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Hypo- and Hyperresponders: Individual Differences in the Response of Serum Cholesterol Concentration to Changes in Diet

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I. Summary

The feeding of cholesterol-rich diets to random-bred animals results in marked interindividual differences in the response of serum cholesterol. Certain animals show only small responses (*hyporesponders*), whereas others develop high degrees of hypercholesterolemia (*hyperresponders*). Inbred strains of rabbits, rats, and mice differing in their sensitivity to dietary cholesterol are available. In these animals, and also in monkeys, the responsiveness to high-cholesterol diets has a strong genetic basis.

The existence of hyper- and hyporesponders also holds in humans, though not as pronounced as in laboratory animals. Repeated trials with the same subjects have shown that persons exist with a consistently low or high response to increased intakes of cholesterol. However, "spontaneous," diet-independent within-person variations in the level of serum cholesterol markedly inflate the between-person variation in the response of serum cholesterol; both variations are of the same order of magnitude.

Hypo- and hyperresponsiveness to dietary cholesterol extends to other hypercholesterolemic components of the diet. In humans and rabbits hyperresponsiveness to dietary cholesterol is associated with responsiveness to dietary saturated fatty acids.

The mechanisms underlying hypo- and hyperresponsiveness to dietary cholesterol have not yet been unraveled. On the basis of available data, we propose that in hyperresponders, compared with hyporesponders, there is a higher hepatic efflux of cholesterol in low-density lipoproteins (LDL), or its precursors, after cholesterol consumption. This may be caused by insufficient inhibition of cholesterol biosynthesis and/or the high capacity of

cholesterol absorption in the hyperresponders. The stimulation of LDL production accounts for the increase in LDL cholesterol in serum. The number of hepatic LDL receptors, which may be already decreased in hyperresponders, will decrease further through down-regulation. The receptor-mediated LDL clearance decreases, but the absolute amount of LDL cholesterol taken up by the cells via the receptor and by the receptor-independent pathway increases because of the increased level of LDL cholesterol. In this way a new equilibrium is reached in which LDL production equals LDL catabolism.

The phenomenon of hypo- and hyperresponsiveness may have implications for counseling subjects who attempt to lower their serum cholesterol by diet. However, identification of true hyper- and hyporesponders is greatly hampered by within-person fluctuations of the level of serum cholesterol. No simple test is available to discriminate hypo- from hyperresponders. As yet, monitoring a person's response to diet should be based on relatively large numbers of serum cholesterol determinations.

II. Introduction

The level of serum cholesterol in humans is sensitive to the type of fat and the amount of cholesterol in the diet. The quantitative effects of these dietary components can be predicted using empirical formulas (Keys *et al.*, 1965 a–d). However, such predictions of serum cholesterol changes only hold for group means and not for individual subjects. It has often been suggested that in certain individuals (hyporesponders) the level of serum cholesterol is relatively insensitive to dietary challenge, whereas in others (hyperresponders) the effect of diet is much more pronounced. This review deals with this phenomenon. Does it exist? If so, then what are the underlying mechanisms?

The concept of hypo- and hyperresponsiveness could at least partly explain the large differences in serum cholesterol levels found between persons on similar diets, especially among affluent populations consuming relatively high amounts of saturated fat and cholesterol. Figure 1 illustrates this suggestion. The high intake of saturated fatty acids and cholesterol by the Finns explains why their *mean* concentration of serum cholesterol is so high compared with the Japanese. However, dietary differences cannot fully account for the wide range of serum cholesterol values found within a fairly homogeneous group such as the middle-aged Finnish men of Fig. 1. Differences in dietary habits tend to be small within an affluent population, and dietary fatty acid and cholesterol intakes of individuals correlate poorly with their serum cholesterol levels (Kahn *et al.*, 1969; Nichols *et al.*, 1976; Niessen *et al.*, 1983). The imprecision of the interview

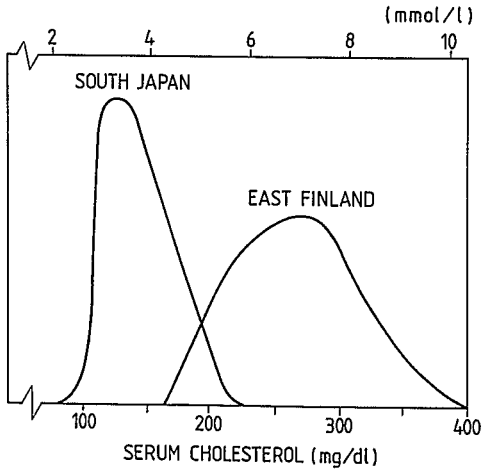

FIG. 1. Serum cholesterol distribution of middle-aged men from southern Japan and from eastern Finland. (Reproduced with permission from *Preventive Medicine* **8**, 612-678, 1979.)

techniques used for measuring nutrient intake in individuals is partly responsible for these low correlations (Marr, 1971; Beaton *et al.*, 1979), but even after correction for this degrading effect (Jacobs *et al.*, 1979), the fraction of variance in serum cholesterol levels explained by differences in diet remains minute. For example, Shekelle *et al.* (1981) reported a correlation of 0.08 between the dietary fatty acids and cholesterol and the serum cholesterol in 1900 middle-aged men. If one assumes that random errors in the assessment of diet and serum cholesterol had attenuated this correlation by a factor of 2—a fairly liberal assumption—then the maximal correlation coefficient was 0.16 and the maximal proportion of variance in serum cholesterol accounted for by variance in dietary fat and cholesterol intake was 0.16^2, or 3%. Thus, between-person differences in susceptibility to a high-fat, high-cholesterol diet rather than differences in dietary habits may be responsible for the wide spread in cholesterol values in the Finns (Fig. 1) and in other populations.

Hypercholesterolemia may of course be due to monogenetic disorders, or occur secondary to other diseases or obesity, but the majority of subjects with mild hypercholesterolemia have no clearly defined defect. Thus the latter category may contain persons who are hyperresponsive to an affluent diet.

The subject of hypo- and hyperresponsiveness is of both practical and scientific interest. Patients with hypercholesterolemia generally receive dietary advice from clinicians in order to lower their serum cholesterol levels. Frequently such advice turns out to be ineffective. Although lack of

compliance may be involved, it is possible that certain patients are insensitive to cholesterol-lowering diets and need a different form of therapy. It is assumed here that subjects hypo- and hyperresponsive to cholesterol-lowering diets are also hypo- and hyperresponsive, respectively, to hypercholesterolemic diets. From the scientific point of view, elucidation of the mechanism underlying hypo- and hyperresponsiveness may shed more light on the relations between dietary components and cholesterol metabolism.

Here we review studies on hypo- and hyperresponsiveness of the serum cholesterol level to various dietary components in several animal species and in humans.

III. Hypo- and Hyperresponsiveness to Dietary Cholesterol in Animals

A. Random-Bred Animals

The addition of cholesterol to the diet of random-bred rabbits elicits a rise of serum cholesterol, but many investigators have noted that there are marked interindividual differences in the extent of the response. This indicates that certain animals are hypo-, and others are hyperresponsive to dietary cholesterol. Figure 2 illustrates this. Such evidence would be more convincing if the same pattern of responses was seen each time after repeated challenges with cholesterol. Figure 3 shows that in random-bred rats the response of serum cholesterol in one experiment is positively correlated ($r = 0.71$, $n = 10$, $p < 0.05$) with the response to a later challenge with the same diet. Thus among random-bred rats there are individuals with a consistently high or low cholesterolemic response to a diet containing cholesterol and cholate.

Many investigators have also documented the existence of hypo- and hyperresponders in monkeys (Clarkson *et al.*, 1971; Eggen, 1976; Malinow, 1979). For example, in random-bred squirrel monkeys (*Saimiri sciureus*) the response of serum cholesterol is stable and reproducible from one experiment to another. Figure 4 illustrates that animals hypo- or hyperresponsive to fortification of the diet with cholesterol showed similar responses after a second challenge 6 months later. Thus responsiveness in these primates seems to be an innate characteristic of the individual animal. Note, however, that these 4 animals represent extremes in that they were selected for hypo- or hyperresponsiveness from a group of 38 animals tested.

B. Inbred Animal Strains

Commonly available breeds of an animal species may differ in sensitivity to dietary cholesterol; thus Adams *et al.* (1972) demonstrated that New

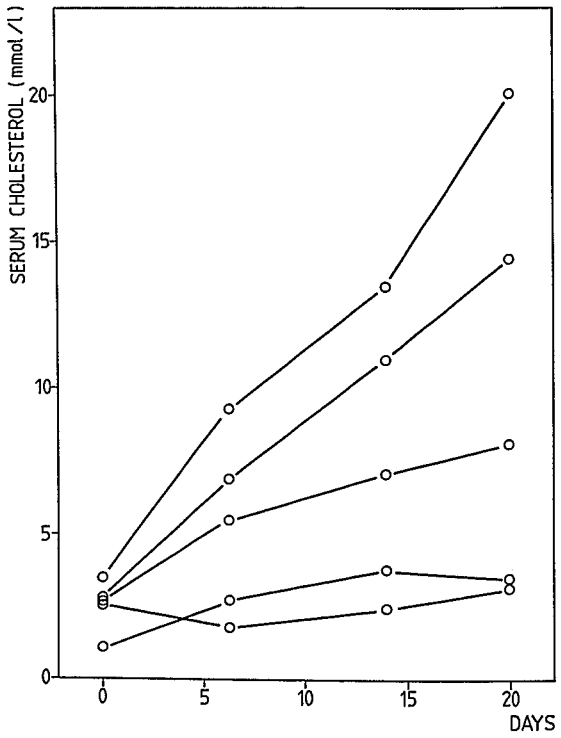

FIG. 2. Effect of dietary cholesterol (0.2%, w/w) on serum cholesterol levels in five individual, random-bred New Zealand White rabbits. Until day 0 of the experiment, the rabbits were fed a cholesterol-free diet. In this experiment, cholesterol was the only dietary variable. (Reproduced with permission from Beynen *et al.*, 1985c.)

Zealand White rabbits are more susceptible to induction of hypercholesterolemia by a high-cholesterol diet than Belted Dutch rabbits.

Extreme differences in the response of serum cholesterol to diet can be found by comparing inbred strains of various animal species. Inbred strains are established by systematic inbreeding through brother-sister matings. After 20 generations this results in animals that are essentially homozygous at all genetic loci including those that determine responsiveness to diet. Van Zutphen and Fox (1977) described inbred strains of rabbits with marked differences in responsiveness to dietary cholesterol. Figure 5 shows the time course of serum cholesterol concentration in three such strains after being transferred to a high-cholesterol diet. It is clear that the strains differ greatly in their sensitivity to dietary cholesterol. This is further illustrated in Fig. 6, which shows the levels of serum cholesterol in male rabbits of six inbred strains. The rabbits were sampled while they were on a commercial rabbit

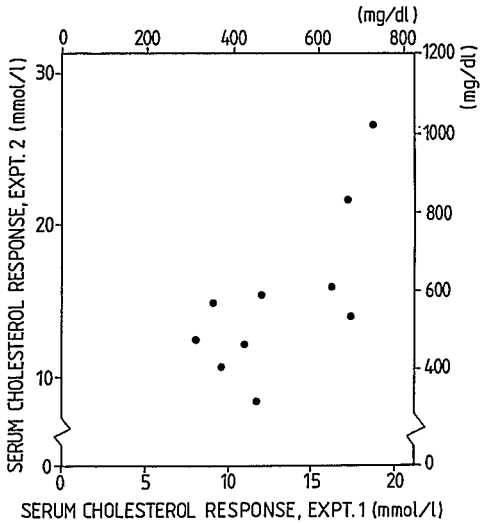

FIG. 3. Correlation between the cholesterolemic responses in two experiments produced by a diet containing cholesterol (2%) and cholate (0.5%) in random-bred female rats. The 10 rats, aged ~4 weeks at the beginning of the experiment, were fed the high-cholesterol diet for 29 days. After that, cholesterol and cholate were removed from the diet for 91 days, and then were added again for another 29 days.

chow, and also after 28 days of receiving the same diet to which 0.5% (w/w) cholesterol had been added. The animals with the most extreme response showed a 5-fold higher increase in serum cholesterol than in the strain with the lowest response.

Hypo- and hyperresponsive rat strains were first described by Okamoto *et al.* (1972). Figure 7 illustrates strain differences in the response of plasma cholesterol in inbred rats. The increase in plasma cholesterol ranged from 75 to 500%. The hypercholesterolemic diets used here were rather extreme in that they contained 2% cholesterol plus 0.5% cholate. Strain differences in the response of plasma cholesterol to this high-cholesterol diet were also seen in inbred mice (Fig. 8). The responses of various strains ranged from 20 to 130%. Roberts and Thompson (1976) have also shown marked differences between mice strains in the cholesterolemic responses to a hypercholesterolemic diet.

Several strains of Show Racer pigeons differing in cholesterol metabolism and susceptibility to atherosclerosis have been produced through selective breeding. Wagner and Clarkson (1974) reported on two selected lines, one hypo-, and the other hyperresponsive. The hyperresponsive animals showed 6-fold higher serum cholesterol levels than the hyporesponders when they were fed pigeon pellets supplemented with 10% lard and 0.5% cholesterol for 6 months.

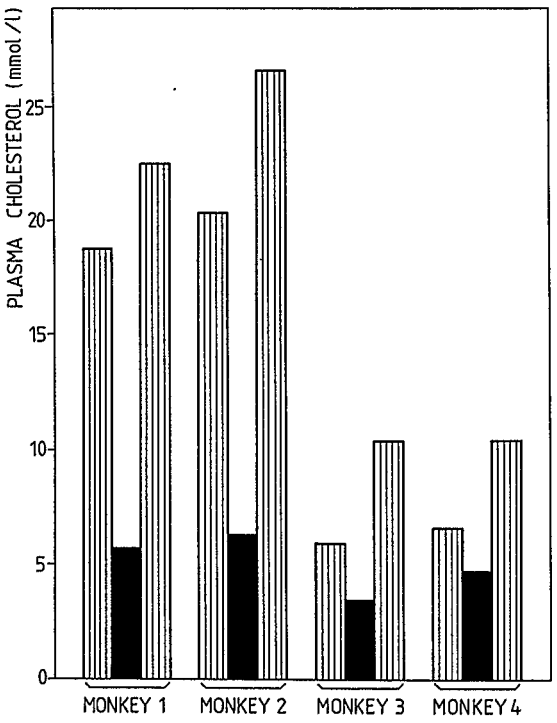

FIG. 4. Plasma cholesterol levels in four male squirrel monkeys fed a diet with 0.5% (w/w) cholesterol (hatched bars) or a cholesterol-free diet (closed bars). The cholesterol-free diet lasted 6 months. Monkeys 1 and 2 are hyper-, and monkeys 3 and 4 are hyporesponders. (From Clarkson *et al.*, 1971; reproduced with permission from Beynen *et al.*, 1985c.)

Thus, in several animal species inbreeding has produced strains that differ vastly in their susceptibility to hypercholesterolemic diets.

