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StAR overexpression decreases serum and tissue lipids in apolipoprotein E-deficient mice

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Abstract

Cholesterol metabolism as initiated by mitochondrial CYP27A1 is a ubiquitous pathway capable of synthesizing multiple key regulatory oxysterols involved in lipids homeostasis. Previously we have shown that the regulation of its activities within hepatocytes is highly controlled by the rate of mitochondrial cholesterol delivery. In the present study, we hypothesized that increasing expression of the mitochondrial cholesterol delivery protein, StAR, is able to lower lipids accumulation in liver, aortic wall, as well as in serum in a well-documented animal model, apoE^{-/-} mice. ApoE^{-/-} mice, characterized by increased serum, liver, and endothelial cholesterol and triglyceride levels by 3 months of age, were infected with recombinant CMV-StAR adenovirus to increase StAR protein expression. Six days following infection, serum total cholesterol and triglycerides had decreased 19 % (P<0.01) and 30% (P<0.01), respectively, with a compensatory 40% (P<0.01) increase in serum HDL-cholesterol in increased StAR expressing mice as compared to controls (no or control virus). Histologic and biochemical analysis of the liver demonstrated not only a dramatic decrease in cholesterol (↓25%; P<0.01), but an even more marked decrease in triglyceride (↓56%; P<0.01) content. En bloc Sudan IV staining of the aorta revealed a >80% (P<0.01) decrease in neutral lipid staining. This study demonstrates for the first time a possible therapeutic role of the CYP27A1-initiated pathway in the treatment of dyslipidemias.

Keywords

lipids; cholesterol; lipoproteins; steroidogenic acute regulatory protein

Introduction

Dyslipidemias, as characterized by abnormal serum lipoprotein profiles, are a risk factor for atherosclerosis (1). Although current strategies have led to significant advances in their treatment, there is great interest to explore new therapeutic strategies to treat and prevent what remains a leading cause of morbidity and mortality throughout the world.

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Steroidogenic acute regulatory protein (StAR/StarD1), an intracellular cholesterol transport protein, was first identified in steroidogenic tissues. This protein is a member of a family that contains a StAR homologue domain that has been found capable of binding and transporting sterols within cells. This family includes the phosphatidylcholine transfer protein (PC-TP/StarD2) (2), MLN64 (StarD3) (3,4), and the newly discovered StarD4, StarD5, and StarD6 (5–8). All of these proteins have a similar structural lipid-binding domain referred to as the StAR-related lipid transfer (START) domain. StAR, first detected in the adrenal, was shown to facilitate cholesterol delivery to the inner mitochondrial cholesterol side-chain cleavage system in the initiation of steroidogenesis (9,10). StAR was initially considered to be confined to steroidogenic tissues such as adrenal gland, ovary, and testicle (7,11). However, StAR has since been found in other tissues/cells inclusive of the liver, brain, endothelium, and macrophages (12–15).

In the liver, StAR has been shown to facilitate cholesterol delivery to the inner membrane of the mitochondria where cholesterol can be oxidized by sterol 27-hydroxylase (CYP27A1) to an important regulatory oxysterol, 27-hydroxycholesterol, before ultimately being metabolized to bile acids (16). Most recently, we have shown that in addition to its metabolism of cholesterol to 27-hydroxycholesterol and subsequently to bile acids, CYP27A1 is also capable of metabolizing cholesterol to 25-hydroxycholesterol with subsequent conversion to its sulfated form (17). Like 27-hydroxycholesterol, both 25-hydroxycholesterol and sulfated 25-hydroxycholesterol are regulators of intracellular lipid metabolism (17–19). Both 27- and 25-hydroxycholesterol are known to down-regulate cholesterol synthesis while stimulating cell cholesterol secretion (18,19). Sulfated 25-hydroxycholesterol has recently been shown to decrease fatty acid synthase as well as activate a nuclear receptor responsible for fatty acid mobilization/secretion (20).

It is possible that increased metabolism of cholesterol as initiated through the CYP27A1 could result in a coordinated lipids lowering response. Increasing StAR expression would be expected to not only increase cholesterol catabolism to bile acids as we have previously shown, but produce key regulatory oxysterols of intracellular lipid homeostasis (12,16,21). In support of this supposition, our recent *in vitro* findings in THP-1 macrophages showed that up-regulation of StAR induced a coordinated mobilization and reduction of cell cholesterol (14). Therefore, it seems reasonable to hypothesize that the StAR/CYP27A1-initiated metabolism of cholesterol is not only a pathway of cholesterol catabolism, but an important intracellular pathway capable of directing intracellular lipid homeostasis.

