INTER-INDIVIDUAL VARIATION IN THE CHOLESTEROLEMIC RESPONSE
TO DIETARY CHOLESTEROL

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INTRODUCTION

Among animals fed cholesterol-rich diets, certain in-
dividuals show only small increases in the level of serum
cholesterol (non-responders or hypo-responders), whereas
others develop high degrees of hypercholesterolemia (hyper-
responders). This phenomenon has been well-established in
animal species such as monkeys (Clarkson, Lofland, Bullock,
Goodman 1971; Eggen 1976), rabbits (Roberts, West, Redgrave,
Smith 1974; Van Zutphen, Fox 1977), rats (Imai, Matsumura
1973; Fig. 1) and pigeons (Wagner, Clarkson 1974). In these
animal species the difference in response to dietary choles-
terol has a strong genetic basis.

As early as 1933 Okey and Stewart reported their work
on the effect of dietary cholesterol on the level of serum
cholesterol in young women. It was found that the mean
serum cholesterol concentration of the subjects increased
somewhat on the cholesterol-supplemented diet, but there
was a considerable variability in individual response. The
striking inter-individual variation in cholesterolemic
response to dietary cholesterol was seen in most experi-
mental studies that followed. In the literature the con-
cept of human hyper- and non-responders to dietary choles-
terol became widely accepted (e.g. Connor, Connor 1972).

In the numerous studies in which the effect of
dietary cholesterol on serum cholesterol in humans was
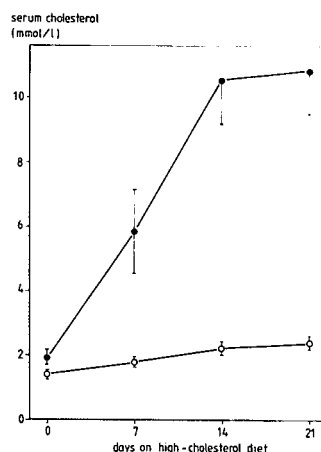assessed (for review, see McGill 1979), the response to

Fig. 1. Plasma cholesterol concentrations of two inbred
strains of rats. At day 0 the animals (4 per strain) were
transferred to the diet containing 2% (w,w) cholesterol and
0.5% cholate. 0, SHR/CpB strain and ●, SD/CpB strain from
the Central Institute for the Breeding of Laboratory Ani-
mals (CPB-TNO), Zeist, The Netherlands. Based on data taken
from Van Zutphen and Den Bieman (1981).

the dietary challenge in a given subject was usually
measured in one study only. The serum cholesterol concen-
tration of one individual fluctuates in time with a coef-
ficient of variation of 5 to 10% (Keys 1967). As a result
an appreciable random error is added to the response of
each subject's serum cholesterol. These error terms average
out when group means are considered, but in the commonly
used designs individuals cannot be reliably classified as
hyper- or hypo-responsive. It is thus not known whether the
observed pattern of cholesterolemic responses among the
subjects studied is reproducible.

Table 1 presents the only data we have been able to
find in the literature concerning subjects who partici-
pated twice in one type of experiment. The experiments were
performed in 1942 by Messinger and coworkers (1950). The
patients with various diseases were fed a daily dietary
supplement of 150 g of egg-yolk powder (providing 3750 mg
cholesterol) emulsified in milk. In the experiments typical
cholesterolemic responses were observed, the increases
ranging from 6 to 31%. Table 1 also shows that the response

Table 1. The effect of consumption of egg-yolk powder on the level of serum cholesterol (mmol/l)

Patient:	AK		JL		EZ		AS	
Exp.:	1	2	1	2	1	2	1	2
Pre-experimental	6.32	7.30	5.13	5.34	4.56	4.61	4.30	4.84
Experimental	7.02	7.72	6.71	5.67	5.36	5.34	5.26	5.83
Change	+0.70	+0.42	+1.58	+0.33	+0.80	+0.73	+0.96	+0.99

Each cholesterol value is the mean of 6 or 7 weekly determinations. The pre-experimental period lasted 48 days, and the experimental periods lasted 42 or 48 days. Data are taken from Messinger,Porosowska and Steele (1950).

was reproducible in only two subjects. Patient JL displayed the highest cholesterolemic response in the first experiment and the lowest response in the second experiment. It follows that the hitherto reported inter-individual variations in the cholesterolemic response to dietary cholesterol cannot be taken as evidence for the existence of human hyper- and non-responders. Theoretically, this variation could be explained by within-person variations in the level of serum cholesterol.

