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Effect of Diets on Lipoprotein Concentrations in Heterozygous Apolipoprotein E-Deficient Mice

D. BOBKOVÁ, E. HONSOVÁ[1], J. KOVÁŘ, R. POLEDNE

Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine and Center for Experimental Cardiovascular Research and [1]Department of Pathology and Anatomy, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

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Summary

Loss of apolipoprotein E synthesis causes increased serum cholesterol concentrations and the sensitivity to high-fat diet in mice. We analyzed the changes in lipoprotein and hepatic structures in apolipoprotein E-deficient mice kept on control diet and cholesterol diets. Basal cholesterolemia of heterozygous (+/-) mice (2.2±0.28 mmol/l) was the same compared to wild-type (+/+) mice (2.3±0.15 mmol/l), but was lower compared to homozygous (-/-) mice (10.3±1.40 mmol/l). In +/- mice, cholesterolemia rose to 3.2 mmol/l on cholesterol diet and to 9 mmol/l on cholate diet, to 3 mmol/l and 3.6 mmol/l in +/+ mice, and to 23.4 mmol/l and 70.5 mmol/l in -/- mice, respectively. While the ratio of cholesterol/triglyceride concentrations in VLDL, IDL and LDL fractions was not increased in +/- mice and +/+ mice, it was increased in -/- mice on control diet. On the cholesterol diet, this ratio rose and was dramatically increased by cholate diet in all groups of mice. Even though cholate supplementation increased cholesterol concentration, it led to substantial toxic changes in hepatic morphology of all animals. In conclusion, one functional apo E allele in +/- mice is effective in keeping serum cholesterol concentrations in normal range on a control diet, but not on the cholesterol and cholate diets.

Key words

Apolipoprotein E • Heterozygous mice • Hyperlipoproteinemia • Hepatotoxicity

Introduction

Apolipoprotein E (apo E) plays an important role in lipoprotein metabolism. As a part of the surface of VLDL, HDL, and remnant-like particles, apo E mediates the binding of these particles to LDL receptors, remnant-like particle receptors (LRP-R), VLDL receptors, and apoER2 receptors (Beisiegel *et al.* 1989, Reardon and Getz 2001). A manifestation of mutation of the apo E gene in humans is dysbetalipoproteinemia (type III hyperlipoproteinemia), characterized by accumulation of βVLDL particles and an increase in total cholesterol (Brewer *et al.* 1983). A relevant model of dysbetalipoproteinemia in man is the homozygous apolipoprotein E-deficient mouse [apo E knockout (KO) homozygotes] (Paigen *et al.* 1994, van Dijk *et al.* 1999). Loss of the ligand for receptors in apo E KO homozygotes is associated with an increase in total cholesterol due to slowed utilization of remnant-like particles and with an increased risk for the development

of atherosclerosis due to LDL particle accumulation (Plump *et al.* 1992, Breslow 1993, 1996). Development of atherosclerotic lesions is also affected by cholesterol reverse transport in which apo E plays a pivotal role. Excessive cholesterol is released from the subendothelial space by means of macrophage-derived apo E (von Eckardstein 1996). An inability to lower excessive body cholesterol as a result of loss of apo E production is associated with development of the atherosclerotic process (Zhu *et al.* 1998). Basal cholesterolemia of apo E KO homozygotes is up to five times higher than that of animals of the same strain without the genetic defect, i.e. about 10 mmol/l. Due to the inability of apo E production, apo E KO homozygotes are highly sensitive to dietary intervention. In these animals, administration of a cholesterol diet leads to an increase in cholesterolemia and the development of macroscopic atherosclerotic lesions (van Ree *et al.* 1994). Unlike apo E KO homozygotes, mice and rats are naturally resistant to any dietary intervention-induced increase in cholesterolemia. Even a long-term admini-stration of cholesterol diet is not associated with the development of hypercholesterolemia. Nevertheless, there are inbred strains with increased susceptibility to atherosclerosis, C57Bl/6, in which lipid streaks in the vessels have been demonstrated. However, the development of these lesions was due to the long-term administration of a high-fat cholesterol-containing diet or another proatherogenic stimulus (Breslow 1996).

Given the low sensitivity of mice and rats to dietary cholesterol, cholate is added to the diet to increase cholesterolemia. Cholate is known to facilitate micelle formation in the intestines, thus enhancing cholesterol absorption. In addition to its effect on cholesterol levels, cholate significantly modulates the formation of bile acids, whose increased levels may have toxic action.

