Mechanism of lipid lowering in mice expressing human apolipoprotein A5

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Abbreviations: APOA5, the human gene; *apoa5*, the rodent gene; ApoAV, the human protein;

ABSTRACT

Recently, we reported that apoAV plays key role in triglycerides lowering. Here, we attempted to determine the mechanism underlying this hypotriglyceridemic effect. We showed that triglyceride turnover is faster in *hAPOA5* transgenic compared to wild type mice. Moreover, both apoB and apoCIII are decreased and LPL activity is increased in postheparin plasma of *hAPOA5* transgenic mice. These data suggest a decrease in size and number of VLDL. To further investigate the mechanism of *hAPOA5* in hyperlipidemic background, we intercrossed *hAPOA5* and *hAPOC3* transgenic mice. The effect resulted in a marked decreased of VLDL triglyceride, cholesterol, apolipoproteins B and CIII . In postprandial state, the triglyceride response is abolished in *hAPOA5* transgenic mice. We demonstrated that in response to the fat load in *hAPOA5*X*hAPOC3* mice, apoAV shifted from HDL to VLDL, probably to limite the elevation of triglycecrides. *In vitro*, apoAV activates lipoprotein lipase. However, apoAV does not interact with LPL but interacts physically with apoCIII. This interaction does not seem to displace apoCIII from VLDL but may induce conformational change in apoCIII and consequently change in its function leading the activation of lipoprotein lipase.

Key words: apoAV, apoCIII, lipoprotein lipase, triglyceride turnover and postprandial.

INTRODUCTION

Recently, two different groups discovered a new apolipoprotein, apoAV [1, 2]. ApoAV is a 366 amino acids protein synthesized by the liver, and in plasma of normolipidemic subjects it is associated with VLDL and mainly with HDL. ApoAV affects triglyceride metabolism, transgenic mice overexpressing *APOA5* had serum triglyceride levels that were one-third of those of control mice, whereas mice deficient in *apoa5* developed hypertriglyceridemia [1]. Moreover, adenoviral overexpression of *APOA5* reduces serum levels of triglycerides and cholesterol in mice [3]. This determinant link between apoAV and triglycerides was supported in several separate human studies through the consistent demonstration of associations between *APOA5* single nucleotide polymorphisms and plasma triglyceride levels [4-11]. Recent studies established a relationship between *APOA5* gene variants and lipids, lipoproteins and apolipoproteins as well as lipoproteins subfractions [12, 13]. Beside the genetic determination of triglyceride levels, the variations in the *APOA5* gene could also influence risk of myocardial infarction [14]. Taken together these mouse and human studies highlight the importance of *APOA5* in the determination of triglyceride plasma levels. Finally, PPARα agonists are known to have hypotriglyceridemic effect, and recent studies have shown that the *APOA5* gene is highly up-regulated by PPARα and FXR [15, 16].

Apolipoprotein CIII plays also an important role in controlling plasma triglyceride metabolism and in determining plasma concentration of atherogenic triglyceride-rich lipoproteins (TRL) [17]. ApoCIII is a 79 amino acids protein synthesized by the liver and the intestine [18], is a component of chylomicrons, very low density lipoproteins (VLDL) and high density lipoproteins (HDL). [19] Plasma concentration of apoCIII is positively correlated with levels of plasma triglyceride [20, 21], Liver perfusion studies demonstrate that apoCIII inhibits the hepatic uptake of TRL and their remnants [22, 23], whereas *in vitro* experiments show that apoCIII inhibits the activity of both lipoprotein lipase (LPL) and hepatic lipase [24-

27]. ApoCIII, therefore modulates the plasma catabolism and clearance of TRL. This is of pathophysiological significance as indicated by angiographic studies showing that plasma lipoprotein distribution of apoCIII is a statically significant independent predictor of the progression or severity of coronary artery diseases (CAD) [28-30]. The role of apoCIII in plasma TRL metabolism has been more defined by the results in transgenic animals [31]. Plasma accumulation of TRL in mice overexpressing *hAPOC3* has been shown to be associated with reduced plasma VLDL and chylomicrons remnants clearance, apparently due to reduced binding of TRL to LDL receptor [32] and to heparan sulfate proteoglycans [33]. The inhibitory effect of C apolipoproteins on the LDL receptor of apo B-containing lipoproteins was also demonstrated [34]. Decreased receptor binding was reversed by addition of exogenous apo E. Immunologicals and cryo-electron microscopy studies have indicated that apoCIII masked some apo B100 epitopes and modified its conformation [33].

