Pathophysiology of apolipoprotein E deficiency in mice: relevance to apo E-related disorders in humans

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ABSTRACT **Apolipoprotein E (apo E) deficiency (or its abnormalities in humans) is associated with a series of pathological conditions including dyslipidemia, atherosclerosis, Alzheimer's disease, and shorter life span. The purpose of this study was to characterize these conditions in apo E-deficient C57BL/6J mice and relate them to human disorders. Deletion of apo E gene in mice is associated with changes in lipoprotein metabolism [plasma total cholesterol (TC) (>+400%), HDL cholesterol (−80%), HDL/TC, and HDL/LDL ratios (−93% and −96%, respectively), esterification rate in apo B-depleted plasma (+100%), plasma triglyceride (+200%), hepatic HMG-CoA reductase activity (−50%), hepatic cholesterol content (+30%)], decreased plasma homocyst(e)ine and glucose levels, and severe atherosclerosis and cutaneous xanthomatosis. Hepatic and lipoprotein lipase activities, hepatic LDL receptor function, and organ antioxidant capacity remain unchanged. Several histological/immunohistological stainings failed to detect potential markers for neurodegenerative disease in the brain of 37-wk-old male apo E-KO mice. Apo E-KO mice may have normal growth and development, but advanced atherosclerosis and xanthomatosis may indirectly reduce their life span. Apo E plays a crucial role in regulation of lipid metabolism and atherogenesis without affecting lipase activities, endogenous antioxidant capacity, or appearance of neurodegenerative markers in 37-wk-old male mice.—Moghadasian, M. H., McManus, B. M., Nguyen, L. B., Shefer, S., Nadji, M., Godin, D. V., Green, T. J., Hill, J., Yang, Y., Scudamore, C. H., Frohlich, J. J. Pathophysiology of apolipoprotein E deficiency in mice: relevance to apo E-related disorders in humans. *FASEB J.* 15, 2623–2630 (2001)**

Key Words: apo E · brain · antioxidants · atherosclerosis · xanthomatosis · lipids

Apolipoprotein (apo) E was first described as a component of very low density lipoprotein (VLDL) particles (1). Apo E is a ligand for low density lipoprotein (LDL) receptors and plays a crucial role in receptor-mediated uptake of all triglyceride (TG) -rich lipoprotein particles including VLDL and chylomicrons and their remnants (2). Further metabolic studies showed that the LDL receptor is not the only receptor involved in catabolism of TG-rich lipoproteins, but that apo E is a ligand for a family of receptors that includes LDL receptor-related proteins (LRP) (3). In this LRP-mediated pathway, apo E plays a role in three different uptake steps: *1)* apo E must be bound to the surface of lipoprotein for delivery of the lipoprotein particle to the receptor; *2)* apo E binds to heparan sulfate proteoglycans to complete the ligand–receptor interaction; and *3)* apo E interacts with the LRP receptor to facilitate uptake process. Abnormalities in each of the above steps result in incomplete metabolism of TG-rich lipoproteins.

Subjects with apo E deficiency or defective apo E may develop type III hyperlipidemia (4, 5). The major plasma lipoprotein in these individuals is a cholesteryl ester-enriched VLDL called β-VLDL (4, 5). Further investigations revealed association of an isoform of apo E, namely, apo E2, with type III hyperlipidemia. Whereas the apo E3 isoform is considered the 'physiological' apolipoprotein, the apo E2 and E4 isoforms alter lipid metabolism. It has also been shown that subjects with apo E4 are more likely to develop neurodegenerative disorders such as Alzheimer's disease (6). Elderly individuals with apo E4 isoform have enhanced decline in cognitive function compared with non-apo E4 carriers of similar age; this difference may be related to cardiovascular risk factors (7). The involvement of apo E in various tissue functions has resulted in detailed studies of its structure and function. Recent advances in DNA technology have allowed creation of many types of genetically modified animal models that closely resemble human disorders.

The apo E gene was the first lipoprotein transport gene to be deleted in mice (8). The β-VLDL particles are major lipoproteins in apo E-KO mice. This lipoprotein profile is believed to play a causal role in acceler-

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ated atherogenesis in this animal model (9). It has been shown that accelerated atherogenesis in apo E-KO mice may be due at least in part to disrupted endogenous antioxidant capacity (10, 11).

