methods

Development of a sensitive ELISA to quantify apolipoprotein CIII in nonhuman primate serum

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Abstract **Apolipoprotein CIII (apoCIII), a major constituent of triglyceride-rich lipoprotein, has been proposed as a key contributor to hypertriglyceridemia on the basis of its inhibitory effects on lipoprotein lipase. Many immunochemical methods have been developed for human apoCIII quantification, including ELISA. However, a sensitive and quantitative assay for nonhuman primates is not commercially available. We developed a sensitive, quantitative, and highly specific sandwich ELISA to measure apoCIII in both nonhuman primate and human serum. Our assay generates a linear calibration curve from 0.01 μg/ml to 10 μg/ml using an apoCIII standard that was purified from cynomolgus monkey serum. It is highly reproducible (intra- and interplate CV < 5% and < 8%, respectively), sensitive enough to distinguish 10% difference of apoCIII present in serum, and has no interference from purified human apolipoprotein AI, AII, B, CI, CII, or E. The same assay can also be used to measure human apoCIII with a linear calibration curve from 0.005 μg/ml to 1 μg/ml using purified human apoCIII as the standard. This fast and highly sensitive ELISA could be a useful tool to investigate the role of apoCIII in lipoprotein transport and cardiovascular disease.**—Wang, Y., Z. Song, J. D. Wagner, C. Pachuk, and R. R. Subramanian. **Development of a sensitive ELISA to quantify apolipoprotein CIII in nonhuman primate serum.** ***J. Lipid Res.*** **2011.** 52: **1265–1271.**

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Human apolipoprotein CIII (apoCIII) is a 79-aa glycoprotein synthesized mainly by the liver and, to a lesser degree, by the small intestine (1, 2). It is a surface component of chylomicrons (CM), very low density lipoproteins (VLDL), and high density lipoproteins (HDL) (1). In various clinical studies, the level of VLDL/LDL-linked apoCIII correlated with the severity of coronary artery disease (CAD) score (2). In vitro experiments have demonstrated that apoCIII inhibits lipoprotein lipase and hepatic lipase and retards clearance of VLDL (1). ApoCIII deficiency results in hypotriglyceridemia in both humans and mice (3). Overexpression of apoCIII in mice results in hypertriglyceridemia (4, 5). These results indicate that lowering serum apoCIII levels may potentially decrease hypertriglyceridemia.

Nonhuman primates are widely employed in studies of lipoprotein metabolism. The close phylogenetic relationship of human and nonhuman primates is reflected in the degree of homology of their major apolipoproteins. A relatively abundant *Macaca fascicularis* (cynomolgus monkey) species of Old World monkey is often used for lipoprotein metabolism studies (6). Herbert et al. (6) identified two forms of apoCIII in *M. fascicularis* that differ in sialic acid content, lack cysteine and isoleucine like human apoCIII, and contain more glycine and less serine than human apoCIII. The predicted amino acid of cynomolgus apoCIII sequence aligned with that of human apoCIII reveals an 87% identity between proteins. The mature cynomolgus apoCIII is 79 residues long and of similar hydrophilicity as its human equivalent. However, the α-helix predicted for the first 40 amino acids of mature human apoCIII is shorter and comprises only amino acids 20-40 in cynomolgus apoCIII (7). These differences in amino acid composition can contribute to the differences in apoCIII protein and anti-apoCIII antibody recognition. Therefore, when immunochemical methods developed for human apoCIII quantification are used to quantify cynomolgus apoCIII, validation is necessary. In a previous study, cynomolgus apoCIII was measured by a Roche Hitachi 717 instrument, and assay reagents were manufactured by Wako Chemicals (8). However, Wako Chemicals has ceased producing reagents for apoCIII measurement, and currently there are no commercial reagents available to precisely measure cynomolgus apoCIII.

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Abbreviations: Apo, apolipoprotein; CAD, coronary artery disease; CV, coefficient of variation; PPAR, peroxisome proliferator-activated receptor; T2DM, type 2 diabetes mellitus.

