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ADRENAL WEIGHT AND SERUM CHOLESTEROL CONCENTRATION IN RABBITS WITH DIFFERENTIAL CHOLESTEROLAEMIC RESPONSE TO CHOLESTEROL FEEDING

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ABSTRACT : The question addressed is whether in rabbits a relationship exists between serum cholesterol response to dietary cholesterol and adrenals autopsy weight. For this purpose we have measured adrenals weight and serum cholesterol levels in backcross and F_2-intercross progeny of a cross between IIIVO/JU (dietary cholesterol resistant) and AX/JU (dietary cholesterol susceptible) rabbits. The animals were fed a commercial, pelleted diet enriched with 0.3% (w/w) cholesterol during a test period which lasted 41 (backcross rabbits) or 78 (F_2-intercross rabbits) days. After the test period hyperresponders with high serum cholesterol levels and hyporesponders with low serum cholesterol levels could be distinguished from normoresponders, both within backcross and F_2-intercross rabbits. A positive relationship between serum cholesterol response and adrenals weight was found for male, but not for female F_2-intercross rabbits. No clear dose-response relationship between serum cholesterol response and adrenals weight was found both for male and female backcross rabbits. It is concluded that the relationship between serum cholesterol response and adrenals weight in rabbits is certainly not a simple one and may depend on gender, duration of the experimental period and the genetic background of the animals.

RESUME : Variation du poids des glandes surrenales et du taux de cholesterol sanguin chez le lapin en réponse au cholesterol alimentaire

La question étudiée est de savoir s'il existe chez le lapin une relation entre l'élévation du taux de cholesterol sanguin liée à l'apport de cholesterol de l'aliment et le poids des glandes surrénales. Pour y répondre, nous avons pesé les glandes surrénales et mesuré le taux de cholestérol sanguin chez les lapins issus du croisement en retour (backcross) ou de la F_2 obtenus avec les souches IIIVO/JU (non sensible au cholesterol alimentaire) et AX/JU (sensible au cholesterol alimentaire). Les animaux ont été nourris avec un aliment commercial granulé enrichi de 0,3% (poids/poids) de cholesterol pendant une période expérimentale de 41 jours pour les lapins en backcross ou de 78 jours pour les lapins F_2. A la fin de la période expérimentale, des sujets hyper-réceptifs avec un taux de cholesterol sanguin élevé, des animaux peu réceptifs avec un taux de cholesterol sanguin faible et des animaux normalement réceptifs ont pu être départagés aussi bien chez les lapins en backcross que chez les lapins F_2. On a trouvé un relation positive entre la réponse du taux de cholesterol sanguin et le poids des glandes surrénales chez les mâles (mais pas chez les femelles) de la F_2. Chez les lapins en backcross, aussi bien pour les mâles que pour les femelles, il n'y a pas de réponse claire de lien entre le taux de cholesterol sanguin et le poids des glandes surrénales .On peut conclure que la relation entre la variation du taux de cholestérol sanguin et le poids de la glande surrénale est complexe et qu'elle peut dépendre du sexe, de la longueur de la période expérimentale et du patrimoine génétique des animaux.

INTRODUCTION

Among humans and between animals of the same species there can be marked differences in serum cholesterol response to an increased intake of cholesterol. Certain individuals (*hyporesponders*) show only small changes in the concentration of cholesterol in the serum, whereas others (*hyperresponders*) develop high degrees of hypercholesterolemia. This phenomenon, which appears to be under genetic control, has been extremely well established in rabbits (BEYNEN *et al.*, 1987). Inbred strains of rabbits with either a high, intermediate or low cholesterolaemic response to high-cholesterol diets have been identified (VAN ZUTPHEN and FOX, 1977).

For a long time it has been known that the sterol content of the adrenals is significantly increased by dietary cholesterol. This leads to hypertrophy with enlargement of cells in the zona fasciculata (FORBES *et al.*, 1964; MORRIS *et al.*, 1966; GWYNNE and HESS, 1978). The hypertrophy is associated with an increment of adrenals weight.

