The effect of old age on apolipoprotein E and its receptors in rat liver

Tharani Sabaretnam · Jennifer O'Reilly ·
Leonard Kritharides · David G. Le Couteur

Received: 4 May 2009 / Accepted: 16 August 2009 / Published online: 7 October 2009
© American Aging Association 2009

Abstract Apolipoprotein E (apoE) is associated with aging and some age-related diseases. The majority of apoE is produced by hepatocytes for the receptor-mediated uptake of lipoproteins. Here, the effects of age on the hepatic expression and distribution of apoE and its receptors were determined using immunofluorescence, Western blots, and quantitative PCR in rat liver tissue and isolated hepatocytes. The expression of apoE mRNA and protein was not influenced

significantly by aging. Immunofluorescence studies in isolated hepatocytes showed that apoE was more likely to be co-localized with early endosomes, golgi, and microtubules in isolated old hepatocytes. The mRNA expression of the receptor involved in sequestration of apoE, heparan sulfate proteoglycan was reduced in old age, without any significant effect on the expression of either the low-density lipoprotein receptor or low density-lipoprotein receptor-related protein. Old age is associated with changes in hepatic apoE intracellular trafficking and heparan sulfate proteoglycan expression that might contribute to age-related disease.

T. Sabaretnam · J. O'Reilly · L. Kritharides ·
D. G. Le Couteur
ANZAC Research Institute, University of Sydney,
Sydney, Australia

T. Sabaretnam · L. Kritharides
Department of Cardiology, Concord Hospital,
Sydney, Australia

T. Sabaretnam · J. O'Reilly · D. G. Le Couteur
Centre for Education and Research on Aging (CERA),
University of Sydney,
Sydney, Australia

L. Kritharides
Centre for Vascular Research, School of Medical Sciences,
University of New South Wales,
Sydney, Australia

D. G. Le Couteur (✉)
Centre for Education and Research on Ageing,
Concord RG Hospital, Hospital Road,
Concord,
Sydney, NSW 2139, Australia
e-mail: dlecoutur@med.usyd.edu.au

Keywords Apolipoprotein E · Liver · Hepatocyte ·
Ageing · Aging · Low-density lipoprotein receptor ·
Low density-lipoprotein receptor-related protein ·
Heparan sulfate proteoglycan

Introduction

Apolipoprotein E (apoE) is a component of many lipoproteins and a ligand for many lipoprotein receptors. The majority of circulating apoE is produced by hepatocytes and it is this hepatic pool that is important for lipid metabolism (Ang et al. 2008; Kolovou and Anagnostopoulou 2007; Smith 2002). ApoE facilitates the uptake of lipoproteins into hepatocytes by receptor-mediated uptake with the low-density lipoprotein receptor (LDLR), low density-lipoprotein receptor-related protein (LRP), and

heparan sulfate proteoglycan (HSPG; Cooper 1997; Yu and Cooper 2001). Most apoE is then re-cycled by secretion into the space of Disse where it is available once more for binding to remnant lipoproteins (Heeren et al. 2001; Ji et al. 1994; Zhu et al. 2005).

ApoE alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) are linked with age-related diseases such as Alzheimer's disease and cardiovascular disease and contribute to the genetic component of longevity (Ang et al. 2008; Jellinger 2002; Smith 2002). The relationship between the concentration of circulating apoE levels with old age and age-related disease has not been fully established, partly because of the confounding effects of genotype, BMI, diet, alcohol, disease, and medications on levels (Braeckman et al. 1998; Sakurabayashi et al. 2001). ApoE concentration increases into middle age, possibly declines in older age (Braeckman et al. 1998; Sakurabayashi et al. 2001) and is associated with dementia and cardiovascular disease, although less influential than the apoE genotype (Siest et al. 2000).

Although apoE is produced primarily by the liver and is associated with aging and age-related disease, little is known about the effects of aging on hepatic apoE. We found that old age is associated with altered distribution of apoE in human and baboon livers. Although overall expression was not increased, there was more cytoplasmic staining and reduced perisinusoidal staining, suggestive of altered trafficking of apoE in hepatocytes (Hilmer et al. 2004). Gee et al.

(2005) found increased hepatic protein and mRNA expression of apoE in livers of old rats, whereas Tollet-Egnell et al. (2001) found no age-related changes using microarray. To further study hepatic apoE and aging, here we investigated the hepatic expression of apoE and its major receptors, then determined whether there are any age-related changes in the intracellular trafficking of apoE.

