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Letter to the Editors

Variability in cholesterol content in serum and aortic tissue in apolipoprotein E-deficient mice is comparable in inbred (129/Sv) and outbred (mixed 129/Sv and C57BL/6) mice

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Dear Editors,

Depending on the breeding strategy used, mice with mutations in lipoprotein metabolism generated by gene targeting in embryonic stem (ES) cells may have a mixed genetic background. Usually the genotype is a composite of 129 (the ES cell donor) and C57BL/6 (the mating partner of the chimeras). As a result, from the F2 generation on, each individual animal can be considered genetically unique. In addition to environmental variability, this genetic heterogeneity could cause variance in an observed phenotype to an unknown extent. It is known that plasma cholesterol levels in 129/J mice are higher than in mice of the strain C57BL/6J [1], whereas the latter are more susceptible to develop diet-induced atherosclerosis than 129/J mice [2]. Unfortunately, the number of genetic loci involved is not known.

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Previously, we have studied the effect of age and gender on serum cholesterol level in apolipoprotein (apo) E-deficient mice with a mixed 129/Sv and C57BL/6 background [3]. To study the effect of genetic heterogeneity, in addition to the created null mutation in the *Apoe* gene, we have compared the cholesterol level in serum and aortic tissue in inbred and outbred apo E-deficient mice fed a regular chow diet. The inbred *Apoe* knock-out mice were obtained by cross breeding the apo E-deficient chimeric males [3] with 129/Sv females (designated 129), whereas the outbreds were F2 or F3 generations from sibling-intercrossed hybrids of 129/Sv and C57BL/6 (designated 129/B6).

Table 1 shows serum cholesterol levels of inbred and outbred apo E-deficient mice and controls at different ages. Serum cholesterol was measured with a colorimetric test using Boehringer Mannheim enzymatic assay kit No. 236691. At 4 weeks of age, serum cholesterol was higher in 129 than in 129/B6 mice. This is most evident in the

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Table 1
Serum cholesterol levels in inbred (129) and outbred (129/B6) apo E-deficient mice with increasing age

		4 weeks			10 weeks			>26 weeks		
		n	Chol (mM)	Coeff. var.	*n*	Chol (mM)	Coeff. var.	*n*	Chol (mM)	Coeff. var.
Inbred (129)										
+/+	♂	22	2.97 ± 0.42[a]	0.14	23	3.35 ± 0.54[c]	0.16	23	2.92 ± 0.72	0.25
+/−	♂	16	2.77 ± 0.41	0.15	16	3.25 ± 0.32[c]	0.10	22	2.85 ± 0.48[b]	0.17
−/−	♂	8	20.98 ± 1.77[a,c]	0.08	7	20.96 ± 2.56[c]	0.12	23	31.56 ± 5.53[a,c]	0.18
+/+	♀	29	2.85 ± 0.60[a,b]	0.21	26	2.79 ± 0.58	0.21	23	2.63 ± 0.56	0.21
+/−	♀	13	2.72 ± 0.44[a]	0.16	14	2.80 ± 0.40	0.14	23	2.76 ± 0.27[a,b]	0.10
−/−	♀	6	26.44 ± 2.55[a]	0.10	6	28.99 ± 6.80[a,b]	0.23	23	25.01 ± 5.92[a]	0.24
Outbred (129/B6)										
+/+	♂	29	2.66 ± 0.44[c]	0.17	14	3.13 ± 0.56	0.18	26	3.15 ± 0.55[c]	0.17
+/−	♂	31	2.57 ± 0.46[c]	0.18	15	3.28 ± 0.41[c]	0.13	31	2.89 ± 0.74[c]	0.26
−/−	♂	30	11.61 ± 2.74	0.24	15	20.63 ± 4.68[c]	0.23	49	20.41 ± 5.06[c]	0.25
+/+	♀	29	2.27 ± 0.38	0.17	14	2.82 ± 0.51	0.18	44	2.63 ± 0.56	0.21
+/−	♀	31	2.23 ± 0.45	0.20	16	2.88 ± 0.55	0.19	22	2.48 ± 0.47	0.19
−/−	♀	30	10.68 ± 1.77	0.17	15	17.71 ± 2.87	0.16	42	15.15 ± 4.25	0.28