In this study, we investigated the mechanism by which apoAV affects lipid metabolism. We showed that apoAV accelerates the VLDL clearance by activating the lipolysis and enhancing VLDL removal. We showed that in hyperlipidemic background apoAV affects also the cholesterol levels in apo B containing lipoproteins. We also reported that apoAV prevents the elevation of triglyceride in response to the fat load. Furthermore, apoAV may activate indirectly the lipoprotein lipase through a physical interaction with apoCIII.

MATERIALS AND METHODS

Animals. Two lines of transgenic mice expressing the human *APOC3* and *APOA5* genes were crossed. Transgenic animals were identified by ELISA using polyclonal antibodies from goat against human apo C-III or ApoAV. Animals were kept under conditions of controlled temperature (20 ± 1°c) and lighting (dark from 8 p.m. to 8 a.m.) in a room of low background noise.

For experiment, both male and female were used. The average age of the mice was 7 weeks.

Bleeding were performed, from 4 hours fasting mice, by the retroorbital plexus under anesthesia with diethyether. Blood samples were mixed with EDTA and kept at 4°c.

Lipids measurements. Plasma was separated through centrifugation for 20 minutes at 4°c. Lipids were determined enzymatically with commercially available kits for cholesterol and triglycerides.

Apolipoprotein measurements. Plasma levels of human apoCIII were measured by kinetic immunonephelemetric system (Immage, Beckman Coulter) using polyclonal antibodies produced against total synthetic apoCIII in goat.

An enzyme-linked immunosorbent sandwich assay was used to measure ApoAV in sera. A pool of two monoclonal anti-human ApoAV antibodies solution, raised in mice by using recombinant protein, was used at 10 µg/mL in PBS 0.1 M, pH 7.2 to coat the wells of the microtiter plates at room temperature overnight. The wells were washed twice with PBS 0.1 M. The remaining sites for protein binding were saturated with 3% BSA/PBS for 1 hour at 37°C. 90 µL of the antigen solution was added to the wells. For quantitation, a pool of human plasma was calibrated and titrated using ApoAV recombinant protein as a primary standard. Then, the pool of human plasma was used for the calibration curve. All dilutions were done in

the blocking buffer (1% BSA/PBS). The antigen solution is incubated for 2 hours at room temperature. The wells were washed four times with PBS. The horseradish peroxidase labeled second anti-ApoAV polyclonal antibody, produced in rabbit using synthetic peptide, was diluted in the blocking buffer and added to the wells. After incubation of 2 hours at 37°C, the plates were washed with several changes of PBS. Prior to developing the enzyme label, 30 mg of o-phenylenediamine (ODP) was dissolved in 20 mL 0.1 M citrate/phosphate buffer and 20 µL of 30% H_2O_2. Then 100 µL of the enzyme substrate solution were added to each micotiter well. After incubation of 30 min at room temperature in the dark, the reaction was terminated by adding 100 µL of HCl l M and the absorbance at 492 nm was measured using a microplate photometer (Dynex Technologies).

Lipoprotein analysis. Plasma lipoproteins from pooled mouse plasma were separated by gel filtration chromatography using a Superose 6HR 10/30 column (Pharmacia LKB Biotechnology). The gel was equilibrated with PBS (10mmol/L) containing 0.1 g/L sodium azide. Plasma were eluted with the buffer at room temperature at a flow rate of 0.2 ml/min. Elution profiles were monitored at 280 nm and record with an analog-recorder chart tracing system (Pharmacia LKB biotechnology). The elution fraction numbers (0.24 ml for each) of the plasma lipoproteins separated by FPLC were VLDL, 10-18. IDL/LDL, 20-30 and HDL, 30-40. Lipids and apolipoproteins in the recovered fractions were assayed as described above.

***In vivo* triglycerides-VLDL metabolism.** *In vivo* [^{3}H]-triglyceride-labeled VLDL turnover studies were based on previously described method [35]. Briefly, [3H]-palmitate acid dissolved in ethanol was evaporated under nitrogen and redissolved in 0.9% NaCl containing 2 mg/ml bovine serum albumin to final a concentration of 1mCi/ml. *hAPOC3* transgenic mice were injected intravenously via the tail vein with 100 µCi of the prepared [3H]-palmitate and

bled from abdominal aorta 25 min after injection. Radiolabeled VLDL were isolated from pooled mouse plasma by ultracentrifugation (d<1.006 g/ml). To study the *in vivo* clearance of labeled VLDL triglycerides, *hAPOA5* transgenic mice and wild type mice were injected intraveinously with 80.000 dpm of [3H]-triglyceride-labeled VLDL. The clearance rate of the radiolabeled VLDL was used to represent VLDL radioactivity.

Triglyceride and lipoprotein lipase activity in postheparin plasma. Mice were injected with heparin and blood samples were taken from each mouse at 5 min after injection. The triglyceride levels are measured as described above. The lipoprotein lipase (LPL) activity was assayed in triplicate using ^{14}C-labeled triolein substrate in 5M and M NaCl as previously described [36].