Methods

Materials

The following antibodies were used: goat anti-apoE polyclonal antibody, anti-mouse immunoglobulin F (ab)2 fragment FITC conjugated raised in sheep (Chemicon, Australia), rabbit anti-human apoE, polyclonal goat anti-rabbit immunoglobulin HRP, rabbit polyclonal antibody to human apoE, goat anti-donkey conjugated-HRP DAPI, affinity-purified rabbit polyclonal anti-goat IgG-peroxidase conjugate (DakoCytomation, Australia), purified human apoE (Biodesign, Australia), mouse monoclonal anti-apoE, anti-EEA1, anti-GM130 (BD Biosciences, Australia), anti-tubulin, Alexa fluorophore 594, Alexa fluorophore 488, Alexa fluorophore 555 (Molecular Probes, Australia). All other reagents were purchased from Sigma-Aldrich (Australia) unless otherwise stated.

Table 1 Primer sequences

Gene		Sequence	Annealing temperature
β ACTIN	F	AGCCATGTACGTAGCCATCC	60
	R	CTCTCAGCTGTGGTGGTGAA	60
GAPDH	F	ACCACAGTCCATGCCATCAC	65
	R	TCCACCACCCTGTTGCTGTA	65
S18	F	CCTTCGCTATCACTGCCATT	60
	R	TGGCCAGAACCTGGCTATAC	60
S29	F	GGGTCACCAGCAGCTGTACT	64
	R	CCGATATCCTTCGCGTACTG	62
LRP	F	CACCTTAACGGGAGCAATGT	60
	R	GTCACCCAGTCTGTCCAGT	64
APOE	F	TGTGAGTGCTATCCGTGACG	62
	R	TATCTGCTGGGTCTGCTCCT	62
LDLR	F	TTCTTCAGGTTGGGGATCAG	60
	R	CAGCTCTGTGTGAACCTGGA	62
HSPG	F	GCAGGACCCTGCTGTAAAAA	60
	R	CAAGTTCGTAGCTTCCTTCG	62

Animals and primary hepatocyte cell isolation

Young mature adult (4–6 months) and old adult (24–26 months) Fischer 344 male rats were obtained from the National Institute of Aging (Bethesda, MD, USA). The study was approved by the Central Sydney Area Health Service Animal Welfare Committee. Livers were perfused with collagenase A and prepared in Hanks buffer without calcium and magnesium (Invitrogen, Australia). Isolated hepatocytes were plated onto coverslips coated with rat-tail collagen and cultured for 36 hr prior to experiments. Viability was assessed by ATP content of cells (determined using a bioluminescent luciferase assay, PerkinElmer, Australia) and mitochondrial activity (determined using 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazoly carbocyanine iodide, Molecular Probes, Invitrogen, Australia). Cells were examined for morphological changes by phase-

contrast microscopy using a Zeiss Axiovert 200 microscope.

Immunofluorescence and co-localization studies of native apoE

Primary hepatocytes were fixed with 4% paraformaldehyde and permeabilized with 0.001% Triton-X 100. Cells were incubated with goat anti-human apoE primary antibody followed by incubation with Alexa fluorophore 561-conjugated anti-goat secondary antibody. In control experiments, fluorescence was shown to be negligible in the absence of primary antibody. Co-localization studies were done with mouse anti-human EEA1, mouse anti-human GM130 Golgi, mouse anti-tubulin. Cells were incubated with the appropriate fluorophore-conjugated secondary antibody; Alexa fluorophore 488-conjugated anti-mouse

Fig. 1 The effect of old age on liver apoE protein and mRNA. There was no statistically significant effect of aging (protein expressed as arbitrary units normalized to cell protein; mRNA are expressed as -fold comparison to β -actin expression)

Fig. 2 Light microscopic appearance of hepatocytes isolated from young (**a**) and old (**b**) rats. In old rats, larger and multinucleate hepatocytes were often apparent. $\times 10$ magnification

IgG, Alexa fluorophore 555-conjugated anti-mouse IgG, Alexa fluorophore 561-conjugated anti-mouse IgG, and DAPI. Single Z-stack images were collected by confocal microscopy using a $\times 100$ oil immersion lens on a Nikon C1 confocal microscope attached to a Nikon TE2000 inverted microscope. Images were analyzed using the Nikon C1 Confocal Viewer program, EZ-C1 version 3.0 (Nikon Corporation, USA). Each conclusion with regards distribution was determined from a group of six

rats. At least six slides per liver isolation were studied, and more than six representative cells per slide were examined. Therefore, each conclusion is based on observations at least 216 cells.

