

## C57/BL6 마우스에서 아데노바이러스 벡터를 이용한 아포지단백 E 과발현이 혈중 지단백 양상에 미치는 영향

박태호<sup>1</sup> · 김영대<sup>1</sup> · 김덕규<sup>1</sup> · 박주인<sup>2</sup>

### The Effect of Apolipoprotein E Overexpression via Adenoviral Vector on the Plasma Lipoprotein Profile in C57/BL6 Mice

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#### ABSTRACT

**Background and Objectives :** Apolipoprotein E (apoE), a 34-kD plasma glycoapolipoprotein, plays a key role in lipoprotein metabolism by facilitating cellular uptake of remnants of triglyceride-rich chylomicrons and VLDL. Various studies using apoE-knockout mice have elucidated the role of apoE in lipolysis, remnant clearance, and atherogenesis. However, the direct in vivo effects of apoE overexpression on lipoprotein metabolism in the presence of endogenous mouse apoE are not yet fully understood. In this study, the technique of adenovirus-mediated gene transfer was employed in order to investigate the in vivo effect of apoE overexpression on lipid and lipoprotein profile in mice. **Materials and Methods :** Recombinant adenovirus (rAd.mApoE) containing mouse apoE cDNA driven by a cytomegalovirus promoter was generated and infused via tail vein in mice fed a normal chow or high cholesterol diet. Plasma lipid and lipoprotein concentration were measured 1 week after gene transfer. **Results :** Among various mouse tissues, rAd.mApoE was concentrated primarily in the liver. ApoE overexpression caused a decrease in the cholesterol and triglyceride concentration in mice fed on normal chow, while inducing a decrease in the triglyceride concentration and an increase in cholesterol in mice fed a high cholesterol diet. As to the lipoprotein profile, the VLDL and LDL fraction were decreased, HDL was increased by apoE overexpression in both groups fed either a normal chow or high cholesterol diet. **Conclusion :** This data suggests that the overexpression of mouse apoE in mice with endogenous apoE may exert an antiatherogenic effect by inducing favorable change in the lipoprotein profile, regardless of diet and consequent plasma lipid level. (Korean Circulation J 2001;31(9):918-929)

**KEY WORDS :** Apolipoprotein E overexpression · Recombinant adenoviral vector · Cholesterol · Lipoprotein · C57/BL6 mouse.

#### 서 론

가

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가 가 apoE가 . Osuga

(high de - nsity lipoprotein, HDL) (C57/BL6) 15) LDL - R knockout rat apoE

(foam cell lesion) 1) transgene apoE가 fatty streak apo E , APOE 16)

가 2) apoE knockout (transgenesis, gene targeting)

E(apolipoprotein E, apo E) apo E가 가

34 - kD C57/BL6 apoE(mApoE)

3) apoE 22 (amphi - pathic helices)

. Apo E (136 150) mApoE

(low density lipoprotein receptor, LDL - R)

(endocytosis) 4) ApoE 방법 및 대상

LDL receptor - related protein(LRP), 5) 재조합 아데노바이러스 벡터의 제조

VLDL 6) , 7) he - Adenoviral shuttle plasmid pAvCvSv<sup>17)</sup>

paran sulfate<sup>8)</sup> lipase<sup>9)10)</sup> apo E Microbix (Microbix Biosystems Inc., Toronto, On - tario, Canada) pXCJL - 1 promoter

가 (embryonic stem cell) polyadenylation signal 가 , hu - man cytomegalovirus promoter translation enh -

(homologous recombination) apoE kno - cker multiple cloning site

ckout 11 - 13) SV40 polyadenylation signal 가 .