DO HUMAN HYPER- AND NON-RESPONDERS TO DIETARY CHOLESTEROL EXIST?

We have carried out repeated dietary trials with a large number of the same subjects in order to address the question whether individuals do exist with a consistently high or low serum cholesterol response to dietary cholesterol. Two types of experiments were performed. In one type, the effect of cessation of egg consumption on serum cholesterol was studied twice in the same subjects. In the other type of experiment, performed with other subjects, the subjects were repeatedly challenged with increased levels of dietary cholesterol.

Cholesterolemic Response to Cessation of Egg Consumption

In 1976 an experiment was carried out by our laboratory
to study the effect on serum cholesterol of removal of eggs
from the diet of subjects with a habitual egg-rich diet
(Bronsgeest-Schoute, Hermus, Dallinga-Thie, Hautvast 1979).
The subjects, who habitually consumed at least one egg/day,
were recruited by advertising. In the subjects serum chole-
sterol was measured (on two occasions within 3 days) while
they were on their habitual diet and again after a 3-week
period during which they were forbidden to consume any eggs
or egg-containing products. In 1982 17 men and 17 women
(aged 23 - 78 yr) out of the 44 subjects studied in 1976
participated again in a trial with exactly the same design
as that in 1976. Three subjects had drastically limited
their egg consumption after participation in the trial of
1976, but on our request they consumed at least one egg/day
during three weeks before the egg-free period.

The habitual diet of the 34 subjects contained 840 ±
437 (mean ± SD) mg cholesterol/day in 1976 and 811 ± 269 mg
in 1982. After cessation of egg consumption these values
were 303 ± 147 mg in 1976 and 245 ± 95 mg in 1982. The con-
tents of macro-nutrients did not change significantly after
removal of eggs from the diet. Table 2 documents that ces-
sation of egg consumption caused a small, but statistically
significant decrease of serum cholesterol levels. This was
seen both in 1976 and 1982. The individual response varied
considerably, the range being -1.27 to +0.51 mmol/l in 1976
(Table 2). The linear correlation coefficient between the
cholesterolemic responses in 1976 and 1982 was found to be
+0.32 (P<0.05).

Cholesterolemic Responses to Egg-Yolk Cholesterol

In 1982 three dietary trials were carried out with one
group of subjects. In the first and second experiment the
subjects consumed 10 mg/MJ cholesterol (on average about
110 mg/day) for 14 days followed by 55 mg/MJ (on average
about 550 mg/day) for another 14 days. Almost all food-
stuffs were supplied daily by our kitchen and intakes
were rigidly controlled. Not supplied was 1 MJ/day, which
the subjects were free to choose from a list of foodstuffs
not containing cholesterol. The diets contained 12% of
energy as protein and 42% as fat (polyunsaturated/saturated

Table 2. The effect of cessation of egg consumption on serum cholesterol measured in 1976 and again in 1982 in the same subjects.

	Serum total cholesterol (mmol/l)	
	1976	1982
On habitual diet	5.88 ± 1.07	6.17 ± 1.25
After cessation of egg consumption	5.72 ± 1.05	5.86 ± 1.13
Change	-0.16 ± 0.42[*]	-0.31 ± 0.35[*]
Range	-1.27 to +0.51	-0.94 to +0.48

Results are means ± SD for 34 subjects. [*] $P<0.05$.

fat ratio = 0.16). The low polyunsaturated/saturated fat ratio served to enhance the effect of dietary cholesterol on serum cholesterol (Bronsgeest-Schoute, Hautvast, Hermus 1979). Cholesterol was added to the diets as egg yolk, and the cholesterol-poor and -rich diets were formulated in such a manner that the cholesterol component was the only variable. Between the first and second experiment there was a period of 4 weeks, during which the subjects resumed their habitual diets. Six months after the second experiment a third experiment was performed. In this experiment the subjects were provided only with certain foodstuffs, namely high-fat bread (polyunsaturated/saturated fat ratio = 0.08), margarine (polyunsaturated/saturated fat ratio = 0.19), salads, salad dressing, cookies and desserts. During the cholesterol-rich period the salads and desserts contained egg yolk. The subjects received 15 mg/MJ of cholesterol for 4 weeks followed by 85 mg/MJ for another 4 weeks. During the first and second experiment blood samples were taken twice on each diet; during the third experiment blood was sampled 6 times on each diet. Serum cholesterol was analysed under conditions of rigid control as described elsewhere (Katan, Van der Haar, Kromhout, Schouten 1982).