C. HERITABILITY OF DIFFERENCES IN RESPONSIVENESS

The studies with inbred strains of rabbits, rats, mice, and pigeons indicate that differences in responsiveness have a genetic basis. This raises the question to what extent differences in response between random-bred animals are genetically determined. The variance of any biological trait is, of course, always made up of genetic and environmental contributions. If the environment is made more uniform, then the apparent heritability of the trait increases, and if the environmental conditions fluctuate a lot from animal to animal then genetic determinants are obscured. Thus heritabilities calculated in different studies usually cannot be compared. Still it is

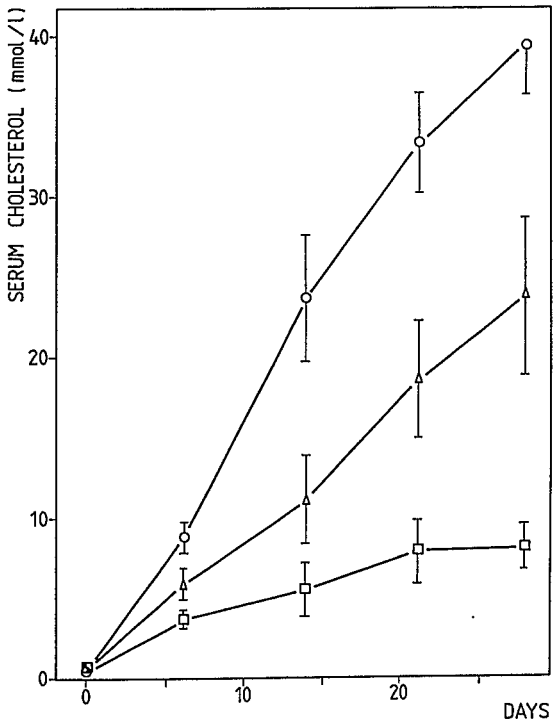

FIG. 5. Effect of dietary cholesterol (0.5%, w/w) on plasma cholesterol concentrations in three inbred strains of rabbits (○, AX/J; △, ACEP/J; □, III VO/J). Results are expressed as means ± SE for five animals per strain. (Based on data taken from Van Zutphen and Fox, 1977.)

legitimate to ask what proportion of the variance in response observed in animal experiments is due to genetic differences, and what proportion is due to metabolic idiosyncrasies that are fixed at another stage, for example, early nutrition.

The extent of the genetic influence on variability in serum cholesterol response to dietary cholesterol has been studied in several animal species. In wild-type squirrel monkeys, Clarkson *et al.* (1971) found that ~65% of the variability in serum cholesterol concentration after cholesterol feeding was attributable to genetic factors. Studies with rhesus monkeys, (*Macaca mulatta*) and stump-tailed macaques (*Macaca arctoides*) confirmed the genetic basis of the sensitivity to dietary cholesterol, but precise heritability estimates could not be given (Clarkson and McMahan, 1980).

Kronfeld *et al.* (1979) fed a high-fat, high-cholesterol diet to racing huskies of which the pedigrees were known for at least four generations. They found that the serum cholesterol concentrations segregated into two distinct

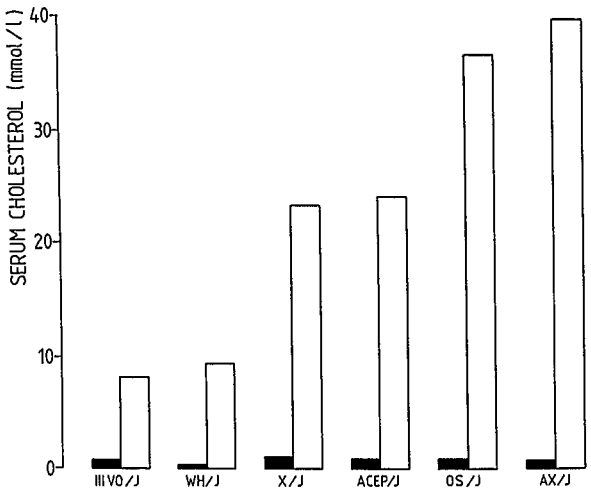

FIG. 6. Effect of 4 weeks of cholesterol feeding (0.5% by weight) on mean plasma cholesterol concentrations of six inbred strains of rabbits (open bars; $n = 5$). Closed bars denote initial levels. (Based on data taken from Van Zutphen and Fox, 1977.)

groups. Of a total of 56 dogs, 14 were identified as hyperresponsive; their serum cholesterol concentration after dietary challenge were 2 to 4 standard deviations higher than the mean. The lineage of 12 of these hyperresponders stemmed from two females, which were unrelated.

Roberts *et al.* (1974) studied a large population of random-bred rabbits, and obtained a wide range of plasma concentrations both on a commercial diet and on a diet containing 0.28% cholesterol. A breeding trial with selected hyper- and hyporesponsive animals showed that the response of plasma cholesterol to dietary cholesterol was inherited. The heritability, estimated from the regression of mean progeny response on midparent response, was found to be 50%. Figure 9 shows the regression of the response of plasma cholesterol to dietary cholesterol in the progeny and the parents.

Imai and Matsumura (1973) used a diet containing 1% cholesterol and 0.2% cholate to breed a hyperresponding strain out of a colony of random-bred Sprague-Dawley/ICL rats. In each generation sisters and brothers were mated, and the litter that showed the highest response was kept and used for further inbreeding. It was found that the progeny became progressively more responsive to the hypercholesterolemic diet (Fig. 10). This effect was more pronounced in the females than in the males. Similar results were obtained by Poledne (1984), who worked with Wistar rats and a diet containing 2% of cholesterol. The selective breeding program did not cause

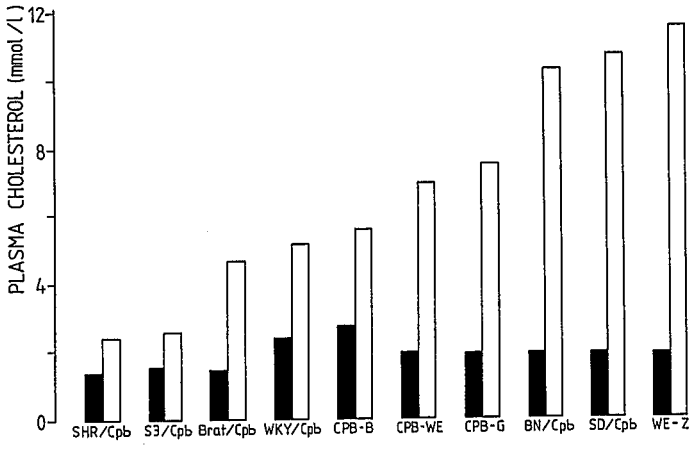

FIG. 7. Mean plasma cholesterol levels in inbred strains of rats sampled on a commercial diet and after feeding a diet containing 2% cholesterol, 0.5% cholate, and 5% olive oil for 21 days (open bars; $n = 4$). Closed bars denote initial values. (Based on data taken from Van Zutphen and Den Bieman, 1981.)

an increase in the plasma cholesterol level on a low-cholesterol commercial diet (Imai and Matsumura, 1973; Poledne, 1984).

Takeuchi *et al.* (1976), who worked with random-bred Wistar-King rats, observed that the offspring of hyporesponsive parents showed a smaller

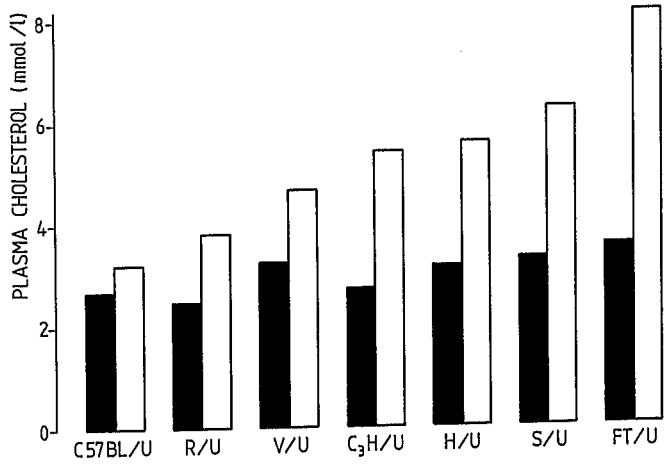

FIG. 8. Mean plasma cholesterol levels in inbred strains of mice fed either a low-cholesterol commercial diet (closed bars) or a diet containing 2% cholesterol, 0.5% cholate, and 5% olive oil (open bars) for 28 days ($n = 6$). (Reproduced with permission from Beynen *et al.*, 1985b.)

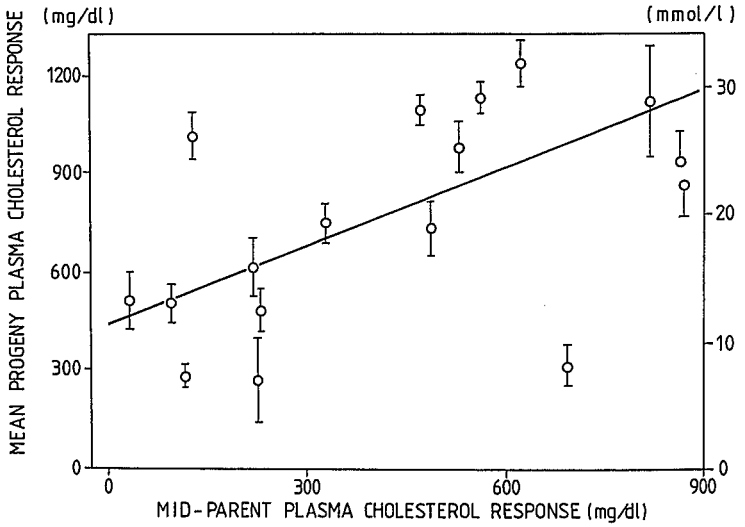

FIG. 9. Relation between mean progeny plasma cholesterol response and mean parental response in rabbits. The response was defined as the increase in plasma cholesterol concentration observed after 3 weeks on a 0.28% cholesterol-containing diet. A total of 135 progeny from 17 litters were studied. Each point represents the mean cholesterolemic response of one litter (±SE). (Reproduced with permission from Roberts *et al.*, 1974.)

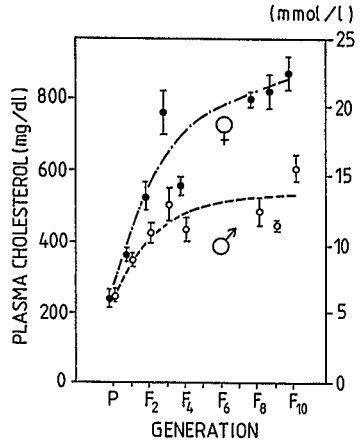

FIG. 10. Mean plasma cholesterol levels in successive generations of inbred male and female rats that were fed a diet containing 1% cholesterol and 0.2% cholate for 4–8 weeks. In each generation the litter with the highest response was kept and used for further inbreeding. (Reproduced with permission from Imai and Matsumura, 1973.)

response to a diet containing 1.5% cholesterol and 0.5% cholate than the offspring of hyperresponsive parents. The basal cholesterol levels were identical in both types of offspring (Takeuchi *et al.*, 1976).

We ourselves studied the inheritance of responsiveness in male rats obtained by crossing a hyperresponsive inbred strain with a hyporesponsive strain (SD/Cpb and SHR/Cpb; Fig. 7). Figure 11 shows the individual responses of the various generations to a diet containing 2% cholesterol and 0.5% cholate. The F_1 hybrids had responses about halfway between those of the parental strains. Animals of the F_2 hybrid had responses scattered over the entire scale. Comparison of the variances of the response of genetically uniform groups (parental strains and F_1 hybrid) and segregating groups (backcrosses and F_2 hybrid) revealed that under these specific conditions >80% of the observed variation could be attributed to additive genetic factors, and that two major genes were involved in the control of the serum cholesterol response (Van Zutphen and Den Bieman, 1983). The studies with monkeys and rabbits described above demonstrated a weaker genetic influence on sensitivity to a hypercholesterolemic diet. However, as pointed out at the beginning of this paragraph, heritability cannot be measured in absolute units but depends on the specific environment in each experiment. Still, it can be concluded that a major part of the variability in response observed in laboratory animals under standard experimental conditions is due to genetic differences.

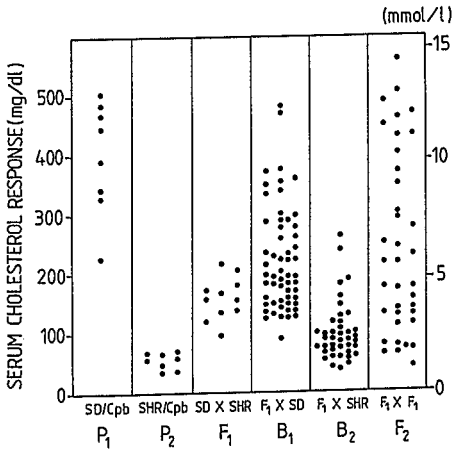

FIG. 11. Individual response of plasma cholesterol after 4 weeks of feeding a diet containing 2% cholesterol and 0.5% cholate to male rats of two fully inbred strains (SD/Cpb and SHR/Cpb) and crosses of these strains. (Reproduced with permission from Van Zutphen and Den Bieman, 1983.)

D. Environmental Contributions to Differences in Responsiveness

There is some evidence that environmental factors can fix responsiveness to diet. The environmental contributions essentially represent early nutrition and/or maternal effects. Roberts and West (1974) performed cross-fostering experiments with hypo- and hyperresponsive rabbits. They found that the young of hyporesponsive parents when suckled on hyperresponsive dams became themselves hyperresponsive in their response of plasma cholesterol to dietary cholesterol. However, offspring of hyperresponsive parents remained hyperresponsive, irrespective of whether they were raised on their natural dams or on hyporesponsive foster dams. These data suggest that under specific conditions the trait for hyperresponsiveness is fixed by the early environment, the composition of the mother's milk possibly being important.

Studies with rats have shown a negative (Reiser and Sidelman, 1972; Hahn and Koldowsky, 1976), a positive (Green *et al.*, 1981; Coates *et al.*, 1983), or no association at all (Kris-Etherton *et al.*, 1979; Hulbron *et al.*, 1982; Beynen *et al.*, 1984c) between early cholesterol or fat feeding and the later response of serum cholesterol to cholesterol-enriched diets. Experiments with baboons (Mott *et al.*, 1982) and observations in humans (McMurry *et al.*, 1982) also disclosed no evidence for such long-term effects.

Results obtained with cholestyramine feeding of guinea pigs do suggest that cholesterol metabolism can be permanently altered. Feeding of cholestyramine to newborn (Li *et al.*, 1979) or recently weaned (Hassan *et al.*, 1982) guinea pigs reduced the cholesterolemic response to cholesterol loading 4–6 weeks later. We ourselves observed no such effect in rats (Beynen *et al.*, 1985a). In addition, we found no effect of early intake of cholestyramine on the accumulation of cholesterol in liver after feeding the high-cholesterol diet in later life. In rats born to dams fed cholestyramine during gestation and early lactation, a significantly increased response of plasma cholesterol to a high-fat, high-cholesterol diet at the age of ∿13 weeks was observed (Innis, 1983). No explanation for this effect can be offered.

In sum, the evidence for an imprinting effect of early nutrition on the later response of serum cholesterol to diet is equivocal. The results obtained with cholestyramine in guinea pigs suggest that responsiveness can be permanently altered, but the conditions under which this occurs remain to be defined.

IV. Hypo- and Hyperresponsiveness to Dietary Cholesterol in Humans

The phenomenon of hypo- and hyperresponsiveness has obviously been well established in various animal species. The existence of human hypo- and hyperresponders to dietary cholesterol has been frequently assumed

(Connor and Connor, 1972; Reiser, 1978), but has proved very difficult to substantiate experimentally. Two major problems are encountered in studies with humans in this area. First, the equivalent of "inbred strains" does not exist in humans; each subject is genetically unique, unless one can enlist identical twins. Second, humans are less sensitive to dietary cholesterol than rabbits or monkeys. Rats may be even more insensitive, but their cholesterolemic response can be enhanced by adding extreme amounts of cholesterol to the diet in combination with the bile acid, cholic acid. The response of serum cholesterol to dietary cholesterol rarely exceeds 20% in humans, even if extreme amounts are given (Messinger *et al.*, 1950), while the spontaneous, diet-independent fluctuations of serum cholesterol level in humans are already of the order of 5–10% (Keys, 1967; Demacker *et al.*, 1982).

