Human apolipoprotein A-II associates with triglyceride-rich lipoproteins in plasma and impairs their catabolism

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Abstract **Postprandial hypertriglyceridemia and low plasma HDL levels, which are principal features of the metabolic syndrome, are displayed by transgenic mice expressing human apolipoprotein A-II (hapoA-II). In these mice, hypertriglyceridemia results from the inhibition of lipoprotein lipase and hepatic lipase activities by hapoA-II carried on VLDL. This study aimed to determine whether the association of hapoA-II with triglyceride-rich lipoproteins (TRLs) is sufficient to impair their catabolism. To measure plasma TRL residence time, intestinal TRL production was induced by a radioactive oral lipid bolus. Radioactive and total triglyceride (TG) were rapidly cleared in control mice but accumulated in plasma of transgenic mice, in relation to hapoA-II concentration. Similar plasma TG accumulations were measured in transgenic mice with or without endogenous apoA-II expression. HapoA-II (synthesized in liver) was detected in chylomicrons (produced by intestine). The association of hapoA-II with TRL in plasma was further confirmed by the absence of hapoA-II in chylomicrons and VLDL of transgenic mice injected with Triton WR 1339, which prevents apolipoprotein exchanges. We show that the association of hapoA-II with TRL occurs in the circulation and induces postprandial hypertriglyceridemia.—** Dugué-Pujol, S., X. Rousset, D. Pastier, N. T. Quang, V. Pautre, J. Chambaz, M. Chabert, and A-D. Kalopissis. **Human apolipoprotein A-II associates with triglyceride-rich lipoproteins in plasma and impairs their catabolism.** ***J. Lipid Res.*** **2006.** 47: **2631–2639.**

Supplementary key words postprandial hypertriglyceridemia • transgenic mice • apolipoprotein A-II knockout mice • chylomicrons • very low density lipoprotein • plasma residence time

High plasma levels of triglyceride-rich lipoproteins (TRLs) are often associated with low HDL cholesterol concentrations, which are correlated with an increased risk of atherosclerosis (1). More recently, hypertriglyceridemia was also established as an independent risk factor for atherosclerosis, because decreasing plasma triglyceride (TG) without changing HDL and LDL cholesterol levels improved endothelial function (2). On the other hand, hypertriglyceridemia and low plasma HDL levels are features of the metabolic syndrome, a cluster of abnormalities comprising insulin resistance, hypertension, glucose intolerance, abdominal obesity, and the preponderance of small, dense LDLs (3, 4). The metabolic syndrome is diagnosed by the presence of any three of these abnormalities and confers an increased risk of cardiovascular disease.

Unexpectedly, we observed that several features of the metabolic syndrome were associated with moderate to high expression of human apolipoprotein A-II (hapoA-II) in transgenic mice generated in our laboratory. The transgenic lines δ and λ expressing hapoA-II at two and three times the normal concentration, respectively, displayed postprandial hypertriglyceridemia, low plasma HDL and apoA-I levels, and a preponderance of small HDLs rich in hapoA-II (5). The high-expressing λ mice also presented mild hypertriglyceridemia after an overnight fast and displayed glucose intolerance (6). Of note, the ratio of hapoA-II to apoA-I in HDL was a key factor regulating the size and number of HDL particles (7). HapoA-II was partly carried by VLDLs in transgenic mice, and apoA-II-containing VLDLs were catabolized in vitro by LPL less efficiently than VLDLs from control mice. Moreover, the addition of hapoA-II to postheparin plasma from control mice dose-dependently inhibited in vitro LPL and HL activities. Because VLDL secretion was comparable among transgenic and control mice, we proposed that the hypertriglyceridemia of transgenic mice resulted from decreased VLDL catabolism by LPL and HL attributable to the presence of hapoA-II in VLDL (5). Indeed, apoA-II is

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Abbreviations: hapoA-II, human apolipoprotein A-II; KO, knockout; mapoA-II, murine apolipoprotein A-II; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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present in VLDL in certain types of dyslipidemia with low plasma HDL levels. Tangier disease patients have very low plasma HDL, and a major fraction of VLDLs transport apoA-II (8). The catabolism of apoA-II-containing VLDLs by LPL is defective, resulting in mild to moderate hypertriglyceridemia. Similarly, 25–70% of VLDLs from patients with type V hyperlipoproteinemia contain apoA-II and are less effective as a substrate for LPL (8). We thus hypothesized that postprandial hypertriglyceridemia and low plasma HDL levels in a subset of subjects with the metabolic syndrome may be associated with the presence of apoA-II in VLDL.

To establish whether the association of hapoA-II with TRL is sufficient to impair their catabolism and provoke hypertriglyceridemia, the present study aimed to *i*) determine the plasma residence time of TRL as a function of hapoA-II expression, and *ii*) establish whether hapoA-II is transferred to TRL in the plasma compartment. This point could not be assessed in our previous study (5), because both hapoA-II and VLDL originated from liver, the major source of VLDLs in the standard chow diet (carbohydrate-rich, with only 5% lipid) (9). Thus, hapoA-II may have associated with VLDLs either in the hepatocyte at some step of the secretory pathway or in the plasma compartment after secretion. Therefore, we dissociated in this study the sites of synthesis of hapoA-II and TRL by inducing chylomicron production after intragastric administration of sunflower oil mixed with [^{14}C]triolein. To determine whether hapoA-II was carried by intestinal TRL in transgenic mice, we analyzed apolipoprotein compositions of chylomicrons and VLDLs of mice receiving the oral lipid load alone or together with Triton WR 1339, a nonionic detergent that coats the surface of lipoproteins and inhibits LPL activity and apolipoprotein exchanges (10, 11). In Triton-treated mice, the transfer of hapoA-II to TRL should be hindered. Finally, to ascertain that the metabolic effects studied were caused by hapoA-II only, we measured plasma TG concentrations after sunflower oil administration to δ and λ transgenic mice deficient in endogenous murine apolipoprotein A-II (mapoA-II) obtained by backcrossing to apoA-II knockout (KO) mice (12).