In the first experiment 94 men and women, aged 18 - 72 years, participated. Dietary cholesterol caused an increase in serum cholesterol of 0.50 ± 0.39 mmol/l (mean ± SD), with individual responses ranging from -0.62 to +1.63 mmol/l. Eighteen non-responders and 23 hyper-responders with mean increases of 0.00 and 0.98 mmol/l of serum cholesterol were

selected to participate in the second experiment. Fifteen of
the non-responders and 17 of the hyper-responders subsequent-
ly took part in the third experiment.

Table 3 shows the results for the 32 subjects who parti-
cipated in all three trials. In experiment 1 the mean in-
creases in serum cholesterol were about zero for the non-
-responders and almost 1 mmol/l for the hyper-responders.
This is not surprising since we had selected the two groups
according to their responses at this stage. In the second
experiment the difference in response between the putative
hyper- and non-responders was less pronounced, but the hyper-
-responders did show a higher mean increase in serum chole-
sterol. The mean increase in serum cholesterol for all the
subjects (n = 41) that participated in experiment 2 was 0.13
mmol/l. This was much lower than in the first and third ex-
periment, suggesting that the subjects had temporarily become
refractory to the effect of egg-yolk cholesterol. It is not
known at present what caused this unexpected observation.
In the third experiment the cholesterolemic response in the
non-responders of experiment 1 was higher than in the previous
experiments, but their response was only half of that seen in
the hyper-responders. Thus, the hyper-responders selected in
experiment 1 consistently displayed a greater cholesterolemic
response in the two further experiments.

In the one type of experiment -cessation of egg consump-
tion- a positive, statistically significant correlation was
found between the cholesterolemic responses in 1976 and 1982.
In the other type of experiment -egg-yolk feeding- the se-
lected hyper-responders consistently showed a higher chole-
sterolemic response than the selected non-responders. It is,
however, obvious that the response per subject is only
partly reproducible from one study to another. Most likely
this is due to the limitations of our studies. During the
study on the effect of cessation of egg consumption on serum
cholesterol the diets were not controlled and only two blood
samples per period were taken. Likewise in experiments 1 and
2 of egg-yolk feeding also only two serum cholesterol deter-
minations per dietary period were performed. Nevertheless,
on the basis of our data, we conclude that human hyper- and
non-responders to dietary cholesterol do exist. However, it
will   not be easy to identify true hyper- and non-respon-
ders. Repeated, long lasting trials and many serum cholesterol
determinations will be needed.

Table 3. Effect of egg-yolk cholesterol on serum cholesterol (mmol/l) in 3 controlled trials with the same subjects.

	Exp. 1		Exp. 2		Exp. 3	
	Non-responders	Hyper-responders	Non-responders	Hyper-responders	Non-responders	Hyper-responders
On low-cholesterol diet	5.14 ± 1.04	5.03 ± 0.84	5.04 ± 1.05	5.26 ± 0.91	5.02 ± 0.96	5.25 ± 0.86
On high-cholesterol diet	5.13 ± 1.04	5.99 ± 0.98	5.10 ± 0.94	5.55 ± 1.05	5.49 ± 0.98	6.07 ± 0.99
Change	-0.01 ± 0.21	0.96 ± 0.27	0.06 ± 0.35	0.28 ± 0.38*	0.47 ± 0.26	0.82 ± 0.35**
Range	-0.62 to +0.22	+0.58 to +1.50	-0.90 to +0.55	-0.31 to +0.92	+0.10 to +0.90	+0.26 to +1.62

Results are expressed as means ± SD for 15 non-responders (9 males and 6 females) and 17 hyper-responders (12 males and 5 females). The cholesterol values on both diets for each individual were the means of two determinations in experiments 1 and 2, and the means of 6 determinations in experiment 3. Change significantly different from that in the non-responders (one-tailed Student's t-test):
* P<0.05, ** P<0.005.