If one is only interested in the mean effect of a certain diet factor on serum cholesterol, then this within-subject variability is usually eliminated by measuring the average response to a dietary challenge in a group of 10–30 subjects. Such a number of subjects yields a reasonably precise estimate of the population mean response. However, if one demands the same precision in estimating the inherent responsiveness of a particular subject, then one theoretically needs to perform 10–30 response experiments with this individual, which is unpracticable. As a result, serious attempts to estimate the extent of differences in responsiveness to dietary cholesterol between individuals have been rare, and where they have been made the results are highly imprecise.

A. Ostensible Variability in Individual Response

In 1926 Mjassnikow, and in 1933 Okey and Stewart reported that the mean serum cholesterol concentration of human subjects increased somewhat on cholesterol-supplemented diets, but that there was a considerable variability in observed individual responses. A similar inter-individual variation in cholesterolemic response was seen in most experimental studies that followed, and the concept of hyper- and hyporesponders to dietary cholesterol became widely accepted. However, in the numerous studies in which the effect of dietary cholesterol on serum cholesterol in humans was assessed (McGill, 1979), the response to the dietary challenge in a given subject was usually measured in one study only. The serum cholesterol concentration of one individual fluctuates with a coefficient of variation of 5–10% around his or her mean value (Keys, 1967; Demacker *et al.*, 1982). These fluctuations are independent of the diet and are of the same order of magnitude as the usual response to dietary cholesterol loads. Such error terms average out when group means are considered, but individual responses cannot be measured precisely enough to allow classification of

subjects as hypo- or hyperresponsive. Table I illustrates this. Six volunteers first abstained from cholesterol-rich products for 10 days, and then took six egg yolks per day for another 10 days. The study was repeated with the same subjects 1 year later. The average response for the group was fairly similar from one experiment to another, but the "hyperresponders" in the first experiment were not necessarily hyperresponders in the second experiment, and neither were those initially classified as hyporesponders consistently unresponsive the second time.

Similar experiments were performed as long ago as 1942 by Messinger and co-workers (1950). They fed patients a dietary supplement of 150 g of egg yolk powder per day emulsified in milk, providing 3750 mg cholesterol. The experiment was repeated in four of these patients, and the response was reproducible in only two of them. The patient who displayed the highest cholesterolemic response in the first experiment showed the lowest response in the second experiment.

These two studies illustrate that the variability in the response to dietary cholesterol observed in single short-term experiments by itself does not prove the existence of human hyper- and hyporesponders, because this variation could be largely explained by random within-person fluctuations in the level of serum cholesterol.

B. Evidence that Human Hypo- and Hyperresponders Do Exist

Inherent differences in responsiveness can be distinguished from chance fluctuations by increasing the number of observations per subject. In dietary trials of long duration, Ahrens *et al.* (1957) and Connor and Connor (1972) observed lasting differences between subjects in the response of serum cholesterol to dietary cholesterol. These studies suggested that true hypo- and hyperresponders do exist, but did not define the extent and prevalence of the phenomenon.

Table I

Changes in Serum Cholesterol Levels in Six Human Volunteers After Daily Consumption of Six Egg Yolks for 10 Days[a]

	Subject (% change in serum cholesterol)					
Experiment	A	B	C	D	E	F
1	+5	−3	+17	+17	+27	+5
2	+16	+12	+26	+25	+4	+3

[a]Twelve months elapsed between experiments 1 and 2; the design was otherwise identical. The preexperimental and experimental serum cholesterol values were both based on two blood samples obtained on successive days. After Katan and Beynen (1983).

We have carried out three controlled dietary trials with the same subjects to address the question whether individuals do exist with a consistently high or low serum cholesterol response to dietary cholesterol. In each trial the volunteers successively consumed a low- and a high-cholesterol diet, the cholesterol component of the diets (provided by egg yolk) being the only variable. Subgroups of putative hypo- and hyperresponding subjects, with mean serum cholesterol increases of 0 and 19%, respectively, were selected from a larger population in a first trial and then underwent a second and third experiment. Although the response in each subject was only partly reproducible, the selected hyperresponders showed significantly higher serum cholesterol responses in the second and third trials than the hyporesponders (Table II). Standardized regression coefficients for individual responses in two experiments ranged from 0.34 to 0.53 ($n = 32$). Figure 12 shows the relationship between the individual responses seen in experiment 1 and 3 ($r = 0.53$; $n = 32$; $p < 0.01$).

Under less controlled conditions we found similar results. In 1976 Bronsgeest-Schoute *et al.* studied the serum cholesterol response to cessation of egg consumption in subjects who habitually consumed at least one egg per day. When eggs were eliminated from the diet, daily cholesterol intake decreased from ∿800 to 300 mg. Mean serum cholesterol fell only slightly (by 3%), but the individual responses varied from −20 to +8%. In 1982, 34 of these subjects were reinvestigated (Beynen and Katan, 1985b), and at our request they again eliminated eggs and egg-containing products from their diet. The differences in serum cholesterol response between individuals were partly reproducible; the individual responses in 1976 and 1982 were positively correlated ($r = 0.32$; $n = 34$; $p < 0.05$).

Table II

EFFECT OF EGG YOLK CHOLESTEROL ON SERUM CHOLESTEROL IN THREE CONTROLLED TRIALS WITH THE SAME SUBJECTS[a]

	Change in serum cholesterol (mmol/liter)	
	Hyporesponders ($n = 15$)	Hyperresponders ($n = 17$)
Selection trial	−0.01 ± 0.21	+0.96 ± 0.27
First reproducibility trial	+0.06 ± 0.35	+0.28 ± 0.38*
Second reproducibility trial	+0.47 ± 0.26	+0.82 ± 0.35**

[a]Results are expressed as means ± SD. Change significantly different from that in the hyporesponders (one-tailed Student's *t*-test): * $p < 0.05$; ** $p < 0.005$. Based on Katan *et al.* (1986).

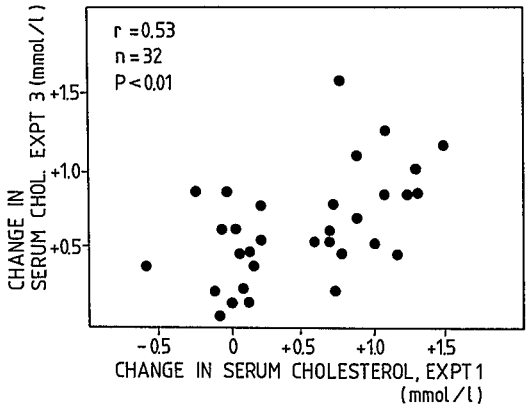

FIG. 12. Relationship between the individual responses of serum cholesterol to dietary cholesterol in repeated experiments. In experiment 1 (selection trial; Table II), the baseline diet provided on average 120 (10 mg/MJ) and the test diet 625 (55 mg/MJ) mg of cholesterol per day; in experiment 3 (second reproducibility trial) these values were 130 (15 mg/MJ) and 990 (85 mg/MJ) mg per day. (Reproduced with permission from *Ernährungs-Umschau* **32**, 356–360, 1985.)

Thus it appears that at least part of the cholesterolemic response to dietary cholesterol in humans is individually determined, although the range of responsiveness is much smaller in humans than in laboratory animals. It is also clear that one will always find subjects who appear hyperresponsive in one experiment and hyporesponsive in another. This is caused by the diet-independent within-person variability of serum cholesterol. In our controlled studies we calculated that the within-person error variance was still responsible for ∿25% of the apparent variance in response between subjects, even if we used 12 independent blood samples to determine each person's response to dietary cholesterol. Thus, it is probably fallacious to characterize a patient as hyper- or hyporesponsive to diet therapy if this is based on the results of a few blood samples only. A large number of serum cholesterol measurements is needed before and after the dietary challenge, and even then the observed response should be interpreted with caution.

V. Hypo- and Hyperresponsiveness to Dietary Components Other than Cholesterol

In humans the nature of the fat in the diet is more important as a determinant of the serum cholesterol concentration than the amount of cholesterol. Thus it is relevant to know whether hypo- and hyperresponders to dietary

fatty acid composition also exist, and whether hyperresponders to dietary cholesterol are also hyperresponsive to saturated fatty acids and other dietary components that affect serum cholesterol levels. Such information may also provide clues to the mechanisms underlying the interindividual variation in the cholesterolemic response to diet.

A. Animals

We have used male rabbits from inbred strains hypo- and hyperresponsive to dietary cholesterol, and measured the response of their plasma 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. Figure 13 documents that 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

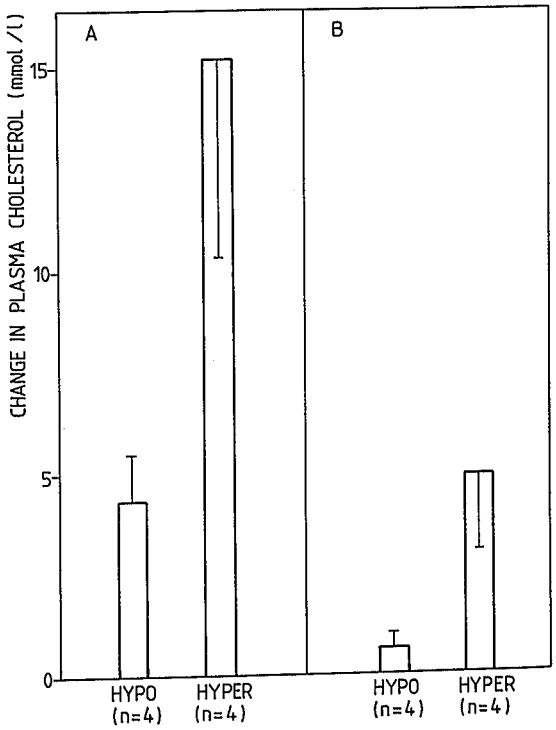

FIG. 13. Plasma cholesterol responses (A) to dietary cholesterol (0.3%, w/w) and (B) to coconut fat versus corn oil in a hypo- and a hyperresponsive inbred strain of rabbits. Results are expressed as means ± SE for four animals per strain; the data in panels A and B refer to the same animals. (Reproduced with permission from Beynen *et al.*, 1985c.)

hyperresponsiveness to dietary cholesterol and to the type of fatty acids coincided. A similar association, though weaker, was found in random-bred rabbits (Beynen *et al.*, 1986b).

In young, growing rabbits, cholesterol-free, semipurified diets containing casein as a protein source produce hypercholesterolemia, but no such effect is observed with soy protein (Kritchevsky, 1979; Beynen *et al.*, 1983a). As shown in Fig. 14, random-bred rabbits hyperresponsive to dietary cholesterol tend to be also hyperresponsive to cholesterol-free casein-containing semipurified diets. This observation has been extended to inbred strains (Beynen *et al.*, 1986a). A rabbit strain hyperresponsive to dietary cholesterol also showed a significantly higher response of plasma cholesterol to casein than the hyporesponsive strain. Thus in rabbits responsiveness to dietary cholesterol coincides with responsiveness to other hypercholesterolemic dietary components.

Hypercholesterolemia in rats is usually elicited by feeding large amounts of dietary cholesterol plus cholic acid. The latter might assist in emulsifying the cholesterol and aid in its absorption. Our classification of hypo- and hyperresponsive rat strains is also based on studies with diets containing 2%

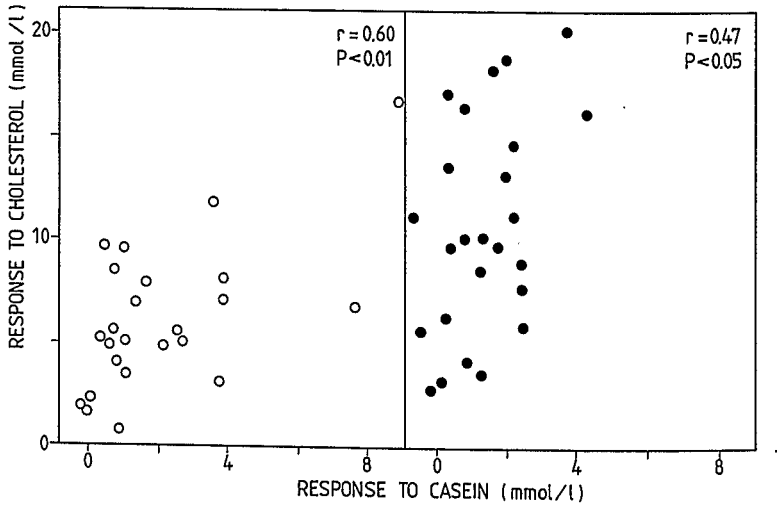

FIG. 14. Correlations between the cholesterolemic responses produced by dietary cholesterol and by dietary casein in rabbits. Rabbits were fed three semipurified diets—namely, a cholesterol-free diet containing 21% (w/w) soy protein, a cholesterol-free diet with 21% (w/w) casein, or a diet containing soy protein plus 0.2% cholesterol. One group (○) of 24 rabbits was fed successively the diet containing soy protein plus cholesterol (25 days), the cholesterol-free soy diet (36 days), and the casein diet (20 days). Another group (●) consisting of 25 animals received consecutively the diet containing casein, soy protein, and soy protein supplemented with cholesterol. At the beginning of the experiment the animals were aged 15 weeks. (Based on data from Beynen *et al.*, 1983b, and reproduced with permission from Beynen and Katan, 1985c.)

cholesterol and 0.5% cholate (Fig. 7). Although rats are notoriously insensitive to dietary cholesterol, we still found that in male rats of a hyperresponsive strain the addition of 1% cholesterol alone to a semipurified diet induced a significantly higher increase in serum cholesterol than in a hyporesponsive strain (Beynen and Katan, 1984). However, the absolute responses were much lower than on the hypercholesterolemic diet containing both cholesterol and cholate. Thus cholate intensifies the difference in cholesterolemic response between the rat strains.

We also made comparisons of the effects of soy protein and casein, and of pectin and cellulose in these two rat strains using cholesterol-free semipurified diets (Beynen and Katan, 1984). Casein caused higher serum cholesterol levels than soy protein, and cellulose caused higher cholesterol levels than pectin. These effects were somewhat greater in the hyper- than in the hyporesponsive strain, but the difference between the strains did not reach statistical significance. Thus so far, in these two rat strains clear differences in responsiveness are seen only on diets containing both cholesterol and cholate.

B. Humans

Experiments of Ahrens *et al.* (1957) showed heterogeneity among patients in the response of serum cholesterol to exchanging saturated fat in the diet with polyunsaturated fat. However, no quantitative data were given on the reproducibility of the individual responses, and thus the contribution of within-patient variability to this apparent heterogeneity cannot be determined.

Jacobs *et al.* (1983) recently reanalyzed data from some of the classical dietary trials performed between 1963 and 1966 by Keys, Grande, and Anderson in Minnesota. In these experiments the amount of cholesterol and the type of fat in the diet varied, and at least two serum cholesterol values per dietary period were known. Analysis of data for 48 subjects who had participated in two or more diet experiments showed that individual diet responsiveness was consistent from experiment to experiment in most men. Quantitative statistical data on the consistency of differences in responsiveness between individuals were not given. However, it was stressed that these differences were small. Most of the men showed a responsiveness within 30% of the value predicted by the formula of Keys (Jacobs *et al.*, 1983), and only 2 of 58 men could reliably be labeled "nonresponder."

Another classical series of experiments, that of the Dutch Unilever group, was reanalyzed by ourselves. This involved nine controlled trials performed in Dutch monasteries in the period of 1963 to 1974 (Vergroesen *et al.*, 1970; Vergroesen and De Boer, 1971; Vergroesen, 1972; Vergroesen and Gottenbos, 1975). In these experiments dietary fat was the only variable;

cholesterol intakes were constant. A total of 130 individuals (82 monks and 48 nuns) had been tested in two or more trials. In these subjects the mean response of serum cholesterol to various polyunsaturated fats was a decrease of 0.57 ± 0.34 mmol/liter (22 ± 13 mg/dl). Analysis of variance showed that significant and consistent individual differences in response were present, but just as in the subjects studied by Keys *et al.* (Jacobs *et al.*, 1983) a major part of the apparent variance between persons was in fact due to random within-subject fluctuations of serum cholesterol levels (M. B. Katan, unpublished).