apoE knockout pXCJL - 1 pBR322 human adenov -

가 irus type 5(Ad5) 5' - inverted terminal repeat(ITR),

5 6 가 cholesterol - rich VL - Ad5 origin of replication, Ad5 encapsidation si -

/ 12) DL - sized lipoprotein apoE mRNA gnal, E1a enhancer multiple cloning site가

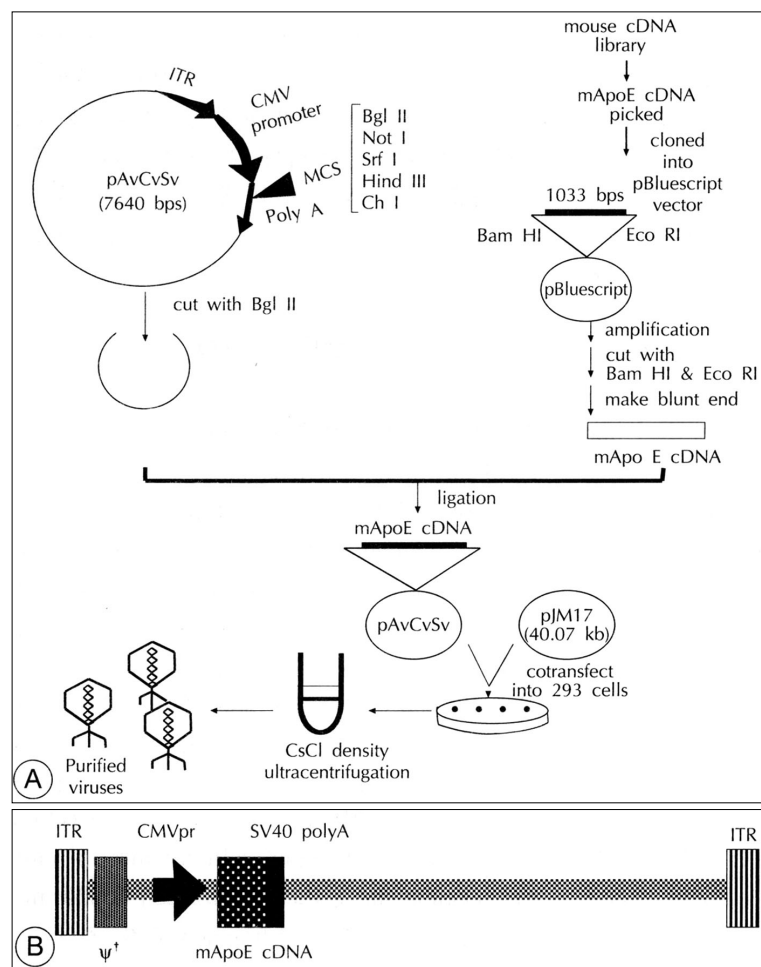
apoE knockout apoE mRNA Ad5 3328 6246 nucleotide 가

50% fragment

13)14) (rAdv.mApoE) full - len - gth mouse apoE cDNA가 pAvCvSv Ad5

DNA pJM17<sup>18)</sup> (hu -  
man embryonic kidney cell) 293<sup>19)</sup> cotr -  
ansfection . pJM17 transfection 실험동물  
가 infectious virions packaging 6 8 (C57/BL6)  
apoE coding cDNA 0.2%(w/w)  
pAvCvSv blunt - ended *Bgl* II site subclone 10%(v/w) coconut oil  
shuttle plasmid(10  $\mu$ g) 10  $\mu$ g su -  
percoiled pJM17 293 calcium phos -  
phate cotransfection . Transfection 0.04%(w/w)  
2 infectious recombinant adenoviral vector pl -  
aques 293 propagation 17) 8  
(polymerase chain reaction, PCR) sc -  
reen mApoE 6 1  $\times 10^{11}$  particles  
cesium chloride density ultracentrifugation EDTA가 phosphate - buffered saline(PBS) 200  $\mu$ l  
1, 2, 4 (retro - orbital plexus)

Fig. 1



**Fig. 1. A :** Schematic diagram for generation of recombinant adenovirus containing mouse apoE cDNA (rAd.mApoE) vector for intravenous injection. The recombinant adenovirus was constructed using adenoviral shuttle plasmid vector, pAvCvSv. pAvCvSv was constructed by addition of promoter and polyadenylation signal in pXCJL-1. The recombinant adenovirus was prepared by cotransfection of pAvCvSv containing full-length mouse apoE cDNA and pJM17 into 293 cells. The recombinant virus (rAd.mApoE) was propagated in 293 cells, screened by PCR for mApoE sequences, and purified by cesium chloride density ultracentrifugation. **B :** The structure of rAd.mApoE. MCS : multiple cloning site, ITR : inverted terminal repeat, CMV pr : cytomegalovirus promoter, SV40 poly A : simian virus 40 poly A tail.

aspartate transaminase(AST) ala - nine transaminase(ALT) kit(Sigma Diagnostics) .