Quantitative real-time PCR

Cells were lysed and total RNA extracted. First-strand cDNA was generated via reverse transcriptase by Oligo-

Fig. 3 Immunofluorescence studies of apoE and early endosomes in hepatocytes isolated from young (**a,b**) and old (**c,d**) rats. ApoE staining is *red*, early endosomes are *green*, and co-localization is shown with *yellow*. Co-localization shown by yellow is more frequent in cells from old livers

dT priming (Invitrogen, Australia). Primers were designed by Proligo (Lismore, Australia, Table 1). Quantitative reverse transcriptase polymerase chain reaction was performed using IQ SYBR Green Supermix and iCycler (Biorad, Australia). Confirmation of a single gene product was carried out through generation of a dissociation curve following each qPCR cycle. A cycle threshold value (C_T) was determined using the iCycler software and quantification of gene products, normalized to the expression of the ribosomal β -actin housekeeping gene, was determined using the comparative C_t ($\Delta\Delta C_t$) method (Pfaffl 2001).

Measurement of apoE protein

Tissue lysate was mixed with sample buffer containing 10 mM dithiothreitol, heated to 100°C for 5 min, and separated by SDS-PAGE (12.5% polyacrylamide

reducing gel). After transfer onto 0.45 μ m nitrocellulose membrane (1 h at 70 V; Amersham Biosciences, Australia) the blot was blocked, incubated with primary antibody to apoE (goat anti-human polyclonal, 1:5,000 v/v), washed and finally incubated with a rabbit anti-goat IgG secondary antibody conjugated to horseradish peroxidase (1:5,000 v/v; Kockx et al. 2004). Proteins were visualized using chemiluminescence, quantified with a BioDocAnalyze system (Biometra, Germany) and expressed as arbitrary units (AU) normalized to cell protein.

Statistical analyses

Results are presented as mean $\pm$ SD. All cell assays were performed in triplicate of $n=3$ –4 livers. Statistical comparisons were performed using the Students t test. A value of $P<0.05$ was considered statistically significant.

Fig. 4 Immunofluorescence studies of apoE and tubulin in hepatocytes isolated from young (a,b) and old (c,d) rats. ApoE staining is red, tubulin staining is green, and co-localization is shown with yellow. Co-localization shown by yellow and is more frequent in cells from old livers

Results

ApoE expression

Quantitative RTPCR did not reveal any statistically significant change in apoE mRNA expression in liver tissue in old rats compared with young rats and results were same regardless of housekeeping genes. Western blots from liver tissue also did not show any reduction in the expression of apoE protein in old age (Fig. 1). Secreted apoE in the media of isolated hepatocytes could not be detected presumably because this fell below the limits of detection of the assay.

Isolated hepatocytes

Old hepatocytes were larger and more likely to be multinucleate upon isolation and culture (Fig. 2). ATP

content of the young hepatocytes was greater than that of the old hepatocytes (196 ± 4 nmol/ μ g protein young vs 117 ± 34 nmol/ μ g protein old, $P \leq 0.001$). Mitochondrial function assayed by JC-1 fluorescence was decreased in old age ($87 \pm 4\%$ functional mitochondrial young vs $71 \pm 6\%$ in old, $P < 0.01$).

Immunofluorescence

In hepatocytes from young rats, apoE fluorescence was located in a homogenous pattern throughout the cytoplasm. In hepatocytes from old rats, the apoE was also located throughout the cytoplasm but tended to be clumped. In hepatocytes from young rats, there was some co-localization of apoE with golgi and microtubules, but not with early endosomes. By contrast, in hepatocytes from the old hepatocytes, there was strong co-localization of apoE with these structures (Figs. 3, 4, 5).

Fig. 5 Immunofluorescence studies of apoE and golgi in hepatocytes isolated from young (a,b) and old (c,d) rats. ApoE staining is red, golgi staining is green, and co-localization is shown with yellow. Co-localization is more frequent in cells from old livers

Table 2 The effects of age on the mRNA expression of lipoprotein receptors in liver tissue (units are -fold comparison to β -actin expression)

	LDLR	LRP	HSPG
Young	6.1 $\pm$ 3.2	0.5 $\pm$ 0.2	35.4 $\pm$ 8.0
Old	5.7 $\pm$ 1.6	0.4 $\pm$ 0.3	16.4 $\pm$ 5.3 *

* $P<0.05$

LDLR, LRP, and HSPG expression

The effects of old age on the mRNA expression of LDLR, HSPG, and LRP are shown in Table 2. There was a significant reduction in the expression of HSPG in old age to about half that seen in young animals. There were no changes with age in either LDLR or LRP.