From the reanalysis of these two sets of experiments it appears that subjects with a consistently high or low response of serum cholesterol to the nature of dietary fatty acids do exist. However, total insensitivity of serum cholesterol to a fat-modified diet is rare, and what is taken to be lack of responsiveness is usually due to random fluctuations and does not constitute a permanent characteristic of the subject in question.

We have addressed the question whether human subjects hypo- or hyperresponsive to dietary cholesterol are also hypo- or hyperresponsive, respectively, to saturated fatty acids in the diet. Twenty-three subjects who participated in the three controlled trials on the effect of dietary cholesterol (see Table II) were also tested for their response to saturated versus polyunsaturated fatty acids. In this experiment cholesterol intake was kept constant at an average of 41 mg/MJ (almost 500 mg/day), but the energy percentage of dietary polyunsaturated fatty acids was kept at 21% for the first 3 and then changed to 5% for the next 3 weeks; the polyunsaturated–saturated fatty acids ratios were 1.91 and 0.22, respectively. The response of serum cholesterol to the change in dietary fatty acid composition in this experiment was positively correlated with the mean response to dietary cholesterol in the three preceding experiments ($r = 0.50$; $n = 23$; $p < 0.05$). This indicates that in humans hyperresponsiveness to dietary cholesterol is associated with hyperresponsiveness to saturated fat.

VI. Serum Lipoproteins in Hypo- and Hyperresponders

Cholesterol in plasma is carried by various lipoproteins. Knowledge of the distribution of cholesterol over lipoproteins in hypo- and hyperresponders, before and after cholesterol loading, may provide clues about the mechanisms underlying the phenomenon of hypo- and hyperresponsiveness. Here, we discuss lipoprotein concentrations in hypo- and hyperresponders. First, the level of serum cholesterol prior to the dietary challenge is considered.

A. Animals

1. Basal Serum Total Cholesterol

In random-bred rabbits initial plasma cholesterol concentration and the increase observed after cholesterol feeding are positively correlated (Roberts *et al.*, 1974; Beynen *et al.*, 1983b). Thus hyperresponders on average have higher basal cholesterol levels than hyporesponders. In contrast, this has not been observed in inbred strains of rabbits (Fig. 6). Likewise, in inbred strains of rats (Fig. 7) and mice (Fig. 8), there appears to be no relation between level of cholesterol and sensitivity to dietary cholesterol. It is possible that these animals were not old enough to detect differences in basal cholesterol values. We have seen that plasma cholesterol increased with age in two inbred strains of rats, the increase being greater in the hyperresponsive strain (Beynen and Katan, 1984; Beynen *et al.*, 1984a).

In monkeys it has been repeatedly found that hyperresponders have higher plasma cholesterol levels than hyporesponders while on a low-cholesterol diet. This holds for squirrel monkeys (Clarkson *et al.*, 1971; Lofland *et al.*, 1972), cynomolgus monkeys (Malinow, 1979), rhesus monkeys (Eggen, 1976; Bhattacharyya and Eggen, 1981), and patas monkeys (Melchior *et al.*, 1984). It is attractive to suppose that the higher cholesterol levels in the hyperresponders are the result of their sensitivity to diet.

2. Basal Lipoprotein Profile

There are already differences in lipoprotein profile between hypo- and hyperresponsive animals while fed a low-cholesterol diet. In African green monkeys (*Cercopithecus aethiops*) Rudel *et al.* (1981) and St. Clair *et al.* (1981) found that basal high-density lipoprotein (HDL) levels were lower in hyper- than in hyporesponsive animals. In contrast, in hyperresponding patas monkeys (*Erythrocebus patas*) and cynomolgus monkeys (*Macaca fascicularis*), basal HDL cholesterol levels were higher than in their hyporesponsive counterparts (Melchior *et al.*, 1984; Malinow, 1979).

In two inbred strains of rabbits we found that on the low-cholesterol, preexperimental diet the hyporesponsive animals had significantly higher HDL cholesterol levels than the hyperresponders (Beynen *et al.*, 1984b).

Thus the basal HDL cholesterol levels may differ in hypo- and hyperresponders, but the available data are limited, and there are discrepancies. More work is required on this point. Especially relevant seems the question of cause and effect with regard to the association between responsiveness to diet and basal HDL level.

3. Effect of Cholesterol Feeding

The increase of serum cholesterol in hyperresponding monkeys after cholesterol feeding is largely located in the LDL fraction. This is especially so when the high-cholesterol diet is fed for longer periods or when the diet contains excessive amounts of cholesterol in combination with saturated fat. Under such conditions hyperresponsive animals show a decline in HDL cholesterol, whereas hyporesponders actually show an increase in HDL. This has been observed in rhesus monkeys (Rudel and Lofland, 1976), cynomolgus monkeys (Malinow, 1979), and patas monkeys (Melchior *et al.*, 1984).

Feeding of cholesterol to two inbred strains of rabbits raised plasma cholesterol concentrations by ∿11 mmol/liter (426 mg/dl) in the hyporesponsive and by 48 mmol/liter (1858 mg/dl) in the hyperresponsive strain (Beynen *et al.*, 1984b); the excess cholesterol in the hyperresponders was contributed mostly by the very-low-density lipoprotein (VLDL) fraction.

Feeding of a high-cholesterol cholate-containing diet to inbred rats caused a decrease in HDL cholesterol concentration and accumulation of cholesterol in VLDL-like particles of density <1.040 g/ml. Both effects were more pronounced in the hyper- than in the hyporesponsive strain (Beynen *et al.*, 1984a).

Thus in animals hyperresponsiveness of serum total cholesterol implies hyperresponsiveness of atherogenic lipoproteins, and a decrease in "protective" HDL.

B. HUMANS

1. Basal Serum Total Cholesterol

Keys *et al.* (1965c) have suggested that the individual response of serum cholesterol to dietary change can be predicted by the initial concentration of serum cholesterol. Men having higher serum cholesterol levels would have a greater response to diet. In an article from the same group (Jacobs *et al.*, 1983), it has been stated that a relationship between a person's responsiveness and intrinsic serum cholesterol level is very difficult to establish, as the latter cannot be directly estimated. Mistry *et al.* (1981) reported a lack of association between individual response to egg yolk cholesterol and the initial level of serum cholesterol.

In our studies (see Table II), average serum cholesterol on the low-cholesterol experimental diets was somewhat higher in the hyper- than hyporesponders. This may be the result of the differential sensitivity to dietary fat. As mentioned above, there is no clear association between initial serum cholesterol and response to diet in individuals.

2. *Basal Lipoprotein Profile*

In our experiments we found a positive correlation between the response of serum total cholesterol to dietary cholesterol and the initial level of HDL cholesterol (Katan and Beynen, 1984). In the experiment where we have measured the response of serum cholesterol to the removal of eggs from the diet (Beynen and Katan, 1985b), we also found a significant correlation between the extent of the response and the HDL cholesterol concentration, measured either on the initial habitual diet ($r = 0.42$; $n = 33$; $p < 0.05$) or on the low-cholesterol, egg-free diet ($r = 0.37$; $n = 34$; $p < 0.05$). In the controlled dietary trial (see Table II) there was also a significant positive correlation between the mean response of serum cholesterol to dietary cholesterol and the cholesterol concentration in the serum HDL_2 fraction isolated by density gradient ultracentrifugation during the low-cholesterol period of the second experiment ($r = 0.41$; $n = 32$; $p < 0.05$). Although the latter correlation was weak, it persisted upon multivariate analysis (Katan and Beynen, 1987).

In contrast to these data, Fisher *et al.* (1983) have reported a negative association between basal HDL cholesterol levels and serum cholesterol response to diets differing in amount of cholesterol and type of fat ($r = -0.69$; $n = 9$; $p < 0.05$). No explanation can be offered for this discrepancy.

3. *Effect of Cholesterol Feeding*

In humans, the increase in cholesterol in serum after cholesterol feeding resides largely in the LDL, although HDL cholesterol levels also increase (Beynen and Katan, 1985a). Cholesterol feeding may also cause the appearance of new species of lipoproteins. These particles are HDL_c, an apoprotein AI-containing lipoprotein enriched with cholesteryl esters and apoprotein E (Mahley *et al.*, 1978), and intermediate-density lipoproteins (IDL) enriched with apoprotein E as well as VLDL enriched with apoprotein E, so-called β-VLDL (Nestel *et al.*, 1982).

Thus cholesterol feeding of humans causes an increase in LDL cholesterol, which is higher in hyper- than hyporesponders. Cholesterol loading also induces the appearance of apoprotein E-rich particles (HDL_c, β-VLDL), but it is not known whether this effect is different in hypo- and hyperresponders.

VII. Metabolic Differences between Hypo- and Hyperresponsiveness to Dietary Cholesterol

A. Overview of Cholesterol Metabolism

The increase in serum cholesterol after cholesterol feeding of humans is due mostly to an increase in LDL cholesterol concentration. Why does

dietary cholesterol cause an increase in LDL cholesterol in hyperresponders, but not or less so in hyporesponders? In order to gain more insight into this, we will first consider how cholesterol metabolism may react to an increased dietary cholesterol intake in general. Figure 15 presents a simplified scheme.

After ingestion, dietary cholesterol reaches the small intestine where it becomes indistinguishably mixed with endogenous cholesterol from bile. A proportion is absorbed, and the remainder is propelled along the intestinal tract and leaves the body with the feces.

In the process of absorption (Fig. 15, step a), dietary cholesterol is incorporated into triglyceride-rich chylomicrons by the intestine, and then enters the plasma via the lymph. The triglycerides are hydrolyzed by lipoprotein lipase, which is located on the capillary endothelial cells in muscle and fat tissue. The cholesterol remains with the chylomicron remnants, which are rapidly taken up by the liver (Fig. 15, step b) via the so-called apoE receptor (Mahley *et al.*, 1981).

Within the hepatocytes the cholesterol provided by chylomicron remnants will expand the cellular cholesterol pools. This elicits two reactions, each aimed at maintaining homeostasis. First, cholesterol synthesis is inhibited (Fig. 15, step c) (Cooper, 1977; Bhattathiry and Siperstein, 1963). Quantitatively, the liver is probably the most important cholesterol-synthesizing organ in humans (Dietschy and Wilson, 1970). Second, the activity of the LDL receptor (apoB, E receptor) is repressed (Fig. 15, step k), and thus the uptake of cholesterol from plasma LDL by the liver is decreased (Slater *et al.*, 1980; Kovanen *et al.*, 1981).

Both these steps aim at reducing the influx of cholesterol into liver pools. But the liver has several more pathways at its disposal to rid itself of excess cholesterol. The cholesterol taken up by the liver with chylomicron remnants may be transferred to the bile (Fig. 15, step d) either as such or after

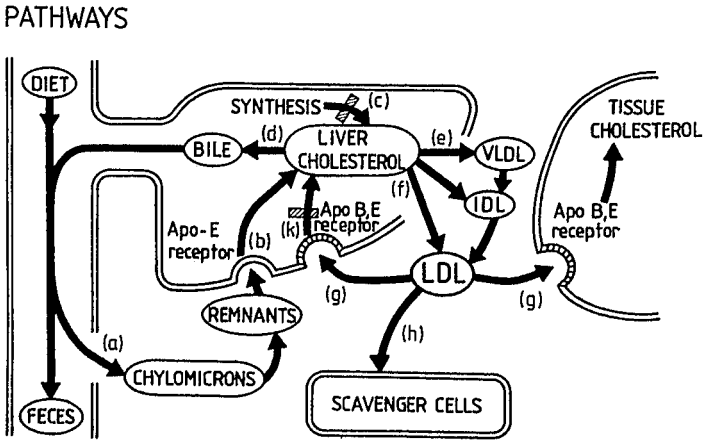

FIG. 15. Pathways of cholesterol metabolism.

conversion into bile acids. The liver may also secrete cholesterol into the blood as a component of VLDL (Fig. 15, step e). In the plasma, VLDL are converted via the IDL into LDL; during this conversion the particle loses triglycerides but cholesterol stays with it. The particle may acquire additional cholesterol esters from HDL in exchange for triglyceride molecules (Chajek and Fielding, 1978). In humans there is also direct LDL synthesis (Fig. 15, step f), which is independent of the VLDL precursor (Kesaniemi *et al.*, 1981). In primates and rats these LDL particles are probably secreted by the liver (Johnson *et al.*, 1983; Swift *et al.*, 1980). Up to 60% of LDL may leave plasma via the LDL receptor pathway originally described by Brown and Goldstein (1977), and denoted by step g in Fig. 15. The fate of the remainder is still obscure, but may involve scavenger cells (Fig. 15, step h).

Hyper- and hyporesponders might differ in each or any of the steps discussed here. Below, we discuss them one by one.

B. Differences in Absorption of Dietary Cholesterol

To start with, hypo- and hyperresponders might differ in their efficiency of absorption of dietary cholesterol (Fig. 16, step 1). If hyperresponders absorbed more cholesterol than hyporesponders, then the increased absorption of cholesterol would cause an enhanced flux of remnant cholesterol into the liver (Fig. 16, step 2) and, as a result, an increased pool of liver cholesterol (Fig. 16, step 3). There are two mechanisms by which this expansion of the liver cholesterol pool may cause an increase of the plasma LDL concentration. First, it may stimulate lipoprotein output by the liver (Fig. 16, step 4), which results in increased formation of LDL (Fig. 16, step 5). The concentration of LDL cholesterol will then increase. If we assume that the absolute rate of LDL clearance from plasma increases with LDL concentration, then plasma LDL will settle at a new, higher level where the clearance rate equals the rate of production.

Second, the increased pool of cholesterol in the liver cells may cause down-regulation of the LDL receptor activity (Fig. 16, step 6) and thus diminish the rate of clearance of LDL from plasma (Fig. 16, step 7). Again, the concentration of LDL in plasma will rise until eventually a new equilibrium is reached between clearance and input.

That manipulation of cholesterol absorption influences plasma LDL concentrations is indicated by studies with a copolymer of maleic acid and an 18-carbon α-olefin, which blocks cholesterol absorption. When the compound was given to human subjects, a significant correlation was found between percentage LDL cholesterol reduction and percentage absorption inhibition (Crouse *et al.*, 1982). But do not innate differences in absorption efficiency explain hypo- and hyperresponsiveness to dietary cholesterol?