Lipoprotein lipase activation : LPL activity was assayed by measuring the amount of NEFA liberated by the action of the bovine lipoprotein lipase (from Sigma, St. Louis, MO, USA). 50µl VLDL enriched or not with recombinant ApoAV was incubated with 50µl of a lipolysis buffer (0.1M Tris buffer pH 8.5, 1.2% albumine bovine essentially NEFA-free w/v) containing bovine LPL (40U/ml) for 60 min at 37°C. The reaction was stopped by the addition of 50µl of 50mM KH_2PO_4, 0.1% Triton X-100, pH 6.9, and the mixture placed on ice. NEFA were measured enzymically with a NEFA-C kit (Wako Chemicals G.m.b.H., Neuss, Germany).

Postprandial triglyceride response : Mice were fasted overnight. After taking a basal blood sample by tail bleeding at t=0, mice received a single bolus of 500 µl of sunflower oil. Additional blood samples were taken from mice at 1, 2, 3, 4, 5, 6, and 7 hours after sunflower

oil administration. Plasma triglyceride levels were measured at the different time points as described above.

Real-Time Biomolecular Interaction Analysis using Biacore 3000 : BIAcore system, sensor chip CM5, HBS-EP buffer and amine coupling kit containing 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC), N-hydroxysuccinimide (NHS) and 1M ethanolamine-HCl pH 8.0 were obtained from Biacore AB, Uppsala, Sweden.

Real time biomolecular interaction analyses were performed by surface plasmon resonance technology using a Biacore® 3000 system. All experiments were conducted at 25°C. The running buffer used was HBS-EP (10mM HEPES pH 7.4, 0.15M NaCl, 3mM EDTA, 0.005% Surfactant P20) at a flow of 10µl/min. Recombinant apoAV was coupled to the dextran-modified gold surface of a CM5 sensor chip by amine coupling as described in the BIAcore system manual. Briefly, the dextran surface of the chip was activated by mixing equal volumes of 400mM EDC and 100mM NHS (freshly prepared in ultrapure water and immediately stored at –20°C) and injecting the mixture over the sensor surface for 15min at a flow rate of 10µl/min. The remaining activated groups were blocked by injection of 1M ethanolamine (pH 8.0) for 7 min at a flow rate of 5µl/min. The immobilisation level was 13000RU corresponding to 13ng/mm^2 of protein. A non-protein, blocked surface (flow cell 1) served as a blank, and sensorgrams for this cell were subtracted from all others.

Samples (30µl) were injected over the chip at a flow rate of 10µl/min, followed by a washing phase with HBS-EP buffer to achieve a steady baseline. After each cycle the chip was regenerated using 5µl of a 0,1%TFA solution. The analysis of the association and dissociation phase was made with the Biacore 3000 Control Software and BIAevaluation Software version 4.0.1.

RESULTS

ApoAV accelerates the VLDL triglyceride turnover.

The marked decrease of triglycerides in *hAPOA5* transgenic mice, suggests that apoAV may influence either the VLDL triglyceride secretion or VLDL triglyceride clearance. First, we measured the VLDL triglyceride production using the Triton WR1339 method in wild type and *hAPO5* transgenic mice. The increase in plasma triglycerides was equal in both mice models (data not shown).

We next investigated whether the VLDL triglyceride decrease in *hAPOA5* transgenic mice is due to enhanced triglyceride clearance. To study this, wild type and *hAPOA5* transgenic mice were injected with [^{3}H]-triglyceride-labeled VLDL. As shown in Fig. 1, triglycerides were more rapidly cleared from the circulation in *hAPOA5* transgenic mice than in wild type.

Postheparin lipolytic activity.

To examine if this enhanced clearance of triglycerides in *hAPOA5* transgenic mice was due to enhanced lipolysis, we determined the postheparin plasma lipase activities. The LPL activities were measured in postheparin plasma (PHP) from wild type and *hAPOA5* transgenic mice using the labeled triolein substrate. The LPL activity in PHP is increased in *hAPOA5* transgenic mice compared to that in wild type mice (Fig. 2).

ApoCIII and apoB levels in hAPOA5 transgenic mice.

To investigate whether the activation of triglyceriderich lipoproteins hydrolysis by LPL is accompanied by a removal of the VLDL particles, we examined the apoCIII and apoB levels in the *hAPOA5* transgenic and wild type mice. Triglycerides are decreased but no significant difference in cholesterol was seen between wild type and *hAPOA5* transgenic mice models

(data not shown) confirming what has been reported before in this model, probably the because cholesterol is mainly in HDL As shown in figure 3, the plasma mouse apoCIII and apoB are decreased in the *hAPOA5* transgenic compared to wild type mice. These data suggest that the overexpression of *APOA5* induces also a decrease of VLDL number.