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To address the accurate determination of cynomolgus apoCIII protein, we developed a sensitive ELISA to measure it. Many immunochemical methods have been developed for human apoCIII quantification, including ELISA, and the original studies provided valuable knowledge to our study (9, 10). The ELISA is sensitive enough to detect a 10% decrease in the amount of apoCIII present in monkey serum, which meets the requirement for apoCIII inhibition studies. In light of the high sensitivity and our ability to measure monkey serum apoCIII accurately, we tested a set of sera available from a previous study of peroxisome proliferator-activated receptor-α (PPAR-α) agonist CP-900691 in cynomolgus monkeys with spontaneous type 2 diabetes mellitus (T2DM) (11). In that study, marked improvements in triglycerides (547 ± 102 to 356 ± 90 mg/dl, $P < 0.01$), HDL cholesterol, lipoprotein index (HDL to nonHDLC ratio), body weight, and C-reactive protein were found with CP-900691 treatment. Using our sensitive ELISA assay, we found a greater than 50% decrease in serum apoCIII in a group of T2DM monkeys with CP-900691 treatment. Our results differed with results from other studies of PPAR-α in cynomolgus monkey; however, researchers did not provide details about the different apoCIII assay methods used (11–16). We believe that a validated assay that can accurately measure apoCIII in nonhuman primates would provide an important tool to study in further detail the actions of lipid-modifying agents targeting dyslipidemia. In this article, we describe an ELISA method to measure serum apoCIII concentrations for human and nonhuman primates.

MATERIALS AND METHODS

Institutional compliance statement

Healthy monkey serum samples used for assay development were purchased from Innovative Research (item ICY-SER), and control human serum samples were purchased from BioServe (Beltsville, MD). Serum from CP-900061-treated cynomolgus monkey was collected at the Wake Forest University Primate Center (Winston-Salem, NC) as previously reported (11). All experimental procedures involving animals were conducted in accordance with Public Health Service policy, and they were approved by and complied with the guidelines of the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences (11).

Selection of antibody pairs for ELISA

Anti-human apoCIII antibodies were purchased from Novus and Abcam; details of the antibodies are listed in **Table 1**. We tested the reactivity of each individual antibody with cynomolgus monkey serum utilizing Western blot to check antibody specificity and Dot blot to check antibody reactivity with the native form of the protein. For Western blot, serum sample was diluted 20-fold with 1× SDS loading buffer and boiled for 10 min. Then 20 μl/lane of diluted serum sample was loaded on NuPAGE gels (4-12% gradient). An amount of 10 ng/lane of purified human apoCIII standard protein (item HAPO-C3, Molecular Innovations) in 1× SDS loading buffer was loaded on the same gel. The standard Western blot method was used to test each antibody for reactivity. Signals from serum samples and human apoCIII were compared and scored as "strong," "medium," or "weak" if the band from cynomolgus apoCIII was stronger, similar to, or weaker than the standard human apoCIII protein on the same gel. If there was no band on the gel, the score was "no" reactivity. For Dot blot analysis, 1 μl of original cynomolgus serum sample and 10 ng of standard human apoCIII in 1 μl volume were loaded on a nitrocellulose membrane. The samples were air dried on the membrane, and routine Dot blot was used. The antibody reactivity to apoCIII was scored in the same way as described above, using the standard human apoCIII signal on the same Dot blot as reference.

ApoCIII ELISA development

The sandwich ELISA was developed by optimizing the capture antibody concentration, detection antibody concentration, blocking buffer, washing buffer, and incubation time. The goal for optimization was to have the lowest detection limit and the largest linear range. Optimized conditions were achieved as follows.