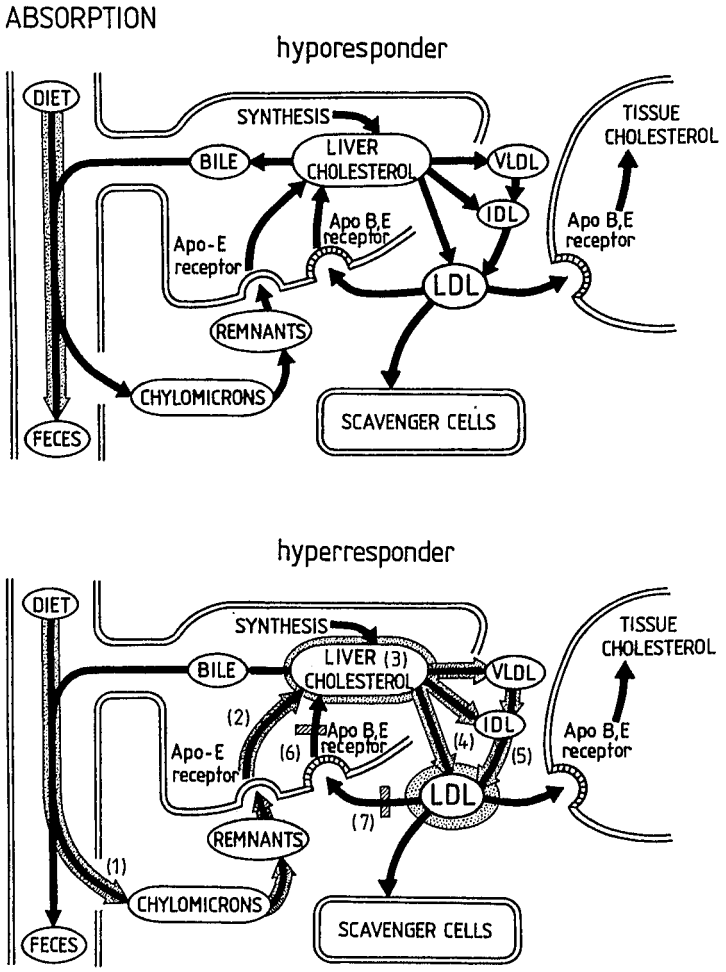

FIG. 16. Enhanced absorption of dietary cholesterol in hyperresponders. The effects are increased liver cholesterol (step 3), increased output of cholesterol by the liver (step 4), depressed LDL receptor activity (step 6), and an increase in LDL cholesterol concentration in plasma. The figure refers to the nonsteady state.

1. Animals

In monkeys individual differences in cholesterol absorption may play a role in the phenomenon of hypo- and hyperresponsiveness. Hyperresponsive squirrel monkeys absorbed cholesterol more efficiently than did their hyporesponsive counterparts (Lofland *et al.*, 1972). St. Clair *et al.* (1981) have shown that in African green monkeys hyperresponders absorbed a significantly higher percentage of dietary cholesterol than hyporesponders

(56 versus 37%). Eggen (1976) and Bhattacharyya and Eggen (1981, 1983) reported similar data for rhesus monkeys. The difference in absorption, however, cannot completely explain the difference in cholesterolemic response between hypo- and hyperresponders. When St. Clair *et al.* (1981) equalized cholesterol absorption in their hypo- and hyperresponding African green monkeys by adding sucrose polyester to the diet of the hyperresponders, the hyperresponsive animals still maintained higher concentrations of plasma cholesterol.

In rats, cholesterol absorption does not explain hypo- and hyperresponsiveness. Takeuchi *et al.* (1976) found that the rate of appearance in the plasma of orally administered labeled cholesterol did not differ between hypo- and hyperresponding rats. We ourselves found in cholesterol balance studies in two inbred strains of rats fed a high-cholesterol diet that cholesterol absorption was actually somewhat higher in the hypo- than in the hyperresponsive strain (Beynen *et al.*, 1984a).

In contrast, in inbred lines of Show Racer pigeons, Wagner and Clarkson (1974) could demonstrate that hyperresponding pigeons absorbed cholesterol more effectively from the diet than hyporesponders did.

Thus increased absorption may be the cause of hyperresponsiveness in some animal strains, but it does not appear to be the only determinant.

2. *Humans*

Accurate determination of cholesterol absorption in humans is difficult. When dietary cholesterol intake is increased, absorption in humans increases in a linear fashion, with ∿40% being absorbed at all intake levels (Quintão *et al.*, 1971; Connor and Lin, 1974; Nestel and Poyser, 1976). Differences in the response to dietary cholesterol between humans showed no clear-cut relation with differences in the proportion absorbed (Quintão *et al.*, 1971; Nestel and Poyser, 1976; Maranhão and Quintão, 1983). However, these studies involved only limited numbers of subjects who were not reliably classified as hypo- and hyperresponsive to dietary cholesterol.

C. Differences in Inhibition of Cholesterol Synthesis

If differences in cholesterol absorption are not the key to hypo- and hyperresponsiveness, it would appear that upon an increase in cholesterol consumption, equal amounts of dietary cholesterol carried by the remnants enter the livers of hypo- and hyperresponders (Fig. 17, step 1). The increased influx of cholesterol into the liver (Fig. 17, step 2) will elevate the concentration of cholesterol in the liver (Fig. 17, step 3) (Quintão *et al.*, 1977), and depress hepatic cholesterol synthesis (Fig. 17, step 4). Hypo- and

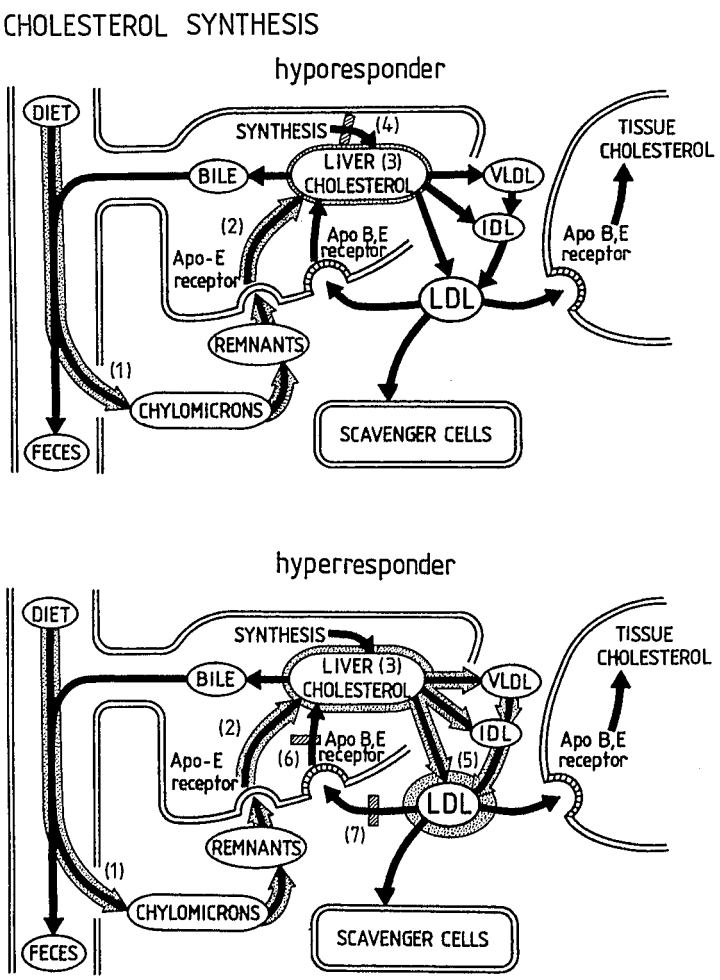

FIG. 17. Lack of inhibition of cholesterol synthesis in hyperresponders. The increased uptake of cholesterol by the liver after cholesterol feeding is compensated in hypo- (step 4), but not in hyperresponders. In hyperresponders, the liver cholesterol pools increase (step 3), which results in enhanced cholesterol output (step 5) and/or depressed LDL uptake (step 7). In the figure the nonsteady state is given.

hyperresponders might differ in the efficiency of this inhibition of cholesterol biosynthesis. Hyperresponders may be unable to compensate for the influx of dietary cholesterol by sufficient suppression of synthesis; as a result the liver cholesterol pool expands further (Fig. 17, step 3), and plasma LDL rises either because of increased excretion of VLDL, IDL, and/or LDL by the liver (Fig. 17, step 5), or because of suppression of LDL

receptors (Fig. 17, step 6), which depresses LDL clearance (Fig. 17, step 7), all exactly as discussed in the previous section under absorption. What is the evidence that hyperresponders are less effective in suppressing endogenous cholesterol synthesis, the primary step in this cascade?

1. Animals

On low-cholesterol diets, hyporesponders of various animal species synthesize cholesterol at higher rates than their hyperresponsive counterparts. This has been shown for rhesus monkeys (Eggen, 1976; Bhattacharyya and Eggen, 1981), squirrel monkeys (Lofland *et al.*, 1972), rats (Takeuchi *et al.*, 1976; Beynen *et al.*, 1984a), and pigeons (Wagner and Clarkson, 1974). The higher rates of cholesterol synthesis in the hyporesponders are in agreement with the observed higher rates of excretion of total endogenous fecal steroids. The higher rates of cholesterol turnover in hyporesponsive animals on a low-cholesterol diet have also been demonstrated directly by determination of the decay of the specific radioactivity of serum cholesterol after the intravenous administration of labeled cholesterol (Lofland *et al.*, 1972; Wagner and Clarkson, 1974; Beynen *et al.*, 1984a).

The greater rate of synthesis of cholesterol in hyporesponding animals theoretically allows more extensive feedback inhibition of cholesterol synthesis. Studies with rhesus monkeys (Bhattacharyya and Eggen, 1981) suggest that this does take place. Cholesterol biosynthesis was assessed in these monkeys by feeding them triparanol, a drug that blocks the conversion of desmosterol into cholesterol, and then determining their plasma desmosterol levels. The rate of accumulation of plasma desmosterol, which is a measure of cholesterol biosynthetic rate, was 50% greater in hypo- than in hyperresponders on a low-cholesterol diet. The addition of cholesterol to the diet caused a decrease in desmosterol accumulation in all monkeys, the decrease being 20% greater in the hypo- than hyperresponders.

Few other investigators have attempted to measure the relation between the rise of plasma cholesterol and the degree of inhibition of endogenous cholesterol synthesis caused by exogenous cholesterol in experimental animals. There is in fact, more information on this relationship in humans than in animals. It is important to note that in studies with animals, especially rats and rabbits, the dietary cholesterol loads, and thus cholesterol absorption, are often of higher orders of magnitude than basal cholesterol synthesis. Thus in these studies it is difficult to see how inhibition of cholesterol synthesis could compensate for the increased absorption of cholesterol.

2. Humans

The conjecture that hypo- and hyperresponders differ in the degree of dietary cholesterol-induced inhibition of cholesterol synthesis is supported

by the work of Nestel and Poyser (1976). These authors studied nine subjects first on a low- and then on a high-cholesterol diet. Figure 18 reveals that the increase in serum cholesterol was related to the decrease in whole-body cholesterol synthesis; the individuals who depressed cholesterol synthesis most markedly showed the smallest increase in serum cholesterol on the cholesterol-rich diet. This agrees with the findings of Mistry *et al.* (1981), who studied the activity of the rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, in freshly isolated blood mononuclear cells. In subjects fed cholesterol the percentage reduction in HMG-CoA reductase was inversely related to the percentage increase in plasma LDL cholesterol concentration ($r = -0.49$; $n = 37$; $p < 0.01$).

In our controlled dietary trial (see Table II) 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 (Fig. 19). This indicates that hyporesponders have higher basal rates of cholesterol synthesis than hyperresponders (Katan and Beynen, 1987).

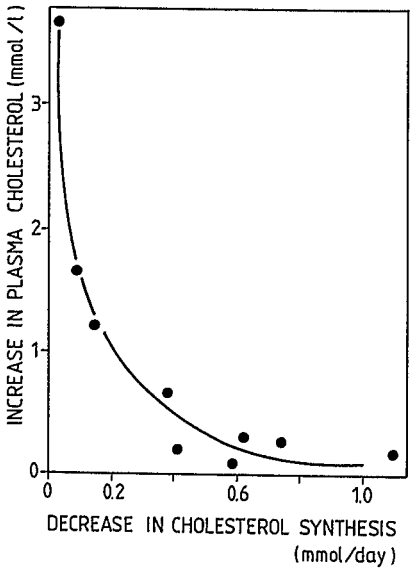

FIG. 18. Relationship between the decrease in whole-body cholesterol synthesis and the increase in serum cholesterol. Subjects consumed 300 mg cholesterol per day for 4–6 weeks and then 800 mg/day for another 4–6 weeks. Feces were collected during the final 8 days of each period. Cholesterol synthesis equals total fecal steroids excretion minus dietary cholesterol intake. Plasma cholesterol concentrations represent the means of two to three times weekly determinations during both periods. (Based on data taken from Nestel and Poyser, 1976, and reproduced with permission from Beynen and Katan, 1985c.)

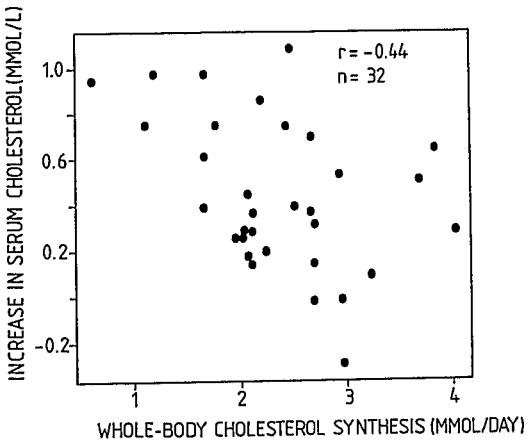

FIG. 19. Relationship between the response of serum cholesterol to increased dietary cholesterol, averaged over the three experiments (see Table II), and whole-body cholesterol synthesis (calculated as fecal steroid excretion minus dietary cholesterol intake) on a low-cholesterol diet (first reproducibility trial; Table II). The intake of cholesterol was on average 110 mg/day.

We were not able to demonstrate a relationship between the decrease in cholesterol synthesis and the increase in serum cholesterol after cholesterol feeding. However, our sterol balance study (Fig. 19) was performed during an experiment in which there was only a small effect of dietary cholesterol on serum cholesterol (first reproducibility trial; Table II).

Studies by Quintão *et al.* (1971) and Maranhão and Quintão (1983) also failed to demonstrate a relationship between the response of serum cholesterol to dietary cholesterol and the degree of suppression of cholesterol synthesis. This may be related to the extremely large changes in cholesterol intake of the patients in these studies. The baseline diets provided <50 mg cholesterol per day, whereas the high-cholesterol diets provided 1350 to $\sim$2500 mg/day. In 10 of the 21 patients studied, cholesterol synthesis on the high-cholesterol diet could not be calculated from the sterol balance data, as their balances (steroid excretion minus intake) were negative. This implies that the body accumulated cholesterol during this period.

Thus there is evidence both in animals and in humans that basal synthesis rates are higher in hypo- than in hyperresponders. This implies that hyporesponders have more room for compensatory decreases in endogenous cholesterol synthesis when cholesterol intake is increased. There is indeed some evidence that they take advantage of this pathway to avoid diet-induced hypercholesterolemia.

The higher basal rates of cholesterol synthesis in hyporesponders, compared with hyperresponders, could be secondary to differences in cholesterol absorption. If human hyporesponders, like their hyporesponsive counterparts among monkeys (Lofland *et al.*, 1972; Eggen, 1976; St. Clair *et al.*, 1981), have a lower efficiency of cholesterol absorption, then their rate of endogenous cholesterol synthesis must be higher than in hyperresponders because, in the hyporesponders, less cholesterol will reach the tissues from the gut, and cholesterol synthesis will be less suppressed.

D. Differences in Excretion of Steroids

In both hypo- and hyperresponders, an increase in cholesterol intake must result in an increase in the hepatic cholesterol pools (Fig. 20, steps 1–3). In hyporesponders one could hypothesize that this triggers an immediate increase in the excretion of cholesterol into bile, either as such or as bile acids (Fig. 20, step 4). Although biliary cholesterol and bile acids will recirculate a number of times, an increase in steroid output from the liver into the gut should in the long run lead to an enhanced loss of steroids with the feces (Fig. 20, step 5). If liver steroid output is not sufficiently responsive to increases in liver cholesterol content, which may be the case in hyperresponders, then the liver pool size will reach a level (Fig. 20, step 3) where cholesterol output with VLDL, IDL, or LDL particles is triggered (Fig. 20, step 6) or LDL receptor activity is diminished (Fig. 20, step 7). As a result, normal concentrations of LDL cholesterol cannot be maintained, and the subject will be hyperresponsive. In this scheme, hyperresponders would be incapable of adequately increasing their fecal output of steroids after cholesterol consumption. What is the experimental evidence for such a mechanism?