Overexpression of human APOA5 in hAPOC3 transgenic improves the hyperlipemia.

To further investigate the effect of apoAV on lipids, we crossed the hyperlipemic model overexpressing *hAPOC3* with *hAPOA5* transgenic mice. Plasma triglycerides are decreased (55% lower), and by contrast to *hAPOA5* transgenic model, total cholesterol levels in the double transgenic mice are also decreased compared to the *hAPOC3* transgenic mice as shown in the table 1. Pool of plasma were fractionnated by FPLC and the lipoprotein analyses showed that the decrease of triglyceride levels occured in VLDL and cholesterol levels occurred only in apoB containing lipoproteins (Fig. 4A and 4B). The plasma hapoCIII, measured by nephelometry, in the double transgenic *hAPOC3*X*hAPOA5* mice is markedly decreased by 60%, compared to *hAPOC3* transgenic mice (Fig. 5A) while *hAPOC3* mRNA level is not affected in both models (data not shown). The plasma mouse apoB in the double transgenic is decreased by 65% compared to *hAPOC3* transgenic mice (Fig. 5B).

We determined the triglyceride levels after heparin injection to *hAPOC3* transgenic mice and *hAPOC3*X*hAPOA5* double transgenic mice. The results, 5 min after heparin injection, are shown in table 2, the triglyceride decreases were 72% (343.9±104.6 to 99.5±44.5 mg/dl) in *hAPOC3*X*hAPOA5* transgenic mice *versus* 46% (909+179.7 to 492.3+139.2 mg/dl) in *hAPOC3* transgenic mice.

These data confirmed the lowering effect of apoAV on triglycerides but also on cholesterol in the hyperlipemic mice model by activating both lipolysis and removal of VLDL.

Postprandial triglyceride response.

To determine if apoAV prevents the increase of triglyceride levels in postpransdial state, wild-type and *hAPOA5* transgenic mice received a single bolus of sunflower oil load after which plasma triglyceride levels were determined over a period of 6 hours. Wild-type mice showed a postprandial increase in plasma triglyceride with a peak at 3 h after sunflower oil administration (Fig. 6). The postprandial response of plasma triglyceride was abolished in the *hAPOA5* transgenic mice.

To further investigate the mechanism of apoAV in the postprandial response, we used *hAPOA5*X*hAPOC3* double transgenic model. We determined the distribution of triglycerides, apoAV and apoCIII in lipoprotein fractions at the basal (0 min) and at the triglyceride peak level (3h). For this purpose, two pools of plasma at 0 and 3h after the fat load were fractionated by FPLC and the cited parameters were analyzed in all fractions. In this model, 3h after the fat load, the triglycerides increased (Fig. 7A) but much less than in *hAPOC3* transgenic mice (data not shown). The total apoAV level is not affected between 0 and 3h but at 0h apoAV is present in VLDL but mainly in HDL, 3h after the fat load apoAV decreased in HDL and increased in triglyceride-rich lipoproteins (Fig. 7B). ApoCIII is increased in triglyceride-rich lipoproteins but did not change in HDL. These results provide evidence of a shift of apoAV from HDL to triglyceride-rich lipoproteins, this shift is not accompanied by displacement of apoCIII.

ApoAV may activate indirectly LPL through an interaction with apoCIII

We determined the effect of apoAV on LPL activity *in vitro*. VLDL particles were enriched with apoAV recombinant protein. The data showed that the LPL activity is increased by 50% with the apoAV enriched VLDL (Fig. 8).

To investigated how apoAV could stimulate LPL activity, we analyzed the interaction of apoAV with LPL, heparan sulfate proteoglycans (HSPG) and apolipoproteins AI, AII, CI and CIII by Biacore 3000 technology. No interaction was detected with HSPG, apoAI, AII and CI (data not shown). In figure 9, the results showed that apoAV does not interact with LPL but interacts physically with apoCIII. According to the results observed above where the shift of apoAV from HDL to triglyceride-rich lipoproteins does not induce a displacement of apoCIII, the interaction of apoAV with apoCIII could induce a change in apoCIII conformation and consequently in its function leading the activation of lipolysis.