MATERIALS AND METHODS

Animals

The animals were housed in animal rooms with alternating 12 h periods of light (7 AM–7 PM) and dark (7 PM–7 AM). They were fed a chow diet (UAR) with free access to food and water. The generation of transgenic mice has been described previously (5). The transgenic lines δ and λ used in this study were backcrossed for at least eight generations to strain C57BL/6J, were hemizygous for the hapoA-II transgene, and were >8 weeks of age. Furthermore, δ and λ transgenic mice, backcrossed to C57BL/6J for at least 10 generations, were bred with mapoA-II-KO mice (12) to generate mice expressing hapoA-II only (subsequently called δKOAII and λKOAII, respectively). ApoA-II-KO mice had previously been backcrossed for 10 generations to strain C57BL/6J. Male and female transgenic mice were used in equal proportions in all studies. C57BL/6J male and female mice (Charles River) of the same age and maintained under the same nutritional conditions were used as controls. HapoA-II was measured in plasma from all animals by immunonephelometry using an antibody (Dade Behring) specific for hapoA-II and not recognizing mapoA-II. The procedures followed were in accordance with institutional guidelines.

Mice were identified by PCR analysis of tail DNA (**Fig. 1**). The δ and λ hapoA-II transgenic lines were identified by the presence of the hapoA-II gene, using as primers oligonucleotides 5′-CGCAGCAACTGTGCTACTCC-3′ and 5′-CTCTCCACACATGGCTCCTT-3′ (13). The δKOAII and λKOAII transgenic mice were identified by the presence of the hapoA-II gene (as above), the presence of the neomycin-resistant (*neo*) gene that replaced the entire mapoA-II gene (12), and the absence of the mapoA-II gene. The *neo* gene was identified using as primers oligonucleotides 5′-CGCCGCTCCCGATTCGCAGCGCATCGC-3′, located in the end portion of the *neo* gene, and 5′-TCTTGGTCTAGAAGCAGCTGGGGTGGGAAGACTG-3′, located in the end of exon 4 of the mapoA-II gene (12). The mapoA-II gene was identified using as primers oligonucleotides 5′-TCCAATCTGCAGAGTCTGATCC-3′ and 5′-TCTTGGTCTAGAAGCAGCTGGGGTGGGAAGACTG-3′ (12).

In vivo clearance of plasma [^{14}C]TG and total TG

A kinetic study was performed with control and hapoA-II transgenic mice fed ad libitum. One control, one δ, and one λ transgenic mice were studied on any given day, between 9:30 AM and 6:00 PM, and six separate experiments were conducted. The production of chylomicrons was induced by intragastric administration of a bolus of 0.4 ml of sunflower oil mixed with [^{14}C]triolein

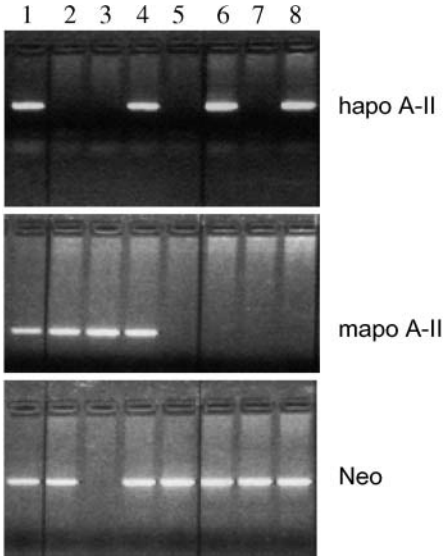

Fig. 1. PCR identification of human apolipoprotein A-II (hapoA-II) transgenic mice deficient in murine apolipoprotein A-II (mapoA-II). In Materials and Methods, the primer oligonucleotides used for the three PCRs performed in tail DNA of each mouse to identify its genotype are listed. Mouse 3 (lane 3) is a control C57BL/6 mouse expressing only mapoA-II; mice 5 and 7 only express the *neo* gene and are deficient in mapoA-II (KOAII); mice 6 and 8 express the hapoA-II and *neo* genes, but not the mapoA-II gene, and are termed δKOAII and λKOAII mice, depending on whether they originated from the δ or λ transgenic line. Mice 1 and 4 have intermediate genotypes: they express hapoA-II and mapoA-II and have one copy of the *neo* gene; therefore, they were not used in any experiment.

(New England Nuclear; specific activity 4.1440 GBq/mmol). Mice received 12×10^6 cpm of [^{14}C]triolein. Six blood samples were taken from each mouse from the jugular vein into EDTA-containing tubes at 4°C: at 0 min (before lipid administration) and at 2, 3, 4, 5, and 8 h after the oral lipid load. In a separate experiment, four transgenic λ mice were studied for up to 24 h. Three blood samples were taken from each mouse at 0 min (before sunflower oil and triolein administration) and at 3 and 24 h after the lipid bolus. The λ mice were deprived of food after triolein administration. Plasma samples were extracted for lipids (14), and lipid classes were separated by TLC and counted. Total plasma TG was measured in duplicate with a commercial kit (Sigma Diagnostics 334-A).

TRL accumulation was also measured in δKOAII and λKOAII mice expressing hapoA-II at levels comparable to the original δ and λ transgenic lines. Control, δKOAII, and λKOAII mice fed ad libitum received an oral lipid bolus of 0.4 ml of sunflower oil. EDTA-plasma samples were taken at 0 min (before lipid administration) and at 3, 5, and 8 h after the oral lipid load, and total plasma TG was measured.

Production rate of chylomicrons and VLDLs after an oral lipid load

Control and transgenic δ and λ mice fasted overnight were injected into the jugular vein with Triton WR 1339 (500 mg/kg) as a 15% (w/v) solution in 0.9% NaCl (10) and then received an intragastric administration of 0.4 ml of sunflower oil. Two blood samples were taken from each mouse into EDTA-containing tubes at 4°C: at 0 min (before Triton and sunflower oil administration) and at 2 h thereafter. Mice received a second Triton injection at 1 h, to maintain a sufficient plasma concentration, because Triton is rapidly taken up and catabolized in the liver lysosomes. Six mice from each group were studied. EDTA-plasma samples were supplemented with 0.005% gentamycin, 1 mM EDTA, and 0.04% Na-azide and protease inhibitors, and chylomicrons and VLDLs were isolated by ultracentrifugation as described (5). TG was measured in total plasma, chylomicrons, and VLDLs from individual mice.