Anti-human apoCIII polyclonal antibody (item ab21032, Abcam) was dissolved in GIBCO DPBS (item 14200, Invitrogen) at a concentration of 10 μg/ml, and 100 μl of the solution was added to each well of high binding ELISA plate (item 655061, Greiner). The antibody was adsorbed to the wells by an overnight incubation at 4°C. After washing, 200 μl of blocking buffer (same as dilution buffer), containing 0.1% BSA and 0.1% (v/v) Tween-20 in DPBS, was added to each well and shaken for 1 h at 200 rpm on a plate shaker at room temperature. Then 50 μl of serum or standard, diluted with dilution buffer, was added to each well and incubated for 2 h at room temperature with shaking. After washing six times with 200 μl/well of washing buffer (0.1% Tween-20 in DPBS buffer), 50 μl/well of biotinylated detection antibody (item ab21024, Abcam) diluted in dilution buffer to a final concentration of 1 μg/ml was added to each well and incubated at room temperature with shaking for 1 h. Then 50 μl/well of 1:100 diluted Streptavidin-HRP (item ab64269-125, Abcam) was added and incubated for 0.5 h at room temperature with shaking. The ELISA plate was washed six times with washing buffer. Next 50 μl/well 1-step Ultra TMB-ELISA (item 34028, Sigma) was added; the color development time was between 5 and 15 min. The reaction was stopped by adding 2N HCL. The absorbance was read on a plate reader at 450 nM.

ApoCIII ELISA performance characteristics

Detection limit and linear range of cynomolgus and human apoCIII. Purified and delipidated cynomolgus apoCIII and human apoCIII proteins were diluted and analyzed by our ELISA assay and compared with the commercially available human apoCIII assay kit from AssayPro (item EA8133-1). GraphPad

TABLE 1. Anti-human apoCIII antibodies used in screening for cynomolgus monkey apoCIII ELISA development

Item	Vendor	Properties	Host	Antigen	Application
NB600	Novus	Polyclonal	Goat	Native human apoCIII	WB, ELISA, IHC
ab23417	Abcam	Polyclonal	Goat	Synthetic peptide	WB, ELISA, IHC
ab77664	Abcam	Polyclonal	Rabbit	Synthetic peptide C terminus	ELISA, IHC
ab76305	Abcam	Monoclonal	Rabbit	Synthetic peptide C terminus	WB, IP
ab21032	Abcam	Polyclonal	Rabbit	Full-length human apoCIII	WB, ELISA
ab21024	Abcam	Polyclonal	Goat	Full-length human apoCIII	WB, ELISA

WB, Western blot; IHC, immunohistochemistry.

Prism 5.0 nonlinear regression sigmoidal dose response curve fit was used to fit the curve, and R^2 was reported on the graph. Two microliters of the same diluted samples were loaded on SDS-PAGE after boiling in SDS loading buffer. Western blot was performed on these proteins using antibody ab21032 (Abcam).

Assay sensitivity. To determine whether our ELISA assay was sensitive enough for cynomolgus apoCIII inhibition studies, a set of cynomolgus serum samples were prepared to mimic from 10% to 90% (in 10% increments) of apoCIII reduction in monkey serum. We used 200-fold diluted cynomolgus serum to start the dilution. Nine vol of 200-fold diluted cynomolgus serum sample plus one vol of blocking buffer is considered 10% apoCIII knockdown, and eight vol of 200-fold diluted cynomolgus serum sample plus two vol of blocking buffer is considered 20% apoCIII knockdown. The same diluted samples were used in ELISA assay and Western blot. ELISA result was analyzed using GraphPad Prism 5.0, linear regression curve fit, and R^2 was reported.

Assay recovery and precision. Purified apoCIII was added to whole serum, and the ELISA procedure was carried out. The total amount of apoCIII in serum was calculated based on the apoCIII standard on the same plate. The intra-plate assay coefficient of variation (%CV) is calculated based on serial diluted cynomolgus serum samples, six replicates (n = 6) for each dilution on the same plate. The inter-plate assay %CV is calculated based on the mean of six replicates on one plate. The same assay was repeated six times (n = 6) over a period of two months.

Cross-reactivity with other human apolipoproteins. Purified human apolipoproteins AI, AII, B, CI, CII, E (items HAPO-A1, HAPO-A2, HAPO-B, HAPO-C1, HAPO-C2, and HAPO-E, Molecular Innovations) were spiked into purified cynomolgus apoCIII, a 1,000-fold excess compared with cynomolgus apoCIII at 50 ng/ml, to determine cross-reactivity of the antibodies.