DISCUSSION

In our previous study, we reported that mice overexpressing *hAPOA5* exibit low plasma triglyceride levels while the mice deficient for *apoa5* are hypertriglyceridemic [1]. However the exact mechanism underlying the low triglyceride levels in *hAPOA5* transgenic mice was not elucidated. The present study aimed to comprehend the mechanisms lowering the triglyceride levels. For this, we performed in vivo VLDL triglyceride turnover studies in wild-type and *hAPOA5* transgenic mice. We used Triton method and found that the decrease of VLDL triglyceride is not due to the decrease of VLDL triglyceride production. The radiolabeled VLDL turnover revealed that the clearance of [^{3}H]-triglyceride-labeled VLDL was significantly enhanced in *hAPOA5* transgenic mice than in their controls, this increase of VLDL triglyceride clearance is consistent with what has been reported in abstracts before [37, 38]. We showed that this enhanced clearance of triglycerides is due in part to the activation of LPL in postheparin plasma. But the activation of LPL observed in postheparin plasma of *hAPOA5* transgenic mice could explain only partly the large effect of apoAV on triglycerides. Next we showed that both apoCIII and apoB are decreased in apoAV transgenic mice indicating an increase of VLDL removal. We attempted to further elucidate the mechanism(s) underlying these phenomena by intercrossing *hAPOA5* transgenic mice with *hAPOC3* transgenic mice to study the effects of *hAPOA5* expression in hyperlipidemic background. Transgenic mice expressing *hAPOC3* have elevated triglycerides levels due the accumulation in plasma of enlarged triglyceride-rich lipoproteins with increased apoCIII and decreased apoE compared to controls [31, 39, 40]. The presence of the enlarged triglyceride-rich lipoproteins is due to a decrease of their clearance. These enlarged triglyceride-rich lipoproteins have an increased residence time in plasma implying a defect of lipolysis in vivo. The overexpression of *hAPOA5* in *hAPOC3* transgenic mice resulted in a marked decrease of triglycerides and apolipoprotein B as well as apolipoprotein CIII. By contrast to *hAPOA5*

transgenic mice, the cholesterol levels are significantly affected in this hyperlipidemic model. The decreased of cholesterol in hAPOA5XhAPOC3 double transgenic mice affected only the apoB containing lipoproteins, this decreas was not observed in *hAPOA5* transgenic mice where the cholesterol is only in HDL. These data indicated that the clearance of the VLDL particles is enhanced in the presence of apoAV. Furthermore, the triglyceride levels in postheparin plasma are decreased by 72% in the double transgenic mice while in *hAPOC3* transgenic mice the decreased is of 46%. Taken together the clearance with lipolysis activation data provide evidence that the mechanism by which apoAV regulates VLDL metabolism *in vivo* may underlie a decrease in VLDL size by activating lipolysis and a decrease in VLDL number by activating their removal.

The effect of apoAV on postprandial hyperlipidemia was investigated in *hAPOA5* transgenic mice. The sunflower induced postprandial hyperlipemia is abolished in *hAPOA5* transgenic mice compared to wild type mice. These data suggest that apoAV may reduce diet-induced hypertriglyceridemia through an increase of dietary triglyceride catabolism rather than inhibition of lipid absorption because apoAV is not expressed in intestin. To further understand the mechanism behind the hypolipemic effect of apoAV, we determined the apoAV distribution before and after the fat load in *hAPOA5*Xh*APOC3* double transgenic mice where the increase of triglyceride in response to the fat load is lower than in *hAPOC3* transgenic mice. The results showed clearly that apoAV shifts from HDL to triglyceride-rich lipoproteins. This shift could counteract the increase of triglyceride by probably making of them a good substrate for lipolysis or/and a good ligand for cellular uptake.

To further investigate the mechanism underlying the effect of apoAV on triglycerides, *in vitro* studies were performed using purified LPL, purified apolipoproteins (apoAI, apoAII, apoCI and apoCIII) and recombinant apoAV. ApoCIII is described to be one of the physiological modulators of VLDL triglyceride metabolism through the inhibition of both LPL and HL-

mediated hydrolysis of VLDL triglycerides [24-27]. *In vitro* VLDL-triglyceride hydrolysis by exogenous LPL was considerably higher with apoAV-enriched VLDL than with the VLDL control. Furthermore, we showed using Biacore 3000 technology and ELISA method that apoAV does not interact neither with LPL nor with coated heparan sulfate proteoglycan. This indirect activation of LPL by apoAV prompted us to address the question on possible relationship between apoAV and apoCIII. Our in vitro studies showed that apoAV does not interact with neither apoCI nor apoAI and apoAII but interacts physically with apoCIII. This interaction does not displace apoCIII from VLDL but may induce conformational change in apoCIII and consequently change in its function leading the activation of lipoprotein lipase. This possible functionally relationship of apoAV and apoCIII is due presumably to physical interaction on the surface of triglyceride-rich lipoproteins.

In summary, the mechanism underlying the marked reduction in plasma triglyceride levels, and also cholesterol levels in hyperlipidemic background, through apoAV involved an activation of lipolysis inducing a change in VLDL size and an acceleration of their removal resulting in a decrease of VLDL number.