Discussion

Old age is associated with changes in the hepatic synthesis and expression of many proteins (Swindell 2008; Tollet-Egnell et al. 2001; Ward and Richardson 1991) and a substantial increase in blood cholesterol and triglyceride concentrations in many species including the Fischer F344 rat used in this study (Liepa et al. 1980; Lingelbach and McDonald 2000; Masoro et al. 1983). However, in this study, we found no significant change in the liver levels of apoE mRNA or protein. This is consistent with our previous immunohistochemical study of aging in human and baboon livers where there was no overall change in the intensity of staining (Hilmer et al. 2004). Likewise, in the livers of aging rats, Tollet-

Egnell et al. (2001) found no age-related changes using microarray to study mRNA expression and Chou et al. (1996) found no change in apoE concentrations. On the other hand, Gee et al. (2005) found an increase in liver apoE mRNA and protein in old F344 rats. Although there is some inconsistency between the studies, there is consensus that liver apoE is not decreased in old age. Therefore, if there is any age-related decrease in circulating apoE as suggested by Braeckman et al. (1998), then this is likely to be secondary to altered hepatocyte secretion and/or increased clearance of apoE.

Most apoE that enters hepatocytes by receptor-mediated uptake is re-cycled via the endosomes and secreted back into the extracellular space of Disse. Some apoE is removed by uptake into lysosomes and additional apoE may be synthesized via endoplasmic reticulum and golgi. Transport between these compartments occurs along the microtubules (Fazio et al. 2000). Hilmer et al. showed altered cellular distribution of apoE in the liver of old humans and baboons. In old age, there was an increase in hepatocyte cytoplasmic staining and a decrease in the perisinusoidal staining, a result consistent with an age-related effect on apoE trafficking and secretion (Hilmer et al. 2004). Here, we found there is further evidence for altered intracellular trafficking of apoE. In old rats, apoE was found to be more strongly co-localized with early endosomes and microtubules compared with young rats. As far as we are aware, the effect of old age on the trafficking of apoE has not been reported previously, however, the results are consistent with reports of altered microtubule function and structure in old age (Cash et al. 2003; Rao and Cohen 1990; Taylor et al. 1992). It is plausible that

Table 3 Published studies of the effects of aging on hepatic LDLR in rodents

Citation	Species	Age range	Assay	Age-related change (% of young value)
(Parini et al. 1999)	Rat	2 vs 18 months	Membrane protein	No change
(Field and Gibbons 2000)	Rat	2 vs 15 months	Membrane protein	58%
(Pallottini et al. 2006)	Rat	3 vs 24 months	Membrane protein	~60%
			Cell protein	~166%
(Walker et al. 1994)	Rat	3 vs 14 months	mRNA	19%
(Bose et al. 2005)	Mouse	4 vs 23 months	mRNA	43%
(Pallottini et al. 2006)	Rat	3 vs 24 months	mRNA	~240%
(Galman et al. 2007)	Rat	6 vs 18 months	mRNA	No change
This study	Rat	4 vs 24 months	mRNA	No change

age-related co-localization of apoE in early endosomes and increased cytoplasmic staining is a consequence of impaired microtubule function in hepatocytes. Such a mechanism would be expected to contribute to age-related dyslipidemia by decreasing available apoE for receptor-mediated uptake of lipoproteins (Hilmer et al. 2004; Le Couteur et al. 2002) indicating that this finding may have important clinical implications. However, it should be noted that isolated hepatocytes were used in this study and it is possible that aging influences the response and viability of cells to the isolation process.