The aim of our study was to investigate the effect of only one functional allele of the apo E gene in apo E KO heterozygotes on lipoprotein levels and on hepatic tissue morphology following a cholesterol diet, and to evaluate the effects of cholate addition to the diet.

Methods

Animals and diets

Heterozygous (+/-) apolipoprotein E KO mice (heterozygotes) (C57Bl/6 strain) (n=50) were used. Homozygous (-/-) apo E KO mice (homozygotes) (n=51) and wild-type mice (+/+) (n=50) with the same genetic background were used as controls. Homozygous apo E KO mice were obtained from the Jackson Laboratory in Maine (USA). All animals (males and females) were two months old.

The animals were fed standard laboratory diet (control diet) or 1 % cholesterol diet (control diet containing 5 % fat and 1 % cholesterol) (cholesterol diet), or cholate diet (1 % cholesterol diet containing 0.5 % sodium cholate) for 21 days. The animals were sacrificed and their blood was collected for lipid analysis, and the liver was used for analysis of liver tissue morphology.

Lipid and plasma lipoprotein analysis

Serum was harvested by whole blood centrifugation or 10 min at 12 000 rpm. Cholesterol and triglyceride concentrations were measured using colorimetric enzymatic assay kits (Boehringer Mannheim Biochemicals, Germany). Pooled serum of all males and females of the group were used for lipoprotein isolation.

Lipoprotein fractions were isolated by sequential ultracentrifugation (VLDL [d<1.006 g/ml] for 18 h, IDL [d=1.006-1.019 g/ml] for 18 h, LDL [d=1.019-1.063 g/ml] for 18 h, HDL [d=1.063-1.210 g/ml] for 20 h) (50.3 rotor, Beckmann, USA) at 39 000 rpm at 8 ^{o}C (Havel *et al.* 1955).

Fast-performance liquid chromatography (FPLC, BioSys 510, Beckmann, USA) was used for isolation of lipoproteins. The column matrix was equilibrated at 0.3 ml/min with a running buffer (50 mM PBS, pH 7.4 containing 0.15 M NaCl, 0.001 M EDTA, and 0.02 % sodium azide). Two hundred µl of serum were loaded on a Superose column (6HR 10/30, Amersham-Pharmacia, United Kingdom) and collected into twenty-one 0.6 ml fractions (Innis-Whitenhouse *et al.* 1998).

The cholesterol and triglyceride contents of each fraction were determined as described above.

Apolipoprotein analysis

Twenty µl of each sample of ultracentrifugation-isolated lipoprotein fractions were loaded on 3-20 % gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE, Mini Protean, 3 Cell, Bio-Rad, USA) (Kotite *et al.* 1995). Protein bands were separated at 8 mA per one gel and stained with Coomassie blue R-250. To estimate the proportion of HDL1 in LDL fraction, apo AI was quantified in corresponding bands in LDL and HDL fractions on gels using a densitometer (GS 710 calibrated imaging densitometer, Bio Rad, USA) and computer program (Quantity One, Bio Rad, USA). Results are expressed as µg of serum apo AI/ml (Kotite *et al.* 1995).

Liver morphology

Sections of hepatic tissue were fixed in 7 % formaldehyde and embedded in paraffin in an Autotechnicon device (TPC 15, Medite, Germany), cut into 4 μm slices (Leica, R), and routinely stained with hematoxylin and eosin. Morphological changes were quantified using a microscope (Olympus PROVIS, Japan) at 200x magnification. Frames were acquired with a digital camera (Olympus DP 12, Japan) connected with PC and processed using a computer program (Adobe Photoshop 4.0, Czech Republic).

Statistical analysis

Unpaired Student's t-test was used to test for differences between all groups. Data are presented as means ± S.D. Differences are considered statistically significant when $P<0.05$.

Table 1. Serum cholesterol in heterozygotes (+/-), homozygotes (-/-) and wild-type mice (+/+) after 3 weeks on the diets.