Apolipoprotein-E mediates the clearance of serum lipids such as cholesterol and triglyceride (22). Apolipoprotein E-deficient (apoE^{-/-}) mice develop hypercholesterolemia as a result of an accumulation of chylomicron remnants, very low-density lipoproteins (VLDL), and intermediate-density lipoproteins (IDL) (23). Mice homozygous for the inactivated apoE gene spontaneously develop hypercholesterolemia and aortic lipid accumulation (24,25). The lipid lesions which develop in the aortas of mice fed a standard rodent chow diet are similar to the early lipid accumulations found in humans (23,26,27). In addition, apoE^{-/-} mice are also known to develop significant hepatic lipid (i.e. cholesterol/triglyceride) accumulation. Therefore, apoE^{-/-} mice provide not only a practical model for the study of early developmental atherosclerosis (28), but an excellent model for the outlined study.

The described trial was simply designed as “an *in vivo* proof of concept” that increasing CYP27A1-initiated cholesterol metabolism as facilitated by the mitochondria-directed cholesterol transport protein, StAR, could represent a novel approach to reduce serum and tissue cholesterol and triglyceride levels. As shown, increased StAR expression led to a coordinated response in serum triglycerides and HDL cholesterol. Furthermore, hepatic

cholesterol was significantly reduced. Of equal importance, neutral lipid levels in the serum, liver, and aorta were also dramatically reduced in this classic animal model of dyslipidemia.

Experimental Procedure

Materials

TRIzol reagent and SuperScript TMIII First-Strand Synthesis System for RT-PCR were purchased from Invitrogen (Carlsbad, CA). Taq DNA Polymerase, dNTP mix and PageRuler Prestained Protein Ladder were purchased from Fermentas MBI (San Diego, CA). Primary antibody against StAR and GAPDH were purchased from Abcam Ltd (Cambridge Science Park, Cambridge, UK) and Kangcheng Bio-Tech (Shanghai, China), respectively. Second antibody against rabbit and mouse IgG were obtained from Kirkegaard & Perry Laboratories (Guildford, UK). SuperSignal West Pico Chemiluminescent Substrate was obtained from Pierce Biotechnology, Inc (Rockford, IL). All other reagents were from Sigma-Aldrich Chemical Co (St. Louis, MO) unless otherwise indicated.

Experimental Animals

C57BL/6J mice and homozygous apoE-deficient (apoE^{-/-}) mice (same inherited background as the C57BL/6J mice) were purchased from the Department of Laboratory Animal Science, Peking University Health Science Center (Beijing, China). The animals were allowed to acclimatize for 1 week while being maintained at a room temperature of 22 ± 2 °C on a 12 h light/dark cycle with free access to standard rodent chow food and water (Standard sustain feed, from Institute of Laboratory Animal Science, Shanghai, China). Housing facilities and all experimental protocols were approved by the Animals Care and Use Committee of Fudan University Shanghai Medical College which adopts the guideline for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Ninety-six C57BL/6J and apoE^{-/-} mice of both sexes at 1, 3 and 5 months old were used to determine serum level of lipids. An additional 27 6-month-old male apoE^{-/-} mice were used to investigate the effect of StAR overexpression on serum, liver, and aortic lipid levels. Prior to and post infusion, the animals dietary intake was not significantly different from either control group. Before sacrificing, the mice were fasted for 12 hours. The mice were then anesthetized (60 mg pentobarbital/kg body weight, intravenously) and euthanized. Blood was collected from the retro-orbital plexus and aliquots of plasma were stored at -70 °C until determination of serum parameters as described below. The liver was excised, weighed, snap frozen in liquid nitrogen, and stored at -70 °C until further analysis. The aorta and liver were harvested for gross and histological examination.

Preparation of Recombinant Adenoviruses

The recombinant adenoviruses encoding StAR were prepared as previously described (29). The virus was then amplified by infecting confluent monolayers of human embryonic kidney 293 cells as previously described (30). Aliquots of the amplified virus were stored at -70 °C until used. The control adenoviruses expressing the enhanced green fluorescence protein were purchased from the Vector Gene Technology Company Ltd (Beijing, China). The infection and particle titers of the viruses were determined using plaque and optical density assays, respectively.