PBS .

DNA 추출

rAd.mApoE 1

가

2 ml

(10 mM Tris, pH 8.0, 1 mM EDTA, 100 mM NaCl, 1% sodium dodecyl sulfate, 1 mg/ml proteinase K) , 55 12

phenol 2 ml

가 1 2 4

8,000 rpm 20

2 3 , PCIA(25 : 24 : 1, phenol/chloroform/isoamyl alcohol )

가 ,

pH 5.5

3 M sodium acetate 100%

가 DNA , 70%

TE

DNA

마우스 조직에서의 재조합 아데노바이러스 벡터의 분포

mApoE cDNA

1

DNA ITR

primer

primer 5' - AG - CCAATATGATAATGAGGGGTG - 3' 5' - TA - CGCGCTATGAGTAACACAAA - 3' primer

adenovirus type 5 left ITR

glyceraldehyde 3 - phosphate dehydrogenase(GAPDH) PCR control

5' - CCCCTATTGACCTCAACTACATGG - 3'

5' - CCTGCTTACCACCTTCTTGAC - 3' primer

10 mM Tris · HCl(pH 8.0), 1

mM MgCl 2, 250 μM dNTP, 5 pmol primer, 5 units Taq polymerase ,

DNA 50 ng 가 50 μl가

94 1 , 57

2 , 72 3 1 30

1.5% 가

혈중 apoE 발현의 측정(western blot analysis)

2 μl 2×SDS

12% SDS - polyacrylamide gel

nitrocellulose membrane

5% skim milk blocking poly - clonal goat anti - human apoE antibody(Chemicon, 1 : 2,000) 1 hors - eradish peroxidase가 conjugation 2 (anti - goat IgG antibody, Pierce, 1 : 3,000)

3 enhanced chemiluminescence(ECL) kit(Amersham) apo E

혈장 지질 농도 및 지단백 측정

0.2 ml 2

Superose 6 column(Pharmacia Biotech Inc., Piscataway, NJ) fast performance liquid chromatography(FPLC) 0.5 ml 30

<sup>20)</sup>

enzymatic kit(Sigma Diagnostics, St. Louis, MO)

## 결 과

아데노바이러스 매개 마우스 apoE의 전달

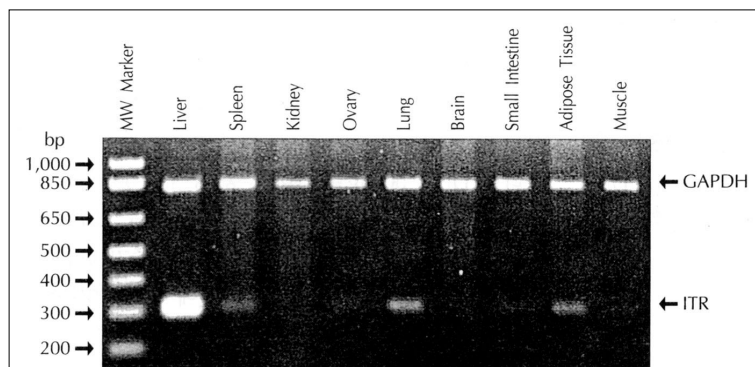
1

가

ITR primer

densitometer

glyceraldehyde phosphate dehydrogenase(GAPDH)