Although apo E-KO mice are now at the center of lipid and atherosclerosis research, some reports indicate their usefulness in studying pathological aspects of the nervous system. For example, Gordon and coinvestigators (12) reported a significant decrease in the activity of choline acetyltransferase in the hippocampus and frontal cortex of the apo E-KO mice compared with their wild-type counterparts. Similarly, decreased brain choline acetyltransferase activity was associated with deficits in working memory of apo E-KO mice. This was accompanied by hyperphosphorylation of π epitopes (13). Using the Morris water maze test, Oitzl et al. (14) showed defective spatial learning and memory of apo E-KO mice vs. controls. Furthermore, a 50% reduction in long-term potentiation with no change in excitatory postsynaptic potential has been reported in apo E-KO mice compared with wild-type counterparts (15). In agreement with these findings, Krugers et al. (16) observed alterations in synaptic plasticity in the hippocampal CA1 of both homozygous and heterozygous apo E mutant mice. Keller et al. (17) reported significant increases in oxidative stress markers (lipid peroxidation, thiobarbituric acid-reactive substances), mitochondrial dysfunction, and caspase activation in the synaptosomes of apo E-KO mice after application of amyloid β-peptide. Clusters of granules were detected in cytoplasm of protoplasmic astrocytes in 18-month-old apo E-KO mice but not in age-matched wild-type mice (18). Other studies revealed significant reductions in synaptic and neuritic markers (as assessed by double immunocytochemical and image analyses) accompanied by a widespread vacuolization of apical dendrites in apo E-KO mice (19). All these changes may be related to altered lipid membrane, particularly phosphatidylcholine, as the result of lack of apo E (20).

In addition to its effects on atherogenesis processes (21), apo E may substantially contribute to the regulation of antioxidant systems (22), inflammatory pathways (23), and other systems (24). These may explain the roles of apo E in brain function (6, 7) and longevity (25). To learn more about the roles of apo E on atherogenesis and to provide additional supportive evidence for its role in other biological systems, we carried out a series of experiments to *1)* further characterize effects of absence of apo E on the quality of plasma lipoproteins by measuring HDL esterification rate and hepatic and lipoprotein lipase activities; *2)* study its effects on endogenous enzymatic antioxidant systems; *3)* investigate its role in appearance of neurodegenerative markers by routinely used histological/immunohistological techniques; and *4)* observe its contribution to life span in C57BL/6J mice. Furthermore, we have investigated the differences in hepatic cholesterol and bile acid synthetic enzyme activities, hepatic LDL receptor function, and plasma homocyst(e)ine and glucose levels (as they play a major role in human atherosclerosis) between wild-type and apo E-KO mice. The experiments were approved by the Animal Care Committee of the University of British Columbia.

MATERIALS AND METHODS

Animals and diets

Male 4- to 6-wk-old C57BL/6J wild-type and apo E-KO mice were purchased from Jackson Laboratory (West Grove, PA). The animals were housed individually under ordinary conditions with 12 h cycle light/dark. The mice had ad libitum access to chow and water. After a 10 day adaptation period, the mice were used for each specific experiment as outlined below.

Plasma lipoprotein profile and glucose concentration

Plasma was prepared from tail blood of male 10- to 12-wk-old apo E-KO (n=20) or C57BL/6J (n=20) mice; plasma cholesterol and triglyceride concentrations were determined by an enzymatic assay as described (9). For determination of lipoprotein cholesterol concentration, aliquots of plasma from apo E-KO (n=20) and C57BL/6J (n=20) were pooled into four samples based on plasma total cholesterol levels (each pooled sample was from five mice). HDL cholesterol concentration was determined after precipitation of apo B-containing lipoprotein with phosphotungstate-MGCl2 (26). Non-HDL cholesterol concentration was calculated by subtracting HDL-C from total cholesterol. The pooled samples before precipitation were used to determine plasma glucose levels by phosphorylation (using hexokinase), followed by dehydrogenation (using glucose-6-phosphate dehydrogenase). These procedures coupled with the reduction of nicotinamide-adenine dinucleotide (NAD) to form 6-phosphoglucono-δ-lactone and NADH. The absorbance of NADH at 340 nm was read and the concentration of glucose in plasma samples was calculated (the amount of NADH is directly proportional to sample glucose concentration).