VAN ZUTPHEN *et al.* (1981) reported that after a 5 week period of feeding a cholesterol-rich diet to the male progeny of crosses between New Zealand White and Vienna White outbred rabbits, there were marked individual differences in serum cholesterol levels and adrenal autopsy weights. Hyperresponders with high serum cholesterol levels had 2 to 3-fold heavier adrenal glands than hyporesponders with low serum cholesterol levels, thus indicating a positive association between serum cholesterol response and adrenals weight. In contrast, in genetically Watanabe rabbits on a low cholesterol diet where serum cholesterol levels are 16 times as high as in New Zealand White rabbits, adrenal cholesterol content was one third that of the adrenal glands of New Zealand White rabbits (HOEG *et al.*, 1985), thus suggesting a negative association between serum cholesterol level and adrenals weight. The low adrenal cholesterol content and high serum cholesterol concentration in Watanabe rabbits are due to a defective LDL-receptor delivery pathway (HOEG *et al.*, 1985).

These conflicting rabbit results prompted us to analyse data collected from backcross and F_2-intercross progeny of a cross between hyporesponsive (IIIVO/JU) and hyperresponsive (AX/JU) inbred rabbits, which had been fed a cholesterol-rich diet. The question addressed is whether rabbits, which differ in serum cholesterol response to dietary cholesterol, also differ in adrenals weight and if so, what the relationship is between these two parameters. On the basis of the aforementioned, such a relationship could provide clues, whether or not the LDL-receptor delivery pathway is involved in the difference in serum cholesterol response between AX/JU and IIIVO/JU rabbits.

MATERIALS AND METHODS

Animals, housing and diets.

At the Department of Laboratory Animal Science (Utrecht, The Netherlands) two rabbit (*Oryctolagus cuniculus*) inbred strains are available: AX/JU which is a dietary cholesterol

Table 1. Characteristics of backcross and F_2-intercross rabbits[#].

Parameter	Backcross rabbits		F_2-intercross rabbits	
	Males	Females	Males	Females
Number of animals	70	63	65	77
Age *(days)*				
Initial	97 ± 9[T]	97 ± 9[T]	108 ± 4	108 ± 5
Final	138 ± 9[T]	138 ± 10[T]	186 ± 6	187 ± 6
Body weight *(g)*				
Initial	2015 ± 181	2012 ± 188[T]	2066 ± 156	2087 ± 154
Final	2410 ± 153[T]	2431 ± 156[T]	2545 ± 215[G]	2684 ± 145
Gain	395 ± 93[T]	419 ± 78[T]	480 ± 176[G]	597 ± 120
Food intake *(g/day)*	99.3 ± 1.1[T]	99.5 ± 0.6[T]	97.0 ± 4.9[G]	99.0 ± 1.4
Adrenal gland weight				
Absolute (mg)				
Left	281 ± 70[T]	270 ± 53[T]	402 ± 96[G]	337 ± 65
Right	235 ± 53[$,T]	224 ± 41[$,T]	338 ± 89[$,G]	287 ± 52[$]
Left plus Right	516 ± 118[T]	494 ± 88[T]	740 ± 181[G]	624 ± 113
Relative (mg/kg body wt)				
Left	117 ± 29[T]	111 ± 23[T]	159 ± 42[G]	126 ± 23
Right	98 ± 22[$,T]	92 ± 18[$,T]	134 ± 39[$,G]	107 ± 19[$]
Left plus Right	215 ± 49[T]	203 ± 38[T]	293 ± 79[G]	233 ± 41
Serum cholesterol level *(mM)*				
Initial	0.7 ± 0.2[G]	1.2 ± 0.3	0.7 ± 0.3[G]	1.1 ± 0.3
Final	29.3 ± 10.0[G]	39.1 ± 10.7[T]	33.6 ± 14.4	32.8 ± 9.6
Change	28.6 ± 9.9[G]	37.9 ± 10.6[T]	32.9 ± 14.3	31.7 ± 9.5
	7.9 - 48.6)	(18.2 - 69.1)	(7.9 - 79.6)	(12.1 - 50.7)

[#] Values are means ± SD. For change in serum cholesterol concentration the range is also indicated in parentheses. Initial and final values refer to the beginning and end of the test period, respectively. For backcross rabbits, final serum cholesterol values refer to day 35 of the test period.

[T] Significant difference ($P<0.025$; two-tailed unpaired Student's *t* test) between backcross and F_2-intercross rabbits of the same sex.

[G] Significant difference ($P<0.025$; two-tailed unpaired Student's *t* test) between male and female rabbits of the same cross.