Effects of sunflower oil and Triton WR 1339 on apolipoprotein composition of TRL

Control and transgenic δ and λ mice were fasted between 9:00 AM and 12:00 noon and then received intragastrically 0.4 ml of sunflower oil. Some of the mice from each genotype were injected with Triton WR 1339 at 15 min after the oral lipid load and again at 1 and 2 h thereafter. All animals were euthanized 3 h after sunflower oil administration. In these experiments, Triton (500 mg/kg as above) was injected in the retro-orbital plexus because this requires only very light anesthesia and thus avoids a high cumulative dose of the anesthetic. Triton has the same efficacy in blocking LPL activity whether injected in the jugular vein or in the retro-orbital plexus.

Three separate experiments were conducted with the six groups of mice (three genotypes + sunflower oil ± Triton). In each experiment, plasma was pooled from 12 control mice receiving sunflower oil only (having the lower chylomicron and VLDL concentrations in plasma) and from 8 mice from the other five groups. Plasma was supplemented with preservatives as described above and ultracentrifuged to isolate chylomicrons and VLDLs. After measuring protein content (15), chylomicrons and VLDLs were delipidated (16), and their apolipoprotein compositions were determined by SDS-PAGE using NuPAGE 4–12% Bis-Tris gels (Novex) (17). Electrophoresis was performed under nonreducing conditions to preserve the dimeric form of hapoA-II. The gels were stained with Coomassie Brilliant Blue (R250).

Statistical analysis

Results are given as means ± SEM. Statistical analysis was performed using GraphPad Prism, and differences were determined with the *t*-test for nonpaired samples after ANOVA.

RESULTS

Effect of hapoA-II expression on plasma residence time of chylomicrons

The transgenic δ and λ mice of this study expressed in the fed state hapoA-II at 50.1 ± 0.06 and 72.4 ± 0.11 mg/dl, respectively (usual concentration in humans, 25–35 mg/dl). To minimize individual variability, TRL residence time was measured in the same mouse between 2 and 8 h after an oral lipid load consisting of sunflower oil mixed with [^{14}C]triolein. Preliminary experiments established that [^{14}C]TG appeared in plasma at 1.5 h after the oral lipid load. In all groups of mice, [^{14}C]TG accounted for 87% of total radioactive plasma lipids.

An important issue in this study was to distinguish between intestinal and hepatic TRL in plasma. Because intestinal and hepatic TRL mix immediately in the circulation upon secretion and both follow the same catabolic pathway initiated by hydrolysis of their core TGs by LPL, it is not possible to distinguish unlabeled intestinal from hepatic TRL in plasma. Besides, intestinal chylomicron production is induced by dietary lipid ingestion, whereas hepatic VLDL production is a constitutive pathway. Therefore, our strategy was to greatly enrich the proportion of intestinal TRL by administration of an oral ^{14}C-labeled lipid bolus, the radioactivity allowing us to set the time of intestinal TRL secretion into the circulation, and to distinguish intestinal from hepatic TRL, at least during the initial time points.

Figure 2 shows the accumulation of [^{14}C]TG and total TG in plasma between 0 and 8 h after triolein administration. In control mice, [^{14}C]TG peaked at 3 h and decreased rapidly thereafter (Fig. 2A). In δ mice, the [^{14}C]TG peak at 3 h was three times greater than that in control mice, decreasing more slowly up to 8 h. In the high-expressing λ mice, [^{14}C]TG was considerably higher than in δ mice already at 2 h, and it continued to increase up to 8 h. The 2 and 3 h time points represented essentially intestinal TRL, as shown by the small apoB-100 content of VLDLs prepared from plasma pools in a separate experiment (see Fig. 4B below). Thereafter, hepatic VLDLs may also contribute to some extent to plasma [^{14}C]TG. Indeed, chylomicron-[^{14}C]TGs are hydrolyzed by LPL, and the released [1-^{14}C]oleic acid is taken up by tissues including the liver, and hepatocytes then partly incorporate exogenous fatty acids into VLDL-TG (18). In humans, [1-^{13}C]palmitic acid administered in a mixed meal started to appear in VLDL-TG after 3 h, whereas ~20% of dietary fatty acids entered the VLDL-TG pool after 6 h (19).

Total plasma TG was substantially greater in transgenic compared with control mice at 0 time, before the lipid bolus (Fig. 2B). This is in accord with the hypertriglyceridemia displayed by hapoA-II transgenic mice in the fed state (5, 7). Similar to [^{14}C]TG, total plasma TG increased

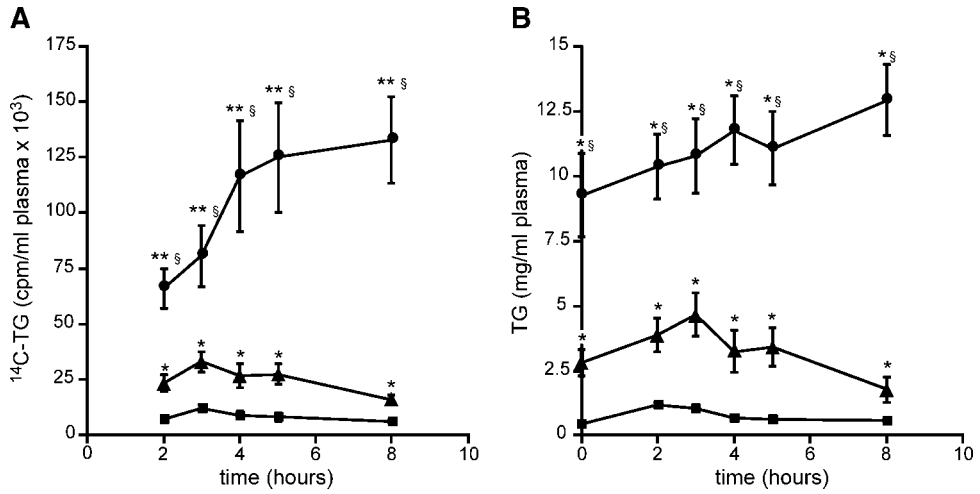

Fig. 2. Plasma residence time of chylomicrons from transgenic and control mice after an oral lipid load. Mice were fed ad libitum on chow diet. Chylomicron-triglyceride (TG) was labeled with [^{14}C]triolein after intragastric administration at 0 time of 0.4 ml of sunflower oil mixed with [^{14}C]triolein. [^{14}C]TG and total TG were measured in 50 μl plasma samples taken from the same mouse at the indicated time points. A: Plasma [^{14}C]TG. B: Plasma total TG of control mice (squares), transgenic δ mice (triangles), and transgenic λ mice (circles). Data represent means ± SEM for six animals in each group. Where not shown, SEM was smaller than the symbols. Statistically significant differences from control mice: * $P < 0.05$, ** $P < 0.001$; statistically significant differences between transgenic δ and λ mice: § $P < 0.001$.

more in transgenic compared with control mice after the oral lipid load. The pronounced and sustained TG accumulation in the circulation clearly indicated that the catabolism of chylomicrons was retarded in both transgenic mouse lines in relation to the expression level of hapoA-II.