Intravenous Infusion of Adenovirus

For the adenovirus infections, the 27 6-month-old apoE^{-/-} mice fed a standard rodent chow diet were equally and randomly divided into 3 groups. Eighteen of mice were tail vein injected with 1×10¹¹ virus particles of Ad-CMV-StAR or Ad-CMV-EGFP. In 9 non-

transduced (control) mice the virus was replaced with the same volume of normal saline (NS). Six days after infection, the animals were briefly anesthetized and euthanized after fasting for 12 hours. Blood was collected for determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lipid levels. Organs were harvested for histology and biochemical analysis. The histological specimens were fixed in 10% formalin. Additional tissue (liver) was quickly frozen in liquid nitrogen and stored at -70°C for further analysis.

Determination of Blood Parameters

The levels of triglyceride (TG), total cholesterol (T-CHO), and HDL cholesterol (HDL-CHO) in serum were measured by enzymatic methods (31) according to the manufacturer's instructions using the detection kit purchased from Rongsheng Biotechnology Company Ltd (Shanghai, China). Serum ALT, AST, and ALP activities were determined by the clinical laboratory at the Longhua Hospital (Shanghai, China).

Histology

After adenoviruses infection, the StAR protein expression in heart, coronary artery and liver were detected by immunohistochemistry by ABC methods as previously described by the authors (13). A portion of liver was harvested and embedded in optimum cutting temperature compound (O.C.T compound, Sakura Finetek, Inc., Torrance, CA), mixed with sucrose (20%), and stored at -80°C . Ten-micrometer cryosections were made lengthwise through the liver lobe. All cryosections were fixed for 1 min in formaldehyde solution, stained for 10 min with Oil Red O (stains lipids red), and counterstained for 1 min with hematoxylin. En face lipid accumulation was determined by removing the aortas from the aortic arch to the ileal bifurcation. The aortas were fixed in 10% neutral buffered formalin. To increase the reliability of lipid quantification, only the aortic segment from left subclavian artery to ileal bifurcation was used for the measurement. The aortas were cut longitudinally, splayed, and pinned in a dish filled with Sudan IV stain for 10 min, destained in 80% ethanol for 5 min, and then photographed. Quantitation of neutral lipid staining was performed by measuring stained area using ImageM software (Department of Physiology and Pathophysiology, Shanghai Medical College, Fudan University, China). The accumulated stained area in a given aorta was calculated as a percentage of the total surface area of the aorta.

Liver Lipid Measurement

Lipids were extracted from liver according to a published method (32). Triglyceride and cholesterol were measured using the same method described previously for blood lipid analysis (31).

Determination StAR Protein Levels in Tissues

Total proteins of heart, lung, liver, spleen, and adrenal gland from the infected mice were isolated using 1×SDS-PAGE sample buffer (50 mM Tris-Cl buffer, pH 6.8, 2% (w/v) SDS, 2% mercaptoethanol, 10% (v/v) glycerol, and 0.1% (w/v) bromophenol blue). Protein was quantitated using the bicinchoninic acid assay purchased from Pierce (Rockford, IL). Fifty micrograms of total protein were separately on 10% SDS-PAGE gels. Western blot analysis of StAR was performed as previously described by the authors (13).

Statistics

Data were reported as mean $\pm$ standard deviation (S.D) and subjected to ANOVA analysis. *F*-test and the *Student-Neuman-Keuls post* test analyses were performed on these data to

analyze the variances and significances between groups. Statistical significance was defined as $P < 0.05$.

Results

Plasma Lipid Levels Increased in ApoE^{-/-} Mice

The absence of apoE^{-/-} in humans and animal models is associated with increases in serum, liver, and aortic lipid levels. To simply confirm the apoE^{-/-} mouse model available as representative and as a viable model for examining the effects of increased StAR expression on serum, aortic, and liver lipids, serum lipids were determined at 1, 3, and 5 months of age and compared to C57BL/6J mice. On a standard rodent chow diet (4.4% fat; 0.06% cholesterol), apoE^{-/-} mice had a significantly higher total cholesterol (T-CHO) and a significantly lower HDL-cholesterol (HDL-CHO) at all time periods vs. age-matched C57BL/6J mice (Table. 1). Serum triglycerides were not significantly different. Of note is that a significant steady decrease in HDL-CHO was observed from month 1 to month 5 in apoE^{-/-} mice. The results indicated that the apoE^{-/-} mice available for study to be a representative and would serve as a viable apoE^{-/-} model. Certain assumptions were made, i.e. that there would be significant liver and aortic lipid accumulation.