		Control diet		Cholesterol diet		Cholate diet	
	Sex	**n**	**mmol/l**	**n**	**mmol/l**	**n**	**mmol/l**
+/-	F	8	2.01±0.229	9	2.66±0.317 **	8	8.42±1.625 $^{+++\,\#\#\#}$
	M	8	2.44±0.084	9	3.68±0.502 *	8	9.58±5.034 $^{+\,\#}$
-/-	F	10	10.46±1.487	8	22.35±1.763 ***	8	66.76±7.199 $^{+++\,\#\#\#}$
	M	9	10.11±1.285	8	24.52±2.227 ***	8	74.17±5.620 $^{+++\,\#\#\#}$
+/+	F	8	2.20±0.097	8	2.62±0.194 **	9	3.67±0.515 $^{++\,\#\#\#}$
	M	8	2.39±0.120	9	3.46±0.163 ***	8	3.46±0.658 $^{\#}$

F (female) and M (male). Results shown are means ± SD of serum cholesterol concentration, n (number of animals). *** $P<0.001$, ** $P<0.01$, * $P<0.05$ as a significant differences between control and cholesterol diet using t-test $^{+++}$ $P<0.001$, $^{++}$ $P<0.01$, $^{+}$ $P<0.05$ as a significant difference between cholesterol and cholate diet using t-test $^{\#\#\#}$ $P<0.001$, $^{\#\#}$ $P<0.01$, $^{\#}$ $P<0.05$ as a significant difference between cholate and control diet using the t-test. The averages of triglyceride concentrations were from 0.95 to 1.55 mmol/l with no significant differences in groups and diets.

Results

Lipoprotein concentrations

Total cholesterolemia ($P>0.05$) as well as lipoprotein concentrations of heterozygotes fed a control diet were comparable with the findings in wild-type mice (Tables 1 and 2). Due to increased basal cholesterolemia ($P<0.001$), lipoprotein concentrations of homozygotes were increased compared with heterozygotes (Tables 1 and 2). The cholesterol/triglyceride ratio in individual lipoprotein fractions in heterozygotes on the control diet did not differ from values seen in wild-type mice. The value of the ratio in the VLDL fraction, 0.3 in heterozygotes, and 0.4 in wild-type mice (Table 2) is consistent with human data. In homozygotes, cholesterol accumulation in lipoproteins resulted in an increased cholesterol/triglyceride ratio in the VLDL fraction to 6.5 (Table 2). Compared to heterozygotes and wild-type mice, HDL levels in homozygotes on the control diet were the lowest (Table 2). Although there was a comparable increase in cholesterolemia in heterozygotes and wild-type mice on a cholesterol diet (Table 1), lipoprotein concentrations changed in heterozygotes only (Table 2). In heterozygotes, there was a 110 % increase in cholesterol concentrations in the VLDL fraction and the cholesterol/triglyceride ratio in this fraction rose to 0.80 (Table 2), whereas in wild-type mice this ratio rose to 0.60 only (Table 2). A 2.3-fold increase in cholesterolemia (Table 1) in homozygotes after cholesterol diet was associated with increased cholesterol concentrations in lipoprotein fractions associated with a rise in the cholesterol/triglyceride ratio (Table 2). The most significant increase in cholesterol concentrations (by 180 %) occurred in the VLDL fraction (Table 2). In heterozygotes, the presence of dietary cholate was associated with an almost threefold increase in cholesterol concentrations (Table 1) compared with cholesterol diet and an increase in VLDL, IDL as well as LDL fractions (Table 2). In wild-type mice, cholesterolemia in response to cholate diet increased by about 40 % in females only (Table 1), and a significant increase in cholesterol concentrations was only seen in VLDL and IDL fractions (Table 2). Compared with the control diet, heterozygotes and wild-type mice on

a cholate diet showed a decrease in HDL fraction concentrations (Table 2). In homozygotes, cholate diet administration was associated, just as in heterozygotes, with an almost threefold increase in cholesterol concentrations (Table 1) and further cholesterol accumulation in lipoproteins (Table 2). Unlike heterozygotes, homozygotes on cholate diet had increased HDL-cholesterol concentrations (Table 2). All groups of animals on the cholate diet consistently showed a rise in the cholesterol/triglyceride ratio in VLDL, IDL, and LDL fractions (Table 2). In heterozygotes and wild-type mice on control and cholesterol diets, but not on a cholate diet, the cholesterol concentrations in males were higher compared to females. In homozygotes, the sex differences in cholesterol concentrations were not found on any diet.

