INTER-INDIVIDUAL VARIATION IN THE CHOLESTEROLEMIC RESPONSE TO DIETARY CHOLESTEROL

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INTRODUCTION

Among animals fed cholesterol-rich diets, certain individuals 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 cholesterol 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 cholesterolemia response to dietary cholesterol was seen in most experimental studies that followed. In the literature the concept of human hyper- and non-responders to dietary cholesterol 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 Animals (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 concentration of one individual fluctuates in time with a coefficient 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 participated 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)

<table>
<thead>
<tr>
<th>Patient:</th>
<th>AK</th>
<th>JL</th>
<th>EZ</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pre-experimental</td>
<td>6.32</td>
<td>7.30</td>
<td>5.13</td>
<td>5.34</td>
</tr>
<tr>
<td>Experimental</td>
<td>7.02</td>
<td>7.72</td>
<td>6.71</td>
<td>5.67</td>
</tr>
<tr>
<td>Change</td>
<td>+0.70</td>
<td>+0.42</td>
<td>+1.58</td>
<td>+0.33</td>
</tr>
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</table>

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 cholesterol 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 contents of macro-nutrients did not change significantly after removal of eggs from the diet. Table 2 documents that cessation 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 foodstuffs 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.

<table>
<thead>
<tr>
<th></th>
<th>Serum total cholesterol (mmol/l)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1976</td>
</tr>
<tr>
<td>On habitual diet</td>
<td>5.88 ± 1.07</td>
</tr>
<tr>
<td>After cessation of egg consumption</td>
<td>5.72 ± 1.05</td>
</tr>
<tr>
<td>Change</td>
<td>-0.16 ± 0.42 &amp;</td>
</tr>
<tr>
<td>Range</td>
<td>-1.27 to +0.51</td>
</tr>
</tbody>
</table>

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 0.08 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 subse-
quent-
ly took part in the third experiment.

Table 3 shows the results for the 32 subjects who partic-
ipated 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 cholesterolemia 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 cholesterolemia
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.

<table>
<thead>
<tr>
<th></th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-responders</td>
<td>Hyper-responders</td>
<td>Non-responders</td>
</tr>
<tr>
<td>On low-cholesterol diet</td>
<td>5.14 ± 1.04</td>
<td>5.03 ± 0.84</td>
<td>5.04 ± 1.05</td>
</tr>
<tr>
<td>On high-cholesterol diet</td>
<td>5.13 ± 1.04</td>
<td>5.99 ± 0.98</td>
<td>5.10 ± 0.94</td>
</tr>
<tr>
<td>Change</td>
<td>-0.01 ± 0.21</td>
<td>0.96 ± 0.27</td>
<td>0.06 ± 0.35</td>
</tr>
<tr>
<td>Range</td>
<td>-0.62 to +0.22</td>
<td>+0.58 to +1.50</td>
<td>-0.90 to +0.55</td>
</tr>
</tbody>
</table>

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 response 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, Poyer 1976) or bile acids (Lin, Connor 1980). Sterol balance studies have shown that on high-cholesterol 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 consequently absorption cannot be regarded as a compensatory mechanism.

Quintão and colleagues (1971) found a marked variation between individuals in the response in compensatory mechanisms to high amounts (up to 4 g/day) of crystalline cholesterol 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 Poyer (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 cholesterol synthesis; the individuals capable of depressing cholesterol synthesis most markedly showed the smallest increase 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 dererepression by preincubation for 72 h in lipoprotein-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 subjects (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
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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 feedback 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 information on rabbits.

In young, growing rabbits cholesterol-free, semipurified diets containing casein as a protein source produce hypercholesterolemia, but no such effect is observed with soybean protein (West, Beynen, Terpstra, Scholz, Carroll, Woodward 1983). There are marked differences between individual rabbits 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 cholesterol 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 containing 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 returned 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 populations. It is important to note that the hyper-responders to egg-yolk cholesterol in our studies (Table 3) had consistently higher mean serum cholesterol values than their non-responding counterparts both on their habitual and on standardized 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 cholesterol 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 cholesteolemic responses produced by dietary cholesterol and 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 (O) 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. (1983).

Saturated 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-responsiveness would help in developing such a test.

ACKNOWLEDGEMENTS

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REFERENCES