ApoCIII protein purification

The human apoCIII purification procedure described by Jackson and Holdsworth (17) was used for cynomolgus monkey serum apoCIII purification. Briefly, triglyceride-rich lipoproteins (d < 1.02 g/ml) was isolated by ultracentrifugation in KBr (22.257 g/l) using a Beckman 45 Ti rotor and tube (item 355622) at 42,000 rpm for 18 h at 8°C. The top layer, which was enriched with triglyceride-rich lipoproteins, was collected. The apolipoproteins were prepared by delipidation with acetone:ethanol (1:1, v/v). The sedimented apolipoproteins were solubilized in 8 M urea solution. The urea-soluble proteins were pooled, and the apoCIII protein was further purified by size exclusion chromatography. The low molecular weight fractions were pooled and further fractioned on anion exchange MonoQ column. The final product was exhaustively dialyzed against 10 mM NH_4HCO_3 and lyophilized. Purity was examined by NuPage, Western blot using anti-apoCIII antibody (ab21032, Abcam), and mass spectrum analysis. Protein concentration was estimated by Bradford method with BSA as standard (18). ApoCIII purification service was provided by Dr. Xiang Yang from ProteinOne (Bethesda, MD).

Sera from CP-900691-treated diabetic monkeys

Method and experimental design have been published previously (11). Briefly, all treatment animals were dosed orally with either vehicle or CP-900691 at 3 mg/kg daily for six weeks. Serum samples were taken before dosing and every two weeks after dosing. Whole-blood samples in EDTA-treated tubes were placed on ice immediately after collection, and then centrifuged. The resulting plasma removed and stored at −80°C. Ten serum samples from five male monkeys in the same treatment group, including pre- and postCP-900691 treatment, were assayed with ELISA and Western blot.

RESULTS

Selection of antibody pair for ELISA

The reactivity of each antibody against cynomolgus serum apoCIII and 10 ng purified human apoCIII was tested by Western and Dot blots. The data is summarized in **Table 2**. Ten nanograms of purified human apoCIII (item HAPO-C3, Molecular Innovations) yielded a strong signal on Western blot and Dot blot developed by all the tested antibodies (data not shown). However, there was a delipidation step in apoCIII protein purification, and whether human apoCIII protein was still in the native conformation is unknown. In contrast, the reactivity of cynomolgus serum apoCIII with different antibodies was very different: NB600 and ab23417 did not yield any band on Western or Dot blot. Antibodies ab77664 and ab76305 did not react with cynomolgus serum apoCIII on Dot blot, but they reacted with human apoCIII on Dot blot. Antibody ab21032 showed strong reactivity with cynomolgus apoCIII on both Western and Dot blots, whereas antibody ab21024 showed weak reactivity with cynomolgus apoCIII on both Western and Dot blots. Antibody ab21032 was chosen as the capture antibody, and ab21024 was used as the detection antibody to develop the sandwich ELISA.

Cynomolgus apoCIII purification

Cynomolgus apoCIII was purified to one band that was reactive with both ab21032 and ab21024 on Western blot. Mass spectrometry analysis showed that the amino acid sequence of the purified protein matched cynomolgus apoCIII amino acid sequence (data not shown). Protein concentration was estimated by Bradford assay (19). This purified protein was used as the standard on every ELISA plate.

ELISA optimization

Detection limit and linear range of cynomolgus and human apoCIII. **Fig. 1A** is the standard curve of purified cynomolgus apoCIII in the dilution buffer containing 0.1% BSA and 0.1% Tween-20 in DPBS. The linear range of this standard curve is from 0.01 μg/mg to 10 μg/ml. Fig. 1B is the standard curve of purified human apoCIII in the same dilution buffer; the linear range of this curve is 0.005 μg/ml to 1 μg/ml. Fig. 1C is the same diluted cynomolgus standard assayed on AssayPro's human apoCIII ELISA kit, which generated a very low $OD_{450\ nm}$ signal. Fig. 1D shows that human apoCIII ELISA kit from AssayPro worked well with human apoCIII detection, with a detection limit of 10

TABLE 2. Reactivity of anti-human apoCIII antibody with cynomolgus apoCIII in serum by Western blot and Dot blot

	Reactivity to Cynomolgus Monkey ApoCIII	
Item	Dot Blot	Western Blot
NB600	No	No
Ab23417	No	No
Ab77664	No	Weak
Ab76305	No	Weak
Ab21032	Strong	Strong
Ab21024	Weak	Weak