To evaluate more accurately the plasma residence time of TRL in the high-expressing λ line, λ mice were fasted overnight and received triolein, and blood was drawn after 3 and 24 h. The 24 h period was chosen because a mild hypertriglyceridemia persisted in some λ mice after an 18 h fast (5, 7). Both [^{14}C]TG (**Fig. 3A**) and total TG (Fig. 3B) returned to basal values at 24 h. This finding is indicative of normal tissue uptake of TRL remnants.

To ascertain that the effects described above were attributable to hapoA-II without any confounding effects of endogenous mapoA-II, we measured TG accumulation in plasma of δKOAII and λKOAII mice gavaged with sunflower oil. Blood samples were taken from the same mouse at all time points studied, as above. **Table 1** shows massive TG accumulation in δKOAII and λKOAII transgenic mice compared with controls that is related to the expression level of hapoA-II. Again, plasma TG of λKOAII mice was maintained at peak levels even at 8 h after the oral lipid load. These results clearly show that hypertriglyceridemia after the oral lipid bolus was induced solely by hapoA-II.

TRL production is not altered in hapoA-II transgenic mice

To validate our hypothesis that the hypertriglyceridemia of transgenic mice after the oral lipid load resulted from impaired TRL catabolism, we measured intestinal TRL production rate after injection of Triton WR 1339, which inhibits TRL-TG hydrolysis by LPL and allows in vivo estimation of TRL production (10, 11). Two hours after sunflower oil administration, total plasma TG was similar among Triton-treated δ and λ transgenic mice and controls (**Table 2**). Chylomicron-TGs were two to three times greater than VLDL-TGs in all groups of mice, and both were comparable among transgenic and control mice. Thus, postprandial hypertriglyceridemia of transgenic mice resulted solely from impaired TRL catabolism.

Association of hapoA-II with TRL

To assess whether hapoA-II is present in intestinal TRL, the apolipoprotein composition of chylomicrons and

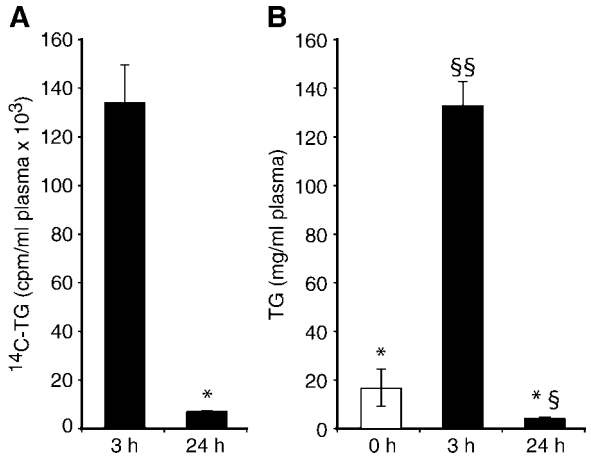

Fig. 3. Plasma TGs of hapoA-II transgenic λ mice return to normal 24 h after an oral lipid load. Four transgenic λ mice received intragastrically 0.4 ml of sunflower oil mixed with [^{14}C]triolein. They were then fasted, and 50 μl plasma samples were taken from the same mouse before the oral lipid load (0 h) and at 3 and 24 h after the lipid bolus. A: Plasma [^{14}C]TG. B: Plasma total TG. Data represent means ± SEM. Significantly different from 3 h values: * $P < 0.001$; significantly different from 0 h values: § $P < 0.05$, §§ $P < 0.001$.

TABLE 1. Plasma TG accumulation after sunflower oil administration to hapoA-II transgenic mice deficient in endogenous apoA-II

Genotype	0 h	3 h	5 h	8 h
Control	0.39 ± 0.06	1.05 ± 0.14	0.47 ± 0.05	0.40 ± 0.05
δKOAII	3.04 ± 0.59[a]	5.34 ± 0.66[b]	4.01 ± 0.48[c]	2.94 ± 0.49[c]
λKOAII	9.70 ± 2.80[a,d]	12.19 ± 1.87[c,e]	12.70 ± 1.89[c,e]	12.92 ± 1.35[c,f]

HapoA-II, human apolipoprotein A-II; KO, knockout; TG, triglyceride. Five mice from each genotype fed ad libitum received 0.4 ml of sunflower oil, as described in Materials and Methods. EDTA-plasma was obtained from each mouse before (0 h) and at 3, 5, and 8 h after the oral lipid load. Plasma TGs are expressed as mg/ml and are means ± SEM. After ANOVA, differences were determined with the *t*-test for nonpaired samples.

[a] $P < 0.05$, compared with control mice.
[b] $P < 0.01$, compared with control mice.
[c] $P < 0.001$, compared with control mice.
[d] $P < 0.05$, between transgenic δKOAII and λKOAII mice.
[e] $P < 0.01$, between transgenic δKOAII and λKOAII mice.
[f] $P < 0.001$, between transgenic δKOAII and λKOAII mice.

VLDLs was analyzed in control and transgenic mice 3 h after administration of either sunflower oil alone or both sunflower oil and Triton, which coats the surface of lipoproteins and impairs exchanges and catabolism by LPL (10, 11). The 3 h time point was chosen because it is the peak of TRL-[^{14}C]TG in plasma and is reportedly the time point with the greatest enrichment of intestinal TRL (19). These experiments were performed with the original δ and λ transgenic lines as well as with the δKOAII and λKOAII lines, with identical results.