1. Animals

Hyporesponding squirrel monkeys have been shown to enhance their fecal bile acid excretion after cholesterol feeding more quickly and to a higher extent than their hyperresponsive counterparts (Lofland *et al.*, 1972). The difference may lie not so much in the final level of bile acid excretion as in the time needed to reach the new plateau, which was shorter for hyporesponders. However, both this study and a later study (Jones *et al.*, 1975) showed that plasma cholesterol reached its maximum concentration before the increase in bile acid excretion occurred. Thus the excretion of cholesterol via enhanced catabolism and excretion as bile acids does not appear to prevent the initial plasma cholesterol increase.

Evidence against a role for bile acid excretion in determining responsiveness in monkeys is furnished by a study of Eggen (1976). The increase in

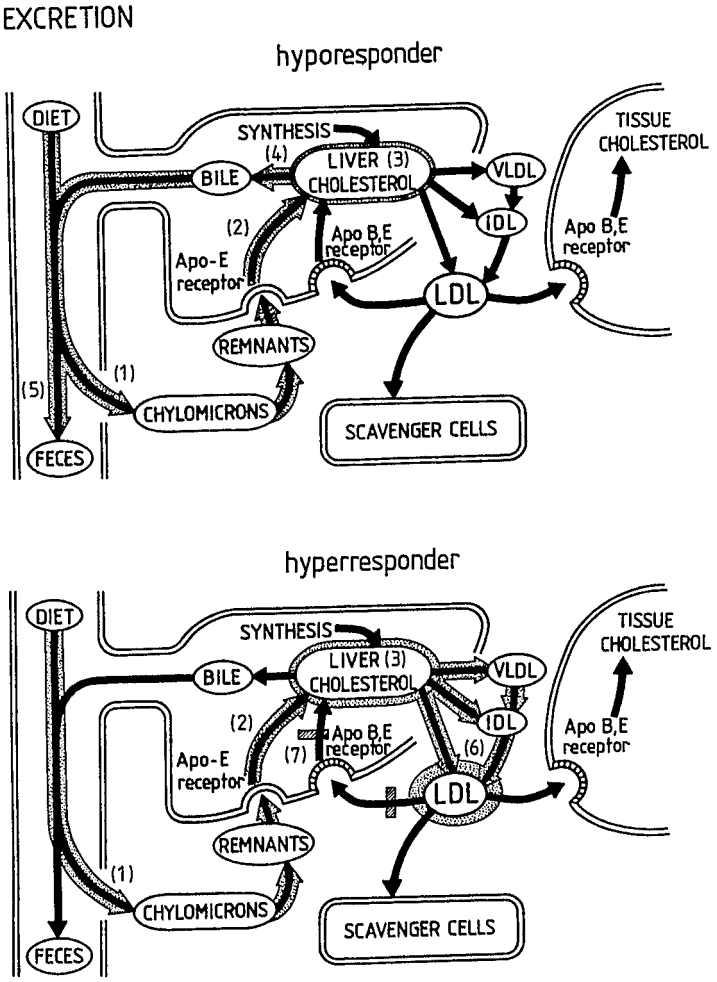

FIG. 20. Lack of increase in the excretion of cholesterol in hyperresponders. Dietary cholesterol causes an increase in liver cholesterol pools (steps 1–3). In hyporesponders, unlike in hyperresponders, this triggers bile acid synthesis (step 4), and excretion with the feces (step 5). In hyperresponders cholesterol output is increased (step 6) and/or LDL uptake decreased (step 7), and during this nonsteady state, LDL cholesterol increases.

fecal bile acid excretion when cholesterol intake was stepped up was greater in hyperresponding rhesus monkeys than in hyporesponders. Parks *et al.* (1977) also concluded from their investigations in African green monkeys that differences in bile acid excretion did not explain the differences in serum cholesterol responsiveness.

In two strains of inbred rats no evidence could be obtained for a relation between fecal excretion of endogenous steroids and the response of serum cholesterol to a high-cholesterol diet (Beynen *et al.*, 1984a). In fact, on the high-cholesterol diet, the hyperresponders excreted more endogenous steroids with the feces than the hyporesponders. There was no difference between hypo- and hyperresponsive rats in the excretion of bile acids.

Hulcher and Margolis (1982) have studied the activity of microsomal cholesterol 7α-hydroxylase (EC 1.14.13.17) in the livers of hypo- and hyperresponding pigeons; this enzyme catalyzes a major regulatory step in the transformation of cholesterol into bile acids. Basal activities of the hydroxylase were about 10-fold higher in hypo- than in hyperresponders. When the animals were fed a high-cholesterol diet, the hydroxylase activity increased in the hyper- but not in the hyporesponders; nevertheless, enzyme activity remained higher in the hyporesponding pigeons. These data suggest that hyporesponsive pigeons have a higher capacity to convert cholesterol into bile acids and possibly also to excrete cholesterol as such.

Thus, hyporesponder pigeons may manage to compensate for an increased cholesterol intake by increasing the conversion of cholesterol into bile acids and the elimination of bile acids from the body via the fecal route. However, this mechanism has not been conclusively demonstrated in other species, including humans.

2. Humans

There is no solid experimental evidence that in humans the individual variability in response is determined by differences in the capacity to stimulate fecal steroid excretion after cholesterol loading (Quintão *et al.*, 1971; Nestel and Poyser, 1976). Consumption of extra cholesterol usually does not lead to enhanced bile acid excretion in humans. Fecal excretion of cholesterol and its bacterial metabolites such as coprostanol is increased upon cholesterol feeding, but most of the increase is due to nonabsorbed dietary cholesterol. Increased elimination of endogenous cholesterol from the body is probably not a major compensatory reaction to cholesterol consumption in humans (Quintão *et al.*, 1971; Nestel and Poyser, 1976). In fact, Nestel and Poyser (1976) found that the excretion of fecal neutral steroids was lower after cholesterol feeding in hypo- than hyperresponders.

In conclusion, hypo- and hyperresponders do not appear to differ primarily in their ability to step up hepatic cholesterol and bile acid output after a cholesterol load.

E. Differences in Receptor-Mediated Clearance of LDL

The increase in hepatic cholesterol pool size (Fig. 21, steps 1–3) after cholesterol consumption could influence plasma LDL levels via the LDL

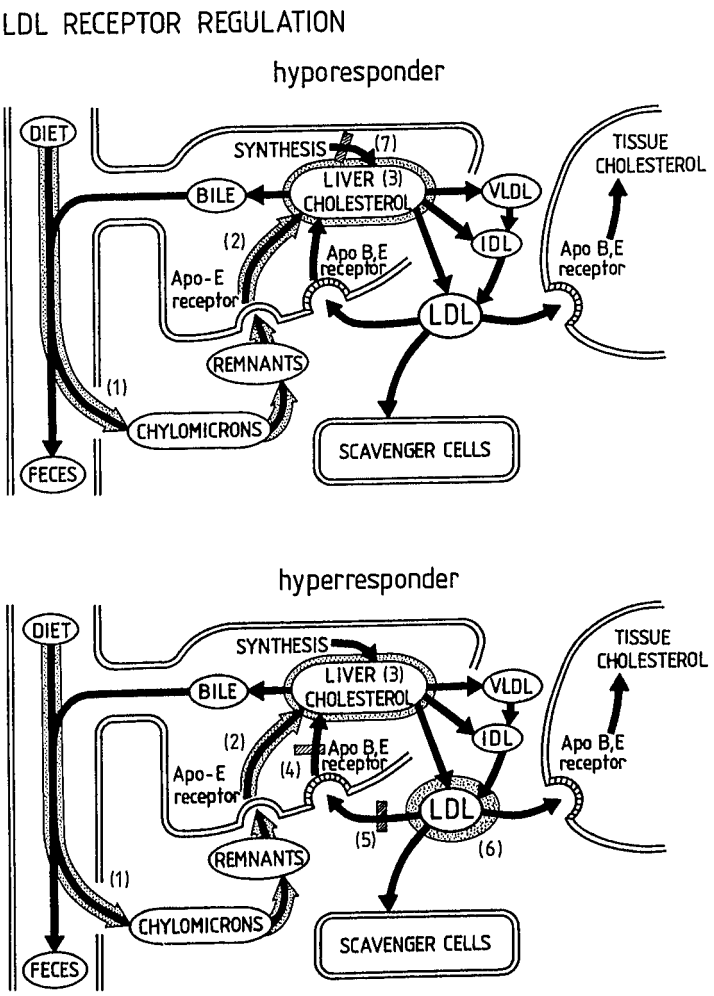

FIG. 21. Enhanced down-regulation of LDL receptor activity in hyperresponders. The increase in liver cholesterol pools (steps 1–3) causes a rapid decrease in receptor activity (step 4) in hyperresponders, whereas in hyporesponders cholesterol synthesis is inhibited (step 7). The nonsteady state is illustrated.

receptor. The regulation of the activity of the LDL receptor (apo B,E receptor) on the cell surface is a major mechanism in cellular cholesterol homeostasis (Brown and Goldstein, 1977). Cells shut off their LDL receptors (Fig. 21, step 4) when their internal cholesterol pool size becomes too large (Fig. 21, step 3). If the liver is the major organ for plasma LDL clearance (step 5), then such a shut-down of hepatic LDL receptors will cause a rise in plasma LDL concentration (Fig. 21, step 6). Either this

mechanism or an increased lipoprotein output from the liver probably explains why plasma cholesterol rises at all after cholesterol consumption. Down-regulation of the hepatic apo B,E receptor after cholesterol feeding has been directly shown in rabbits (Kovanen *et al.*, 1981; Slater *et al.*, 1980). In humans cholesterol feeding has also been shown to decrease the receptor-mediated fractional clearance of LDL (Packard *et al.*, 1983). Cholesterol suppression of LDL receptor activity has been demonstrated with blood mononuclear cells (Mistry *et al.*, 1981; Applebaum-Bowden *et al.*, 1984).

Does the degree of down-regulation induced by dietary cholesterol differ between hypo- and hyperresponders? Hyporesponders would then fail to decrease their number of hepatic apo B,E receptors after cholesterol loading. As a result their liver cholesterol pool will expand (Fig. 21, step 3), and in the long term they will have to adjust their cholesterol synthesis (Fig. 21, step 7) or their biliary excretion.

We have argued in Section VII,D that differences in biliary excretion do not explain differences in responsiveness. As a consequence, the receptor hypothesis for responsiveness implies that in hyporesponders the influx of cholesterol into the liver after a dietary cholesterol load leads to such a rapid adjustment of cholesterol synthesis (Fig. 21, step 7) that LDL receptor activity and LDL influx from plasma are not affected. Thus this mechanism is simply a different way of expressing the cholesterol synthesis hypothesis discussed in Section VII,C; in hyporesponders cholesterol-synthesizing enzymes are the first to sense the increase in hepatic cholesterol stores, and in hyperresponders it is LDL receptor synthesis that reacts first.

What information do we have on LDL receptor activity in hypo- and hyperresponders?

1. Animals

Guertler and St. Clair (1977) have studied the *in vitro* rates of cholesterol synthesis and esterification by cultured skin fibroblasts from hypo- and hyperresponding squirrel monkeys. Incubation of the fibroblasts with LDL resulted in stimulation of cholesterol esterification and inhibition of cholesterol synthesis, the percentage effects being similar in cells from hypo- and hyperresponders. This observation suggests that hyperresponsive monkeys are not receptor defective. However, the key question is whether there is a difference between hypo- and hyperresponders in the degree of suppression of hepatic LDL receptor activity when liver cholesterol pool size increases after cholesterol feeding. This question cannot be answered by the data reported by Guertler and St. Clair (1977).

2. Humans

Mistry *et al.* (1981) have demonstrated that human hyperresponders to dietary cholesterol have a lower maximal capacity for LDL receptor activity in blood mononuclear cells than hyporesponders. The increment in plasma cholesterol concentrations after egg yolk feeding was negatively associated with the LDL receptor activity ($r = -0.74$; $n = 18$; $p < 0.001$) measured before the dietary challenge in derepressed blood mononuclear cells (Fig. 22). This suggests that the maximally attainable rate of receptor-mediated catabolism is lower in hyperresponders. A similar conclusion can be derived by combining the results of the trial of Ginsberg *et al.* (1981), in which the response to dietary cholesterol was minimal, with those of Packard *et al.* (1983), who observed a large response of serum cholesterol to a dietary load. In the hyperresponders of Packard *et al.* (1983), the baseline fractional clearance rate of LDL is lower than in the hyporesponders of Ginsberg *et al.* (1981) (Table III). Thus hyperresponders apparently have a reduced number of LDL receptors, possibly also in the liver.

These observations do not support the hypothetical mechanism outlined above, but they do not refute it either; hyperresponders may still show a

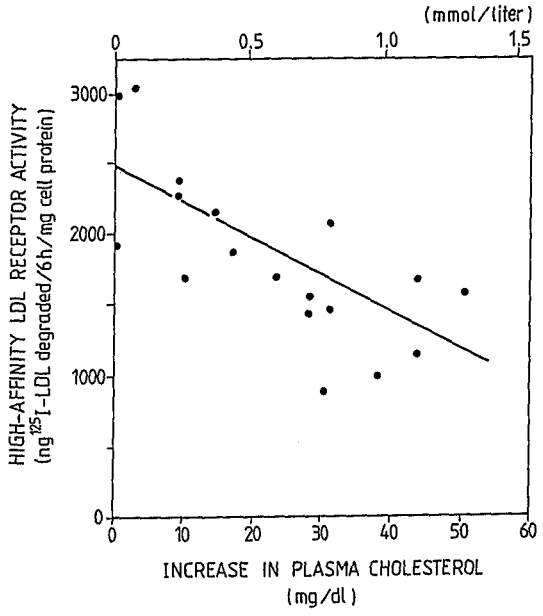

FIG. 22. Relationship between the increment in plasma cholesterol concentration produced by consuming six egg yolks daily for 14 days and the LDL receptor activity of derepressed blood mononuclear cells collected immediately before the commencement of cholesterol feeding. (Reproduced from *The Journal of Clinical Investigation,* 1981, Vol. 67, p. 499 by copyright permission of The American Society for Clinical Investigation.)

Table III

EFFECTS OF CHOLESTEROL FEEDING ON THE SERUM CHOLESTEROL RESPONSE AND LDL METABOLISM IN HYPER- AND HYPORESPONSIVE SUBJECTS[a]

	Hyporesponders (n = 5)	Hyperresponders (n = 7)
Expected mean serum cholesterol response (mmol/liter)	0.40	0.75
Observed mean serum cholesterol response (mmol/liter)	−0.04	1.47
Range (mmol/liter)	−0.50 to 0.37	0.58 to 2.39
Total fractional catabolic rate of apo-LDL on low-cholesterol diet (% per day; mean ± SD)	52 ± 10	35 ± 6
Change in production rate of apo-LDL after cholesterol feeding (mg/kg/day; mean ± SD)	+0.06 ± 3.51	+3.06 ± 2.00

[a]The discrimination between hypo- and hyperresponders is based on the difference between the expected response (calculated with formula of Keys, 1965a-d) and the observed cholesterolemic response. The hyporesponders consumed 36 mg cholesterol/MJ followed by 120 mg/MJ; for the hyperresponders these values were 26 and 213 mg/MJ, respectively. All other nutrients were kept constant. Data for the hyporesponders are taken from Ginsberg *et al.* (1981), those for the hyperresponders are taken from Packard *et al.* (1983).

more extensive decrease in LDL receptor activity even if their maximal attainable activity or their initial clearance rate is lower than that in hyporesponders. Mistry *et al.* (1981) observed that the high-cholesterol diet caused a striking reduction in the mean LDL receptor activity of freshly isolated mononuclear cells, but they did not report a relation between change in receptor activity and in plasma LDL concentration induced by diet. Applebaum-Bowden *et al.* (1984) have since showed that the percentage increase in LDL cholesterol after cholesterol loading was negatively correlated with the percentage decrease in LDL receptor activity in blood mononuclear cells ($r = -0.80$; $n = 6$; $p = 0.06$). Thus hyporesponders indeed showed less of a depression in LDL receptor activity. However, the number of subjects was small, and if instead of the change in LDL cholesterol levels obtained on the last day of each diet phase, the percentage change in the reported mean individual cholesterol levels over the full dietary periods is used, then the correlation with the change in LDL receptor activity falls to 0.32. In addition, the observed relation still does not prove that oversuppression of LDL receptors is the primary cause of hyperresponsiveness to dietary cholesterol. A rise in plasma LDL caused by other factors would still cause cells to stem the influx of LDL by down-regulating their receptors, and this reaction would be most pronounced in those in

whom plasma LDL showed the largest increase, i.e., the hyperresponders. This reasoning implies that it is difficult experimentally to prove or disprove involvement of LDL receptor activity in the phenomenon of hypo- and hyperresponsiveness.