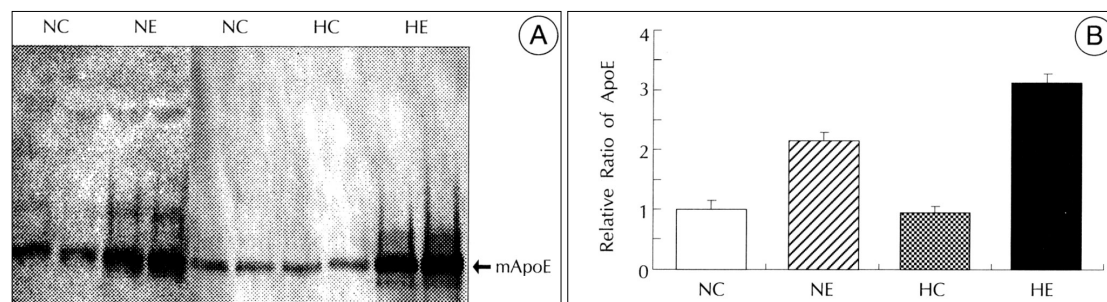


**Fig. 2.** Tissue distribution of rAd.mApoE vector. DNA (50 ng) prepared from the various tissues of mice injected with rAd.mApoE was used as template. The presence of adenoviral inverted terminal repeat (ITR) in various tissues was detected by PCR. The endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as a PCR control.

**Table 1.** AST/ALT in plasma of PBS-injected mice and recombinant virus (rAd.mApoE) injected mice

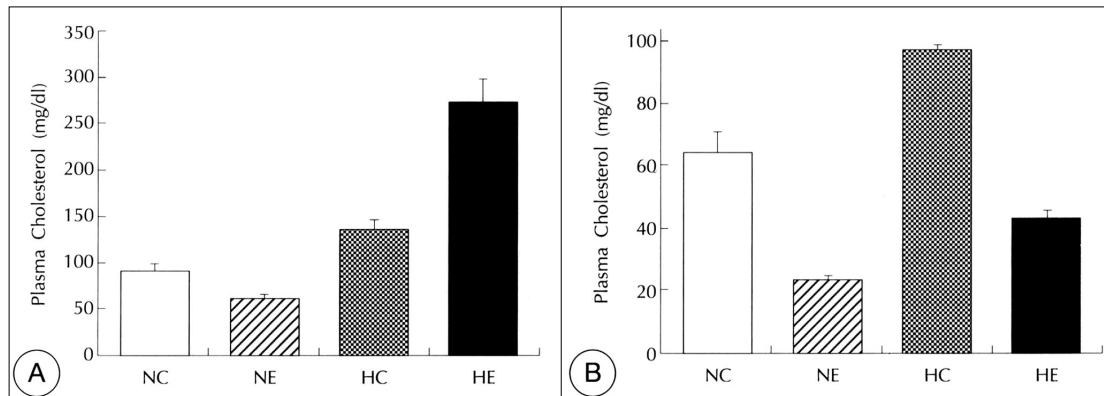
Weeks after injection	AST (IU/L)		ALT (IU/L)	
	PBS injected	Virus injected	PBS injected	Virus injected
0	36.63 ± 10.42	38.45 ± 8.76	17.00 ± 10.42	17.89 ± 6.35
1	19.47 ± 1.54	18.82 ± 0.88	8.73 ± 0.81	13.91 ± 0.38
2	25.99 ± 5.50	359.16 ± 124.54	13.83 ± 5.91	515.27 ± 175.27
4	29.08 ± 4.79	102.18 ± 34.34	5.90 ± 2.02	92.93 ± 58.87

Plasma was collected from PBS-injected mice and recombinant virus-injected mice at indicated times after 6 hours fast and AST and ALT were assayed by kit (Sigma Diagnostics). Values represent mean ± SD, n = 6  
AST : aspartate transaminase, ALT : alanine transaminase