The functional role of apoE is mediated by its interaction with hepatocellular membrane lipoprotein receptors. In particular, the hepatic uptake of chylomicron remnants is facilitated by interactions between apoE in the lipoprotein and three receptors found in the space of Disse: the LDLR, LRP, and HSPG (Cooper 1997; Yu and Cooper 2001). The hepatic clearance of lipoproteins, especially chylomicron remnants is impaired in old age (Le Couteur et al. 2002), which could be secondary to changes in the expression and interactions between apoE and its receptors. There have been some previous reports on the effect of aging on the hepatic LDLR in rodents (Table 3). The results are not consistent between studies and this presumably reflects differences in methodology, species, and ages. Our results support the contention that expression of LDLR does not change substantially with old age. Therefore, the age-related reduction in the hepatic clearance of remnants is more likely secondary to other mechanisms such as age-related changes in apoE we report here, or the uptake of lipoproteins across the liver endothelium (Hilmer et al. 2005; Le Couteur et al. 2002, 2008). The effect of aging on the other receptors has not been widely reported. Field and Gibbons (2000) reported that LRP protein in the hepatocyte membrane fell in old rat livers to 45% of values seen in younger rats, however, we did not detect any changes in LRP mRNA expression. However, in old age, there was a substantial reduction in the expression of HSPG mRNA to about half the level seen in young animals. As far as we are aware, the effect of aging on hepatic HSPG has not been reported previously. Because HSPG facilitates apoE-mediated uptake of lipoproteins (MacArthur et al. 2007), this might be mechanistically associated with age-related dyslipidemia.

In conclusion, old age was associated with evidence of altered hepatocyte trafficking of apoE and decreased expression of one of its receptors HSPG. These changes may contribute to some age-related disorders associated with apoE.

Acknowledgments We acknowledge funding from the Australian National Health and Medical Research Council and the Ageing and Alzheimer's Research Foundation.

References

- Ang LS, Cruz RP, Hendel A, Granville DJ (2008) Apolipoprotein E, an important player in longevity and age-related diseases. *Exp Gerontol* 43:615–622
- Bose C, Bhuvaneshwaran C, Udupa KB (2005) Age-related alteration in hepatic acyl-CoA: cholesterol acyltransferase and its relation to LDL receptor and MAPK. *Mech Ageing Dev* 126:740–751
- Braeckman L, De Bacquer D, Rosseneu M, De Backer G (1998) The effect of age and lifestyle factors on plasma levels of apolipoprotein E. *J Cardiovasc Risk* 5:155–159
- Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu X, Raina AK, Vinters HV, Tabaton M, Johnson AB, Paula-Barbosa M, Avila J, Jones PK, Castellani RJ, Smith MA, Perry G (2003) Microtubule reduction in Alzheimer's disease and aging is independent of tau filament formation. *Am J Pathol* 162:1623–1627
- Chou SC, Cheng B, Hornick TR, Kowal J, Abraham S (1996) Apolipoprotein E is increased in aged rat kidney. *Biochem Mol Biol Int* 38:847–854
- Cooper AD (1997) Hepatic uptake of chylomicron remnants. *J Lipid Res* 38:2173–2192
- Fazio S, Linton MF, Swift LL (2000) The cell biology and physiologic relevance of ApoE recycling. *Trends Cardiovasc Med* 10:23–30
- Field PA, Gibbons GF (2000) Decreased hepatic expression of the low-density lipoprotein (LDL) receptor and LDL receptor related protein in aging rats is associated with delayed clearance of chylomicrons from the circulation. *Metabolism* 49:492–498
- Galman C, Matasconi M, Persson L, Parini P, Angelin B, Rudling M (2007) Age-induced hypercholesterolemia in the rat relates to reduced elimination but not increased intestinal absorption of cholesterol. *Am J Physiol Endocrinol Metab* 293:E737–E742
- Gee JR, Ding Q, Keller JN (2005) Modulation of apolipoprotein E and interleukin-1 β in the aging liver. *Exp Gerontol* 40:409–415
- Heeren J, Grewal T, Jackle S, Beisiegel U (2001) Recycling of apolipoprotein E and lipoprotein lipase through endosomal compartments in vivo. *J Biol Chem* 276:42333–42338
- Hilmer SN, Warren A, Cogger VC, Fraser R, McLean AJ, Sullivan D, Le Couteur DG (2004) The effect of aging on the immunohistochemistry of apolipoprotein E in the liver. *Exp Gerontol* 39:53–57

