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Biometrical genetic analysis of serum cholesterol response and basal serum HDL cholesterol level in the rabbit

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Introduction

The supplement of cholesterol to the diet of random-bred rabbits elicits a rise of serum cholesterol, but there are marked interindividual differences in the extent of the serum cholesterol response. Rabbits showing only small changes in the concentration of serum cholesterol (hyporesponders) and animals showing high degrees of hypercholesterolemia (hyperresponders) could be distinguished from normoresponders (*Beynen et al.* 1987). Inbred strains of rabbits differing in their sensitivity to dietary cholesterol have also been described (*Van Zutphen & Fox* 1977), thus indicating that differences in serum cholesterol responsiveness in rabbits has a genetic basis. Hyper- and hyporesponders to dietary cholesterol have also been found within several other mammalian species, including man, though not as pronounced as in rabbits (*Beynen et al.* 1987). In an attempt to analyze the genetic control of the serum cholesterol response in the rabbit, a number of crosses (F_1, F_2 and backcrosses) between hyporesponsive and hyperresponsive inbred rabbits were produced and tested for their susceptibility for dietary cholesterol. In addition to serum cholesterol response, we also measured the basal serum HDL cholesterol level (as percentage of serum total cholesterol concentration), since in the two inbred strains of rabbits, when on a low-cholesterol diet, serum HDL cholesterol concentrations are lower in hyper- than in hyporesponders (*Meijer* 1991).

Materials and Methods

Animals, housing and diets:

At the Department of Laboratory Animal Science (Utrecht, The Netherlands) two rabbit (*Oryctolagus cuniculus*) inbred strains are maintained: AX/JU which is a dietary cholesterol susceptible (*hyperresponding*) strain and IIIVO/JU which is a dietary cholesterol resistant (*hyporesponding*) strain (*Van Zutphen & Fox* 1977, *Meijer* 1991). The strains originated from the Jackson Laboratory colony, Bar Harbor, ME, USA (*Fox* 1975) and are the two extreme opposite responding strains out of the six previously tested strains (*Van Zutphen & Fox* 1977). The two inbred strains are maintained by brother-sister mating. The coefficient of inbreeding (F) >0.95 for both strains. To produce F_1-hybrids matings were made between IIIVO/JU females and an AX/JU male. The F_1-hybrids were subsequently intercrossed (brother-sister mating; F_2 rabbits) or backcrossed to both parental strains. Male F_1-hybrids were mated with their IIIVO/JU mothers (backcross to the IIIVO/JU strain = BC_1) and female F_1-hybrids were mated with the AX/JU father (backcross to the AX/JU strain = BC_2). We have tested 12 (6 ♂♂ and 6 ♀♀) IIIVO/JU rabbits (P_1), 81 animals (40 ♂♂ and 41 ♀♀) of the AX/JU strain (P_2), 71 (38 ♂♂ and 33 ♀♀) F_1-hybrids, 57 (26 ♂♂ and 31 ♀♀) BC_1-progeny, 76 (44 ♂♂ and 32 ♀♀) BC_2-progeny and 142 (65 ♂♂ and 77 ♀♀) F_2 rabbits. After weaning at the age of 10 weeks, all rabbits were fed a commercial, pelleted, natural-ingredient diet (LKK-20®, Hope Farms BV, Woerden, The Netherlands) and were housed individually in stainless steel cages with wire mesh bases (Ruco BV, Waalre, The Netherlands) as described previously (*Beynen et*

al. 1989). The chemical composition of the commercial rabbit diet has been described elsewhere (*Van Lith et al.* 1995). The cages, which were randomized with respect to gender and litter origin, were located in a room with controlled lighting (light from 07.00 to 19.00 hours), temperature (16-19°C) and relative humidity (55-65%). At 12-16 weeks of age, the diet of the rabbits was changed from the commercial diet to the same diet to which 0.3 g of cholesterol (Solvay-Duphar BV, Weesp, The Netherlands) per 100 g diet had been added. The cholesterol was added without exchange with another food component. The cholesterol-rich diet was fed during the test period which lasted 35 days. Restricted amounts of diet were fed. The daily amount of pellets was 100 g for all rabbits. Acidified tap water was provided *ad libitum*. The rabbits were allowed to practice coecotrophy. Food intake was recorded once a week throughout the entire test period. The cholesterol-rich diet was stored at 4°C until feeding. The various cohorts of rabbits were tested between September 1992 and May 1995. For each class of rabbits (P_1, P_2, F_1, BC_1, BC_2 or F_2) the results from the various cohorts of rabbits did not differ and were pooled.