Recombinant StAR Gene Was Successfully Expressed in ApoE^{-/-} Mice

To determine if StAR protein was being overexpressed following mouse infection with Ad-CMV-StAR, 6 days following infection selected organs were screened for StAR protein expression by immunohistochemistry and Western blotting. After infection, StAR expression was higher in the mice infused with adenovirus encoding CMV-StAR than the other two groups in heart, coronary artery and liver tissue. The increases of StAR in the heart and coronary arteries were marginal (Fig. 1a). StAR protein was detected in the adrenal gland, spleen, liver, and lung of controls (NS: normal saline; Ad-CMV-EGFP), with large amounts of StAR found in the spleen and adrenal gland. Following Ad-CMV-StAR infection, StAR protein was found in all tested tissues with the largest increases found in the liver, a known reservoir for infecting adenovirus (Fig 1b).

Liver Transaminases

Serum ALT, AST, and ALP activity levels in mice were measured to determine if there was liver injury following adenovirus infection. No significant differences were observed among the three groups of NS, Ad-CMV-StAR and Ad-CMV-EGFP (data not shown).

Transduction of Ad-CMV-StAR Decreases Lipid Accumulation in the Serum and Liver of ApoE^{-/-} Mice

Sera—Six days following infection with Ad-CMV-StAR, the serum T-CHO and TG were 19% ($P < 0.01$) and 30% ($P < 0.01$) lower in apoE^{-/-} mice receiving Ad-CMV-StAR than in those receiving NS or Ad-CMV-EGFP, respectively. In contrast, there was a dramatic reversal in the HDL-CHO levels with HDL-CHO in their plasma about 40% higher ($P < 0.01$) than controls (Table 2).

Liver tissues—Liver sections stained with Oil Red O to evaluate the hepatic accumulation of lipids showed that the lipid levels were significantly less in the livers of Ad-CMV-StAR mice as compared to NS and Ad-CMV-EGFP mice (Fig. 2a). To confirm these results, lipids were extracted from the liver tissues and measured using commercial colorimetric kits for both cholesterol and triglycerides (Rongsheng Biotechnology Company Ltd, Shanghai, China). The hepatic cholesterol and triglyceride concentrations in the Ad-CMV-StAR mice decreased 25% ($P < 0.01$) and 56% ($P < 0.01$) as compared to NS and Ad-CMV-EGFP mice,

respectively (Fig. 2b and 2c). There were no significant differences in hepatic cholesterol and triglyceride levels between the NS and Ad-CMV-EGFP groups (Fig. 2b and 2c).

Transduction of StAR Reduces Aortic Neutral Lipid Accumulation in ApoE^{-/-} Mice

A previous study has shown that apoE^{-/-} mice will develop visible aortic lipid accumulation at 20 weeks on a normal mouse diet (23). We found the microscopic presence of aortic lipid accumulation by the 3rd month with accumulation grossly visible by the 5th month in apoE^{-/-} mice fed a similar diet. Six days following Ad-CMV-StAR transduction, examination en face of aortas from Ad-CMV-StAR injected mice using ImageM software revealed significantly less neutral lipid than in NS or Ad-CMV-EGFP mice (Fig. 3a). The amount of lipid accumulation in aortas between NS and Ad-CMV-EGFP mice was found to not be different (Fig 3a). The accumulated area of lipid stained lesions had decreased from $21.03 \pm 2.66\%$ and $19.42 \pm 2.39\%$ in NS and Ad-CMV-EGFP mice, respectively, to $3.74 \pm 1.57\%$ (Fig. 3b).

Discussion

Hyperlipidemia is an important risk factor for atherosclerosis. The treatment response to current medications is frequently inadequate. Therefore, there remains great interest to develop new therapeutic strategies. Based upon recent observations, it was hypothesized that facilitating mitochondria-directed cholesterol transport could elicit in an *in vivo* model a sequence of metabolic events resulting in both serum and tissue cholesterol lowering. The current study was designed to explore this hypothesis by increasing expression of StAR, a mitochondrial directed cholesterol transport protein, in a well-described animal model of dyslipidemia.