A low capacity of the LDL receptor pathway by itself probably does not cause increased sensitivity to dietary cholesterol. Patients with familial hypercholesterolemia, who have drastically reduced numbers of LDL receptors in all tissues, have been shown to produce cholesterolemic responses to dietary cholesterol which are similar to those of healthy subjects (Martin and Nestel, 1979; Connor and Jagannathan, 1973).

F. Differences in LDL Production

As discussed above, the remnant cholesterol taken up by the liver (Fig. 23, steps 1–3) may be secreted into the blood as a component of VLDL. In plasma, VLDL are converted into IDL and then into LDL. The liver may also secrete IDL and LDL directly, independently from the VLDL precursor. Evidence that these IDL particles secreted by the liver are enriched in dietary cholesterol has been found in the cholesterol-fed dog (Melchior *et al.*, 1981). Thus another possible pathway for hyperresponsiveness is that in hyperresponders dietary cholesterol causes higher rates of LDL production than in hyporesponders (Fig. 23, step 4). This may then cause accumulation of LDL cholesterol in the plasma (step 5), the concentration of which will rise until a new level is reached where LDL catabolism equals LDL production.

Kesäniemi and Grundy (1982) have suggested that the rate of LDL production is the major determinant of LDL cholesterol concentration in humans. It was found that differences in LDL cholesterol levels between subjects on their habitual diets were directly correlated with differences in the rate of appearance of the apoprotein of LDL, apoB, in the LDL density fraction. By analogy, hyperresponders may increase their rates of LDL production when increased amounts of cholesterol reach the liver. In hyporesponders the VLDL synthesis pathway would have a higher threshold, and the influx of cholesterol into the liver would instead be compensated by a decrease in synthesis (Fig. 23, step 6). Increased flow into the bile is an alternative possibility, but this is less likely, as argued in Section VII,D. The evidence for a difference in hepatic lipoprotein production between hypo- and hyperresponders is summarized below.

1. Animals

Cholesterol feeding of African green monkeys increased production of IDL-like, or light LDL particles by their perfused livers (Johnson *et al.*,

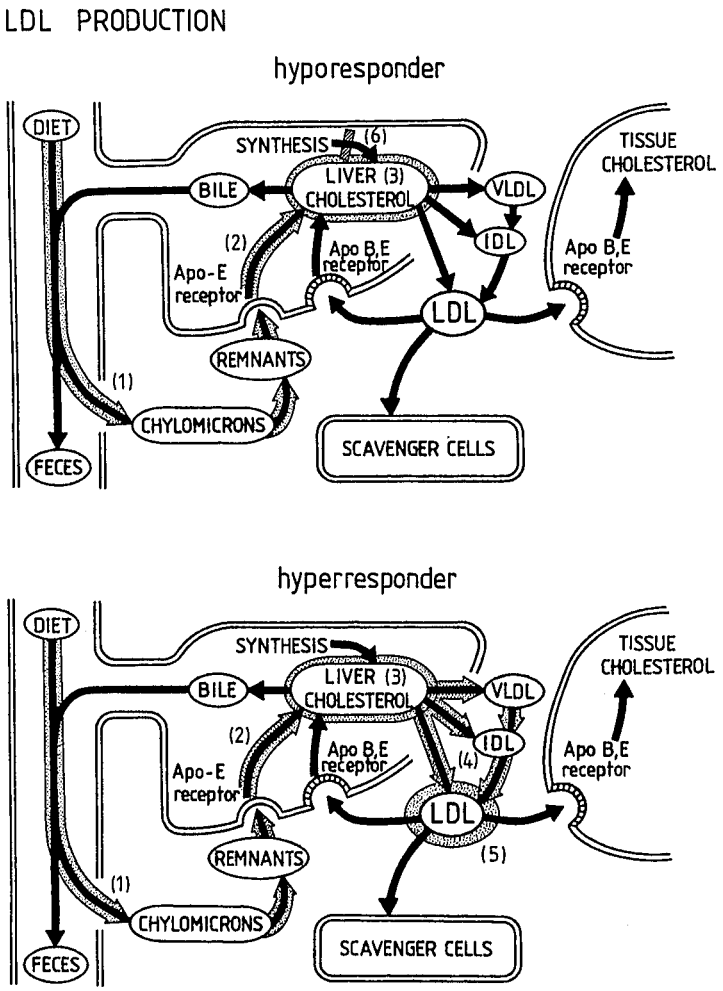

FIG. 23. Increased LDL production in hyperresponders. The increase in liver cholesterol pools (steps 1–3) causes increased hepatic cholesterol output in hyperresponders (step 4), while in hyporesponders cholesterol synthesis is depressed (step 6). Nonsteady-state condition is given in hyperresponder.

1983). Furthermore, in the same study cholesterol output by the perfused liver was positively correlated with the plasma cholesterol concentration of the animal when it was on the high-cholesterol diet (Fig. 24).

2. *Humans*

A role for hepatic lipoprotein secretion in determining responsiveness is supported by comparing the studies of Packard *et al.* (1983) and Ginsberg *et*

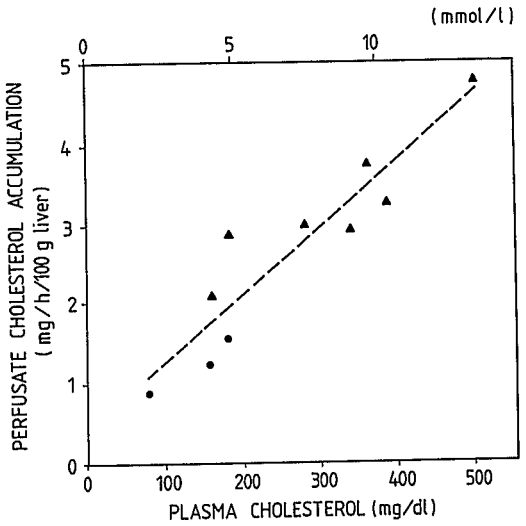

FIG. 24. Relationship between the level of plasma cholesterol in African green monkeys on a high-cholesterol diet and the rate of accumulation of cholesterol in the perfusate of their isolated livers. (Reproduced from *The Journal of Clinical Investigation,* 1983, Vol. 72, p. 226 by copyright permission of The American Society for Clinical Investigation.)

al., (1981). In the hyperresponders studied by Packard *et al.* (1983), unlike the hyporesponders of Ginsberg *et al.* (1981), there was a pronounced increase in LDL production after cholesterol feeding (Table III). The dietary cholesterol-induced enhancement of LDL synthesis in hyperresponders may involve direct synthesis of LDL or IDL by the liver. Nestel and Billington (1983) 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 (Fig. 25).

Thus both in monkeys 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.

G. Differences in Accumulation of Cholesterol in the Body

A human subject who is given an isocaloric diet with an increased cholesterol content will sooner or later reach a steady state where the rate of efflux of cholesterol and its metabolites from the body equals the rate of influx from the diet and from biosynthesis. However, in dietary trials as commonly performed this steady state is often not reached, and thus apparent hyporesponders might be storing their excess dietary cholesterol in tissues other than plasma for the duration of the experiment (Fig. 26). There is indeed evidence that this happens in trials with dietary cholesterol.

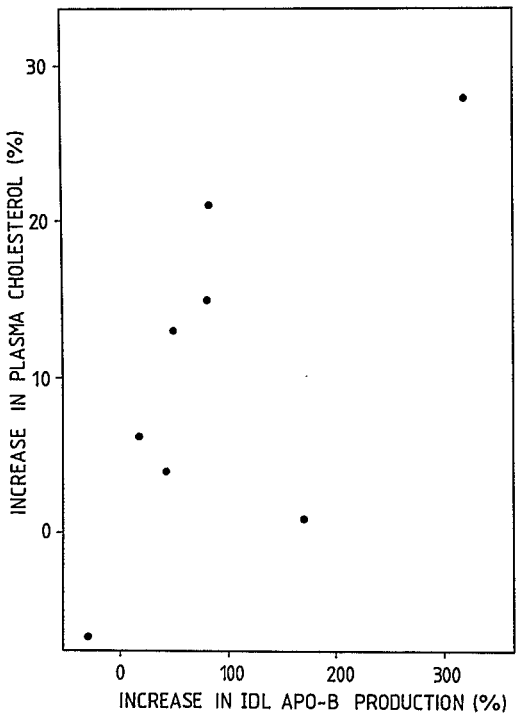

FIG. 25. Relationship between percentage change in plasma cholesterol concentrations and percentage change in IDL apoprotein B formation in human subjects after an increase in cholesterol intake of about 200 to 1500 mg/day ($r = -0.70$; $n = 8$; $p < 0.05$). (Based on data from Nestel and Billington, 1983.)

1. Animals

Massive amounts of cholesterol accumulate in the liver of cholesterol-fed rabbits, and even more so in rats. West and Roberts (1974) reported that livers of hypo- and hyperresponsive rabbits had similar cholesterol concentrations when the animals received a low-cholesterol diet. However, after a period of cholesterol loading, the livers of hyporesponders contained more cholesterol than those of hyperresponders. We made similar observations in two inbred strains of rabbits: the cholesterol content of the livers of cholesterol-fed hyporesponsive rabbits tended to be higher than that of hyperresponders (Beynen *et al.*, 1985e). On the other hand, there was no such difference in a study with hypo- and hyperresponding rabbits obtained from selected crosses between New Zealand White and Vienna White rabbits (Van Zutphen *et al.*, 1981).

The concentration of cholesterol in adipose tissue is directly correlated with the degree of hypercholesterolemia in cholesterol-fed rabbits (Ho *et*

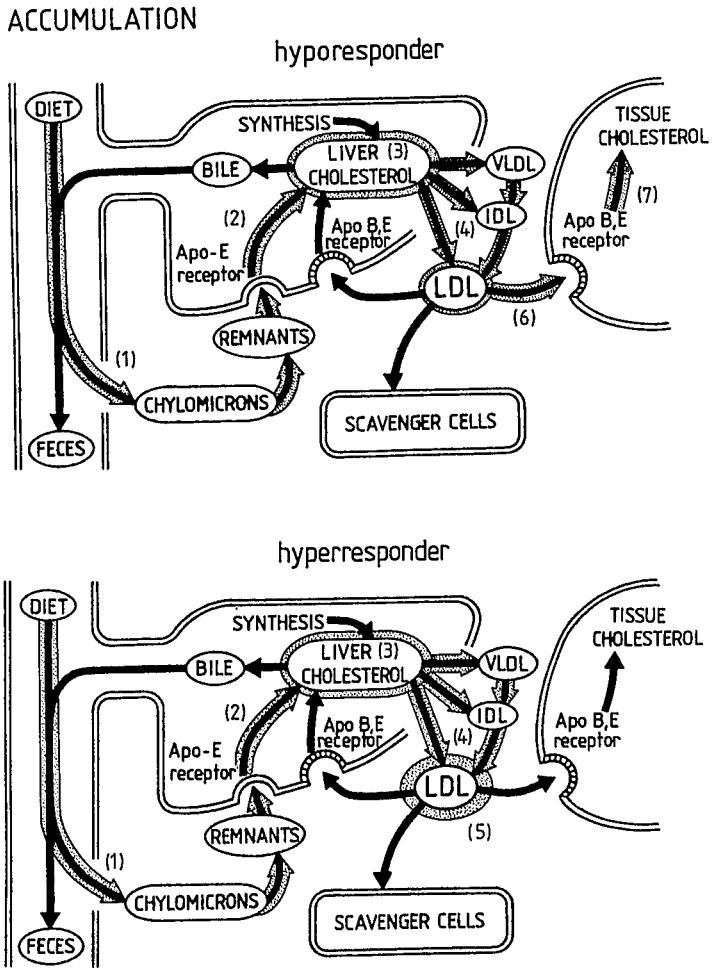

FIG. 26. Lack of enhanced storage of cholesterol in the tissues of hyperresponders. Liver cholesterol (steps 1–3) is secreted (step 4), and stays in the LDL fraction in hyperresponders (step 5), whereas in hyporesponders it is directly transported to tissues (steps 6–7). It goes without saying that the figure presents the initial stage of cholesterol feeding.

al., 1974) and squirrel monkeys (Raymond *et al.*, 1976). Thus accumulation of cholesterol in adipose tissue does not protect the animal from a rise in plasma cholesterol. As a consequence, individual differences in the net transfer of cholesterol to the adipose tissue cannot explain differences in the response of plasma cholesterol levels to dietary cholesterol.

Rats of an inbred hyporesponsive strain fed high-cholesterol cholate-containing rations accumulated more cholesterol in their livers than rats of a

hyperresponsive strain. Although the difference did not reach statistical significance (Beynen *et al.*, 1984a), the capacity to store cholesterol in the liver may be an important mechanism in the regulation of serum cholesterol levels in these rats. After the animals had been fed cholesterol for 24 days, the difference between the hypo- and hyperresponsive rats in the amounts of liver cholesterol (1510 versus 1140 μmol) far exceeded the opposite difference in the total amount of cholesterol in serum (17 versus 45 μmol).

2. *Humans*

Sterol balance studies have demonstrated that on high-cholesterol diets there can be a net storage of cholesterol in the human body (Quintão *et al.*, 1971; Lin and Connor, 1980). In these studies cholesterol accumulation in the body could occur while plasma cholesterol levels were essentially unchanged; there was no correlation between the cholesterol increments in tissues and those in plasma (Quintão *et al.*, 1971).

Sterol balance data do not indicate where cholesterol accumulates in the body. Quintão *et al.* (1977) used liver biopsies to show that dietary cholesterol causes an increase in liver cholesterol in humans. Unfortunately, this study gives no information about whether there is a difference in hepatic cholesterol accumulation between hypo- and hyperresponders.

Mistry *et al.* (1981) reported that egg yolk feeding caused a significant increase in the cholesterol content of blood mononuclear cells, but the correlation between this increase and the change in individual plasma cholesterol was not reported.

Adipose tissue is a major site of cholesterol storage in humans, but, as in animals, there appears to be no evidence that storage in this tissue prevents accumulation of cholesterol in plasma. On the contrary, several observations indicate that the amount of cholesterol in adipose tissue rises with elevations of circulating cholesterol (Krause and Hartman, 1984). Thus cholesterol storage in adipose tissue cannot be regarded as a compensatory mechanism that keeps plasma levels down in the face of increased intakes of cholesterol.

H. Conclusions

The mechanism underlying hypo- and hyperresponsiveness is obviously still obscure, and it is probably heterogeneous. It is possible that in hyperresponders, there is a higher efflux of cholesterol in the form of IDL and LDL particles from the liver after cholesterol consumption than in hyporesponders. The cause of this higher output of cholesterol by the liver may be that in hyperresponders the activity of the cholesterol-biosynthetic pathway is not suppressed sufficiently; this would then be the primary defect in hyperresponders.