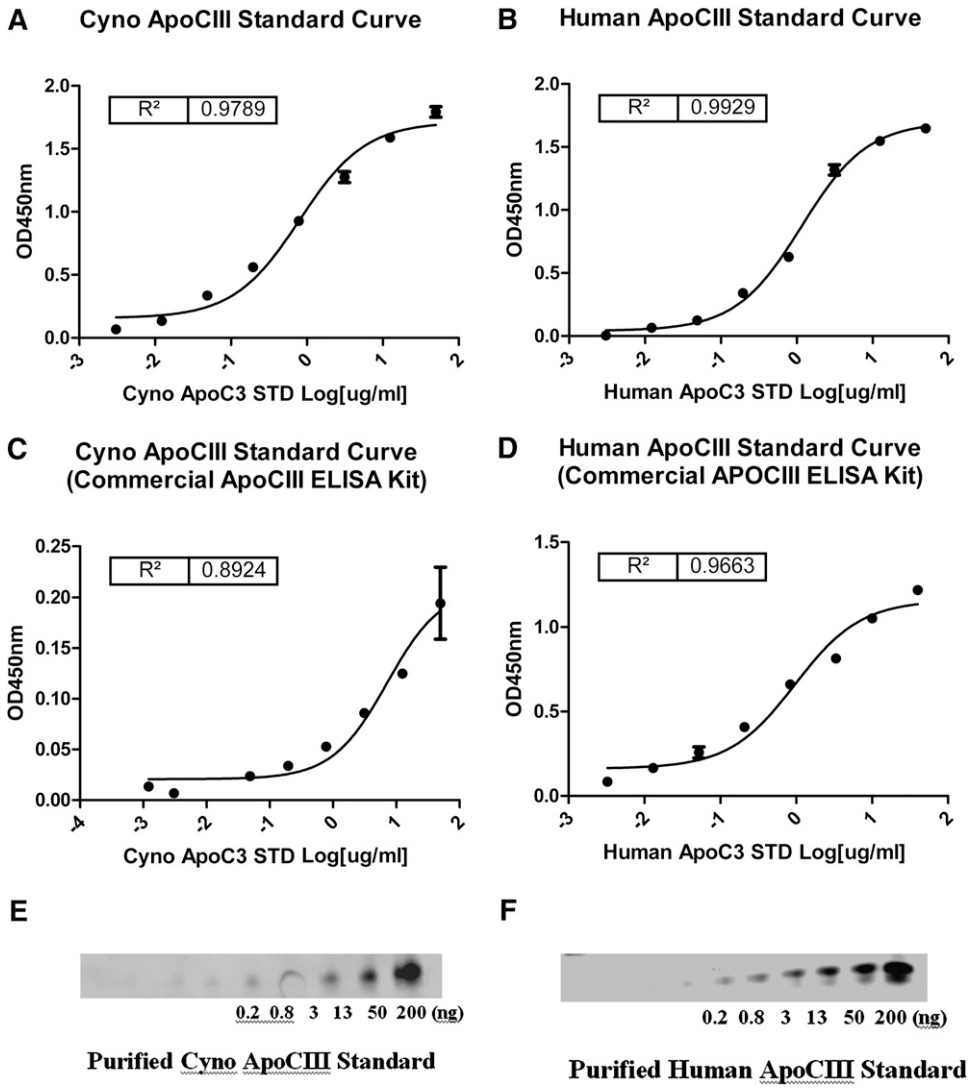

Fig. 1. ApoCIII ELISA can accurately detect and quantitate both human and nonhuman primate apoCIII. A-D: Purified apoCIII from cynomolgus monkey and human serum were tested using both the ELISA described in this article and a commercially available apoCIII ELISA kit. Standard curves were generated to assess accurate detection of serum apoCIII. E, F: Western blots validate serial dilutions of purified apoCIII protein tested by the ELISA methods.

ng/ml and linear range from 50 ng/ml to 10 μg/ml. Fig. 1E and F show Western images of the same serial diluted standard apoCIII protein from cynomolgus and human. Western blot was developed using antibody Abcam 21032, which was used as the capture antibody on the ELISA plate in this study, indicating strong antigen-antibody recognition for both cynomolgus and human apoCIII protein. This is perhaps one of the reasons that the ELISA developed in our study can be used for both human and cynomolgus apoCIII detection, whereas the ELISA kit from AssayPro is specifically designed for human apoCIII protein.