Blood sampling and chemical analyses:
On the day before blood sampling any remaining food was removed at 16 hours. Blood sampling was performed between 8.00 and 10.00 hours. Samples of blood were taken from the marginal ear vein. Blood was collected in tubes without anticoagulant. To collect serum, the blood in the tubes was allowed to clot for 30 min at room temperature. Serum was prepared by low-speed centrifugation (10 min, 3000 g, room temperature) and was analyzed the same day. Cholesterol in the serum was measured enzymatically according to *Siedel et al.* (1983), using a kit (Monotest®) supplied by Boehringer Mannheim GmbH (Mannheim, Germany). Serum HDL cholesterol was measured as Mg^{2+}-dextran-sulphate soluble cholesterol (*Warnick et al.* 1982). The inter- and intra-assay coefficients of variation for serum cholesterol determination always fell within the limits as prescribed by the manufacturer. Total and HDL cholesterol concentrations in the serum are measured in mM.

Biometrical genetic and statistical analyses:
The biometrical genetic analysis has been performed according to the procedure described by *Festing* (1976). The variances of the segregating generations BC_1, BC_2, and F_2 are due both to genetic and environmental factors, whereas the variances of P_1, P_2 and F_1 are mainly due to environmental factors. Biometrical genetic analysis of the data requires normal within-group distributions and equal variances in the P_1, P_2 and F_1 groups. The Kolmogorov-Smirnov one-sample test was used to check normal distribution of the data. All data within groups were found to be normally distributed. However, Bartlett's test indicated that the variances of the traits under study were not equal in the P_1, P_2 and F_1 populations. Thus a transformation which equalizes the variances was necessary. The following transformations were applied:

$$y = (ax + b)^c \quad (1)$$

$$y = {}^{10}\log(x + d) \quad (2)$$

where y is the transformed trait value, x is the untransformed trait value, and a, b, c and d are constants. Equation (1) was used for the serum cholesterol response and equation (2) was used for basal serum HDL cholesterol level (as percentage of serum total cholesterol concentration). After transformation all within-group data were still normally distributed. Thus, the transformed data may now be used for biometrical genetic analysis assuming that genetic factors also have additive effects on this scale. Support for this assumption can be obtained by further scale testing. The following relationships between means of the nonsegregating and segregating groups should hold:

$$2BC_1 - P_1 - F_1 = 0 \quad (A)$$

$$2BC_2 - P_2 - F_1 = 0 \quad (B)$$

$$4F_2 - 2F_1 - P_1 - P_2 = 0 \quad (C)$$

were P_1, etc. refer to the (transformed) means of each of the six classes.
The (transformed) variances of these three equations are as follows:

$$V_{(A)} = 4V_{BC1} + V_{P1} + V_{F1}$$

$$V_{(B)} = 4V_{BC2} + V_{P2} + V_{F1}$$

$$V_{(C)} = 16V_{F2} + 4V_{F1} + V_{P1} + V_{P2}$$

These variances can then be used to determine whether the equations (A), (B) or (C) differ significantly from zero. The scales of measurement are