Determination of HDL fractional esterification rate (FER_{HDL})

Supernatant of apolipoprotein B-precipitated plasma samples were used (pooled samples as described above). Trace amount of ^{3}H-cholesterol was added to samples using a paper disc. Spontaneous transfer of the label proceeds at 4°C for 18 h. The paper disc was removed and the samples incubated at 37°C in a shaking water bath for 30 min. Lipids were extracted and applied on thin-layer chromatography plates. Radioactivity of esterified and unesterified cholesterol fractions was counted using a liquid scintillation counter. FER_{HDL} was calculated as the difference between the percentage of labeled esterified cholesterol before and after incubation. Details of this method have been described elsewhere (26).

Hepatic cholesterol and bile acid synthesis enzyme activities

The various enzyme activities were measured using published methods (27–29). For HMG-CoA reductase activity, an aliquot of the microsomal preparation was incubated with 3-hydroxy-3-methyl-3-[^{14}C]glutaryl-CoA for 15 min at 37°C in a buffer containing an NADPH-generating system and ^{3}H mevalonolactone as an internal recovery standard. The ^{14}C-labeled product was determined by thin-layer chromatography and liquid scintillation spectrometry. Microsomal choles-

terol 7α-hydroxylase activity and mitochondrial sterol 27-hydroxylase were measured by isotope incorporation methods using [^{14}C]cholesterol as substrate (28, 29).

Hepatic LDL receptor function

Liver membranes were used to determine the function of LDL receptors by measuring specific binding of ^{125}I-labeled LDL from either human (LDL_H) or cholesterol-fed apo E-KO mice (LDL_M) as described (27). LDL particles were separated by ultracentrifugation at a density 1.019–1.063 g/ml and VLDL particles at a density of 1.006–1.019 g/ml and labeled with ^{125}I by the iodine monohydrochloride method, as described previously (27). The LDL receptor-mediated binding was determined as the difference between total and nonspecific binding (measured in the absence and presence of 40-fold excess unlabeled lipoprotein, respectively).

Hepatic and lipoprotein lipase activities

One hundred fifty units per kg body weight of heparin were injected into the abdomen of both wild-type and apo E-KO mice. Ten minutes later, blood samples were taken from the hearts of anesthetized animals. Blood samples were kept on ice, plasma separated, and used to measure lipoprotein lipase and hepatic lipase activities as described (9). Labeled triglyceride ([^{3}H]triolein) was incubated with plasma samples and liberated fatty acids ([^{3}H]oleate) were quantified by liquid scintillation counting.

Homocyst(e)ine measurements

Blood samples were taken from the anesthetized mice and quickly centrifuged for plasma preparation. Plasma samples were analyzed for total homocyst(e)ine concentrations by a standard HPLC method in the clinical laboratory at St. Paul's Hospital. An internal standard was added to plasma samples, then all disulfide bonds were reduced by tri-n-butylphosphine. Proteins were precipitated by perchloric acid and the supernatant was derivatized with a thiol-specific fluorogenic probe, SBD-F (ammonium 7-fluorobenzo-2-oxa-1, 3-diazole-4-sulfate) for 1 h at 60°C. The samples were analyzed using an HPLC system with a reversed-phase column and fluorescence detector.

Enzymatic antioxidant capacity

Red blood cells were separated from plasma by centrifugation and washed twice with isotonic saline. The kidney, heart, and lung were removed and frozen. Organs were homogenized; aliquots of organ homogenates, plasma, and red cells were analyzed for activities of glutathione peroxidase (GPx), glutathione reductase (GRed), superoxide dismutase (SOD), and catalase (9).

Histology

Brains from six apo E-KO and six C57BL/6J at age 37 wk were removed from the skulls and placed in 10% phosphate-buffered formalin. A transverse section was made and the distal parts were processed for paraffin embedding. Sections (4 μm) were prepared and stained with standard hematoxylin and eosin (H&E) (30), Congo red (30) for evaluation of amyloid plaque formation, Bielchowsky (30) for axonal examination, and Luxol fast blue (30) for myelin examination.

