low HDL levels on the development of atherosclerosis and hypertriglyceridemia associated with the low HDL level.

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