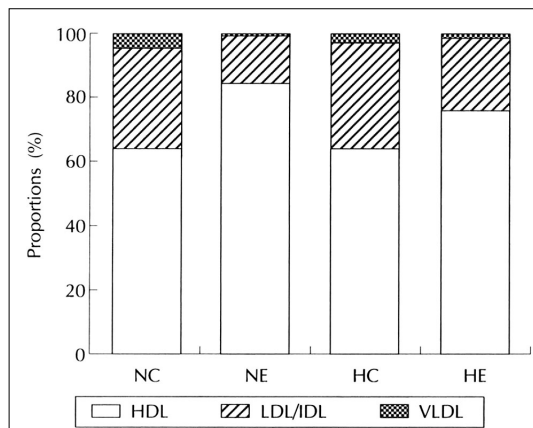


**Fig. 3.** A : ApoE immunoblot of mouse plasma. 2  $\mu$ l of plasma samples from mice subjected to SDS-PAGE, followed by Western blotting analysis with anti-human apoE antibody. NC : samples from mice fed on normal chow, NE : samples from mice fed on normal chow injected with rAd.mApoE, HC : samples from mice fed on high cholesterol diet, HE : samples from mice fed on high cholesterol diet injected with rAd.mApoE. B : Each band was quantitated by densitometric analysis. Values are expressed as relative ratio of apoE and expressed as the mean ± SD, n = 6.

1.452, 0.132, 0.212, (Table 1).  
0.123, 0.212, 0.  
075 가 apoE  
(Fig. 2). 1  
가  
apoE western blot  
mA -  
AST ALT 가가 2 poE cDNA  
apoE PBS



**Fig. 4.** A : Effect of rAd.mApoE injection on plasma cholesterol and triglyceride concentrations of mice fed on normal chow or high cholesterol diet. Total cholesterol (A) and triglyceride concentrations (B) were measured on day 7 after i.v. injection of rAd.mApoE or PBS into mice fed on normal chow or high cholesterol diet. All mice were fasted for 6 hours prior to bleeding. Bars represent mean  $\pm$  SD, n = 6.



**Fig. 5.** Effect of rAd.mApoE injection on plasma lipoprotein fractions of mice fed on normal chow or high cholesterol diet. NC : normal chow-fed mice, NE : normal chow-fed mice on day 7 after rAd.mApoE injection, HC : high cholesterol-fed mice, HE : high cholesterol-fed mice on day 7 after rAd.mApoE injection.

2 3 가 (Fig. 3A and 3B).  
가  
.  
장기간의 고콜레스테롤 식이에 의한 혈중 콜레스테롤 농도 및 중성지방 농도의 변화  
6 8 8  
(TC ; 88.2  $\pm$  7.9 mg/

dl, TG ; 64.4  $\pm$  7.4 mg/dl)  
(133.1  $\pm$  11.4 mg/dl)  
1.5 가 (96.4  $\pm$  1.3 mg/dl)  
1.5 가 (Fig. 4A and 4B).

정상식이를 한 마우스에서 mApoE 과발현에 의한 혈중 콜레스테롤 농도 및 중성지방 농도의 변화

	mApoE	PBS
		(88.2 $\pm$ 7.9 mg/dl)
mApoE	1	
(61.3 $\pm$ 5.0 mg/dl)	30.5%	(Fig. 4A).
mApoE	1	(24.0 $\pm$ 1.9 mg/dl)
PBS		mApoE (64.4 $\pm$ 7.4 mg/dl)
4 mg/dl)	62.7%	(Fig. 4B).

장기간 고콜레스테롤 식이를 한 마우스에서 mApoE 과발현에 의한 혈중 콜레스테롤 농도 및 중성지방의 변화  
mApoE 1 (270.0  $\pm$  27.1 mg/dl)

	mApoE	PBS
		(133.1 $\pm$ 11.4 mg/dl)
가	(Fig. 4A).	mApoE (43.5 $\pm$ 3.4 mg/dl)
1		mApoE (96.4 $\pm$ 1.3 mg/dl)
	PBS	

4 ± 1.3 mg/dl) 54.9% (Fig. 4B).

장기간의 고콜레스테롤 식이에 의한 혈중 지단백 분획의 변화

FPLC

가

(Figs. 5 and 6A).