Alternatively, the primary defect in hyperresponders could be greater efficiency of cholesterol absorption. This would cause a higher influx of dietary cholesterol into the liver of hyperresponders. This in turn could result in a higher output of cholesterol by the liver of hyperresponders.

The stimulation of the production of LDL accounts for the increase in the concentration of LDL cholesterol in hyperresponders. The number of LDL receptors, which is already decreased in hyperresponders, will decrease further through down-regulation (Brown *et al.*, 1981), as shown in blood mononuclear cells (Mistry *et al.*, 1981; Applebaum-Bowden *et al.*, 1984). As a result, the receptor-mediated fractional clearance of LDL decreases (Packard *et al.*, 1983), but the absolute amount of LDL cholesterol delivered to the cells by the receptor pathway increases somewhat because the concentration of substrate (LDL) is increased (Packard *et al.*, 1983). The rise in LDL production will also increase LDL clearance via the receptor-independent scavenger pathway (Packard *et al.*, 1983). In this way a new equilibrium is reached in which LDL production equals LDL catabolism. The fractional clearance rate by the scavenger cells is not affected by dietary cholesterol (Packard *et al.*, 1983), and therefore the decrease in total fractional catabolic rate in hyperresponders (Table III) is entirely accounted for by the decrease in receptor-mediated fractional clearance of LDL (Packard *et al.*, 1983).

VIII. Miscellaneous Characteristics of Hypo- and Hyperresponders to Dietary Cholesterol

As shown in the preceding section, differences in the known pathways of cholesterol metabolism can partly explain the observed differences between individuals in responsiveness of serum cholesterol to diet. However, a careful comparison of hypo- and hyperresponsive strains or individuals may reveal differences in other, less obvious attributes, and study of these may help to understand what causes hypo- and hyperresponsiveness. One of such characteristics, the concentration of HDL cholesterol in plasma, has already been discussed above (Section VI). In this section we briefly discuss the plasma arylesterases, body mass index, and habitual cholesterol intake.

A. Plasma Arylesterases

The plasma of vertebrate animals contains enzymes that can hydrolyze artificial fatty acid esters of aromatic alcohols; these enzymes are called arylesterases (EC 3.1.1.2). The enzymes differ in pH optimum and specificity for artificial substrates, including the chain length of the esterified fatty acid. Arylesterase-containing zones can be detected after starch or

polyacrylamide gel electrophoresis by incubation of the gel in a buffer-containing substrate and an agent to visualize enzyme activity. The physiological function of plasma arylesterases is still obscure, but their activity is associated with hypo- and hyperresponsiveness in several inbred strains of laboratory animals.

1. Arylesterase Isoenzymes

The relation between arylesterases and plasma cholesterol responsiveness was first noted in rats by Okamoto *et al.* (1972). The presence of an isoenzyme of high mobility on starch-gel electrophoresis was found to be associated with a diminished response of serum cholesterol to a high-cholesterol diet. Further work with rats showed that the cholesterolemic response was low in six of seven inbred strains that displayed the zone with high mobility (which in rats is called Es-1), whereas the absence of the enzyme was associated with the development of high degrees of hypercholesterolemia after cholesterol feeding in two of three inbred strains (Van Zutphen and Den Bieman, 1981). Similar results were obtained in six inbred strains of rabbits (Van Zutphen and Fox, 1977). Hyporesponsive rabbit strains displayed a high-mobility band on electrophoresis (called Est-2 here), but the hyperresponders did not. The *Est-2* genetic locus of the rabbit is assumed to be homologous with the *Es-1* locus in the rat (Fox and Van Zutphen, 1979). Figure 27 shows the zymogram of esterases in plasma from hypo- and hyperresponsive rabbit and rat strains.

2. Plasma Total Arylesterase Activity

The electrophoretic esterase pattern gives only qualitative information. We have therefore measured quantitative plasma esterase activities on low- and high-cholesterol diets in inbred strains of rabbits, rats, and mice. The qualitative difference in esterase pattern between hypo- and hyperresponsive rabbits (Fig. 27) corresponds with a quantitative difference in the plasma total esterase activity. The baseline plasma esterase activity was significantly higher in the inbred rabbit strain which is hyporesponsive to dietary cholesterol than in the hyperresponsive strain (Beynen *et al.*, 1984b). Cholesterol feeding (0.5% cholesterol) increased plasma total esterase activities in both strains but the activity in the hyporesponders remained higher than in the hyperresponsive rabbits (Beynen *et al.*, 1984b). Similar data were found in two inbred rat strains with high or low response of serum cholesterol to a diet containing 2% cholesterol and 0.5% cholate (Beynen *et al.*, 1984d). In seven inbred strains of mice there was no clear relation between the plasma esterase pattern after gel electrophoresis and the response of plasma cholesterol to the diet containing 2% cholesterol and

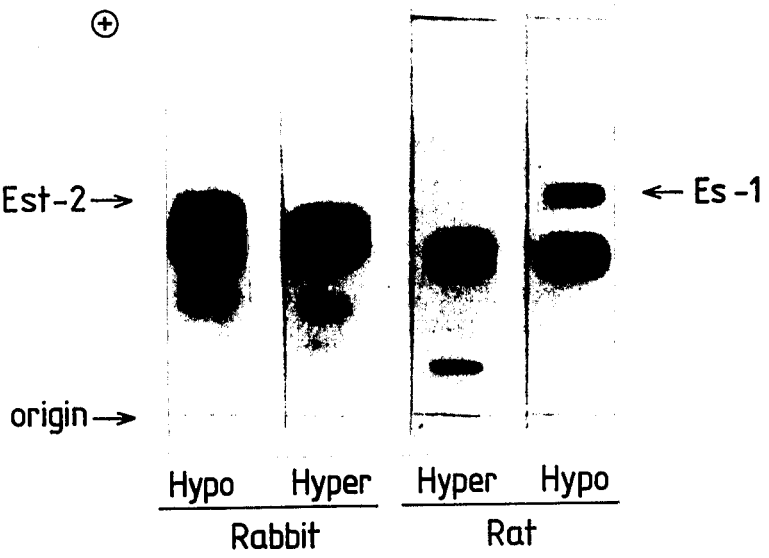

FIG. 27. Electrophoresis on starch gels of arylesterases in plasma from inbred strains of rats and rabbits on a low-cholesterol commercial diet. Naphthylpropionate was used as substrate for visualizing enzyme activity. (Reproduced with permission from Beynen *et al.*, 1985c.)

0.5% cholate (Beynen *et al.*, 1985d). Likewise there was no association between plasma total esterase activity on the low-cholesterol diet and the plasma cholesterol response to the high-cholesterol diet. However, in all strains plasma total esterase activity was increased upon cholesterol feeding (Beynen *et al.*, 1985d).

There is thus some evidence that arylesterases are associated with cholesterol metabolism and with the response to dietary cholesterol in the selected strains of rabbits and rats, but the evidence for such a role in the inbred strains of mice is inconclusive. It should be realized that plasma of the laboratory animals used contains at least 10 different arylesterases, most of them probably not related to cholesterol metabolism. It is therefore desirable to study and measure the various esterases separately.

We can only speculate about the role of arylesterases in cholesterol metabolism. One interpretation of the data for the inbred rats and rabbits is that a low esterase activity causes an increased susceptibility to dietary cholesterol, whereas induction of plasma esterase activity is required to compensate for cholesterol loading. It is also possible that the increase in esterase activity results from a release of esterases from the intestine induced by dietary cholesterol. In rats, Lewis and Hunter (1966) found that injection of fat into the stomach

caused a marked increase in the activity of esterases of high electrophoretic mobility in the intestinal lymph, and later also in the serum. The possible role of these esterases in cholesterol absorption remains to be elucidated. Alternatively, the increase in plasma esterase activity after cholesterol feeding may be an artifact due to cell damage in the liver. The use of inbred strains of animals with defined, but different plasma esterase patterns, may help to elucidate the functions of plasma arylesterases.

B. Obesity

People who are obsese have on the average slightly higher serum cholesterol levels than lean persons do (Albrink *et al.*, 1980). Obese Zucker rats are more responsive to hypercholesterolemic diets than their lean counterparts (Beynen *et al.*, 1983c). However, there is no evidence that obese humans are more susceptible to the effect of dietary cholesterol. On the contrary, Bronsgeest-Schoute *et al.* (1979) found that in free-living subjects who stopped eating eggs, the body mass index (weight/height^2) was negatively associated with the serum cholesterol reduction after cessation of egg consumption in 1976 ($r = -0.43$; $n = 34$; $p < 0.05$), but the relation was not found in our experiment in 1982 with the same group of subjects (Beynen and Katan, 1985b). However, in controlled laboratory experiments (Katan and Beynen, 1987) we also observed that a low body mass index is associated with a high serum cholesterol response to an increase in dietary cholesterol ($r = -0.50$; $n = 32$; $p < 0.05$). Thus human hyperresponders to dietary cholesterol appear to be on average leaner than hyporesponders.

How can this surprising finding be interpreted? First, as discussed in Section VII,C, there is some evidence that a low response to dietary cholesterol may be due to a large compensatory decrease in the rate of whole-body cholesterol synthesis. Individuals capable of depressing cholesterol synthesis most markedly showed the smallest increase in serum cholesterol on a cholesterol-rich diet. As cholesterol turnover is increased in human obesity (Nestel *et al.*, 1973), it could be that in obese subjects there is a wider range over which cholesterol synthesis can be down-regulated in response to an increased cholesterol intake. Likewise, the increase in cholesterol synthesis when dietary cholesterol is removed, may be greater in obese subjects. Thus in obese subjects changes in cholesterol intake may be effectively compensated by changes in cholesterol synthesis, which makes such persons hyporesponsive to dietary cholesterol. In any case, again it appears that high rates of basal cholesterol turnover are associated with a low response of serum cholesterol to dietary cholesterol (see Section VII,C). Storage of cholesterol in their fat tissue probably is not an important factor in determining sensitivity to dietary cholesterol (Section VII,G). Finally, the observed relationship could be spurious, and obesity could be acting as a surrogate variable for some other, more powerful determinant of responsiveness.

C. Habitual Cholesterol Intake

As discussed in Section III,D, the question whether in animals the response to hypercholesterolemic diets can be conditioned by diet or drug treatment in early life has not been settled. Similar experiments in humans have not yet been performed, but we do have some evidence for a relation between habitual cholesterol intake and responsiveness. In our controlled experiments, responsiveness to dietary cholesterol was found to be significantly and negatively correlated with the habitual cholesterol consumption before or in between experimental periods. The relation persisted upon multiple linear regression analysis (Katan and Beynen, 1987). Thus, egg eaters were less responsive to dietary manipulation. In view of the small number of subjects, these results should be interpreted with caution, and data on other samples of subjects are urgently needed. Mistry *et al.* (1981), at least, found no significant relation of the response of plasma cholesterol to egg yolk consumption with habitual cholesterol intake.

IX. Practical Considerations

We have reviewed the evidence that animals or humans exist with an unusually high or low responsiveness of serum cholesterol to dietary cholesterol and other dietary constituents. Though less pronounced than in animal models, this phenomenon of hyper- and hyporesponsiveness does appear to exist in humans. In addition, there is evidence that hyperresponsiveness to dietary cholesterol coincides with hyperresponsiveness to other hypercholesterolemic components of the diet, including saturated fatty acids.

The phenomenon of hyper- and hyporesponsiveness to diet may be of significance, since the known disorders such as familial hypercholesterolemia account for only a small percentage of the prevalence of hypercholesterolemia within affluent populations. It is important to note that the hyperresponders to egg yolk cholesterol in our studies (see Table II) had slightly, but consistently higher mean serum cholesterol values than their hyporesponding counterparts, both on their habitual and on standardized diets (Section VI,B,1). This may be the result of the differential sensitivity to dietary cholesterol and to saturated fat.

Another point of practical interest concerns the seemingly inevitable rise of serum cholesterol with age. In our experiments with subjects who ate at least one egg per day we found a mean increase with age of serum cholesterol over a period of 6 years of $\sim$0.3 mmol/liter (12 mg/dl). In men, the individual increase in serum cholesterol with age and the sensitivity of serum cholesterol to cessation of egg consumption were associated (Fig. 28). The correlation ($r = 0.42$; $n = 16$; $p = 0.11$) failed to reach statistical significance; in the women these variables were not correlated ($r = -0.14$;

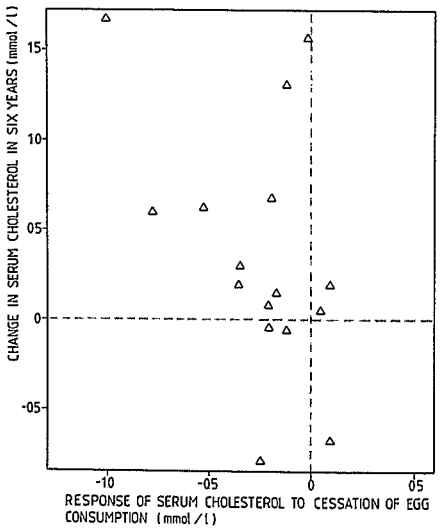

FIG. 28. Relationship between the short-term cholesterolemic response when egg eating is stopped and the change in serum cholesterol with age from 1976 to 1982 in men. The response of egg consumption is the mean of that in the trials of 1976 and 1982. (Reproduced with permission from Beynen and Katan, 1985b.)

$n = 15$). Although the evidence is thus hardly solid, it is tempting to speculate that the increase in serum cholesterol with age proceeds faster in hyperresponders than in hyporesponders, and that this is the result of a difference in sensitivity to diet.

How many persons among the population are truly hypo- or hyperresponder? The distribution of individual responsiveness can be best described relative to the group mean response of serum cholesterol. Assuming a normal distribution and using the between-person variation of the response corrected for within-person fluctuations of serum cholesterol, we calculated from our controlled trials (see Table II) that 10% of subjects will have a response of less than half of the mean response. Another 10% may have a responsiveness of $>150\%$ of the mean. The distribution of responses is thus quite narrow. These figures are very similar to those presented by Jacobs *et al.* (1983) for diets that differed both in the amount of cholesterol and the type of fat. Thus most subjects will show some response to a cholesterol-lowering diet, provided that adherence is good and the number of serum cholesterol measurements is sufficient.

Nevertheless, some subjects will respond only marginally or not at all, and this may have implications for counseling subjects who attempt to lower their serum cholesterol by diet. On the other hand, some subjects are

extremely sensitive to dietary saturated fat and cholesterol, and it is imperative that they restrict their intakes.

Identification of extreme hyper- and hyporesponders is greatly hampered by spontaneous, diet-independent within-person fluctuations of the level of serum cholesterol. Up until now, no simple test is available which discriminates hyper- from hyporesponders. Attempts to develop a rapid egg tolerance test (Mjassnikow, 1926; Sodhi *et al.*, 1979, 1981; Katan and Beynen, 1983) have met with uniform failure, both because the response of serum cholesterol to increased cholesterol intake develops too slowly and because of the within-subject fluctuations stressed throughout this review.

An improved understanding of the mechanism of hyper- and hyporesponsiveness should help in developing a better test. This may be complicated by the possible heterogeneity of human hyper- and hyporesponders in terms of underlying mechanisms. Furthermore, the between-person variation in response to diet may involve a considerable interaction between genotype and environmental factors, assuming that the magnitude of the response is genetically determined as it is in animal species. Genetic studies in humans have not been performed yet, but in our repeated studies the distributions of individual responses to dietary cholesterol did not show discrete subgroups, even though the subjects had originally been drawn from the opposite tails of the response distribution of the first experiment (see Table II). The use of genetically defined animals with different sensitivity to diet may be of help in developing a simple test to discriminate human hypo- from hyperresponders. Until that time, determination of responsiveness to diet requires large numbers of serum cholesterol determinations, and careful monitoring of dietary adherence.

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