[$] Significantly different from the left adrenal gland ($P<0.05$; two-tailed paired Student's *t* test).

susceptible (hyperresponding) strain and IIIVO/JU which is a dietary cholesterol resistant (hyporesponding) strain (VAN ZUTPHEN and FOX, 1977; MEIJER, 1991). The strains originated from the Jackson Laboratory colony, Bar Harbor, ME, USA (FOX, 1975). The two inbred strains are maintained by brother-sister mating. The coefficient of inbreeding (F) is > 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 either intercrossed by brother-sister mating (F_2-intercross; n = 142; 65 males and 77 females) or backcrossed to both parental strains. Male F_1-hybrids were mated with their IIIVO/JU mothers (backcross to the IIIVO/JU strain; n = 57; 26 males and 31 females) and female F_1-hybrids were mated with the AX/JU father (backcross to the AX/JU strain; n = 76; 44 males and 32 females). In the present study, the progeny of the latter two crosses was indicated as backcross rabbits. Since, we produced backcrosses to both parental strains in about equal amounts and pooled the data from the two types of backcrosses, it is likely that the genetic background of the F_2-intercross and backcross population are similar. 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 previously described (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 rabbits were transferred 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 obtained cholesterol-rich diet was fed during the test period, which lasted 40-43 days for backcross animals and 63-84 days for F_2-intercross rabbits. 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 caecotrophy. Food intake was recorded once a week throughout the entire test periods. In Tables 1-3 (see RESULTS AND DISCUSSION) food intake (g/day) is expressed as mean ± SD over all days of the test period. The various cohorts of rabbits were tested between May 1993 and May 1994. For each cross of rabbits (backcross or F_2-intercross) the results from the various cohorts of rabbits did not differ and were pooled. One batch of cholesterol-rich diet was used throughout the entire experiment and this diet was stored at 4°C until feeding. Measurement of the body weights were performed weekly on the same day between 08.00 and 10.00 hours.

Table 2. Characteristics of backcross rabbits for the different serum cholesterol groups[#].

Parameter	Gender	Serum cholesterol group			Sign.[*]
		Low	Intermediate	High	
Number of animals	*Males*	23	23	24	
	Females	21	21	21	
Age *(days)*					
Initial	*Males*	98 ± 10	96 ± 9	95 ± 8	
	Females	96 ± 11	98 ± 7	97 ± 10	
Final	*Males*	139 ± 10	138 ± 10	137 ± 9	
	Females	137 ± 11	139 ± 8	139 ± 11	
Body weight *(g)*					
Initial	*Males*	2012 ± 194	2024 ± 153	2010 ± 199	
	Females	1966 ± 196	2045 ± 149	2025 ± 214	
Final	*Males*	2374 ± 153	2434 ± 166	2421 ± 141	
	Females	2395 ± 182	2443 ± 122	2454 ± 160	
Gain	*Males*	362 ± 79	411 ± 100	412 ± 93	
	Females	430 ± 74	398 ± 66	430 ± 91	
Food intake *(g/day)*	*Males*	99.1 ± 1.5	99.4 ± 0.6	99.3 ± 1.1	
	Females	99.4 ± 0.9	99.7 ± 0.2	99.6 ± 0.3	
Adrenal gland weight					
Absolute (mg)	*Males*	494 ± 137	547 ± 113	508 ± 99	
	Females	452 ± 77	522 ± 73	506 ± 101	S
Relative (mg/kg body wt)	*Males*	209 ± 58	225 ± 47	210 ± 39	
	Females	190 ± 35	215 ± 34	207 ± 42	
Serum cholesterol level *(mM)*					
Initial	*Males*	0.66 ± 0.17	0.77 ± 0.23	0.78 ± 0.17	
	Females	1.17 ± 0.24	1.26 ± 0.26	1.24 ± 0.35	
Final	*Males*	18.0 ± 3.5	29.3 ± 2.9	40.4 ± 5.0	s
	Females	27.4 ± 6.0	39.5 ± 2.4	50.5 ± 5.5	s
Change	*Males*	17.4 ± 3.4	28.5 ± 2.8	39.6 ± 4.9	s
	Females	26.2 ± 5.9	38.2 ± 2.3	49.2 ± 5.6	s
	Males	(7.9 - 21.6)	(21.8 - 32.0)	(32.6 - 48.6)	
	Females	(18.2 - 34.3)	(34.6 - 43.0)	(43.1 - 69.1)	

[#] Values are means ± SD. For change in serum cholesterol concentration the range is also indicated in parentheses. Initial and final values refer to the beginning and end of the test period, respectively. Final serum cholesterol values refer to day 35 of the test period.
[*] Significance ($P<0.05$) between the three serum cholesterol groups of the same sex based on one-way analysis of variance (S) or based on Kruskal-Wallis test (s).

Blood sampling, tissue collection and chemical analyses.