Cholesterol metabolism as initiated via mitochondrial CYP27A1 is subsequently metabolized to key regulatory oxysterols of cholesterol homeostasis; and within the liver, continued catabolism to bile acids. For proper function, inner mitochondrial cholesterol levels must be tightly controlled, and it has been suggested that the key function of CYP27A1 is to initiate the metabolism of cholesterol to a metabolite that can be more easily mobilized out of the mitochondria. As CYP27A1 has been detected in all examined peripheral tissues as well as the liver, others have suggested that the conversion of cholesterol to 27-hydroxycholesterol as important under conditions of cholesterol excess. Under these conditions it has been proposed that the 27-hydroxylation can facilitate a process of reverse cholesterol transport of cholesterol from peripheral tissues to the liver with 27-hydroxycholesterol's subsequent elimination as bile acids (16,33–36). In support of this hypothesis, CTX (Cerebrotendinous xanthomatosis), characterized by the inability of cells to 27-hydroxylate sterols, is associated in humans with early dementia, premature atherosclerosis, and cataracts due to abnormal tissue sterol accumulation (37,38). As a function of these observations, CYP27A1 was overexpressed in transgenic mice, but interpreted as having little appreciable effect on lipid metabolism (39). We also found that following overexpression of CYP27A1 in hepatocyte cultures only a modest increase in the rates of bile acid synthesis was observed as compared to the 8 to 10-fold increase consistently seen following CYP7A1 overexpression (40). This suggested that inner mitochondrial substrate availability could be the rate-determining step in the CYP27A1-initiated pathway.

As described, StAR has been shown to not only facilitate cholesterol delivery to the inner mitochondrial cholesterol side-chain cleavage system in the initiation of steroidogenesis, but that increasing its expression would markedly increase steroidogenesis (9,10). Applying this concept to hepatocytes, we demonstrated that increased StAR expression markedly increased the rates of bile acid synthesis (BAS) in both *in vitro* and *in vivo* models (16,21).

Our inability to up-regulate bile acid synthesis in CYP27A1^{-/-} mouse hepatocytes demonstrated that StAR's effects were mediated through the CYP27A1-initiated pathway (17).

Recently, Ren et al. demonstrated for the first time that CYP27A1 also metabolizes cholesterol to 25-hydroxycholesterol. 25-hydroxycholesterol, in a manner like 27-hydroxycholesterol, can down-regulate cholesterol synthesis, stimulate cholesterol secretion and stimulate fatty acid synthesis (17,18). Sulfated 25-hydroxycholesterol, a newly uncovered and subsequent metabolite of 25-hydroxycholesterol, like its precursor, also down-regulates cholesterol synthesis (17–19). However, in direct contrast to 25-hydroxycholesterol and 27-hydroxycholesterol, sulfated 25-hydroxycholesterol down-regulates fatty acid synthetase (19). Furthermore, it is capable of activating PPAR γ , a nuclear receptor known to play a role in the redistribution body fat as well as increase tissue insulin responsiveness (41,42). All three oxysterols are increased following increased StAR expression as evidenced by their increased cytosolic and nuclear levels (16,18,21,30). Furthermore, in addition to their intestinal lipid solubilization properties, bile acids, an end product of cholesterol/27-hydroxycholesterol metabolism, are also known regulators of key steps of lipid homeostasis (43–45).

Based on these observations, we hypothesized that increased cholesterol metabolism as initiated by the CYP27A1 pathway and stimulated by increased StAR expression would result, not only in increased cholesterol elimination as bile acids, but elicit a homeostatic response as a function of increased levels of at least three known regulatory oxysterols. More specifically, these oxysterols, working in a coordinate fashion, should lead to decreased cholesterol synthesis and increased cholesterol export; responses that would be expected to decrease serum and tissue cholesterol levels. Although not originally considered, a metabolic response in fatty acid metabolism should have been entertained given how tightly cholesterol and fatty acid metabolism are interwoven.

The apoE^{-/-} mouse was chosen as the animal model to explore this hypothesis as it is a well studied model of atherosclerosis that on a rodent chow diet will develop serum and tissue dyslipidemia as it ages. The model was first confirmed with serum lipid analysis. As predicted, based upon prior *in vivo* studies in rats and mice, 6 month old mice tolerated the level of increased StAR expression for 6 days without any detectable adverse effects or dietary differences from controls. Increased StAR expression led to dramatic normalization of both serum and tissue cholesterol levels in apoE^{-/-} mice. Serum and tissue triglyceride levels demonstrated an even greater response. These responses demonstrate for the first time a possible therapeutic role for the CYP27A1-initiated pathway in the treatment of dyslipidemias.