Serum cholesterol levels at age 4 weeks, 10 weeks and more than 26 weeks are given as mean ± S.D. *n*, number of animals analyzed; coeff. var., coefficient of variation (S.D. divided by the mean); +/+, control mice; +/−, heterozygous apo E-deficient mice; −/−, homozygous apo E-deficient mice.
[a]Significant difference in serum cholesterol level ($P < 0.05$) as compared with outbred mice of same genotype using an unpaired *t*-test.
[b]Significant difference in standard deviation ($P < 0.05$) as compared with outbred mice of same genotype using an unpaired *t*-test.
[c]Significant difference in serum cholesterol level ($P < 0.05$) as compared with female mice of same genotype using an unpaired *t*-test.

homozygous apo E-deficient females, with serum cholesterol of 26.44 ± 2.55 mM and 10.68 ± 1.77 mM in the 129 and 129/B6 groups, respectively. With increasing age, only the homozygous deficient inbred mice exhibit higher cholesterol levels than their outbred counterparts. Differences in serum cholesterol influenced by gender are most prominent for the homozygous null mutants. 129/B6 homozygous males display higher serum levels than females, whereas in 129 mice, this is only the case at an older age (> 26 weeks). At a younger age, homozygous 129 females show higher serum cholesterol levels than males.

Much to our surprise, we observed no clear difference in variance of the serum cholesterol levels between 129 and 129/B6 mice. Thus, even though the genetic variation is absent in the inbred groups, this does not result in a significantly smaller standard deviation, when compared with the outbred groups. Mice deficient in apo C3 have also been studied on a mixed 129/B6 and on a pure 129 background [4]. Although the authors did not comment on it, in that study the variability in plasma cholesterol also seemed to be similar for the inbred and the outbred groups, corroborating our results.

Atherosclerosis is a multifactorial process, in which multiple genes are expected to be involved. To find out whether the genetic heterogeneity in 129/B6 mice influences the development of atherosclerosis, we quantified the degree of atherosclerosis in 129/B6 and 129 mice by determining cholesterol content in aortic tissue. Nielsen and co-authors described that aortic total cholesterol as a measure of atherosclerosis severity in cholesterol-fed rabbits is just as good a measure as aortic esterified cholesterol [5]. Therefore, we determined aortic total cholesterol in our animals. Aorta was isolated from the aortic arch up to and including the thoracic aorta, and stripped from surrounding fat tissue. The vessel was minced with a pestle and mortar, and lipids were extracted according to Bligh and Dyer [6]. Lipids were dissolved in ethanol and total cholesterol was measured with a colorimetric test as mentioned above for serum cholesterol.

Table 2
Cholesterol content in aortic tissue of inbred (129) and outbred (129/B6) apo E-deficient mice

	Inbred	Outbred
+/+	1.15 ± 0.17 (3)[a]	2.50 ± 0.42 (3)
−/−	12.29 ± 3.81 (6)	13.68 ± 2.01 (8)

Cholesterol levels in aorta of male control (+/+) and male homozygous apo E-deficient (−/−) mice are given in μg/mg wet weight of aortic tissue (mean ± S.D.) (number of animals analyzed). The control animals were 6 months of age, the homozygotes 13 months.
[a]Significant difference in cholesterol level ($P < 0.05$) as compared with outbred mice of same genotype using an unpaired t-test.

As is shown in Table 2, there is a clear difference in cholesterol level in aorta of control and apo E-deficient animals. However, within the homozygotes there is no difference between the absolute cholesterol content in aorta of 129/B6 and 129 males, nor in the variance. In other words, genetic heterogeneity in apo E-deficient mice does not cause a greater variability in total cholesterol in aortic tissue of outbred mice when compared with their inbred counterparts.

In conclusion, we have demonstrated that the use of an inbred strain of mice [129] does not reduce the variance in serum and aortic cholesterol levels, when compared to outbred mice (129/B6). This would suggest that environmental differences between the individual animals, rather than the variability in genetic background between the two strains of mice, are a major source of the observed variance in cholesterol levels.

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