정상식이 마우스와 장기간의 고콜레스테롤 식이 마우스에서의 mApoE 과발현에 의한 혈중 지단백의 분획 변화

mApoE

, mApoE

가 (Figs. 5 and 6B).

mApoE

, mApoE

가 (Figs. 5 and 6C).

고 찰

ApoE  
ylomicron)

(glycoprotein)  
(ch -

ocytes)

(macrophage)

(astr -

(pleiotropism)

3)21)22)

apoE

DNA

endosome

10<sup>4</sup> 10<sup>5</sup>

(progeny)

. DNA

가

4

(E1 - E4)가

,

E1

24)

25)

293

가

. E1 E3

가

apoE

가

293

APOE 가

apoE

apoE

knockout

LDL - R knockout

apoE

apoE가

mApoE

C57/BL6

foam cell lesion

2)

. mApoE cDNA

mApoE

mApoE

80 90 nm envelope가

icosahedral capsid

36 kb

DNA(linear, double - stranded DNA)

가

1)

sequence E1

, 2)

가(up to 10<sup>12</sup>

가

particles/ml)

, 3)

cDNA

가

( 7.5 kb),

4) retrovirus

, 5)

가

. ApoE

, 5)

가

. ApoE

(astr -

endosome

10<sup>4</sup> 10<sup>5</sup>

(progeny)

. DNA

가

4

(E1 - E4)가

,

E1

24)

25)