Three hours after sunflower oil administration, small amounts of chylomicrons and VLDLs were present in control mice, as expected from the rapid TG clearance shown in Fig. 2. Using a greater number of animals in this group (12 instead of 8), we analyzed VLDL apolipoproteins (**Fig. 4B**), whereas chylomicron apolipoproteins were too low (data not shown). On the contrary, transgenic mice displayed appreciable amounts of chylomicrons and VLDLs, again in accord with the prolonged TG accumulation in plasma (Fig. 2). Both chylomicrons (Fig. 4A) and VLDLs (Fig. 4B) of δKOAII and λKOAII mice transported apoB-48, apoE, apoCs, and an appreciable amount of hapoA-II. The presence of apoA-I in VLDL is probably indicative of a greater intestinal production after the oral lipid load. Indeed, we consistently detected apoA-I in TRL of mice fed a high-fat diet (unpublished data), which increases intestinal TRL production (9).

TABLE 2. TG-rich lipoprotein production after sunflower oil and Triton WR 1339 administration

Genotype	Total Plasma-TG	Chylomicron-TG	VLDL-TG
Controls	4.93 ± 0.64	2.88 ± 0.37	1.00 ± 0.13
hAIItg-δ	5.09 ± 0.93	2.95 ± 0.48	1.16 ± 0.16
hAIItg-λ	5.23 ± 1.15	2.05 ± 0.54	1.25 ± 0.28

Six mice from each genotype received sunflower oil and Triton WR 1339, as described in Materials and Methods. EDTA-plasma was obtained from each mouse before (0 h) and 2 h after sunflower oil and Triton administration. TG values of total plasma, chylomicrons, and VLDLs are expressed as mg/ml and are differences between 2 h and 0 h measurements. They are means ± SEM and were analyzed by ANOVA followed by Tukey's multiple comparison test. Differences among experimental groups were not statistically significant.

After sunflower oil and Triton administration, control mice displayed increased chylomicron and VLDL amounts, attributable to the inhibition of TRL catabolism by LPL. Thus, TRL of Triton-treated control and transgenic mice contained similar apolipoprotein amounts. Irrespective of genotype, chylomicrons and VLDLs essentially carried apoB-48 and little apoB-100, which is indicative of a great enrichment of intestinal TRL (Fig. 4A, B, respectively). The small apoB-100 amount of TRL corresponds to the presence of a small proportion of hepatic VLDL, because VLDL secretion is a constitutive pathway and cannot be stopped in vivo. Of note, the impairment of apolipoprotein exchanges by Triton resulted in the complete absence of hapoA-II in TRL. Interestingly, apoE and apoCs were also absent from TRL of animals treated with Triton for 3 h, implying that these apolipoproteins essentially associate with TRL in the plasma compartment. We verified that VLDL prepared from chow-fed or overnight-fasted δ and λ transgenic mice not gavaged with sunflower oil also contained hapoA-II in the absence but not in the presence of Triton (**Fig. 5**). Because VLDLs of chow-fed mice are essentially of hepatic origin, they contain great amounts of apoB-100. Finally, VLDLs from transgenic mice were incubated in vitro without or with Triton for 15 min, either at room temperature or at 37°C. VLDL apolipoprotein composition was not modified by in vitro addition of Triton (**Fig. 6**).

Together, these observations strongly suggest that TRLs of transgenic mice acquire hapoA-II after their secretion in the plasma compartment. The presence of hapoA-II in chylomicrons correlates with the hypertriglyceridemia measured after the oral lipid load and thus may account for the impaired hydrolysis of their TGs by LPL.

DISCUSSION

This study focused on the mechanisms of hapoA-II association with TRL and the effects on TRL catabolism. We show that TRLs produced after an oral lipid load accumulated in the plasma of hapoA-II transgenic mice but were rapidly cleared from the circulation of control mice. In transgenic mice, chylomicrons (produced by the

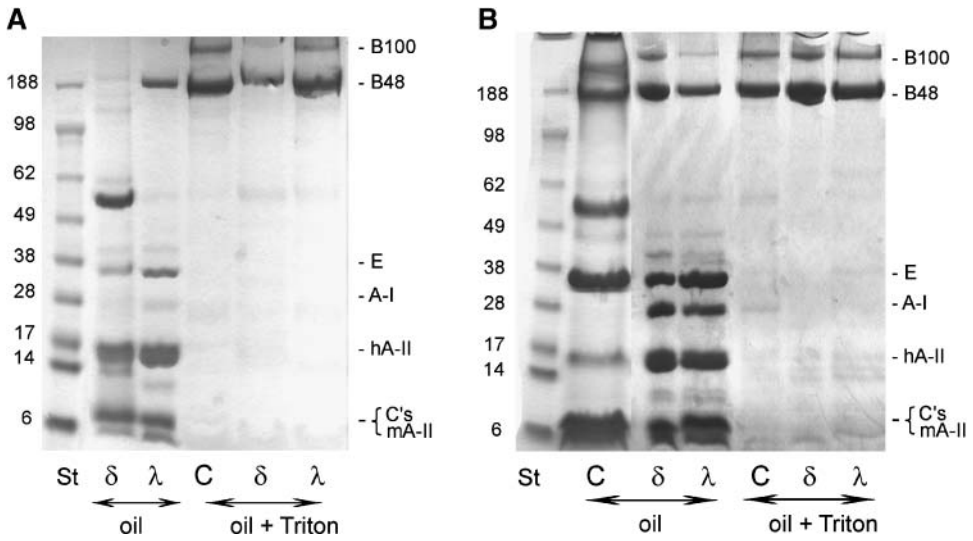

Fig. 4. Apolipoprotein composition of chylomicrons and VLDLs isolated 3 h after an oral lipid load. Mice were fasted between 9 AM and 12 noon and then received intragastrically 0.4 ml of sunflower oil. Some of the mice in each group were injected with Triton at 15 min after gavage. Plasma pools were prepared from 12 control mice receiving only the lipid bolus and from 8 mice in all other groups. The same results were obtained with the original δ and λ mice and with the δKOAII and λKOAII mice. Findings from one of three separate experiments with similar results are shown, using δKOAII and λKOAII mice. Chylomicrons (A) and VLDLs (B) were prepared by ultracentrifugation and delipidated, and their apolipoproteins were subjected to electrophoresis (12–15 μg protein/lane) using 4–12% Bis-Tris gradient gels with MES-SDS buffer (NuPAGE; Novex). The gels were stained with Coomassie blue. The small band at ~17 kDa is not hapoA-II, as assessed by Western blotting with an antibody specific for hapoA-II. St, molecular mass standards (Novex), with the corresponding sizes of the markers shown at left in kDa; C, control mice; δ, transgenic δ mice; λ, transgenic λ mice.

intestine) carried hapoA-II (produced by the liver), strongly suggesting apoA-II transfer to TRL in the circulation. The absence of hapoA-II in TRL of transgenic mice injected with Triton, which coats the surface of lipoproteins, thereby hindering exchanges (11), confirmed that hapoA-II associates with TRL in plasma. Together, these results demonstrate that association of hapoA-II with TRLs in the circulation impairs their catabolism and induces postprandial hypertriglyceridemia, the extent and duration of which depend on the expression level of hapoA-II. Any confounding effects of endogenous mapoA-II were excluded by the use of hapoA-II transgenic mice deficient in mapoA-II.