UNDERLYING MECHANISMS

The individual variability in the cholesterolemic res-
ponse to dietary cholesterol must lie in differences in the
efficiency of compensatory mechanisms. In man the responses
to increased amounts of ingested cholesterol are generally
a diminished synthesis of cholesterol in the body and an
enhanced excretion of neutral steroids (Quintão, Grundy,
Ahrens 1971; Nestel, Poyser 1976) or bile acids (Lin, Connor
1980). Sterol balance studies have shown that on high-chole-
sterol diets there can also be a net storage of cholesterol
in the body (Quintão, Grundy, Ahrens 1971; Lin, Connor 1980).
Cholesterol absorption increases with cholesterol intake in a
linear fashion (Quintão, Grundy, Ahrens 1971) and consequent-
ly absorption cannot be regarded as a compensatory mechanism.

Quintão and colleagues (1971) found a marked variation
between individuals in the response in compensatory mecha-
nisms to high amounts (up to 4 g/day) of crystalline chole-
sterol in the diet. No clear relationship could be detected
between the increases in plasma cholesterol concentrations
and the effectiveness of the metabolic compensatory mechanisms.
Nestel and Poyser (1976) studied nine subjects on a low-
-cholesterol diet (about 300 mg cholesterol/day) and on a
high-cholesterol diet (about 800 mg cholesterol/day), the
increase in dietary cholesterol being derived from dried egg-
-yolk powder. Their data reveal that the increase in serum
cholesterol is related to the decrease in whole-body chole-
sterol synthesis; the individuals capable of depressing
cholesterol synthesis most markedly showed the smallest in-
crease in serum cholesterol while on the cholesterol-rich
diet (Fig. 2).

Mistry and coworkers (1981) have measured LDL (low-
density lipoprotein) receptor activity in blood mononuclear
cells after derepression by preincubation for 72 h in lipo-
protein-deficient medium. The mononuclear cells were isolated
from subjects while on their habitual diet. The same subjects
then consumed six egg yolks daily (which corresponds to about
1500 mg cholesterol/day) for 14 days. It was found that the
increment in plasma cholesterol concentrations in the sub-
jects (n = 18) was negatively associated with LDL receptor
activity (r =-0.74; P<0.001). Thus, it would appear that
hyper-responders have less LDL receptor activity at their
blood mononuclear cells than non-responders. This would imply
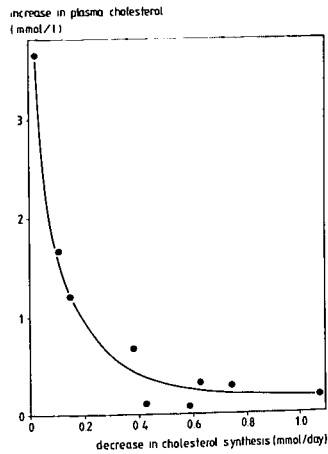that hyper-responders have a reduced capacity to catabolize

Fig. 2. Relationship between the decrease in whole-body cholesterol synthesis and the increase in serum cholesterol in subjects that increased their cholesterol intake from about 300 mg/day to 800 mg cholesterol/day. The dietary periods lasted 4-6 weeks, and feces were collected during the final 8 days of each period. Cholesterol synthesis equals total fecal sterols excreted minus dietary cholesterol. Plasma cholesterol concentrations were the means of two to three times weekly determinations during both periods. Based on data taken from Nestel and Poyser (1976).

LDL, the lipoprotein which carries most of the excess of plasma cholesterol upon cholesterol feeding. Since cholesterol synthesis is subject to feed-back inhibition by cholesterol derived from LDL taken up by the receptor-mediated pathway (Brown, Goldstein 1976), this mechanism of down-regulation of cholesterol synthesis would be defective in hyper-responders. Indeed, Fig. 2 illustrates that in subjects with the most pronounced increases in plasma cholesterol the reduction in cholesterol synthesis is negligible.