The hearts were also fixed and processed as described above. Aortic roots were located in the hearts and serial sections were cut. The extent and severity of atherosclerosis were assessed by H&E and Movat's pentachrome staining (9).

Immunohistology

Additional brain sections were used for detection of markers of neurodegenerative disease by immunohistochemistry procedures. Antibodies against glial fibrillary acidic protein (GFAP) (31), amyloid precursor protein (APP) (32), neurofilament (NF) (33), and ubiquitin (33) were included (these markers are routinely used for the histological assessment of neurodegenerative disorders in humans). The immunohistochemical staining was performed in the Department of Pathology, University of Miami, according to standard procedures (34). Briefly, 4 μm paraffin sections were dewaxed at 37°C for 18 h, followed by immersion in xylene for 10 min. Slides were then rehydrated in decreasing grades of ethanol and exposed to 6% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. After washing in PBS, aliquots of the primary antibodies were added to each slide and incubated for 45 min at 22°C in a humidity chamber. Characteristics of each antibody used were as follows: polyclonal antibodies (DAKO, Carpinteria, CA) against GFAP and ubiquitin at optimal concentrations of 1:3000 and 1:250, and monoclonal antibodies (DAKA and Boehringer Mannheim, Indianapolis, IN) against APP-A4 and NF at optimal concentrations of 1:500 and 1:200, respectively. After a PBS wash, the slides were sequentially incubated with the biotinylated secondary antibody (25 min), and avidin-biotin-peroxidase complex (25 min). Diaminobenzidine was used as chromogenic substrate (6 min). The slides were counterstained with hematoxylin, dehydrated through gradients of ethanol, and mounted with synthetic resin. The negative control slides were treated in the same manner except for replacement of the primary antibodies with respective nonimmune sera. Positive immunohistochemical reactions were observed as dark brown granules in the cytoplasm or cytoplasmic processes of the cells. All brain sections were reviewed by an independent certified specialist in neuropathology in a blinded fashion.

Statistical analysis

Data were analyzed using the two-tailed Student's t test at a significance level of $P < 0.05$; results are expressed as mean ± SD.

RESULTS

Body weight

Figure 1 shows body weight gain of wild-type and apo E-KO mice over a 33 wk period. Both groups of mice had a similar weight gain.

Plasma lipids, lipoprotein profile, HDL esterification rate, and lipase activities

Data on lipoprotein profiles and other lipid-related measurements in C57BL/6J and apo E-KO mice are presented in **Table 1**. Apo E-KO mice had a fourfold increase in plasma total cholesterol levels and a twofold increase in plasma TG levels vs. their wild-type counterparts.

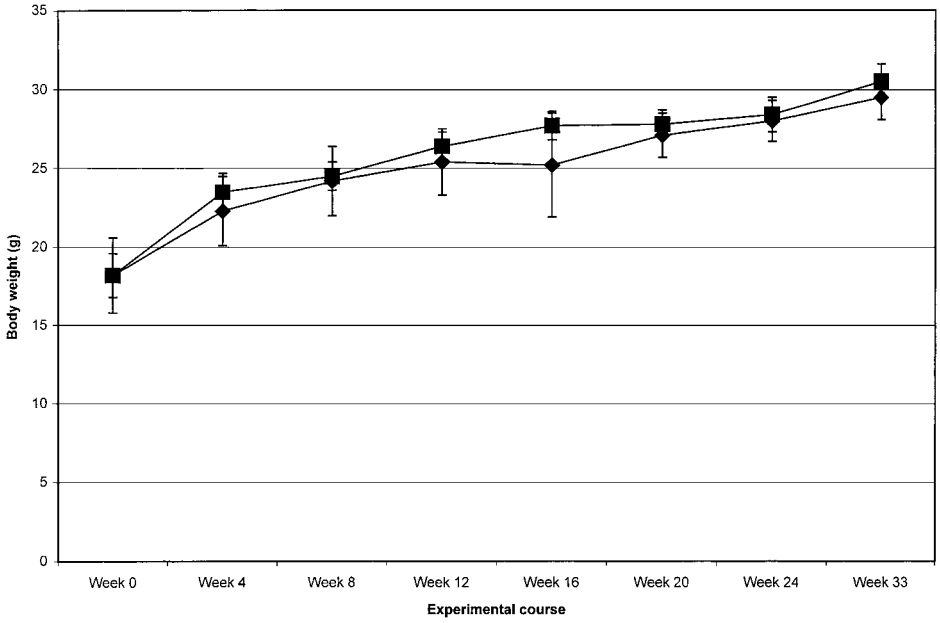

Figure 1. Comparison of body weight gain (mean±SD, g) between C57BL/6J (n=10, square symbol) and apo E-KO mice (n=10, diamond symbol) over a 33 wk period.