- Hilmer SN, Cogger VC, Fraser R, McLean AJ, Sullivan D, Le Couteur DG (2005) Age-related changes in the hepatic sinusoidal endothelium impede lipoprotein transfer in the rat. *Hepatology* 42:1349–1354
- Jellinger KA (2002) Alzheimer disease and cerebrovascular pathology: an update. *J Neural Transm* 109:813–836
- Ji ZS, Fazio S, Lee YL, Mahley RW (1994) Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. *J Biol Chem* 269:2764–2772
- Kockx M, Rye KA, Gaus K, Quinn CM, Wright J, Sloane T, Sviridov D, Fu Y, Sullivan D, Burnett JR, Rust S, Assmann G, Anantharamaiah GM, Palgunachari MN, Katz SL, Phillips MC, Dean RT, Jessup W, Kritharides L (2004) Apolipoprotein A-1-stimulated apolipoprotein E secretion from human macrophage is independent of cholesterol efflux. *J Biol Chem* 279:25966–25977
- Kolovou GD, Anagnostopoulou KK (2007) Apolipoprotein E polymorphism, age and coronary heart disease. *Ageing Res Rev* 6:94–108
- Le Couteur DG, Fraser R, Cogger VC, McLean AJ (2002) Hepatic pseudocapillarisation and atherosclerosis in ageing. *Lancet* 359:1612–1615
- Le Couteur DG, Warren A, Cogger VC, Smedsrod B, Sorensen K, de Cabo R, Fraser R, McCuskey RS (2008) Old age and the hepatic sinusoid. *Anat Rec* 291:672–683
- Liepa GU, Masoro EJ, Bertrand HA, Yu BP (1980) Food restriction as a modulator of age-related changes in serum lipids. *Am J Physiol* 238:E253–E257
- Lingelbach LB, McDonald RB (2000) Description of the long-term lipogenic effects of dietary carbohydrates in male Fischer 344 rats. *J Nutr* 130:3077–3084
- MacArthur JM, Bishop JR, Stanford KI, Wang L, Bensadoun A, Witztum JL, Esko JD (2007) Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J Clin Invest* 117:153–164
- Masoro EJ, Compton C, Yu BP, Bertrand H (1983) Temporal and compositional dietary restrictions modulate age-related changes in serum lipids. *J Nutr* 113:880–892
- Pallottini V, Martini C, Cavallini G, Donati A, Bergamini E, Notarnicola M, Caruso MG, Trentalance A (2006) Modified HMG-CoA reductase and LDLr regulation is deeply involved in age-related hypercholesterolemia. *J Cell Biochem* 98:1044–1053
- Parini P, Angelin B, Rudling M (1999) Cholesterol and lipoprotein metabolism in aging: reversal of hypercholesterolemia by growth hormone treatment in old rats. *Arterioscler Thromb Vasc Biol* 19:832–839
- Pfaffl M (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acid Res* 29:e45
- Rao KM, Cohen HJ (1990) The role of the cytoskeleton in aging. *Exp Gerontol* 25:7–22
- Sakurabayashi I, Saito Y, Kita T, Matsuzawa Y, Goto Y (2001) Reference intervals for serum apolipoproteins A-I, A-II, B, C-II, C-III, and E in healthy Japanese determined with a commercial immunoturbidimetric assay and effects of sex, age, smoking, drinking, and Lp(a) level. *Clin Chim Acta* 312:87–95
- Siest G, Bertrand P, Herbeth B, Vincent-Viry M, Schiele F, Sass C, Visvikis S (2000) Apolipoprotein E polymorphisms and concentration in chronic diseases and drug responses. *Clin Chem Lab Med* 38:841–852
- Smith JD (2002) Apolipoproteins and aging: emerging mechanisms. *Ageing Res Rev* 1:345–365
- Swindell WR (2008) Comparative analysis of microarray data identifies common responses to caloric restriction among mouse tissues. *Mech Ageing Dev* 129:138–153
- Taylor L, Jones AL, Schmucker DL (1992) Does aging affect liver microtubules? *Proc Soc Exp Biol Med* 199:441–445
- Tollet-Egnell P, Flores-Morales A, Stahlberg N, Malek RL, Lee N, Norstedt G (2001) Gene expression profile of the aging process in rat liver: normalizing effects of growth hormone replacement. *Mol Endocrinol* 15:308–318
- Walker RF, Ness GC, Zhao Z, Bercu BB (1994) Effects of stimulated growth hormone secretion on age-related changes in plasma cholesterol and hepatic low density lipoprotein messenger RNA concentrations. *Mech Ageing Dev* 75:215–226
- Ward W, Richardson A (1991) Effect of age on liver protein synthesis and degradation. *Hepatology* 5:935–949
- Yu KC, Cooper AD (2001) Postprandial lipoproteins and atherosclerosis. *Frontiers Biosci* 6:332–354
- Zhu MY, Hasty AH, Harris C, Linton MF, Fazio S, Swift LL (2005) Physiological relevance of apolipoprotein E recycling: studies in primary mouse hepatocytes. *Metabolism* 54:1309–1315