EFFECTS OF DIETARY COMPONENTS OTHER THAN CHOLESTEROL ON SERUM CHOLESTEROL IN HYPER- AND NON-RESPONDERS TO DIETARY CHOLESTEROL

It is important to know whether hyper-responders to dietary cholesterol are also hyper-responsive to other dietary

components, as such information may provide further clues to
the mechanisms underlying the differential cholesterolemic
response to cholesterol. At present, this information is not
available for human hyper-responders, but we do have some in-
formation on rabbits.

In young, growing rabbits cholesterol-free, semipurified
diets containing casein as a protein source produce hyper-
cholesterolemia, but no such effect is observed with soybean
protein (West, Beynen, Terpstra, Scholz, Carroll, Woodward
1983). There are marked differences between individual rab-
bits in the cholesterolemic response to casein (Beynen, Den
Engelsman, Scholz, West 1983). We have addressed the question
whether animals hyper- and hypo-responsive to dietary choles-
terol are also hyper- and hypo-responsive, respectively, to
dietary casein. For this purpose, the same rabbits were fed
the semipurified diet containing cholesterol and that con-
taining casein, each for a period of about 3 weeks. Between
these periods the hypocholesterolemic diet containing soy
protein was fed, so that the serum cholesterol levels re-
turned to base-line values. Significant correlations were
found between the cholesterolemic responses to cholesterol
and casein (Fig. 3). Thus, at least in rabbits, the concept
of hyper- and non-responders to dietary cholesterol also
extends to another hypercholesterolemic dietary factor.

PRACTICAL CONSIDERATIONS

The phenomenon of hyper- and non-responsiveness to
dietary cholesterol is probably of major significance since
the known disorders such as familial hypercholesterolemia and
combined hyperlipemia account for only a small percentage of
the variation in serum cholesterol concentrations within po-
pulations. It is important to note that the hyper-responders
to egg-yolk cholesterol in our studies (Table 3) had consis-
tently higher mean serum cholesterol values than their non-
responding counterparts both on their habitual and on stan-
dardized experimental diets. This may be the result of the
differential sensitivity to dietary cholesterol and to
dietary fat.

There is considerable evidence that high serum choles-
terol levels cause atherosclerotic diseases. In order to
lower serum cholesterol it is widely recommended to limit
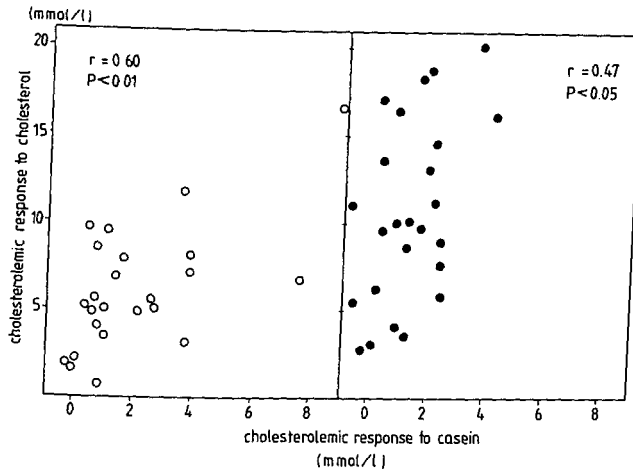cholesterol intake and to increase the intake of polyunsa-

Fig. 3. Correlations between the cholesterolemic responses
produced by dietary cholesterol and casein in rabbits.
Rabbits were fed three semipurified diets, namely a chole-
sterol-free diet containing 21% (w,w) soy protein, a chole-
sterol-free diet with 21% (w,w) casein, or a diet con-
taining soy protein plus 0.2% cholesterol. One group (0)
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 supple-
mented with cholesterol. At the beginning of the experiment
the animals were aged 15 weeks. Based on data from Beynen
et al. (1983).

turated fats at the expense of saturated fats. However, such
dietary advice would probably only be effective in the hyper-
-responding segment of the population. A simple test for the
identification of hyper- and non-responders would therefore
be desirable. Up until now, however, no simple test is
available which discriminates hyper- from non-responders.
An improved understanding of the mechanism of hyper-respon-
siveness would help in developing such a test.

ACKNOWLEDGEMENTS

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