Table 2. Lipoprotein concentration in heterozygotes (+/-), homozygotes (-/-) and wild-type mice (+/+) after 3 weeks on the diets

	Control diet			**Cholesterol diet**			**Cholate diet**		
	TC	**TG**	**TC/TG**	**TC**	**TG**	**TC/TG**	**TC**	**TG**	**TC/TG**
	(mmol/l)			**(mmol/l)**			**(mmol/l)**		
+/-									
VLDL (<1.006 g/cm^3)	0.21	0.62	0.3	0.44	0.56	0.8	5.20	0.50	10.4
IDL (1.006-1.019 g/cm^3)	0.10	0.19	0.5	0.29	0.31	0.9	2.70	0.27	10.0
LDL (1.019-1.063 g/cm^3)	0.25	0.15	1.7	0.44	0.13	3.4	1.87	0.07	26.7
HDL (1.063-1.210 g/cm^3)	1.78	0.40	4.5	2.27	0.53	4.3	1.63	0.23	7.1
-/-									
VLDL (<1.006 g/cm^3)	3.44	0.53	6.5	9.79	0.34	28.8	27.88	0.61	45.7
IDL (1.006-1.019 g/cm^3)	1.21	0.12	10.1	2.06	0.11	18.7	10.71	0.36	29.8
LDL (1.019-1.063 g/cm^3)	1.80	0.12	15.0	3.19	0.10	31.9	11.45	0.08	143.1
HDL (1.063-1.210 g/cm^3)	1.34	0.17	7.9	1.50	0.30	5.0	2.10	0.62	3.4
+/+									
VLDL (<1.006 g/cm^3)	0.20	0.49	0.4	0.27	0.48	0.6	0.76	0.45	1.7
IDL (1.006-1.019 g/cm^3)	0.11	0.15	0.7	0.17	0.11	1.5	0.42	0.16	2.6
LDL (1.019-1.063 g/cm^3)	0.33	0.15	2.2	0.41	0.10	4.1	0.48	0.10	4.8
HDL (1.063-1.210 g/cm^3)	1.93	0.35	5.5	1.74	0.32	5.4	1.40	0.35	4.0

TG/TC (the ratio of cholesterol/triglyceride concentrations), TC (cholesterol), TG (triglyceride). Pooled serum (females and males) were used for lipoprotein isolation using ultracentrifugation.

Lipoprotein profile

The findings of changes in lipoprotein concentrations, assessed by ultracentrifugation, were confirmed by FPLC. The lipoprotein profile of heterozygotes on a control diet did not differ from that in wild-type mice (Fig. 1). Almost 80 % of total cholesterol was carried in the HDL fraction. In homozygotes, HDL fraction on control diet accounts for only 13 % of total cholesterol. Compared with findings in heterozygotes, cholesterol concentrations in individual lipoprotein fractions of homozygotes on a control diet were several times higher. In heterozygotes, cholesterol diet administration was followed by redistribution of total cholesterol among the individual lipoprotein fractions, and an increase in VLDL, IDL, and LDL fractions, whereas the lipoprotein profile remained unaltered in wild-type mice (Fig. 1). Homozygotes on a cholesterol diet developed a significant rise in VLDL and IDL fractions. The addition of cholate into the diet modulated the lipoprotein profile in all groups of animals. Compared to wild-type mice, heterozygotes showed more pronounced changes in the lipoprotein profile characterized by a rise in VLDL, IDL, and LDL fractions. Consistent with data from ultracentrifugation, heterozygotes and wild-type mice on cholate diet showed a decrease in the HDL fraction. Cholate diet administration to homozygotes was associated with an increase in cholesterol concentrations in VLDL, IDL, and LDL fractions (Fig. 1).