adequate if each of the equations deviated less than two times the SD from zero (*Festing* 1976). Estimates of heritability and the number of loci can then be made. Heritability (in narrow sense; h^2) was estimated by the ratio of the additive genetic variance to the total phenotypic variance in the F_2 generation ($h^2 = V_A/V_{F2}$). The additive genetic variance was derived from the difference between two-times the variance of the F_2 generation and the sum of the variances of the two backcross generations ($V_A = 2V_{F2} - V_{BC1} - V_{BC2}$)(*Festing* 1976). The estimate of the number of loci (n) involved in the genetic determination of the average difference between the AX/JU and IIIVO/JU inbred strains was calculated as the ratio of the squared difference between these two averages to eight-times the additive genetic variance ($n = [P_1 - P_2]^2/8V_A$)(*Festing* 1976). The assumptions underlying this estimate of the number of loci are additive gene effects, unlinked loci and no epistasis. Furthermore, the formula requires the assumption that one parental strain carries all of the "minus" genes and the other all of the "plus" genes. Any deviation will tend to cause underestimation of the number of loci. Thus, the value of n is usually thought to represent the minimum number of segregating loci.

Statistically significant differences were found between male and female average serum cholesterol concentrations at day 0 and 35 in several of the crosses (two-tailed Student's t test, $P<0.05$; data not shown). Therefore, all of the biometrical genetic analyses were performed as two analyses, one for the males and one for the females. Two-side probabilities were estimated throughout, and the level of statistical significance was preset at $P<0.05$. All statistical analyses were carried out according to *Steel & Torrie* (1981) using the SPSS PC+ computer program (*SPSS Inc.* 1990).

Results and Discussion

Serum cholesterol response:

Results for serum cholesterol response are summarized in Table 1. The response is calculated as the difference between serum total cholesterol concentration at day 35 (data not shown) and day 0 (data not shown) and also as the quotient of serum total cholesterol concentration at day 35 and serum total cholesterol concentration at day 0. On average, serum cholesterol response differs between the parental strains by a factor 7-10. In the genetically homogeneous populations (P_1, P_2 and F_2) fe-

Table 1. Descriptive statistics of the untransformed serum cholesterol response in rabbits after 35 days of feeding a cholesterol-rich diet.

		Serum cholesterol response [Day 35-Day 0]			Serum cholesterol response [Day 35/Day 0]		
Class	N	Mean±SEM	*V*	Range	Mean±SEM	*V*	Range
Male rabbits							
P_1	6	3.6±0.6	2.4	1.6- 5.5	5.8±0.9	4.4	3.4- 8.6
P_2	40	26.4±0.7	21.5	19.9-39.7	43.6±1.6	103.7	13.2-65.4
F_1	38	25.6±1.4	69.5	13.1-43.0	46.2±2.2	186.1	27.5-78.1
BC_1	26	28.7±2.2	130.6	11.3-48.6	36.8±2.9	216.1	14.4-60.2
BC_2	44	28.6±1.4	81.4	7.9-44.5	44.1±2.1	194.5	15.4-80.3
F_2	65	24.2±1.3	116.4	8.2-61.6	35.9±1.9	222.8	12.7-81.3
Female rabbits							
P_1	6	3.7±0.4	1.2	2.2- 5.1	4.8±0.5	1.4	3.3- 6.3
P_2	41	38.6±0.9	30.9	22.7-48.2	37.0±1.1	46.6	26.0-54.9
F_1	33	42.1±1.1	43.0	32.0-56.0	37.0±1.9	118.1	21.3-56.8
BC_1	31	35.3±2.3	162.4	18.2-69.1	32.7±2.6	210.7	13.7-72.3
BC_2	32	40.4±1.3	56.0	19.7-52.8	34.1±1.7	95.4	21.6-65.0
F_2	65	30.5±1.1	94.7	11.8-56.3	28.6±1.0	71.9	15.2-55.9

Table 2. Scaling tests and descriptive statistics of the transformed serum cholesterol response in rabbits after 35 days of feeding a cholesterol-rich diet.

