Atherosclerosis VIII. G. Crepaldi et al, editors.

INBRED RABBIT STRAINS AS MODELS FOR HUMAN HYPO- AND HYPERRESPONDERS TO DIETARY CHOLESTEROL

MARTIJN B. KATAN[1], GERT W. MEIJER[2] and ANTON C. BEYNEN[1, 2]

[1]Department of Human Nutrition, Agricultural University, P.O. Box 8129, 6700 EV Wageningen
[2]Department of Laboratory Animal Science, State University, P.O. Box 80.166, 3508 TD Utrecht (The Netherlands)

INTRODUCTION

An increase in cholesterol intake elicits a range of responses of serum cholesterol concentration among humans. Although most of this variation is due to irreproducible variations, some of it is due to reproducible, innate differences (1). The metabolic basis for this phenomenon has not yet been unravelled (2). Although significant with regard to risk for coronary heart disease, the range of true differences in serum cholesterol response in man is relatively small (1). This hampers progress in the understanding of underlying mechanisms. The verification responsiveness in man through repeated dietary trials (1) is extremely tedious. Also, invasive studies cannot be performed. These problems can be overcome with the use of animal models. In this communication we present evidence that specific inbred strains of hypo- and hyperresponsive rabbits are a valuable model.

Table 1 shows the levels of serum cholesterol in male rabbits of two inbred strains on an essentially cholesterol-free diet (Day 0), and also after 4 weeks of receiving the same diet to which various amounts of cholesterol had been added. The hyperresponsive strain (AX/JU) always displayed a greater response of serum cholesterol than the hyporesponsive strain (IIIVO/JU).

Amounts of cholesterol in the diet as low as 0.08% (w/w) already discriminate between the serum cholesterol responses in the two rabbit strains. We consider these cholesterol loads more physiological than the large amounts that are commonly used. On the basis of sterol balance studies we conclude that these low amounts can still be compensated for by down regulation of cholesterol synthesis, and there is no excessive accumulation of liver cholesterol. In contrast, rats and mice are notoriously insensitive to dietary cholesterol and diets containing high amounts of both cholesterol (2.0%) and cholate (0.5%) have to be used. Although the feeding of such diets identifies hypo- and hyperresponsive strains of inbred rats and mice (5, 6), they also induce liver damage such as bile duct proliferation and fibrosis (7). Liver function may also be impaired as evidenced by increased activities in serum of the liver function indicators, alanine and aspartate aminotransferase (8).

TABLE 1

EFFECT OF DIETARY CHOLESTEROL ON SERUM CHOLESTEROL CONCENTRATIONS IN TWO INBRED STRAINS OF RABBITS

Results are expressed as means ± SE for 6 animals per strain, and refer to different experiments. (3, 4).

Strain	Dietary cholesterol (%, w/w)	Serum cholesterol (mmol/l)	
		Day 0	Day 28, 31
Hypo	0.08	0.6 ± 0.0	1.1 ± 0.1
Hypo	0.3	0.9 ± 0.1	6.7 ± 1.4
Hypo	0.5	0.7 ± 0.1	8.0 ± 1.9
Hyper	0.08	0.7 ± 0.1	1.7 ± 0.2
Hyper	0.3	0.7 ± 0.2	29.0 ± 1.6
Hyper	0.5	0.6 ± 0.1	33.6 ± 3.1

RESPONSE TO DIETARY FAT TYPE

In man, dietary saturated fatty acids are more cholesterolemic than polyunsaturated fatty acids. The response of serum cholesterol to saturated fat differs among human subjects (9), and it tends to be positively correlated with the response to dietary cholesterol (10).

In male rabbits from our two inbred strains hypo- and hyperresponsive to dietary cholesterol, we measured the response of serum cholesterol to saturated fatty acids provided by coconut fat versus polyunsaturated fatty acids from corn oil. Cholesterol-free, semipurified diets were used, and the fat source was the only dietary variable. The replacement of corn oil by coconut fat elicited a significantly higher response of plasma cholesterol in the hyper- than in the hyporesponsive rabbits. Thus in these inbred rabbit strains hypo- and hyperresponsiveness to dietary cholesterol and to the type of fatty acids coincided (10, 11), just as it did in man.

DIFFERENCES IN CHOLESTEROL METABOLISM BETWEEN HYPO- AND HYPERRESPONDERS

In the inbred strains, a higher cholesterol absorption, as measured by the [^{3}H]cholesterol/[^{14}C]β-sitosterol method, was found in the hyperresponders (12). This could cause an increased flux of cholesterol into the liver after cholesterol feeding, which is supported by the observed higher concentrations of hepatic cholesterol in the hyperresponsive rabbits (Table 2).

TABLE 2

LIVER CHOLESTEROL CONCENTRATIONS IN HYPO- AND HYPERRESPONSIVE RABBITS

Results expressed as means ± SD for 4 to 8 animals.