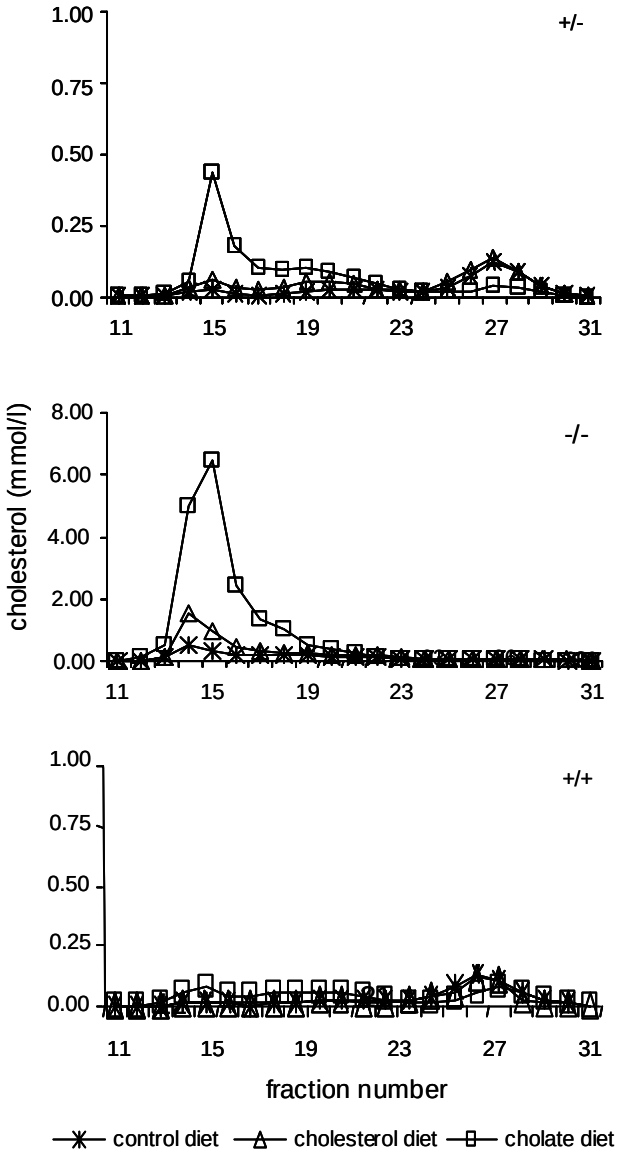

Fig. 1. FPLC profile of serum lipoproteins from heterozygotes (+/-), homozygotes (-/-) and wild-type mice (+/+). The 200 µl of pooled serum (females and males) were loaded on Superose column and were collected into twenty-one 0.6 ml fractions. Fractions 14-17 represent VLDL and IDL, fractions 18-23 LDL and fractions 25-31 HDL.

fraction of heterozygotes makes up only 5 % of apo AI concentration in HDL fraction, it makes about 63 % in the LDL fraction of homozygotes. Cholesterol diet administration to heterozygotes was associated with an increase in apo AI concentrations in LDL and HDL fractions (Table 3). No significant changes occurred in wild-type mice, confirming the data obtained from ultracentrifugation. Although apo AI concentration in the HDL fraction of homozygotes did not change significantly, there was a rise of apo AI concentrations in the LDL fraction. Cholate diet consumption was associated with an increase of apo AI concentration in the LDL fraction in heterozygotes, but apo AI concentration in wild-type mice remained unaltered. Like heterozygotes, homozygotes on a cholate diet showed an increase in apo AI concentration in LDL fraction (Table 3).

Table 3. Concentration of apoliprotein Al (apo Al) in LDL and HDL fractions in heterozygotes (+/-), homozygotes (-/-) and wild type mice (+/+) after 3 weeks on the different diets.

Diets		Control	Cholesterol	Cholate
Genotype			apo Al (µg/ml)	
+/-	LDL	10	47	275
	HDL	190	356	
-/-	LDL	82	365	515
	HDL	130	137	
+/+	LDL	26	7	6
	HDL	242	265	

Densitometer and computer program were used for quantification of apo Al in lipoprotein fractions isolated using ultracentrifugation and electrophoresed on 3-20 % polyacrylamide-SDS gels. Concentration of apo Al in HDL fractions were not evaluated on cholate diet. The values corresponded to µg of apo Al/ml of serum.

Apolipoprotein concentrations

Apo AI concentrations in the HDL fraction of heterozygotes on a control diet were lower compared with those in wild-type mice but higher compared with homozygotes (Table 3). The sum of total apo AI concentrations in HDL and LDL fractions of heterozygotes on a control diet was lower compared with findings made in wild-type mice. Compared with heterozygotes, total apo AI concentrations in homozygotes are similar; however, they do differ in the relative proportions on apo AI in LDL fractions. While on a control diet, the apo AI concentration in the LDL

Liver morphology

Morphological lesions in hepatic tissue from mice on cholate diet were compared with the findings obtained in mice on control and cholesterol diets. Tiny fatty inclusions were demonstrated in the hepatic tissue of all animals on the control diet (Fig. 2). Compared with findings on the control diet, heterozygotes and wild-type mice on cholesterol diet did not show any progression of these changes. The findings in the hepatic tissue of homozygotes on a cholesterol diet were consistent with the findings made on the control diet; but activated liver macrophages (Kupffer cells) were present. Heterozygotes

on a cholate diet developed a large number of lipid droplets resulting in a typical pattern on progressive liver steatosis. Compared with the findings made on the cholesterol diet, wild-type mice on the cholate diet did not show progression of hepatic lesions. The hepatic tissue of homozygotes on the cholate diet showed, in addition to the presence of lipid droplets, a host of modified Kupffer cells (foam macrophages), which are the equivalent to the foam cells of atherosclerotic plaques. Toxic injury characterized by the formation of small granulomatoid clusters was demonstrated in the hepatic tissue of all animals fed the cholate diet (Fig. 2).