Serum cholesterol response								
	Male rabbits [Day 35/Day 0]				Female rabbits [Day 35-Day 0]			
Class	N	Mean±SEM	*V*	Range	N	Mean±SEM	*V*	Range
	Transformation: $y=^{10}log(x+2)$				*Transformation:* $y=^{10}log(x+5)$			
P_1	6	0.88±0.05	0.0150	0.73-1.02	6	0.93±0.02	0.0029	0.86-1.00
P_2	40	1.65±0.02	0.0125	1.18-1.83	41	1.64±0.01	0.0036	1.44-1.73
F_1	38	1.67±0.02	0.0145	1.47-1.90	33	1.67±0.01	0.0036	1.57-1.79
BC_1	26	1.56±0.03	0.0283	1.22-1.79	31	1.58±0.02	0.0191	1.37-1.87
BC_2	44	1.64±0.02	0.0186	1.24-1.92	32	1.65±0.01	0.0059	1.39-1.76
F_2	65	1.55±0.02	0.0273	1.17-1.92	77	1.53±0.01	0.0144	1.23-1.79

Scaling tests	
Male rabbits	Female rabbits
Bartlett's test [$V_{P1} \approx V_{P2} \approx V_{F1}$]: P=0.896	Bartlett's test [$V_{P1} \approx V_{P2} \approx V_{F1}$]: P=0.958
(A) = +0.57±0.38 [+1.51*SD]	(A) = +0.57±0.29 [+1.96*SD]
(B) = -0.03±0.32 [-0.08*SD]	(B) = -0.00±0.18 [-0.02*SD]
(C) = +0.33±0.72 [+0.46*SD]	(C) = +0.23±0.50 [+0.46*SD]

male rabbits when compared with male rabbits have higher serum cholesterol responses (two-tailed Student's *t* test, P<0.05). This corroborates earlier investigations using New Zealand White rabbits (*Hromadova & Hacik* 1984). Similar gender effects with respect to cholesterolaemic response have also been described for rats (*Imai & Matsumura* 1973). For both sexes, irrespective of how the serum cholesterol response is expressed, the mean of the response in F_1-hybrids did not differ significantly of the mean response in the AX/JU (P_2) strain (two-tailed Student's *t* test, P>0.05). Thus, suggesting genetic dominance.

To carry out straightforward biometrical genetic analyses, it was necessary to logarithmically transform the data. For male rabbits we used the serum cholesterol response data expressed as quotient and for females as the difference between serum cholesterol level at day 35 and day 0. The scaling tests indicated that the scales of measurement were adequate (Table 2). The narrow sense heritability (h^2) was calculated as 0.28 in males and 0.27 in females. Thus, indicating that under these experimental conditions environmental factors have still a major impact on the serum cholesterol response in rabbits. In contrast to using the (logarithmically transformed) individual response data as used in the study with six inbred rabbit strains (*Van Zutphen & Fox* 1977), we calculated an intra-class correlation (r_I i.e. the proportion of the total variation that is accounted for by differences between strains; another way of measuring heritability) of 0.77. Furthermore, *Van Zutphen & Den Bieman* (1983) estimated the h^2 in male rats to be 0.86. Possibly, maternal and/or seasonal influences have played a significant role in the present study with rabbits.

Although estimates of the number of loci are rather imprecise (*Zeng et al.* 1990) they probably indicate whether a small or large number of loci is involved. The number of loci was estimated as 10 in the male data and 16 in the female data. In contrast, only two major loci were found to control the individual variation of the serum choles-

Table 3. Descriptive statistics of the untransformed basal serum HDL cholesterol level in rabbits.

Basal serum HDL cholesterol level [% of serum total cholesterol]								
	Male rabbits				Female rabbits			
Class	N	Mean±SEM	*V*	Range	N	Mean±SEM	*V*	Range
P_1	6	74±3	38	63-79	6	74±2	14	70-78
P_2	40	30±1	63	18-51	41	35±2	98	18-71
F_1	38	52±1	27	43-67	33	40±1	58	24-61
BC_1	26	61±1	44	42-72	31	53±1	62	35-68
BC_2	44	44±2	130	21-67	32	36±2	133	16-73
F_2	65	52±1	145	24-74	77	48±1	113	24-75

terol response in rats (*Van Zutphen & Den Bieman* 1983).