In another distinct 33 wk study, we observed that plasma total cholesterol levels in apo E-KO mice increased over time but decreased slightly in C57Bl/6J mice, most likely due to decreased endogenous cholesterol synthesis (see the data on HMG-CoA reductase activity). For example, plasma cholesterol levels at wk 0 in apo E-KO mice were sixfold greater than those in corresponding wild-type controls; this difference increased to 13-fold by wk 29 of the experiment.

Whereas the majority of cholesterol in wild-type mice is in HDL particles, apo B-containing lipoproteins, particularly β-VLDL (9), are the principal source of plasma cholesterol in apo E-KO mice. This results in >90% decreases in HDL/TC or HDL/LDL ratios.

TABLE 1. *Comparison of various plasma analytes (mean ± SD) between wild-type and apo E-KO C57BL/6J mice*

Plasma analytes	C57BL/6J (n)	Apo E-KO (n)
Total cholesterol (mmol/l)	3.0 ± 0.5 (20)	12.8 ± 2.6** (20)
HDL-cholesterol (mmol/l)	1.4 ± 0.2 (4#)	0.3 ± 0.3** (4#)
Non-HDL-cholesterol (mmol/l)	1.9 ± 0.4 (4#)	12.1 ± 2.8** (4#)
HDL/TC ratio	0.43 ± 0.07 (4#)	0.03 ± 0.03** (4#)
HDL/LDL ratio	0.77 ± 0.19 (4#)	0.03 ± 0.03** (4#)
Triglyceride (mmol/l)	0.8 ± 0.4 (20)	1.5 ± 0.5** (20)
FER_{HDL} (%/hr)	46.5 ± 5 (4#)	103.3 ± 27** (4#)
Hepatic lipase (mU)	78 ± 24 (6)	54 ± 12 (6)
Lipoprotein lipase (mU)	163 ± 71 (6)	146 ± 51 (6)
Homocysteine (umol/l)	6.4 ± 0.5 (5)	4.9 ± 0.3* (6)
Glucose (mmol/l)	15.2 ± 0.2 (4#)	13.4 ± 1.1* (4#)

* $P < 0.05$; ** $P < 0.01$; #, aliquots of plasma from 20 mice were pooled in 4 samples (5 mice with comparable plasma total cholesterol level in each sample); FER_{HDL}, cholesterol esterification rate in plasma depleted of apo B-containing lipoproteins; results are expressed as mean ± SD. These data are from 10- to 12-wk-old mice.

These changes are accompanied by a marked increase in HDL esterification rate and comparable activities of postheparin hepatic and lipoprotein lipase.

Plasma homocyst(e)ine and glucose levels

Apo E-KO mice have significantly lower plasma total homocyst(e)ine and glucose levels than wild-type mice. Increased levels of homocyst(e)ine and glucose are associated with atherosclerotic vascular disease in humans (35, 36). The reason and significance of reduced levels of these potentially atherogenic chemicals in this animal model of atherosclerosis are unknown.

Organ antioxidant capacity

Our results (**Table 2**) indicate a significant variation in the endogenous antioxidant components among various tissues from either group of mice. For example, kidney had the highest GPx, GRed, and catalase activity whereas their activities were minimal in plasma except for catalase, which is not detectable in plasma. Similarly, activity of SOD was high and comparable among the heart, kidney, and lung vs. low activity in red cells and plasma. Unlike significant organ-related variations, we did not detect noticeable differences in antioxidant activities when apo E-KO and wild-type mice were compared.