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REFERENCES

[1] L. A. Pennacchio, M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin, An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing, Science 294 (2001) 169-173.

[2] H. N. van der Vliet, M. G. Sammels, A. C. Leegwater, J. H. Levels, P. H. Reitsma, W. Boers, and R. A. Chamuleau, Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration, J Biol Chem 276 (2001) 44512-44520.

[3] H. N. van der Vliet, F. G. Schaap, J. H. Levels, R. Ottenhoff, N. Looije, J. G. Wesseling, A. K. Groen, and R. A. Chamuleau, Adenoviral overexpression of apolipoprotein A-V reduces serum levels of triglycerides and cholesterol in mice, Biochem Biophys Res Commun 295 (2002) 1156-1159.

[4] B. E. Aouizerat, M. Kulkarni, D. Heilbron, D. Drown, S. Raskin, C. R. Pullinger, M. J. Malloy, and J. P. Kane, Genetic analysis of a polymorphism in the human apoA-V gene: effect on plasma lipids, J Lipid Res 44 (2003) 1167-1173.

[5] L. Baum, B. Tomlinson, and G. N. Thomas, APOA5-1131T>C polymorphism is associated with triglyceride levels in Chinese men, Clin Genet 63 (2003) 377-379.

[6] K. Endo, H. Yanagi, J. Araki, C. Hirano, K. Yamakawa-Kobayashi, and S. Tomura, Association found between the promoter region polymorphism in the apolipoprotein A-V gene and the serum triglyceride level in Japanese schoolchildren, Hum Genet 111 (2002) 570-572.

[7] S. Martin, V. Nicaud, S. E. Humphries, and P. J. Talmud, Contribution of APOA5 gene variants to plasma triglyceride determination and to the response to both fat and glucose tolerance challenges, Biochim Biophys Acta 1637 (2003) 217-225.

[8] L. Masana, J. Ribalta, J. Salazar, J. Fernandez-Ballart, J. Joven, and M. C. Cabezas, The apolipoprotein AV gene and diurnal triglyceridaemia in normolipidaemic subjects, Clin Chem Lab Med 41 (2003) 517-521.

[9] J. Ribalta, L. Figuera, J. Fernandez-Ballart, E. Vilella, M. Castro Cabezas, L. Masana, and J. Joven, Newly identified apolipoprotein AV gene predisposes to high plasma triglycerides in familial combined hyperlipidemia, Clin Chem 48 (2002) 1597-1600.

[10] L. A. Pennacchio, M. Olivier, J. A. Hubacek, R. M. Krauss, E. M. Rubin, and J. C. Cohen, Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels, Hum Mol Genet 11 (2002) 3031-3038.

[11] P. J. Talmud, E. Hawe, S. Martin, M. Olivier, G. J. Miller, E. M. Rubin, L. A. Pennacchio, and S. E. Humphries, Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides, Hum Mol Genet 11 (2002) 3039-3046.

[12] P. J. Talmud, S. Martin, M. R. Taskinen, M. H. Frick, M. S. Nieminen, Y. A. Kesaniemi, A. Pasternack, S. E. Humphries, and M. Syvanne, APOA5 gene variants, lipoprotein particle distribution, and progression of coronary heart disease: results from the LOCAT study, J Lipid Res 45 (2004) 750-756.

[13] M. A. Austin, P. J. Talmud, F. M. Farin, D. A. Nickerson, K. L. Edwards, D. Leonetti, M. J. McNeely, H. M. Viernes, S. E. Humphries, and W. Y. Fujimoto, Association of apolipoprotein A5 variants with LDL particle size and triglyceride in Japanese Americans, Biochim Biophys Acta 1688 (2004) 1-9.

[14] J. A. Hubacek, Z. Skodova, V. Adamkova, V. Lanska, and R. Poledne, The influence of APOAV polymorphisms (T-1131>C and S19>W) on plasma triglyceride levels and risk of myocardial infarction, Clin Genet 65 (2004) 126-130.

[15] N. Vu-Dac, P. Gervois, H. Jakel, M. Nowak, E. Bauge, H. Dehondt, B. Staels, L. A. Pennacchio, E. M. Rubin, J. Fruchart-Najib, and J. C. Fruchart, Apolipoprotein A5, a crucial determinant of plasma triglyceride levels, is highly responsive to peroxisome proliferator-activated receptor alpha activators, J Biol Chem 278 (2003) 17982-17985.

[16] X. Prieur, H. Coste, and J. C. Rodriguez, The human apolipoprotein AV gene is regulated by peroxisome proliferator-activated receptor-alpha and contains a novel farnesoid X-activated receptor response element, J Biol Chem 278 (2003) 25468-25480.