Basal serum HDL cholesterol level:

Serum HDL cholesterol concentration (at day 0; data not shown) is expressed as the percentage of serum total cholesterol concentration (at day 0; data not shown). Table 3 shows the baseline serum HDL cholesterol level. In the genetically homogeneous populations there was no gender effect with respect to basal serum HDL cholesterol (two-tailed Student's *t* test; P>0.05). IIVO/JU (P_1) rabbits had 2-2.5 times higher serum HDL cholesterol levels than AX/JU (P_2) rabbits. This is consistent with earlier work (*Meijer* 1991). For both sexes, the mean of the serum HDL cholesterol level in F_1-hybrids was in-between the

Table 4. Scaling tests and descriptive statistics of the transformed basal serum HDL cholesterol level in rabbits.

Basal serum HDL cholesterol level [% of serum total cholesterol]								
	Male rabbits				Female rabbits			
Class	N	Mean±SEM	*V*	Range	N	Mean±SEM	*V*	Range
	Transformation: $y=(1.5x-14)^{1.5}$				*Transformation:* $y=(x-22)^2$			
P_1	6	968±55	17857	725-1077	6	2685±157	148366	2265-3169
P_2	40	181±17	10932	44-500	41	277±62	159512	2-2413
F_1	38	508±16	9247	360-804	33	363±59	114207	3-1555
BC_1	26	681±25	16328	342-917	31	1028±86	226930	168-2075
BC_2	44	386±28	34885	73-812	32	325±86	236864	1-2572
F_2	65	522±26	43312	105-950	77	778±63	308841	3-2806

Scaling tests	
Male rabbits	Female rabbits
Bartlett's test [$V_{P1} \approx V_{P2} \approx V_{F1}$]: P=0.572	Bartlett's test [$V_{P1} \approx V_{P2} \approx V_{F1}$]: P=0.623
(A) = -114±304 [-0.38*SD]	(A) = -993±1082 [-0.92*SD]
(B) = +83±400 [+0.21*SD]	(B) = +9.74±1105 [+0.01*SD]
(C) = -78±871 [-0.09*SD]	(C) = -578±2389 [-0.24*SD]

means of the serum HDL cholesterol level in both parental strains. Since for both sexes the variances of the P_1, P_2 and F_1 populations were not similar, the data were transformed in order to equalize the variances. A polynomial transformation was applied for both sexes. The scaling tests indicated that this kind of transformation produced scales of measurement which were adequate (Table 4).

Using the transformed data, estimates of the heritability and number of loci involved in individual variation in basal serum HDL cholesterol can be made. The narrow sense heritability (h^2) is 0.82 for male and 0.50 for female rabbits. It was calculated that only two (data of males) or five (data of females) loci are involved in the control of basal serum HDL cholesterol level in rabbits.

Conclusions

In conclusion, the present study reveals that under the described experimental conditions about 30% of the individual variation of the serum cholesterol response in rabbits is under the control of 10 to 16 loci. For basal serum HDL cholesterol level, 50 to 80% of the observed individual variation could be attributed to additive effects of two to five genes. Molecular- and biochemical-genetic studies are in progress to identify and localize the chromosomal regions associated with these traits in rabbits (*Van Lith & Van Zutphen* 1994, *Van Lith et al.* 1996).

Summary

The inheritance of the susceptibility for dietary cholesterol (serum cholesterol response) and of basal serum HDL cholesterol level in the rabbit have been studied by measuring serum total cholesterol levels and HDL cholesterol levels in animals from crosses between hyperresponding and hyporesponding inbred strains. The serum cholesterol response and basal serum HDL cholesterol levels of the resulting six populations (the two parental strains, F_1-hybrid, two backcrosses and F_2) were subjected to a biometrical genetic analysis to determine the number of loci involved and the (narrow sense) heritability of the two traits. The study revealed that 30% of the individual variation of the serum cholesterol response is under genetic control and that 10 to 16 loci may be involved. The heritability for basal serum HDL cholesterol levels was 50 to 80%, whereas two to five loci may be involved in regulating the basal serum HDL cholesterol level in rabbits.

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