Life span

Whereas laboratory wild-type mice can live free of disease for several years, maintaining apo E-KO mice is not as feasible. These mice usually develop progressive skin lesions, mainly in the form of eruptive xanthomas on the shoulder and back areas (37). Lipids and extracellular matrix are predominant components of these lesions. In our experimental setting, the severity

TABLE 2. *Comparison of organ antioxidant activities between wild-type and apo E-KO mice*[a]

Enzyme	GPx (nmol/min/mg protein)		GRed (nmol/min/mg protein)		SOD (units/mg protein)		Catalase (k/mg protein)	
Mice	Apo E-KO	C57BL/6J	Apo E-KO	C57BL/6J	Apo E-KO	C57BL/6J	Apo E-KO	C57BL/6J
Plasma	3.7 ± 0.4	3.4 ± 0.1	2.2 ± 0.4	2.0 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	NA	NA
RBC	44.1 ± 3.6	46.6 ± 4.5	2.1 ± 0.1	2.2 ± 0.1	5.3 ± 0.3	4.9 ± 0.5	0.071 ± 0.030	0.056 ± 0.008
Heart	30.4 ± 3.3	26.4 ± 2.5	11.3 ± 0.8	11.6 ± 1.2	50.9 ± 4.1	48.1 ± 5.3	0.002 ± 0.002	0.003 ± 0.001
Lung	75.0 ± 25.2	47.3 ± 5.1	59.7 ± 7.4	45.4 ± 5.8	55 ± 5.1	51.9 ± 3.2	0.023 ± 0.006	0.013 ± 0.006
Kidney	174.3 ± 6.1	151.3 ± 6.4	90.4 ± 1.6	84.7 ± 4.5	48.9 ± 3.9	49.4 ± 8.3	0.167 ± 0.015	0.147 ± 0.006

[a] Three mice in each group; results are expressed as mean ± SD. Data are from 10- to 12-wk-old mice.

of these lesions did not allow keeping the animals for more than 18 months. During this period, three of eight apo E-KO mice died (~35%). Coronary artery disease may contribute to these sudden deaths, as a histological examination revealed advanced atherosclerotic lesions. Representative photomicrographs of such lesions show massive atheromas with abundant cholesterol crystals, neutral lipids, and diminished extracellular matrix in both aortic roots and coronary arteries. Inflammatory elements along with evidence for rupture of lesions are also present (**Fig. 2**).

Histochemistry/immunohistochemistry evaluation of the brain

H&E staining showed no pathological changes in the brain from apo E-KO and wild-type mice. Scattered eosinophilic neurons in hippocampus, clusters of Purkinje cells in cerebellum with similar characteristics, and some shrunken neurons in hemispheres were noted in both groups of mice; these observations appear to be artifacts. Various stains revealed no plaques, tangles, or inclusions in the brain sections from either wild-type or apo E-KO mice. Eosinophilic neurons observed with H&E stain showed diffuse cytoplasmic silver impregnation. All brain sections examined with ubiquitin, APP, and Congo red were negative for these stains. NF staining showed a normal pattern of neurofilament staining in all areas of the brains from either group of mice. Similarly, normal pattern of GFAP positivity was observed in the brain of both groups.

Hepatic cholesterol and bile acid synthetic enzyme activities, cholesterol content, and LDL receptor function

The activity of cholesterol synthesis enzyme HMG-CoA reductase was significantly (−30%, $P<0.05$) reduced in 8-month-old C57BL/6J mice vs. 2-month-old mice; this was associated with lower hepatic cholesterol content. However, aging did not induce a significant effect on the activity of bile acid synthetic enzymes cholesterol 7α-hydroxylase or sterol 27-hydroxylase. Deletion of apo E gene further reduced the activity of rate-limiting enzyme HMG-CoA reductase (up to 60%) along with a 15% increase in cholesterol content in 8-month-old mice. These data are summarized in **Table 3**.