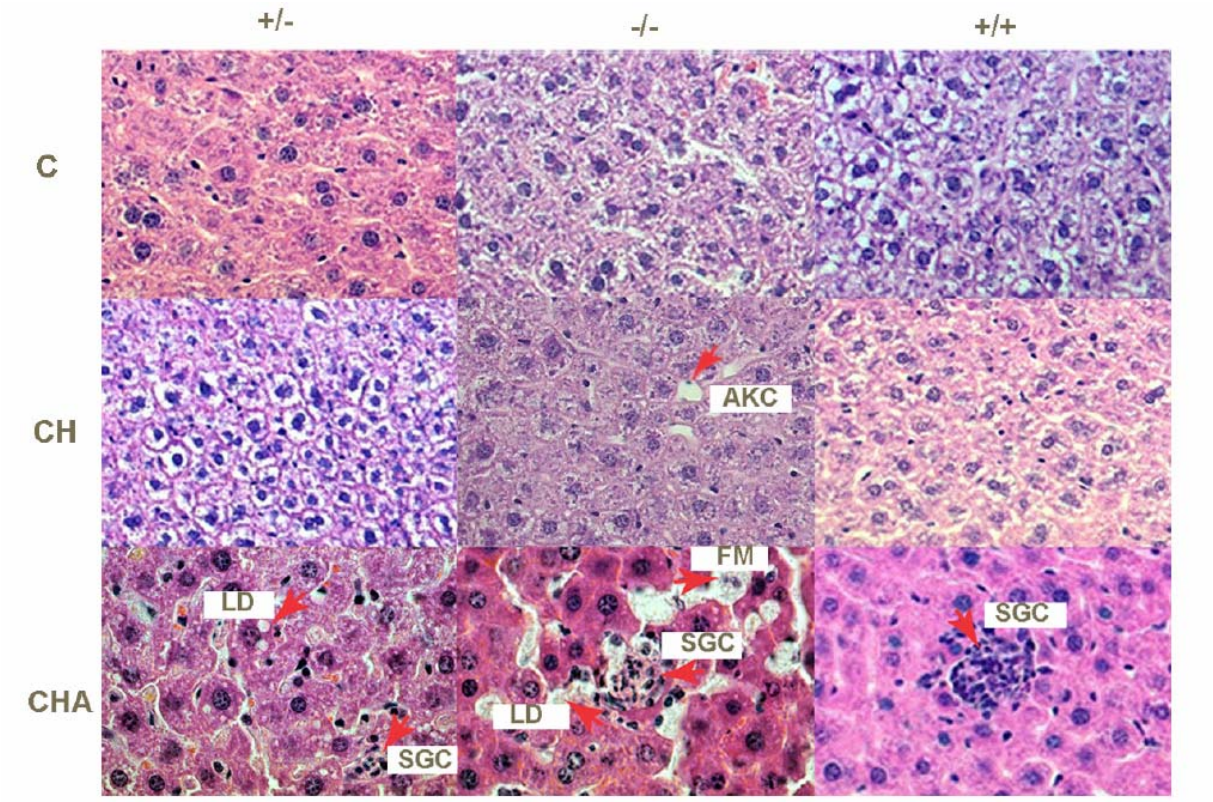

Fig. 2. Morphological changes in hepatic tissue in heterozygotes (+/-), homozygotes (-/-) and wild-type mice (+/+) mice after 3 weeks on diet C (control diet), CH (cholesterol diet) and CHA (cholate diet). LD (lipid droplet), AKC (activated Kupffer cell), FM (foam macrophage), SGC (small granulomatoid cluster). Sections of hepatic tissue were routinely stained with eosin and hematoxylin. Morphological changes were quantified using a microscope (magnification x200).