Assay sensitivity. Serially diluted human and cynomolgus sera were tested using our ELISA. We carried out a 4-fold serial dilution of serum samples in PBS containing 0.1% Tween-20, and subsequently, 50 μl/well of each dilution was tested by ELISA. The human serum dilution curve has a linear range from -5.0×10^0 to -2.0×10^0 dilution (**Fig. 2A**) and cynomolgus serum dilution curve has a linear range from -4.5×10^0 to -2.0×10^0 dilution (Fig. 2B). We tested the ELISA sensitivity using cynomolgus serum samples serially diluted to represent decrements of 10% in apoCIII protein. Then we compared the ELISA (Fig. 2C) and Western blot (Fig. 2D) on a set of cynomolgus serum samples that had 10% difference in apoCIII concentration. The linear relation observed in cynomolgus and human sera indicated that the apoCIII antigenic sites were exposed to both antibodies when sera were diluted in PBS buffer containing 0.1% Tween-20.

Recovery and precision. Purified apoCIII was added to whole serum, and the ELISA procedure was carried out. Recovery of apoCIII was 104 ± 5% (n = 6). We also determined the precision of the ELISA by testing serum samples with six replicates on the same plate (n = 6) to calculate

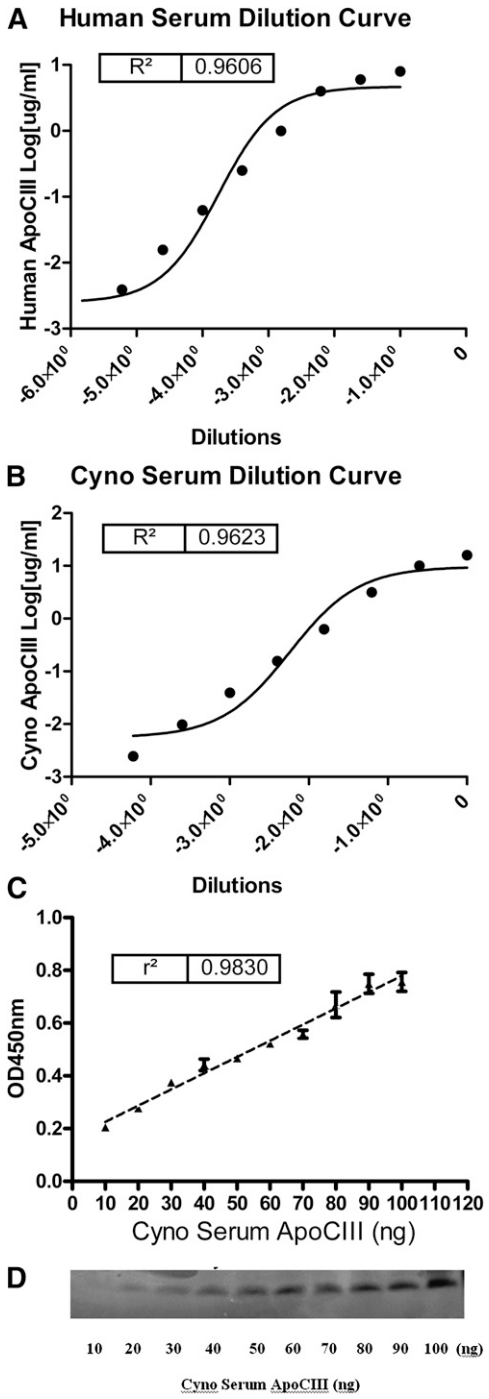

Fig. 2. Determination of apoCIII ELISA sensitivity and linearity. A, B: ApoCIII ELISA was performed on serially diluted human serum and cynomolgus serum. In brief, we carried out a 4-fold serial dilution of serum samples in PBS containing 0.1% Tween-20, and then 50 μl/well of each dilution was tested by ELISA. We tested the ELISA sensitivity using cynomolgus serum samples serially diluted to represent decrements of 10% in apoCIII protein. C, D: Comparison of ELISA and Western blot on a set of cynomolgus serum samples that have 10% difference in apoCIII concentration.

intra-plate %CV. The same assay was performed six times (n = 6) over a period of two months for inter-plate %CV calculation. The intra-plate CV is within 5% for different dilutions, and the inter-plate CV is within 8% (**Table 3**).