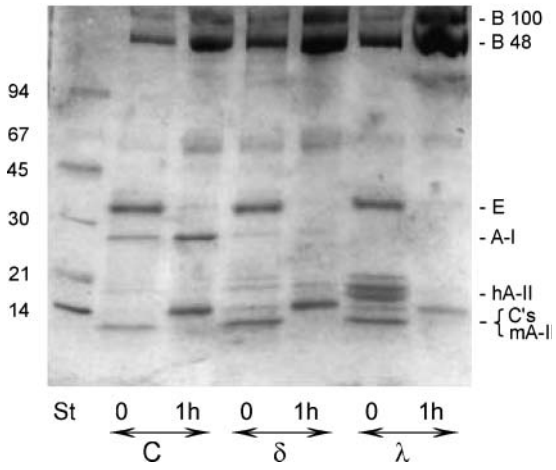

Fig. 5. VLDL apolipoprotein composition of Triton-treated mice not receiving an oral lipid load. VLDLs were prepared from hapoA-II transgenic and control mice fed a standard chow diet before and 1 h after Triton injection. Under these conditions, VLDL is mainly produced in the liver. VLDL was delipidated, and apolipoproteins were analyzed by SDS-PAGE using 4–20% gradient gels (Bio-Rad). St, low molecular mass standards (Pharmacia Amersham Biotechnologies) with the corresponding sizes of the markers shown at left in kDa; C, control mice; δ, transgenic δ mice; λ, transgenic λ mice.

The main objective of this study was to investigate whether the association of hapoA-II with TRLs is sufficient to impair their catabolism and induce hypertriglyceridemia. Because apoA-II is synthesized in liver, we sought to determine whether apoA-II would associate with intestinal TRLs and impair their catabolism. Thus, in this study, mice received a radioactive oral lipid bolus that served the dual purpose to set the time of food intake and to help differentiate intestinal from hepatic TRLs. The questions now arise of up to which time point do plasma [^{14}C]TGs originate from intestine, and from which time point onward do they also originate from liver? Indeed, part of the fatty acids resulting from TRL-TG hydrolysis are taken up by the liver, esterified, and secreted as VLDL-TG. Secretion of exogenous fatty acids can account for 30% of VLDL-TG secreted by hepatocytes (18). A recent study in humans showed that [1-^{13}C]palmitic acid included in a mixed meal started to appear in chylomicron remnants at 1.5 h after the meal and peaked after 3 h, whereas there was at least a 1.5 h delay before the label appeared in the

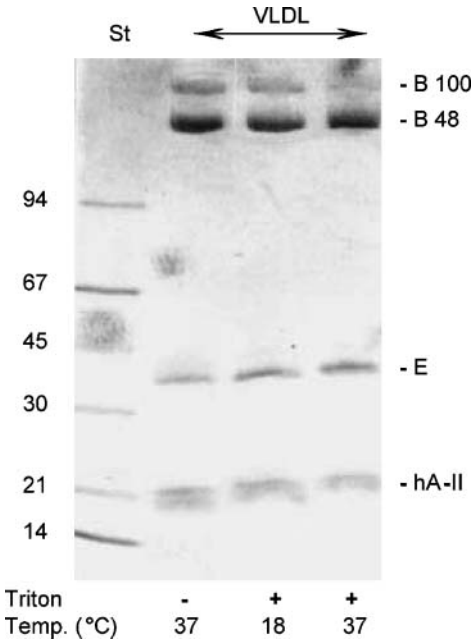

Fig. 6. Triton does not modify in vitro VLDL apolipoprotein composition. VLDLs isolated from transgenic mice fed ad libitum were incubated with or without Triton (10 mg/ml) for 15 min, either at room temperature or at 37°C. VLDL apolipoproteins were delipidated and analyzed by SDS-PAGE using 4–20% gradient gels (Bio-Rad). St, low molecular mass standards; Temp, incubation temperature.

VLDL-TG pool (19). Thus, it is reasonable to assume that in our study, plasma TG is essentially of intestinal origin at 3 h, at least in control mice. Plasma TG clearance is greatly decreased in transgenic δ and λ mice compared with controls, not only 3 h after triolein but also at later time points. Furthermore, the decrease in TG clearance is related to plasma hapoA-II content, because it is far greater in λ versus δ mice. The decreased TG clearance in transgenic mice is in favor of intestinal TG accumulation for longer time points. In the high-expressing λ line, we even observed a lack of decline of plasma radioactive and total TG up to 8 h after administration of the oral lipid load, suggesting minimal hydrolysis by LPL and thus a minimal contribution of hepatic VLDL.

An important question is whether plasma TG accumulation resulted solely from the association of hapoA-II with TRL or whether this effect was also attributable to endogenous mapoA-II. Both hapoA-II and mapoA-II expression in transgenic mice induces hypertriglyceridemia, which is more pronounced in hapoA-II compared with mapoA-II transgenic mice (5, 7, 13, 20). However, hapoA-II expression decreases plasma HDLs that have smaller particle sizes and very low apoA-I contents (5, 7, 13), whereas high mapoA-II expression results in higher concentrations of large HDLs rich in apoA-I and mapoA-II (20). To answer this question, we used δKOAII and λKOAII transgenic mice deficient in mapoA-II. These mice display similar HDL and apoA-I decreases as well as the same preponderance of small HDLs carrying large hapoA-II amounts as the original δ and λ lines (A. D. Kalopissis, D. Pastier, and X. Rousset, unpublished results). The occurrence of plasma TG accumulation after an oral lipid bolus in δKOAII and λKOAII transgenic mice clearly established that hapoA-II is the protein responsible for a dose-dependent induction of hypertriglyceridemia. Interestingly, injection of hapoA-II, either in a lipid-free form or in reconstituted spherical HDLs, into rabbits (a species deficient in apoA-II) resulted in TG enrichment of the endogenous VLDL and HDL (21), establishing the hypertriglyceridemic effect of hapoA-II in vivo.