[17] H. N. Hodis, and W. J. Mack, Triglyceride-rich lipoproteins and the progression of coronary artery disease, Curr Opin Lipidol 6 (1995) 209-214.

[18] H. B. Brewer, Jr., R. Shulman, P. Herbert, R. Ronan, and K. Wehrly, The complete amino acid sequence of alanine apolipoprotein (apoC-3), and apolipoprotein from human plasma very low density lipoproteins, J Biol Chem 249 (1974) 4975-4984.

[19] C. Lenich, P. Brecher, S. Makrides, A. Chobanian, and V. I. Zannis, Apolipoprotein gene expression in the rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues, J Lipid Res 29 (1988) 755-764.

[20] M. L. Kashyap, L. S. Srivastava, B. A. Hynd, P. S. Gartside, and G. Perisutti, Quantitation of human apolipoprotein C-III and its subspecie by radioimmunoassay and analytical isoelectric focusing: abnormal plasma triglyceride-rich lipoprotein apolipoprotein C-III subspecie concentrations in hypertriglyceridemia, J Lipid Res 22 (1981) 800-810.

[21] G. Schonfeld, P. K. George, J. Miller, P. Reilly, and J. Witztum, Apolipoprotein C-II and C-III levels in hyperlipoproteinemia, Metabolism 28 (1979) 1001-1010.

[22] F. Shelburne, J. Hanks, W. Meyers, and S. Quarfordt, Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat, J Clin Invest 65 (1980) 652-658.

[23] E. Windler, and R. J. Havel, Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver, J Lipid Res 26 (1985) 556-565.

[24] R. M. Krauss, P. N. Herbert, R. I. Levy, and D. S. Fredrickson, Further observations on the activation and inhibition of lipoprotein lipase by apolipoproteins, Circ Res 33 (1973) 403-411.

[25] P. K. Kinnunen, and C. Ehnolm, Effect of serum and C-apoproteins from very low density lipoproteins on human postheparin plasma hepatic lipase, FEBS Lett 65 (1976) 354-357.

[26] C. S. Wang, W. J. McConathy, H. U. Kloer, and P. Alaupovic, Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III, J Clin Invest 75 (1985) 384-390.

[27] W. J. McConathy, J. C. Gesquiere, H. Bass, A. Tartar, J. C. Fruchart, and C. S. Wang, Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III, J Lipid Res 33 (1992) 995-1003.

[28] D. H. Blankenhorn, P. Alaupovic, E. Wickham, H. P. Chin, and S. P. Azen, Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts. Lipid and nonlipid factors, Circulation 81 (1990) 470-476.

[29] H. N. Hodis, W. J. Mack, S. P. Azen, P. Alaupovic, J. M. Pogoda, L. LaBree, L. C. Hemphill, D. M. Kramsch, and D. H. Blankenhorn, Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin, Circulation 90 (1994) 42-49.

[30] E. Koren, C. Corder, G. Mueller, H. Centurion, G. Hallum, J. Fesmire, W. D. McConathy, and P. Alaupovic, Triglyceride enriched lipoprotein particles correlate with the severity of coronary artery disease, Atherosclerosis 122 (1996) 105-115.

[31] K. Aalto-Setala, E. A. Fisher, X. Chen, T. Chajek-Shaul, T. Hayek, R. Zechner, A. Walsh, R. Ramakrishnan, H. N. Ginsberg, and J. L. Breslow, Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles, J Clin Invest 90 (1992) 1889-1900.

[32] H. H. van Barlingen, H. de Jong, D. W. Erkelens, and T. W. de Bruin, Lipoprotein lipase-enhanced binding of human triglyceride-rich lipoproteins to heparan sulfate: modulation by apolipoprotein E and apolipoprotein C, J Lipid Res 37 (1996) 754-763.

[33] C. Y. Yang, Z. W. Gu, N. Valentinova, H. J. Pownall, B. Lee, M. Yang, Y. H. Xie, J. R. Guyton, T. N. Vlasik, J. C. Fruchart, and et al., Human very low density lipoprotein structure: interaction of the C apolipoproteins with apolipoprotein B-100, J Lipid Res 34 (1993) 1311-1321.

[34] V. Clavey, S. Lestavel-Delattre, C. Copin, J. M. Bard, and J. C. Fruchart, Modulation of lipoprotein B binding to the LDL receptor by exogenous lipids and apolipoproteins CI, CII, CIII, and E, Arterioscler Thromb Vasc Biol 15 (1995) 963-971.