TABLE 3. Precision of the cynomolgus apoCIII ELISA assay

ApoCIII Concentration	Intra-assay CV (n = 6)	Inter-assay CV (n = 6)
ng/ml	%	%
250	4.98	4.77
83	3.72	7.12
30	1.23	7.88
9	2.55	6.45
3	4.32	2.11
1	2.65	2.12

Cross-reactivity with other human apolipoproteins. In normal human plasma, the apolipoprotein levels are apoA1, 90-130 mg/dl; apoAII, 30-50 mg/dl; apoB, 90 mg/dl; apoCI, 4-7 mg/dl; apoCII, 3-8 mg/dl; apoCIII, 8-15 mg/dl; and apoE, 5 mg/dl. In human plasma, apoCIII levels are at most 10-fold lower than other apolipoproteins. Cynomolgus apoCIII at 50 ng/ml gives a strong signal with our ELISA assay. Purified human apolipoproteins AI, AII, B, CI, CII, and E at 50 μg/ml each were spiked into purified cynomolgus apoCIII, a 1000-fold excess compared with apoCIII at 50 ng/ml, to determine cross-reactivity of the antibodies. These spiked apolipoproteins generated signals in our ELISA that are less than 1% of the signal generated by 50 ng/ml of cynomolgus apoCIII alone; therefore, we considered it a lack of cross-reactivity (data not shown).

Effect of PPAR-α agonist CP-900691 on diabetic monkey serum apoCIII

Cynomolgus monkeys with spontaneous T2DM, who were maintained on daily insulin therapy, were dosed orally with CP-900691 daily for 10 weeks. We measured the predose and postdose (week 6) samples and obtained a greater than 50% reduction of apoCIII protein in the treated monkey sera using apoCIII ELISA. The result was further confirmed by Western blot analysis (**Fig. 3**).

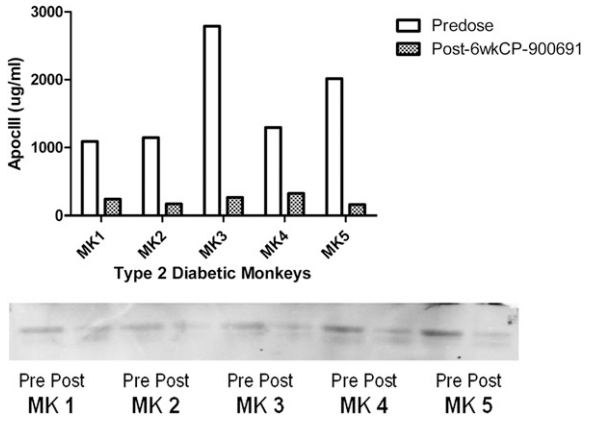

Fig. 3. CP-900691, a PPAR-α agonist, decreases serum apoCIII protein. Type 2 diabetic monkeys were treated daily with 3 mg/kg CP-900691 for six weeks. Pretreatment and post-treatment sera were analyzed with the ELISA described in this article and Western blot. Results demonstrate that the ELISA can identify significant decreases in apoCIII protein, which was confirmed by Western blot.

DISCUSSION

We describe the development of an ELISA that is highly specific for cynomolgus apoCIII protein and that also detects human apoCIII. The ELISA developed for cynomolgus monkey apoCIII reached the sensitivity of 10 ng/ml, with a linear range from 0.01 μg/ml to 10 μg/ml. At present, there is no other cynomolgus apoCIII-specific assay kit to compare with ours. The quality of our ELISA assay is comparable to the commercially available human apoCIII assay kit from AssayPro, although our ELISA has higher sensitivity for both cynomolgus and human apoCIII. From the antibody pair screening experiment, it is important to note that the reactivity of anti-human apoCIII antibodies is different between cynomolgus monkey apoCIII and human apoCIII. Although we often assume that antibodies generated for human proteins cross react with nonhuman primate proteins (and vice versa), in this case, the predicted amino acid of the cynomolgus apoCIII sequence aligned with that of human apoCIII revealed a 87% identity between both proteins. Therefore, validation must be performed to ensure cross-reactivity when an immunochemical method is used.