What is the mechanism underlying the association of hapoA-II, a major HDL apolipoprotein, with TRL? The first possibility is that hapoA-II, synthesized mainly in the liver (5, 13), associates with nascent VLDL in the hepatocyte and is secreted in the circulation on VLDL. The second possibility is that hapoA-II is secreted by hepatocytes as a lipid-poor apolipoprotein and associates partly with TRL in the plasma compartment. In our first study, δ and λ transgenic mice displayed hypertriglyceridemia when fed a low-fat chow diet, with accumulation of large VLDLs containing hapoA-II and apoB-100 that are solely produced in liver (5). Therefore, the hepatic origin of apoA-II and VLDL did not allow determination of the site of association of apoA-II with TRL. In this study, the sites of synthesis of TRL and apoA-II were dissociated through the induction of intestinal chylomicron production by an oral lipid load. The presence of hapoA-II on chylomicrons at 3 h after the oral lipid load, when the majority of TRLs are of intestinal origin (as explained above), clearly shows that hapoA-II transfers to TRL in the circulation. This was further confirmed by the absence of hapoA-II in TRLs of transgenic mice receiving triolein and Triton WR 1339, which coats the surface of lipoproteins and hinders apolipoprotein exchanges and LPL accessibility (10). Although we cannot exclude the possibility that some apoA-II molecules may also associate with nascent VLDLs in the hepatocyte before secretion, this is unlikely because hapoA-II was absent from VLDLs of Triton-treated transgenic mice not gavaged with triolein (Fig. 5).

Because the intestinal TRL production rate was comparable among transgenic and control mice, it follows that hapoA-II expression only affected TRL catabolism. Coupled with the observation that TRL carried hapoA-II, this strongly suggests that hapoA-II on the surface of TRL inhibits LPL activity, probably by hindering LPL access to the lipoprotein surface. Indeed, addition of hapoA-II to postheparin plasma of control mice inhibited LPL and HL activities in a dose-dependent manner, and VLDL-TG from hapoA-II transgenic mice were hydrolyzed less efficiently by LPL compared with VLDL from control mice (5). Interestingly, in the course of a paradoxical hypertriglyceridemia obtained by fenofibrate treatment of independently established high-expressing hapoA-II transgenic mice, a similar defect in catabolism by LPL of hapoA-II-carrying VLDLs was reported (22). The apoA-II-KO mouse fits, in an inverse manner, our hypertriglyceridemic mice expressing hapoA-II. When apoA-II-KO mice were crossed with apoE-KO mice, which display dramatic accumulation of cholesterol-rich remnants, VLDL clearance was greatly

increased (12). Thus, when apoA-II was absent, both LPL and HL apparently functioned at maximal activities.

An important issue is whether apoA-II influences plasma TG levels in humans. The common −265T/C polymorphism in the promoter region of the hapoA-II gene was shown to be associated with a small decrease in plasma apoA-II in human carriers of the −265C/C allele compared with the −265T/T allele (23). The −265C allele reduced the basal rate of transcription in transfection studies compared with the −265T allele. Moreover, oral fat tolerance tests provided evidence that subjects homozygous for the −265C allele have faster clearance of postprandial large VLDLs compared with subjects homozygous for the −265T allele (23). Thus, small variations in plasma apoA-II levels are sufficient to influence postprandial triglyceridemia. Besides, variation of apoA-II plasma levels was associated with plasma TG in familial combined hyperlipidemia (24), whereas VLDL from patients with Tangier disease or type V dyslipidemia who carry apoA-II was less efficiently catabolized by LPL (8).

What is the physiological relevance of the presence of apoA-II in TRL? A careful review of the literature reveals that apoA-II is partly/totally transported by TRL in metabolic situations with low/extremely low plasma HDL concentrations, such as *i*) Tangier disease and type V hyperlipoproteinemia patients, their VLDL carrying apoA-II (8), and *ii*) LPL-KO mice rescued at birth by transient adenovirus-mediated expression of LPL in liver. Adult LPL-KO mice practically lack HDL, and both apoA-II and apoA-I are present in TRL (25). We propose that a low number of HDL particles may not accommodate all newly secreted, lipid-poor apoA-II, so that part of apoA-II associates with TRL. The resulting impairment of TRL catabolism may participate in the increased cardiovascular risk conferred by low plasma HDL. Indeed, pronounced postprandial lipemia after a fatty meal impaired endothelium-dependent dilation of the brachial artery in men, which represents a surrogate functional marker for early atherosclerotic disease (26). Conversely, vascular smooth muscle function significantly improved after decreasing plasma TG by fenofibrate treatment, without changes in confounding lipoproteins or insulin resistance (2). Further studies are needed to establish the effects of hapoA-II-induced postprandial hypertriglyceridemia in vascular endothelial cell metabolism.

In conclusion, a moderate overexpression of hapoA-II in mice led to postprandial accumulation of intestinal TRL for several hours, in a manner comparable to the accumulation of hepatic VLDL we reported previously (5, 7). The transfer of hapoA-II on the surface of TRL in the plasma compartment is probably the main mechanism underlying defective TRL catabolism by LPL and may be linked to low plasma HDL content (5, 7). The impaired LPL activity in turn results in decreased availability of TRL surface components for HDL formation, thus contributing to lower plasma HDL concentrations. Thus, we have identified in hapoA-II transgenic mice a novel mechanism of postprandial hypertriglyceridemia that is related to low plasma HDL levels.