Hepatic LDL receptor function was significantly reduced in 8-month-old C57BL/6J mice compared with 2-month-old counterparts. Receptor-mediated binding of iodinated mouse LDL to hepatic membrane contributed to 78% of total LDL binding in young mice vs. 54% in old mice. LDL receptor activity in apo E-KO was

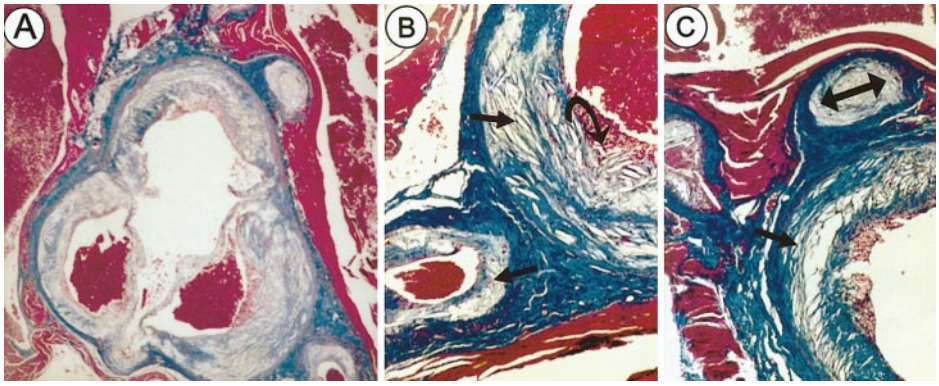

Figure 2. Representative photomicrographs of serial transverse sections at the level of aortic root from an apo E-KO mice that died suddenly at 17 months of age. Gigantic vulnerable lipid-rich atherosclerotic lesions with diminished extracellular matrix volume and lack of strong plaque caps are seen in all three valve cusps of the aortic root (*A*). *B, C)* Several characteristic features of these vulnerable lipid-rich lesions in major epicardial coronary arteries and aortic root; straight arrows show extensive neutral lipids and abundant cholesterol clefts; the down-curved arrow illustrates evidence for plaque rupture and presence of inflammatory cells; the right-left arrow show complete obstruction of a major epicardial coronary artery by an exclusively lipid plaque. These vulnerable lesions may explain sudden death in these animals. These features of the atherosclerotic lesions resemble those in humans that usually lead to clinical events. Movat's pentachrome stain; *A*, ×10; *B, C*, ×25.

TABLE 3. *Hepatic cholesterol content, cholesterol and bile acid synthetic enzyme activities, and LDL receptor function in C57BL/6J and apo E-KO mice*[a]

Groups	HMGR[b]	Cyp7a1[b]	Cyp27a[b]	LDL receptor binding[b]	Cholesterol content[c]
C57BL/6J, 2-month-old	200 ± 44 (6)	24 ± 10 (8)	19 ± 7 (6)	1.1 ± 0.4 (6)	27 ± 5 (8)
C57BL/6J, 8-month-old	126 ± 12* (5)	28 ± 3 (5)	15 ± 2 (5)	0.7 ± 0.1* (5)	21 ± 3* (5)
Apo E-KO, 8-month-old	60 ± 29** (5)	38 ± 5** (5)	20 ± 2** (5)	0.9 ± 0.3 (5)	31 ± 4** (5)

[a] Data are expressed as mean ± SD; HMGR, HMG-CoA reductase: Cyp7a, cholesterol 7α-hydroxylase, Cyp27a, sterol 27-hydroxylase; [b] pmol/mg/min; [c] mg/mg membrane protein; [d] μmol/mg protein. * $P < 0.05$ compared to 8-month-old C57BL/6J; ** $P < 0.05$ compared to 8-month-old C57BL/6J; the number of animals (n) is presented in parentheses.

comparable to that in corresponding wild-type counterparts. Receptor-mediated binding accounted for 87% of total binding in apo E-KO mice.

DISCUSSION

In addition to its role in lipoprotein metabolism, apo E may have other functions such as antioxidant (22), anti-platelet aggregation (38), anti-proliferative effects (39), and immunomodulation properties (23). Possible effects on human life span (25) and the onset of Alzheimer's disease (40) have been also reported.

In this study, we have provided evidence for the relevance of apo E to the development of the above-mentioned conditions in C57Bl/6J mice. Absence of apo E in mice is associated with a fourfold increase in plasma total cholesterol levels and a twofold increase in plasma triglyceride levels (Table 1), similar to humans with apo E deficiency (41). Severe hypercholesterolemia (TC=11, VLDL-C=7.2, LDL-C=3.2, mmol/l) and hypertriglyceridemia (TG=3.7 mmol/l) was associated with severe xanthomatosis, including numerous tuberoeruptive xanthomas on knees and elbows of a 30-year-old human with apo E deficiency; surprisingly, this subject was free of clinical symptoms of atherosclerosis (41).