On the day before blood sampling and/or before sacrificing the animals, any remaining food was removed at 16.00 hours. Samples of blood were taken from the lateral ear vein or via heart punction. Blood samples were drawn on days 0 and 35 from both backcross and F_2-intercross rabbits and at the end of the experimental period (day 63-84) from the F_2-intercross rabbit

s. Blood was collected in tubes without anticoagulant. To collect serum, the blood in the tubes was allowed to clot at room temperature. Serum was prepared by low-speed centrifugation (10 min, 3000 g, 3°C) and was analyzed the same day. Total cholesterol in the serum was measured enzymatically according to SIEDEL *et al.* (1983), using a kit (Monotest®) supplied by Boehringer Mannheim GmbH (Mannheim, Germany). The inter- and intra-assay coefficients of variation for serum cholesterol determination always fell within the limits as prescribed by the manufacturer. At the end of the test period, the fasted rabbits were anaesthetized by an intravenous injection of hypnorm® (Janssen Pharmaceutica BV, Tilburg, The Netherlands) sufficient to reach the surgical phase (approximately 0.5 ml/rabbit). Subsequently, the animals were killed by cardiac exsanguination and the adrenal glands were rapidly removed and weighed. Blood sampling and sacrificing was done in random order.

Statistical analyses.

Results are presented as means ± SD. The Kolmogorov-Smirnov one-sample test was used to check normality of the data. All results within groups were found to be normally distributed. Student's one-sample *t* test for paired data was used to compare the weight of left and right adrenal glands. The significance of the differences between groups was calculated by one-way analysis of variance (for equal variances) or Kruskal-Wallis test (for unequal variances) with serum cholesterol as factor (Tables 2 and 3) or with unpaired Student's *t* tests (Table 1). In case of the analyses of variance, the homogeneity of variances was checked using Bartlett's test. The unpaired Student's *t* tests were performed with pooled (for equal variances) or separate (for unequal variances) variance

Table 3. Characteristics of F_2-intercross rabbits for the different serum cholesterol groups[#].

Parameter	Gender	Serum cholesterol group			Sign.[*]
		Low	Intermediate	High	
Number of animals	*Males*	21	22	22	
	Females	25	26	26	
Age *(days)*					
Initial	*Males*	108 ± 4	109 ± 4	108 ± 4	
	Females	108 ± 7	108 ± 3	109 ± 4	
Final	*Males*	188 ± 5	188 ± 5	185 ± 5	
	Females	187 ± 8	187 ± 4	189 ± 5	
Body weight *(g)*					
Initial	*Males*	2020 ± 152	2092 ± 159	2084 ± 165	
	Females	2123 ± 172	2041 ± 129	2098 ± 155	
Final	*Males*	2544 ± 170	2618 ± 146	2503 ± 288	
	Females	2700 ± 170	2672 ± 110	2692 ± 140	
Gain	*Males*	524 ± 110	526 ± 128	419 ± 233	
	Females	577 ± 111	631 ± 112	594 ± 126	
Food intake *(g/day)*	*Males*	98.7 ± 2.8	99.0 ± 1.3	94.6 ± 6.5	
	Females	99.1 ± 1.2	99.4 ± 1.2	98.9 ± 1.4	
Adrenal gland weight					
Absolute (mg)	*Males*	669 ± 134	729 ± 181	816 ± 184	S
	Females	635 ± 128	632 ± 111	601 ± 99	
Relative (mg/kg body wt)	*Males*	262 ± 43	278 ± 65	334 ± 100	s
	Females	235 ± 44	236 ± 37	224 ± 38	
Serum cholesterol level *(mM)*					
Initial	*Males*	0.62 ± 0.21	0.68 ± 0.22	0.81 ± 0.31	
	Females	0.97 ± 0.24	1.13 ± 0.24	1.29 ± 0.29	
Final	*Males*	17.8 ± 5.5	33.4 ± 4.2	49.0 ± 9.2	s
	Females	22.0 ± 4.3	32.9 ± 2.7	43.3 ± 4.7	s
Change	*Males*	17.1 ± 1.2	32.7 ± 4.2	48.2 ± 9.3	s
	Females	21.0 ± 4.4	31.7 ± 2.7	42.0 ± 4.6	s
	Males	(7.9 - 24.7)	(24.9 - 38.5)	(38.9 - 79.6)	
	Females	(12.1 - 27.5)	(28.0 - 36.4)	(36.5 - 50.7)	