Dietary cholesterol (%, w/w)	Liver cholesterol (μmol/g)	
	Hypo	Hyper
0.01	4.5 ± 0.4	6.3 ± 1.2
0.3	41.8 ± 9.1	73.4 ± 7.5

On the low-cholesterol diet, whole body synthesis of cholesterol, as calculated from sterol balances, was markedly higher in the hypo- than the hyperresponsive rabbits (12). In our controlled dietary trials with humans (13) we found that whole body cholesterol synthesis, measured as the cholesterol balance, was negatively associated ($r=-0.44$; $n=32$; $p<0.05$) with the responsiveness of serum cholesterol to dietary cholesterol. Thus there is evidence both in animals and in humans that basal synthesis rates are higher in hypo- than in hyperresponders. This could imply that hyporesponders have more room for compensatory decreases in endogenous cholesterol synthesis when cholesterol intake is increased. The higher basal rates of cholesterol synthesis in hyporesponders, compared with hyperresponders, could be secondary to differences in cholesterol absorption. In the hyporesponders less cholesterol will reach the tissues from the gut, because of their lower efficiency of cholesterol absorption, and therefore cholesterol synthesis will be less suppressed.

Theoretically, the increased cholesterol pools in the liver of hyperresponders may lead to increased secretion of VLDL, IDL and/or LDL. Isolated perfused livers from cholesterol-fed rabbits were found to secrete more cholesterol than livers from rabbits fed a low-cholesterol diet. Livers of hyperresponsive rabbits tended to release cholesterol at higher rates than those of hyporesponders. Most of the secreted cholesterol was associated with VLDL. Enhanced hepatic lipoprotein secretion might also occur in human hyperresponders. In the human responders studied by Packard et al. (14), unlike the hyporesponders of Ginsberg et al. (15), there was a pronounced increase in LDL production after cholesterol feeding. The dietary cholesterol-induced enhancement of LDL synthesis in hyperresponders might involve direct synthesis of LDL or IDL by the liver. Nestel and Billington (16) have shown that in humans

cholesterol feeding caused an increase in IDL apoB production, and that this increase was directly correlated with the rise in serum cholesterol. Thus both in our rabbits and humans, hyperresponders may have increased rates of LDL cholesterol production after cholesterol feeding, and this may explain the elevated concentrations of LDL cholesterol in hyperresponders.

TABLE 3
RATES OF CHOLESTEROL SECRETION BY PERFUSED LIVERS OF HYPO- AND HYPERRESPONSIVE RABBITS
Means ± SD for 4 to 8 perfused livers

Dietary cholesterol (%, w/w)	Cholesterol secretion rate (μmol/100 g liver/hr)	
	Hypo	Hyper
0.01	2.0 ± 2.8	15.6 ± 5.2
0.3	5.4 ± 2.8	26.8 ± 8.6

In summary, our two inbred strains of rabbits and human hypo- and hyperresponders share many characteristics. We feel that the use of the rabbit strains may contribute to describe the metabolic basis for human hypo- and hyperresponsiveness to dietary cholesterol, especially with respect to those aspects that are experimentally inaccessible in man. In addition, the rabbit strains may be used to study the genetic basis of hypo- and hyperresponsiveness.

REFERENCES

1. Katan MB, Beynen AC, De Vries JHM, Nobels A (1986) Am J Epidemiol 123:221
2. Beynen AC, Katan MB, Van Zutphen LFM (1987) Adv Lipid Res 22:115
3. Van Zutphen LFM, Fox RR (1977) Atherosclerosis 28:435
4. Beynen AC, Katan MB, Van Zutphen LFM (1984) Comp Biochem Physiol 79B:401
5. Van Zutphen LFM, Den Bieman MGCW (1981) J Nutr 111:1833
6. Beynen AC, Lemmens AG, De Bruijne JJ, Ronai A, Wassmer B, Von Deimling O, Katan MB, Van Zutphen LFM (1987) Atherosclerosis 63:239
7. Beynen AC, Danse LHJC, Van Leeuwen FXR, Speijers GJA (1986) Nutr Rep Int 34:1079

8. Beynen AC, De Bruijne JJ, Katan MB (1985) Atherosclerosis 58:149

9. Jacobs DR Jr, Anderson JT, Hannan P, Keys A, Blackburn H (1983) Arteriosclerosis 3:349

10. Katan MB, Beynen AC (1986) Prog Lipid Res 25:491

11. Beynen AC, West CE, Katan MB, Van Zutphen LFM (1986) Nutr Rep Int 33:65

12. Beynen AC, Lemmens AG, Glatz JFC, Katan MB, Van Zutphen LFM (1987) In: Paoletti R, Kritchevsky, D, Holmes WL (eds) Drugs Affecting Lipid Metabolism. Springer Verlag, Berlin, pp. 122-125

13. Katan MB, Beynen AC (1987) Am J Epidemiol 125:387

14. Packard CJ, McKinney L, Carr K, Shepherd J (1983) J Clin Invest 72:45

15. Ginsberg H, Le N-A, Mays C, Gibson J, Brown WV (1981) Arteriosclerosis 1:463

16. Nestel PJ, Billington T (1983) Metabolism 32:320