Our data indicate that increased plasma cholesterol levels cause a significant decrease in hepatic cholesterol synthesis and significant up-regulation of bile acid formation via classical cholesterol 7α-hydroxylase-initiated pathway, but no significant alterations in hepatic LDL receptor function. These observations suggest a non-LDL receptor-mediated uptake of circulating lipoproteins by hepatocytes. These changes were accompanied by development of severe atherosclerotic lesions and cutaneous xanthomatosis in apo E-deficient mice, most likely due to extremely high plasma cholesterol levels, diminished HDL cholesterol, and the presence of less antiatherogenic (small) HDL particles as indicated by increased FER_{HDL} (42, 43). Apparently, up-regulation of cholesterol catabolism via bile acid synthesis is a compensatory mechanism in the presence of severe hypercholesterolemia. However, it is not sufficient to prevent development of the atherosclerotic lesions.

Our data indicate that body weight gain is independent of apo E and is not affected by the consequences of apo E deficiency in mice. However, apo E may directly or indirectly affect the life span of mice. Indeed, ~35% of the apo E-KO mice died suddenly over an 18 month of study course. Advanced coronary atherosclerotic disease was the likely cause of sudden death in these animals. Similarly, severe eruptive skin lesions indirectly shorten their life span.

Abnormalities in apo E along with oxidative stress are considered the major cause of numerous disorders, including neurodegenerative disease (44) and atherosclerosis (45). Our results indicate that apo E deficiency is associated with severe and advanced atherogenesis in mice, but not with significant alterations in endogenous enzymatic antioxidant activities or abnormalities in brain histology. Lack of any significant histological alterations and the absence of neurodegenerative markers in the brain tissue indicates that apo E deficiency does not cause an early brain dysfunction in male mice. However, our observations cannot rule out the possible role of apo E deficiency in the late onset of brain dysfunction. Similar to advanced atherosclerotic lesions in the aorta and coronary arteries, brain vessels may be involved at a later stage resulting in brain dysfunction. It should be noted that other studies reported functional alterations (15, 16) and age-dependent congophilic inclusions (18) in the brain of apo E-KO mice. However, these studies did not specify either gender or diet. On the other hand, we studied the brains of 37-wk-old male mice individually housed and fed regular mouse chow containing 4.5% fat. Similar to our findings, Bales et al. (46) did not observe amyloid deposits in the brain of 6-month-old apo E-KO mice. Further investigations by cross-breeding apo E-KO mice with transgenic mice overexpressing a human mutant amyloid precursor protein gene (V717F) provided solid evidence that apo E is critical for amyloid deposition and neuritic plaque formation in mice (46). The brains of apo E-KO mice did not show plaque or tangle-like changes when treated with antibodies against β-amyloid and Tau2 (19).

In conclusion, our data indicate that the absence of apo E significantly influences cholesterol metabolism in mice, which may be similar to apo E deficiency/abnormalities in humans. Male apo E-KO (37-wk-old) mice fed low-fat chow apparently have normal brain histology and do not show evidence of neurodegenerative disorders such as formation of amyloid plaques and abnormalities in neurofilament patterns. Similarly, the

brain sections from either wild-type or apo E-KO mice did not stain with Congo red, ubiquitin, or APP. Deletion of apo E gene does not influence plasma and organ antioxidant activities or affect lipase activities. On the other hand, apo E-KO mice have lower plasma homocyst(e)ine and glucose levels; the cause and significance of these findings are unknown. Development of advanced atherosclerosis and cutaneous xanthomas are the major morbidity observed in apo E-KO mice; these may directly or indirectly reduce their life span. This study also supports the notion of using FER_{HDL} as a predictive measure for the risk of CHD. Although apo E-KO mice have abnormalities of lipid metabolism that are related to atherogenesis in humans, distinct differences with regard to endogenous antioxidant systems and development of markers of neurodegenerative disease may exist between apo E-KO mice and humans with apo E-abnormalities. FJ

We would like to thank Elena Neumann, MD, FRCPC (Vancouver, BC) for her valuable assistance on the evaluation of various mouse brain sections in a blinded fashion.

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Received for publication June 20, 2001.
Accepted for publication August 27, 2001.