Discussion

We have demonstrated that total cholesterolemia as well as lipoprotein concentrations in heterozygotes on the control diet were comparable with the concentrations seen in wild-type mice and lower than those in homozygotes (van Ree *et al.* 1994, van Eck *et al.* 1997). It is therefore clear that one functional allele of the apolipoprotein E gene in heterozygotes is enough to maintain the cholesterolemia at concentrations consistent with those seen in wild-type mice. Lipoprotein isolation by ultracentrifugation confirmed that the composition of lipoprotein fraction in heterozygotes on the control diet, as evaluated using the cholesterol/triglyceride ratio, did not differ from that in wild-type mice. Compared with homozygotes, the ratio was lower in heterozygotes. Whilst the VLDL of heterozygotes and wild-type mice had a composition similar to that seen in humans (Gotto *et al.* 1986), and equivalent to a cholesterol/triglyceride ratio of 0.4, the cholesterol/triglyceride ratio in homozygous mice was 6.5 as a result of cholesterol accumulation in VLDL particles. The complete loss of the ability to synthesize apolipoprotein E in homozygotes on the control diet was associated with cholesterol accumulation in the VLDL fraction and an increase in cholesterolemia, while the presence of a single functional apolipoprotein E allele in heterozygotes was enough to maintain normal lipoprotein concentrations. Whereas cholesterol diet administration raised cholesterolemia in all groups of animals, lipoprotein composition was

changed significantly only in heterozygotes and homozygotes. Increased VLDL particle concentrations in the circulation of heterozygotes and homozygotes is associated with their increased conversion to IDL particles as precursors to LDL particles. Conversion of IDL particles to LDL particles also increases LDL particle concentrations in the circulation. Cholesterol accumulation is associated with a change in the cholesterol/triglyceride ratio, with the most significant rise being observed in the VLDL fraction. This finding confirms that, although one functional apolipoprotein E allele is present in heterozygotes, it is not able to prevent the increase in cholesterolemia after increasing alimentary cholesterol. Cholate diet administration to heterozygotes, compared with the control diet, results in a threefold rise in cholesterolemia, and increased VLDL, IDL, and LDL concentrations. The most significant rise in heterozygotes can be seen with cholesterol concentration in the VLDL fraction. Although these particles are referred to, according to their floating density, as VLDL, they are consistent, in terms of their composition, with remnant particles referred to as β VLDL (Guyton 1999). Although the cholesterolemia in wild-type mice varies only in females by about 40 %, the concentrations of VLDL, IDL and LDL rose. Just as in heterozygotes, homozygotes showed a threefold rise in cholesterolemia and an increase of cholesterol concentrations in all lipoprotein fractions. The rise in lipoprotein concentrations in all groups of animals on the cholate diet can be explained by enhanced intestine cholesterol absorption (Wang *et al.* 1999).

We have demonstrated that HDL fraction accounts for almost 80 % of total cholesterol in heterozygotes and wild-type mice. These findings support the notion that heterozygotes do not differ from wild-type animals. The HDL fraction of homozygous apo E KO mice contains as little as 13 % of total cholesterol (van Ree *et al.* 1994, van Eck *et al.* 1997), which can be explained by cholesterol accumulation in VLDL, IDL and LDL fractions. Although cholesterol diet does not dramatically change the cholesterol concentrations in heterozygotes, redistribution of total cholesterol among the individual lipoprotein fractions occurs as evidenced by an increase in VLDL, IDL, and LDL fractions. In wild-type mice, the lipoprotein profile remains unaltered. In homozygotes, VLDL and IDL cholesterol concentrations increase. Our data indicate that the loss of apolipoprotein E in heterozygotes as well as in homozygotes on cholesterol diet is not enough to prevent cholesterol accumulation in lipoproteins, manifesting itself in total cholesterol redistribution among lipoprotein fractions. All groups of animals on the cholate diet develop alterations in the lipoprotein profile associated with increases in VLDL, IDL, and LDL fractions. The change in the lipoprotein profile is likely to be associated with the action of cholate on cholesterol absorption.