293

가

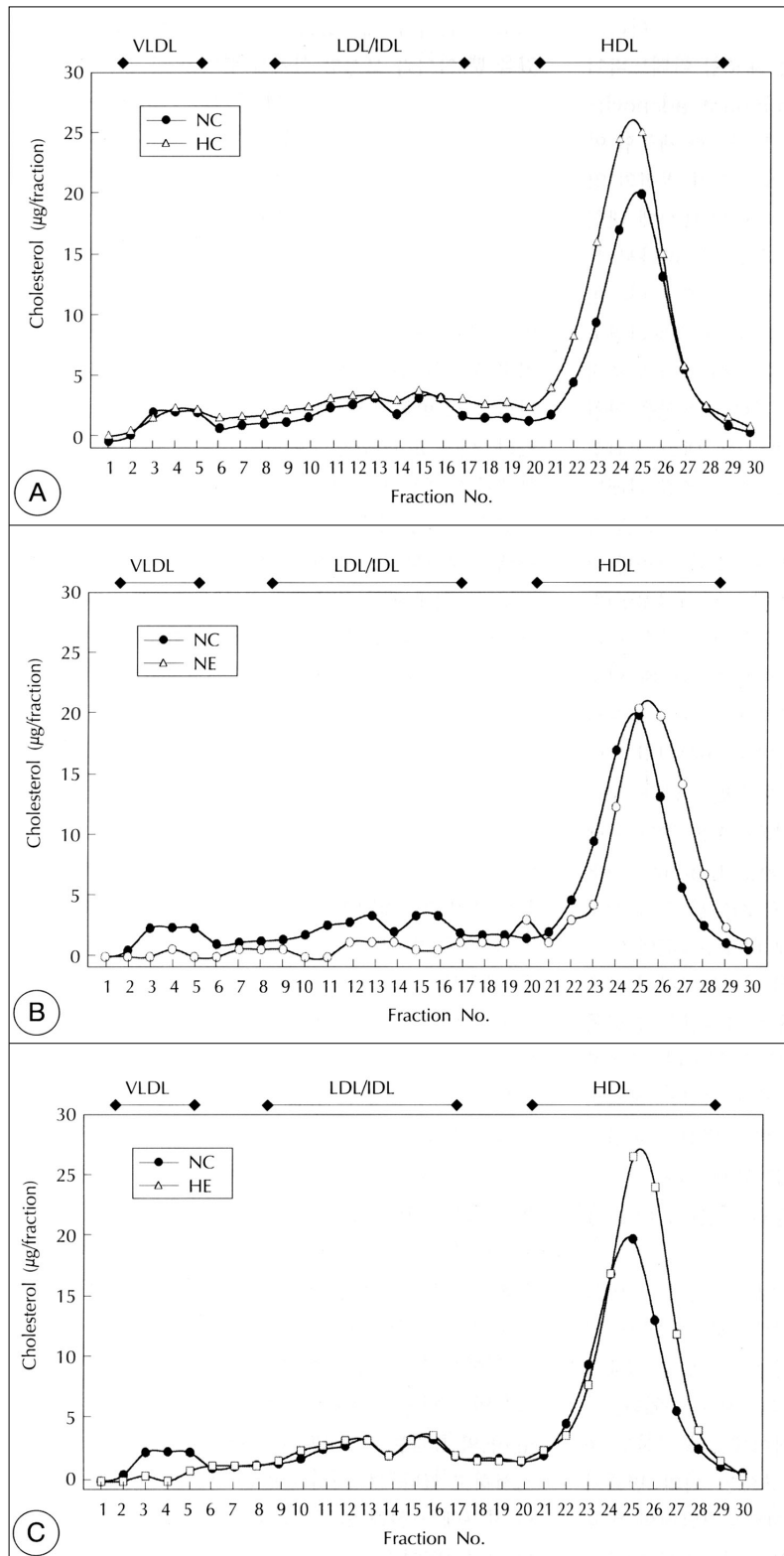
. E1 E3

가

apoE

가

293



**Fig. 6.** FPLC profiles of plasma lipoproteins from mice fed on normal chow or high cholesterol diet after the injection of rAd.mApoE or PBS. Plasma samples (200  $\mu$ l) were separated by gel filtration chromatography using a HPLC/FPLC system with two Superose 6 columns connected in series. FPLC fractions 1 - 30 were collected and cholesterol concentrations were measured in each fraction using enzymatic assay kits from Sigma Diagnostics. A : Graph shows representative FPLC profiles of plasma cholesterol of normal chow-fed mice and high cholesterol-fed mice. B : Graph shows representative FPLC profiles of plasma cholesterol of normal chow-fed mice and on day 7 after the injection of rAd.mApoE. C : Graph shows representative FPLC profiles of plasma cholesterol of normal chow-fed mice and high cholesterol-fed mice on day 7 after the injection of rAd.mApoE. FPLC quantitation was performed in 6 animals for each treatment group.



(replication - defective  
recombinant adenovirus)  
<sup>26)</sup> .  
, mApoE  
<sup>27)</sup> . APOE 가  
1 mApoE 가 가  
. apoE  
. *in vitro* apoE가  
<sup>29)30)</sup> , *in vivo*  
AST ALT 가가 2 apoE  
. apoE  
<sup>31)</sup> .  
. apoE  
. rat  
4 8 apoE 가  
<sup>28)</sup> 가  
<sup>32)</sup> LDL - R knockout  
<sup>33)</sup> , Watana -  
hu - be - heritable hyperlipidemic(WHHL) rabbit  
man apoE Western blot apoE  
<sup>34)</sup> . apoE knockout  
가  
human apoE  
가  
<sup>35)36)</sup> .  
apoE Huang <sup>37)</sup>  
apoE 가  
. 가  
60% apoE  
, .  
가 . apoE  
가  
<sup>33)34)</sup> .  
가 가 apoE  
<sup>38)</sup> .  
가 . C57/BL6 apoE

가

가 . apoE가

apoE

·

C57/BL6

·

· null virus control gene

PBS

35)

·

mApoE cDNA

mApoE

가

가

·

mApoE apoE가

가 .

C57/BL6

apoE

방 법 :

apoE cDNA  
(rAd.mApoE)

APOE

결 과 :

rAd.mApoE 1

. ApoE

가

가 .

가 .

결 론 :

## 요약

연구목적 :

E(apolipoprotein E, apoE) 34 - kD  
(glycoapolipoprotein)  
(triglyceride - rich chylomicron)  
(VLDL) (remnant)

apoE                      apoE

knockout

apoE가

중심 단어 :

E .

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