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Figure 1. Successful gene transduction of StAR in apoE^{-/-} mice fed with normal diet
 The StAR expression in paraffin imbedded slices of heart, coronary artery and liver were detected by immunohistochemistry as shown in panel a. Western blot of StAR protein levels in different major organs after StAR overexpression as shown in panel b. **NS**: apoE^{-/-} mice injected with normal saline. **EGFP**: apoE^{-/-} mice injected with control adenovirus encoding CMV-EGFP. **StAR**: apoE^{-/-} mice injected with adenovirus encoding CMV-StAR. Bar = 10 μm

Figure 2. Transduction of Ad-CMV-StAR decreases lipid accumulation in the liver of apoE^{-/-} mice fed with normal diet

a: Oil Red O-stained liver sections (bar = 10 μ m) of apoE^{-/-} mice. **b:** Total hepatic cholesterol. **c:** Hepatic triglyceride. Data are means $\pm$ S.D. ** represent $P < 0.01$ versus NS mice. ## represent $P < 0.01$ versus Ad-CMV-EGFP mice. **NS:** apoE^{-/-} mice injected with normal saline (n = 11). **EGFP:** apoE^{-/-} mice injected with control adenovirus encoding CMV-EGFP (n = 9). **StAR:** apoE^{-/-} mice injected with adenovirus encoding CMV-StAR (n = 9).

Figure 3. Effect of transduction of StAR on aortic lipid accumulation in apoE^{-/-} mice
a: En face staining of Sudan IV-stained aortas of mice fed with normal diet. Arrows indicate neutral lipid stained plaques. **b:** Quantification of neutral lipid staining presented as percentage of total en face aorta surface area of mice fed with normal diet (see “Methods”). ** represent $P < 0.01$ versus NS. ## represent $P < 0.01$ versus Ad-CMV-EGFP. NS, EGFP and StAR represent apoE^{-/-} mice injected with normal saline, control adenovirus encoding CMV-EGFP, and adenovirus encoding CMV-StAR, respectively.

Table 1

Increased plasma lipid levels in apoE^{-/-} mice^a

Mice	Total cholesterol		Triglyceride		HDL-cholesterol	
	Female	Male	Female	Male	Female	Male
Wild type						
1 M	1.98±0.14	2.32±0.25	1.96±0.83	1.60±0.54	1.29±0.02	1.73±0.05
3M	2.08±0.14	2.22±0.21	1.18±0.59	1.26±0.19	1.52±0.17	1.74±0.21
5M	1.76±0.34	2.31±0.11	0.94±0.41	0.97±0.95	1.15±0.16	1.97±0.43
apoE^{-/-}						
1M	7.55±3.42 *	7.53±3.26 *	0.84±0.53 *	1.31±0.52	0.63±0.33 *	0.95±0.20 *
3M	9.58±1.55 *	7.81±3.88 *	1.08±0.64	1.50±0.46	0.56±0.15 *	0.78±0.41 *
5M	9.98±0.97 *	8.96±0.67 *	1.02±0.25 *	1.54±1.29	0.36±0.15 *	0.53±0.31 *

^aValues are mmol/L.

ApoE^{-/-} ; apolipoprotein E-deficient; Values are presented as means±S.D; n =6.

Statistical analyses were performed by one-way ANOVA within each age group.

* P <0.01 versus wild type C57BL/6J mice at the same age.

Table 2The level of plasma lipids in apolipoprotein E-deficient mice after StAR overexpression ^a

	NS	Ad-CMV-EGFP	Ad-CMV-StAR
T-CHO	13.53±1.17	13.76±1.21	11.01±0.62 ^{**###}
TG	1.25±0.30	1.12±0.08	0.82±0.14 ^{**#}
HDL-CHO	0.73±0.08	0.67±0.13	1.00±0.19 ^{**###}

^aValues are mmol/L.

Values are presented as means±S.D;

NS: apoE^{-/-} mice injected with normal saline. Ad-CMV-EGFP: apoE^{-/-} mice injected with control adenovirus encoding CMV-EGFP. Ad-CMV-StAR: apoE^{-/-} mice injected with adenovirus encoding CMV-StAR.

^{**} P <0.01 versus NS group^{##} P <0.01 versus Ad-CMV-EGFP group[#] P <0.05 versus Ad-CMV-EGFP group.