Apo AI concentration in the HDL fraction of heterozygotes on the control diet is lower than in wild-type mice. Compared to homozygotes, apo AI concentration in the HDL fraction of heterozygotes is higher. This finding suggests that loss of the apolipoprotein E gene allele is associated with a decrease in apo AI concentration in HDL fraction. In heterozygotes, apo AI concentration in LDL fraction accounts for only 5 % of the apo AI concentration in HDL, while it is about 63 % in homozygotes. Cholesterol accumulation in HDL particles of homozygotes presumably results in the formation of large HDL particles with a markedly decreased density, referred to as HDL1 (de Silva *et al.* 1994). During ultracentrifugation isolation, HDL1 particles move along with LDL particles and are hence included into this fraction, thus overestimating apo AI concentration in the LDL fraction. Although apo AI concentration in the HDL fraction of heterozygotes is higher than in homozygotes, cholesterol accumulation associated with HDL1 particle formation does not occur in their HDL particles. The changes of apo AI concentrations in LDL and HDL fractions in all groups of animals on the cholesterol diet are consistent with the data obtained by ultracentrifugation. It can be assumed that the rise of apo AI concentrations in the LDL and HDL fractions of heterozygotes are related to the increase of cholesterol concentration in these fractions. Administration of a cholate diet results in a rise of apo AI concentration in the LDL fraction of heterozygotes. While wild-type mice do not show an increase in apo AI concentrations in the LDL fraction, such an increase does occur in both homozygotes and heterozygotes. A possible explanation for the rise in apo AI concentration in the LDL fraction of heterozygotes is that cholesterol accumulation and formation of HDL1 particles occur in these animals, just as in homozygotes on a cholate diet.

Tiny lipid inclusions are noted in the hepatic tissue of all groups of animals on the control diet. Given the presence of these alterations in all groups of animals, this finding must be regarded as a physiological picture of hepatic tissue in mice. Cholesterol diet administration to heterozygotes and wild-type mice did not result in

marked alterations in liver morphology, which is consistent with the finding of only a mild increase in cholesterolemia. Although homozygotes did not show an increased incidence of hepatic inclusions, there appeared activated macrophages, Kupffer cells. A likely explanation for the activation of hepatic macrophages is the loss of apolipoprotein E synthesis. As a result of the loss of a receptor ligand, IDL particles and, partly, also LDL particles (despite their increased circulating concentrations) can not bind to the conventional low-density lipoprotein receptor and low-density lipoprotein receptor-related protein in the liver. A possible pathway for their removal from the circulation is the binding of IDL and LDL particles to scavenger receptors of liver macrophages as an alternative pathway for eliminating increased concentrations of circulating cholesterol. Administration of the cholate diet to heterozygotes resulted in the development of liver steatosis characterized by the presence of a large number of lipid droplets. Cholate action presumably leads to enhanced cholesterol absorption from the intestine. A part of the absorbed cholesterol is transported, by IDL and LDL particles, to the liver where these particles bind to LDL particles and, especially, to LRP receptors. Excessive cholesterol transport into the liver results in cholesterol accumulation in hepatocytes and in the development of progressive liver steatosis. Because of the small increase in cholesterolemia, no progression of hepatic alterations occurred in wild-type mice on the control diet compared to the cholesterol diet. After the cholate diet, homozygotes showed, in addition to lipid droplets, the presence of a large number of cholesterol-rich modified Kupffer cells, foam macrophages (Zhang *et al.* 1994). The presence of Kupffer cells is related to the alternative pathway of cholesterol removal from the circulation. Because of the loss of apolipoprotein E, liver macrophages are unable to get rid of the accumulated cholesterol, giving rise to foam macrophages (an equivalent to the foam cells of the subendothelial cells of blood vessels). In all groups of animals, cholate diet administration was associated with toxic damage to hepatic tissue. Cholate action results not only in an increased intestinal cholesterol absorption, but also increased bile acid concentrations. Increased bile acid concentrations leads to the formation of small granulomatoid clusters in hepatic tissue creating the picture of toxic liver damage.

We have confirmed, in this study, that basal cholesterolemia of apo E knockout heterozygotes does not differ from values obtained in wild-type mice. Although cholesterol diet administration results, in apo E KO heterozygotes, in only a negligible rise in cholesterolemia compared with apo E KO homozygotes, the lipoprotein spectrum changes significantly and the concentrations of atherogenic fractions IDL and LDL increase. We have demonstrated that the addition of cholate to cholesterol diet increases the cholesterolemia of animals and changes lipoprotein concentrations; however, it is associated with the onset of toxic injury to hepatic tissue, a fact decreasing the appropriateness of its use.

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Reprint requests

D. Bobková, Mgr., Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine, Vídeňská 1058/9, 140 21 Prague 4, Czech Republic. Fax: +420-241721574. E-mail: dabq@medicon.cz