[35] M. C. Jong, V. E. Dahlmans, P. J. van Gorp, M. L. Breuer, M. J. Mol, A. van der Zee, R. R. Frants, M. H. Hofker, and L. M. Havekes, Both lipolysis and hepatic uptake of VLDL are impaired in transgenic mice coexpressing human apolipoprotein E*3Leiden and human apolipoprotein C1, Arterioscler Thromb Vasc Biol 16 (1996) 934-940.

[36] P. H. Iverius, and J. D. Brunzell, Human adipose tissue lipoprotein lipase: changes with feeding and relation to postheparin plasma enzyme, Am J Physiol 249 (1985) E107-114.

[37] F.G. Schaap, P.J. Voshol, P.C Rensen, H.N. van der Vliet, R.A. Chamuleau, N.M. Maeda, L.M. Havekes, A.K. Groen and K.W. van Dijk, Abstr. American Heart Association, abstr. 1215, 2003

[38] I. Grosskopf, N. Baroukh, S-J. Lee, E.M. Rubin, L.A. Pennacchio and A.D. Cooper, Abstr. American Heart Association, abstr. 1217, 2003.

[39] Y. Ito, N. Azrolan, A. O'Connell, A. Walsh, and J. L. Breslow, Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice, Science 249 (1990) 790-793.

[40] H. V. de Silva, S. J. Lauer, J. Wang, W. S. Simonet, K. H. Weisgraber, R. W. Mahley, and J. M. Taylor, Overexpression of human apolipoprotein C-III in transgenic mice results in an accumulation of apolipoprotein B48 remnants that is corrected by excess apolipoprotein E, J Biol Chem 269 (1994) 2324-233

FIGURE LEGENDS

Fig. 1. Clearance of [^{3}H]-triglyceride-labeled VLDL in wild type (open circles) and *hAPOA5* transgenic (dark circles) mice. The disappearance of the labeled VLDL was followed by counting the plasma radioactivity expressed as the percentage of the injected dose. The values are mean±SD of 5 mice per group. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).

Fig. 2. Plasma postheparin LPL activity in wild type (open bar) and *hAPOA5* transgenic mice (dark bar). The LPL activities were measured using labeled triolein substrate. The values are are mean±SD of 5 mice per group. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).

Fig. 3. Mouse apoCIII (A) and mouse apoB (B) in wild type (open bar) and *hAPOA5* transgenic mice (dark bar). Both apos were determined by nephelometry method. The values are mean±SD of 8 mice per group. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).

Fig. 4. Plasma triglycerides (A) and cholesterol (B) distribution after gel permeation chrpmatography (FPLC) of pooled plasma from *hAPOC3* transgenic mice (open circles) and *hAPOA5XhAPOC3* double transgenic mice (dark circles). The values are mean±SD of 7 mice per group.

Fig. 5. Plasma human apoCIII (A) and mouse apoB (B) in *hAPOC3* transgenic mice (open bar) and *hAPOA5*X*hAPOC3* transgenic mice (dark bar). The values are mean±SD of 12 mice per group. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).

Fig. 6. Fat loading test in wild type (open circles) and *hAPOA5* transgenic mice (dark circles). The triglycerides are measured before and after a single bolus of sunflower oil. The values are mean±SD of 5 mice per group.

Fig. 7. Triglycerides (A), human apoAV (B) and mouse apoCIII (C) distribution after gel permeation chromatography (FPLC) of pooled from *hAPOA5*X*hAPOC3* transgenic mice 3 hours after a single bolus of 500 µl of sunflower oil. The values are mean±SD of 5 mice per group.

Fig. 8. Effect of recombinant apoAV on the LPL-mediated lipolysis of human VLDL.

The recombinant apoAV-enriched VLDL was obtained by incubating a volume of human VLDL solution corresponding to 1mg of triglycerides with 60µg of recombinant apoAV or with PBS for control during 1hour at 37°C. The lipolysis assay was performed as indicated in Material and Methods. The results are expressed as average of three experiments. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).

Fig. 9. Sensogram of interaction in real time of LPL and apoCIII on the immobilized recombinant apoAV. Real time biomlecular interaction analyses were performed by surface plasmon resonnance technology using a Biacore 3000 system.

Table 1. Fasting plasma lipid levels in *hAPOC3* and *hAPOA5XhAPOC3* transgenic mice. Total cholesterol and triglyceride are measured in the plasma of 4 hours fasted mice. The values are mean±SD of 8 mice per group. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).

Table 2. Postheparin plasma triglyceride levels in *hAPOC3* and *hAPOA5XhAPOC3* transgenic mice. Triglyceride levels are measured in the plasma of 5min heparin injected mice. The values are mean±SD of 5 mice per group. Statistically significant differences between groups versus control were obtained with an anova one way test and are indicated by *asterisks* (*:$p<0.05$, **:$p<0.01$, and ***:$p<0.001$).