[#] Values are means ± SD. For change in serum cholesterol concentration the range is also indicated in parentheses. Initial and final values refer to the beginning and end of the test period, respectively.
[*] Significance ($P<0.05$) between the three serum cholesterol groups of the same sex based on one-way analysis of variance (S) or based on Kruskal-Wallis test (s).

estimates. The equality of variances was then tested using a F test. To take into account the greater probability of a type I error due to multiple comparisons, the level of significance for the unpaired Student's *t* tests was pre-set at $P < 0.025$ (Table 1) instead of $P < 0.05$, according to Bonferroni's adaptation. In all other cases, the probability of a type I error < 0.05 was taken as the criterion of significance. Between selected parameters, Pearson's linear correlation coefficient (r) or Spearman's coefficient of rank correlation (R) were calculated; significance was assessed by a two-tailed test. Two-side probabilities were estimated throughout. All statistical analyses were carried out according to STEEL and TORRIE (1981) using a SPSS PC+ computer program (SPSS, 1990).

RESULTS AND DISCUSSION

Age, body weight and food intake.

At the beginning of the test period F_2-intercross rabbits were on average 11 days older than backcross rabbits (Table 1). Initial body weight was significantly correlated with initial age ($r = 0.5790$, $P < 0.001$, $n = 275$), thus as a consequence, group mean initial body weight of F_2-intercross rabbits when compared with backcross rabbits was slightly higher. For females this difference reached the level of statistical significance. Female rabbits initially had a similar body weight as male rabbits. Although the duration of the test period was about 2 times longer for F_2-intercross rabbits (78 ± 4 days, $n = 42$) when compared with backcross rabbits (41 ± 1 days, $n = 133$), final body weight and body weight gain were only slightly higher. Thus, at the age of approximately 20 weeks the rabbits are already in the horizontal part of the growth curve. Male when compared with female F_2-intercross rabbits had lower final body weight and a reduced weight gain. The reduced weight gain in male F_2-intercross rabbits was associated with diminished food intake. In contrast, male and female backcross rabbits had identical final body weight and weight gain. The genetic background of backcross and F_2-intercross rabbits is assumed to be identical in the present study (see MATERIALS AND METHODS), thus it might be concluded that the sex difference in body weight is age

dependent. Similar gender differences in body weight for rabbits of race III and race X, at least when mature, have been described by CRARY and SAWIN (1960). In the rabbit the female when mature is usually the larger of the two sexes, which differs from most other mammals. F_2-intercross rabbits consumed slightly, but significantly, less and /or spilled more food than backcross rabbits.

Adrenal gland weight.

In keeping with earlier results using New Zealand White and hybrid-ZIKA rabbits (DRESCHER and BREIG, 1993), in all our animals left adrenal glands were significantly heavier (absolute and relative) than the right adrenal glands (Table 1). Male rabbits had higher group mean absolute and relative adrenal gland weights than female rabbits; this gender effect was statistically significant for the F_2-intercross rabbits. A similar sex difference in adrenal gland weight was also reported by MYERS *et al.* (1981). In contrast, in most other mammalian species, the adult female adrenal gland is larger than the adult male adrenal gland (LIDDLE, 1981). Backcross animals when compared with F_2-intercross rabbits had lower absolute and relative adrenal weights. It has been reported that the weight of the adrenals is significantly raised with prolonged intake of dietary cholesterol (FORBES *et al.*, 1964). The genetic background of backcross and F_2-intercross rabbits is assumed to be identical in the present study (see MATERIALS AND METHODS), thus the effect of type of cross on adrenal gland weight is due to the increased duration of the experimental period for F_2-intercross rabbits.

Serum cholesterol response.

Female rabbits when compared with male rabbits have higher initial serum cholesterol levels (Table 1). This is in line with earlier observations using the parental strains AX/JU and IIIVO/JU (BEYNEN *et al.*, 1984). After feeding the cholesterol-rich diet for 35 days, female backcross rabbits had still higher serum cholesterol concentrations than male backcross rabbits. For F_2-intercross rabbits, serum cholesterol concentration of females after 35 days of feeding the cholesterol-rich diet (31.6 ± 9.9 mM, n = 77) were also significantly higher than those of males (24.8 ± 10.9 mM, n = 65) (two-tailed unpaired Student's *t* test, $P < 0.05$). This corroborates earlier investigations using New Zealand White rabbits (HROMADOVA and HACIK, 1984). Similar gender effects with respect to cholesterolaemic response have also been described for rats (IMAI and MATSUMURA, 1973). However, the present gender effect might be transient, since it disappeared when the duration of the test period for F_2-intercross rabbits was increased to 78 ± 4 days (Table 1, final values serum cholesterol concentration of F_2-intercross rabbits). The serum cholesterol response is expressed as the absolute increment of serum cholesterol level during the test period. As would be expected, both within backcross and F_2-intercross rabbits, hyperresponders with high changes in serum cholesterol concentration and hyporesponders with low changes in serum cholesterol concentration could be distinguished from normoresponders (Table 1, ranges in change in serum cholesterol concentration).