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REFERENCES

1. Miller, G. J., and N. E. Miller. 1975. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet.* **1:** 16–19.
2. Capell, W. H., C. A. DeSouza, P. Poirier, M. L. Bell, B. L. Stauffer, K. M. Weil, T. L. Hernandez, and R. H. Eckel. 2003. Short-term triglyceride lowering with fenofibrate improves vasodilator function in subjects with hypertriglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* **23:** 307–313.
3. Haffner, S. M. 2006. The metabolic syndrome: inflammation, diabetes mellitus, and cardiovascular disease. *Am. J. Cardiol.* **97 (Suppl.):** 3A–11A.
4. Grundy, S. M., J. I. Cleeman, S. R. Daniels, K. A. Donato, R. H. Eckel, B. A. Franklin, D. J. Gordon, R. M. Krauss, P. J. Savage, S. C. Smith, Jr., et al. 2006. Diagnosis and management of the metabolic syndrome. An American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Curr. Opin. Cardiol.* **21:** 1–6.
5. Boisfer, E., G. Lambert, V. Atger, N. Q. Tran, D. Pastier, C. Benetollo, J-F. Trottier, I. Beaucamps, M. Antonucci, M. Laplaud, et al. 1999. Overexpression of human apolipoprotein A-II in mice induces hypertriglyceridemia due to defective very low density lipoprotein hydrolysis. *J. Biol. Chem.* **274:** 11564–11572.
6. Sauvaget, D., V. Chauffeton, S. Dugué-Pujol, A. D. Kalopissis, I. Guillet-Deniau, F. Foufelle, J. Chambaz, A. Leturque, P. Cardot, and A. Ribeiro. 2004. In vitro transcriptional induction of the human apolipoprotein A-II gene by glucose. *Diabetes.* **53:** 672–678.
7. Pastier, D., S. Dugué, E. Boisfer, V. Atger, N. Q. Tran, A. van Tol, M. J. Chapman, J. Chambaz, P. M. Laplaud, and A. D. Kalopissis. 2001. Apolipoprotein A-II/A-I ratio is a key determinant in vivo of HDL concentration and formation of pre-beta HDL containing apolipoprotein A-II. *Biochemistry.* **40:** 12243–12253.
8. Alaupovic, P., C. Knight-Gibson, C-S. Wang, D. Downs, E. Koren, H. B. Brewer, Jr., and R. E. Gregg. 1991. Isolation and characterization of an apoA-II-containing lipoprotein (LP-AII:B complex) from plasma VLDL of patients with Tangier disease and type V hyperlipoproteinemia. *J. Lipid Res.* **32:** 9–19.
9. Kalopissis, A. D., S. Griglio, and X. Le Liepvre. 1982. Intestinal very low density lipoprotein secretion in rats fed various amounts of fat. *Biochim. Biophys. Acta.* **711:** 33–39.
10. Otway, S., and D. S. Robinson. 1967. The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Physiol.* **190:** 321–332.
11. Schotz, M. C., A. Scanu, and I. H. Page. 1956. Effect of Triton on lipoprotein lipase of rat plasma. *Am. J. Physiol.* **188:** 399–402.
12. Weng, W., and J. L. Breslow. 1996. Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. *Proc. Natl. Acad. Sci. USA.* **93:** 14788–14794.
13. Marzal-Casacuberta, A., F. Blanco-Vaca, B. Y. Ishida, J. Julve-Gil, J. Shen, S. Calvet-Marquez, F. Gonzalez-Sastre, and L. Chan. 1996. Functional lecithin:cholesterol acyltransferase deficiency and high density lipoprotein deficiency in transgenic mice overexpressing human apolipoprotein A-II. *J. Biol. Chem.* **271:** 6720–6728.
14. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
16. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions of ethanol of the small molecular weight peptides of the

serum very low density and high density lipoproteins: relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44:** 576–588.
17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
18. Kalopissis, A. D., S. Griglio, M. I. Malewiak, R. Rozen, and X. Le Liepvre. 1981. Very low density lipoprotein secretion by isolated hepatocytes of fat-fed rats. *Biochem. J.* **198:** 373–377.
19. Heath, R. B., F. Karpe, R. W. Milne, G. C. Burdge, S. A. Wootton, and K. N. Frayn. 2003. Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *J. Lipid Res.* **44:** 2065–2072.
20. Hedrick, C. C., L. W. Castellani, C. H. Warden, D. L. Puppione, and A. J. Lusis. 1993. Influence of mouse apolipoprotein A-II on plasma lipoproteins in transgenic mice. *J. Biol. Chem.* **268:** 20676–20682.
21. Hime, N. J., K. J. Drew, K. Wee, P. J. Barter, and K-A. Rye. 2006. Formation of high density lipoproteins containing both apolipoprotein A-I and A-II in the rabbit. *J. Lipid Res.* **47:** 115–122.
22. Ribas, V., X. Palomer, N. Roglans, N. Rotllan, C. Fievet, A. Tailleux, J. Julve, J. C. Laguna, F. Blanco-Vaca, and J. C. Escolà-Gil. 2005. Paradoxical exacerbation of combined hyperlipidemia in human apolipoprotein A-II transgenic mice treated with fenofibrate. *Biochim. Biophys. Acta.* **1737:** 130–137.
23. Van't Hooft, F. M., G. Ruotolo, S. Boquist, U. de Faire, G. Eggertsen, and A. Hamsten. 2001. Human evidence that the apolipoprotein A-II gene is implicated in visceral fat accumulation and metabolism of triglyceride-rich lipoproteins. *Circulation.* **104:** 1223–1228.
24. Allayee, H., L. W. Castellani, R. M. Cantor, T. W. A. de Bruin, and A. J. Lusis. 2003. Biochemical and genetic association of plasma apolipoprotein A-II levels with familial combined hyperlipidemia. *Circ. Res.* **92:** 1262–1267.
25. Strauss, J. G., S. Frank, D. Kratky, G. Hämmerle, A. Hrzenjak, G. Knipping, A. von Eckardstein, G. M. Kostner, and R. Zechner. 2001. Adenovirus-mediated rescue of lipoprotein lipase-deficient mice. *J. Biol. Chem.* **276:** 36083–36090.
26. Gaenzer, H., W. Sturm, G. Neumayr, R. Kirchmair, C. F. Ebenbichler, A. Ritsch, B. Föger, G. Weiss, and J. R. Patsch. 2001. Pronounced postprandial lipemia impairs endothelium-dependent dilation of the brachial artery in men. *Cardiovasc. Res.* **52:** 509–516.