The antibody pair selected in our ELISA reacts with monkey serum apoCIII under mild conditions (PBS with 0.1% Tween-20), which could avoid sample pretreatment steps, such as heating, organic solvent extraction, ultracentrifugation fractionation, size exclusion fractionation, etc., which are commonly used in apolipoprotein-related assays to increase the solubility and antigen exposure of apolipoproteins. These pretreatment steps may increase the potential of polymerization, precipitation, and degradation of the tested proteins. However, the pretreatment test should be considered in the assay validation.

Schultze et al. (14) have used the Roche Hitachi 717 instrument and reagents manufactured by Wako Chemicals for cynomolgus apoCIII measurement. However, after Wako Chemicals discontinued the apoCIII assay reagents, we could not find any detailed assay method or apoCIII level in cynomolgus studies. In contrast, in recent human apoCIII studies, increasing evidence points to the key role that apoCIII plays in triglyceride-rich lipoprotein metabolism, presumably because with the human apoCIII assay kit, the apoCIII level in human serum can be measured accurately. Due to the lack of proper assay methods for nonhuman primate apoCIII, the role of apoCIII in triglyceride-rich lipoprotein metabolism in nonhuman primates could not be revealed. Increasingly, therapeutics developed to treat triglyceridemia and CAD will be tested for efficacy using nonhuman primates. The development of a sensitive apoCIII ELISA for nonhuman primates is critical in testing the mechanism of action.

Our ELISA detected cynomolgus apoCIII in sera from T2DM monkeys treated with a potent and selective PPAR-α agonist, where improvements in triglycerides, HDL cholesterol, lipoprotein index, and body weight had been reported in a previous study (11). Our ELISA added to those findings by demonstrating that the PPAR-α agonist can lower apoCIII by greater than 50%. Our results were confirmed by Western blot. However, there was a discrepancy when we compared our results with those from other PPAR-α agonist-related studies. We postulate that the discrepancy may due to the use of the human apoCIII-specific ELISA to measure monkey apoCIII protein. We showed that we obtained a very low apoCIII signal when we used a commercially available human apoCIII ELISA kit (Fig. 1). We used Western blot to confirm the decrease in apoCIII protein and revealed that the commercial ELISA alone would have underestimated the cynomolgus apoCIII level. Schultze et al. found moderate decreases (39.6 ± 2.7 μg/ml baseline to 31.6 ± 3.0 μg/ml on day 7) in serum apoCIII level in young adult male cynomolgus monkeys treated with PPAR-α agonist for two weeks, as measured by Wako Chemicals apoCIII reagent on a Roche Hitachi 717 instrument (14). Chakrabarti et al. reported a significant reduction in serum apoCIII in high-fat-fed rats treated with Ragaglitazar (PPARα and PPARr agonist); apoCIII was measured by the immunoturbidometric method using a kit from Daiichi Pure Chemicals Co. (12). Using Western blot analysis, Qu et al. reported a 50% decrease in serum apoCIII in Syrian golden hamsters fed a high-fructose diet treated with fenofibrate (15). Using automated immunoprecipitation analysis, Winegar et al. found a 29% reduction of apoCIII (from baseline 7.93 ± 1.06 mg/dl to 5.60 ± 0.77 mg/dl) in obese rhesus monkeys treated with fenofibrate (16). All these different results could be due to different animal models. However, from our experience, using validated antibody is extremely important in immunochemical methods to ensure the true value of serum apoCIII.

In conclusion, to understand the role of apoCIII in hypertriglyceridemia and develop effective therapeutics for hypertriglyceridemia, development of a reliable and sensitive method to quantify apoCIII is very important. Our ELISA is a highly sensitive method that detects apoCIII protein in both cynomolgus monkeys and humans, and it could serve as a valuable tool for translational research.

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