Because of differences in adrenal gland weight for backcross and F_2-intercross rabbits and for males and females (Table 1), the data were analyzed separately. As a primary analysis, the distribution of change in serum cholesterol concentration was divided into tertiles (i.e. approximately into hypo-, normo- and hyperresponders) and the three serum cholesterol groups were compared for both sexes and both types of crosses (Tables 2 and 3).

Backcross rabbits: hypo-, normo-, and hyperresponders.

Table 2 shows the characteristics of male and female backcross rabbits for the three serum cholesterol groups. Both for males and females, age, body weight and food intake were not significantly different between the three serum cholesterol groups. Only for females, absolute, but not relative, adrenal gland weight differed slightly, but significantly, between the three groups. The intermediate serum cholesterol groups had the heaviest adrenal glands. Both for females and males, initial serum cholesterol levels were not significantly different between the three serum cholesterol groups. Thus, there were no significant associations between group mean initial serum cholesterol level and group mean serum cholesterol response (males: $r = 0.9011$, $P = 0.285$, $n = 3$; females: $r = 0.7572$, $P = 0.453$, $n = 3$).

F_2-intercross rabbits: hypo-, normo-, and hyperresponders.

Table 3 illustrates the characteristics of male and female F_2-intercross rabbits for the three serum cholesterol groups. Both for females and males there were no significant differences in age, body weight and food intake between the three serum cholesterol groups. For males, but not for females, increased serum cholesterol response coincides with increased

Table 4. Associations (Spearman's coefficient of rank correlation) between individual serum cholesterol response and adrenal gland weight.

	Backcross rabbits		F_2-intercross rabbits	
	Males	**Females**	**Males**	**Females**
Number of animals	70	63	65	77
		Absolute adrenal gland weight		
R	0.0574	0.2053	0.3955	-0.0321
P-value	0.637	0.107	0.001	0.783
		Relative adrenal gland weight		
R	0.0487	0.1511	0.4229	-0.0455
P-value	0.689	0.237	0.001	0.696

absolute and relative adrenal gland weight. For male rabbits there tended to be a positive association between group mean adrenal gland weight and group mean serum cholesterol response ($r = 0.9942$, $P = 0.068$, $n = 3$). For both sexes hyporesponders had lowest and hyperresponders highest group mean initial serum cholesterol levels. For females, but not for males, there was a significant linear correlation between group mean initial serum cholesterol level and group mean serum cholesterol response (males: $r = 0.9777$, $P = 0.135$, $n = 3$; females: $r = 0.9999$, $P = 0.007$, $n = 3$).

Backcross and F_2-intercross rabbits: individual associations.

Additional individual analysis (by Spearman rank correlation) has been performed in order to reveal a possible relationship between serum cholesterol response and absolute and relative adrenal gland weight. The results are summarized in Table 4. Only for male F_2-intercross rabbits there was a weak, but statistically significant, positive association. Since no significant negative relationships were found, the results suggest that differences in LDL-receptor pathway between AX/JU and IIIVO/JU rabbits, if any, may not explain the difference in serum cholesterol response between the two strains.

CONCLUSIONS

In sum, the present study shows that for male, but not for female, F_2-intercross rabbits there is a clear significant positive relationship between serum cholesterol response and adrenal weight (Tables 3 and 4). This is consistent with the work of VAN ZUTPHEN *et al.* (1981) using the male progeny of crosses between New Zealand White and Vienna White rabbits and showing that adrenal weights after dietary cholesterol challenge were more increased in hyper- than hyporesponders. In contrast, both for male and female backcross rabbits there is no clear dose-response relationship between serum cholesterol response and adrenal weight. The lack of a relationship in male backcross rabbits might be due to the relatively short experimental period of 40-43 days (Tables 2 and 4). Combining the present findings with the results of VAN ZUTPHEN *et al.* (1981) and HOEG *et al.* (1985), it can be concluded that if there is a relationship between serum cholesterol response and adrenal weight in rabbits, it is certainly not a simple one and may depend on gender, duration of the experimental period and genetic background of the animals.

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