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INTER-INDIVIDUAL VARIATION IN THE RESPONSE OF SERUM CHOLESTEROL TO CHANGES IN DIET

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Evidence is presented that persons exist with a consistently high (hyperresponders) or low (hyporesponders) response of the concentration of serum cholesterol to a change in diet. Possible mechanisms underlying hypo- and hyperresponsiveness are discussed on the basis of data available from both human and animal studies. 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 spontaneous, diet-independent within person fluctuations in the level of serum cholesterol. As yet, monitoring a person's response to diet should be based on relatively large number of serum cholesterol determinations.

KEY WORDS: hyperresponders — hyporesponders — serum cholesterol — dietary cholesterol — dietary fatty acids

The type of fat and the amount of cholesterol in the diet affect the level of serum cholesterol in man. The effects of these dietary components can be predicted using empirical formulas (14–16). However, such predictions of serum cholesterol changes only hold for group means and not for individual subjects. It has been proposed (9) that in certain individuals (hyporesponders) the level of serum cholesterol is insensitive to diet, whereas in others (hyperresponders) there are pronounced effects. Only recently this concept has been substantiated by experimental evidence in man. In this communication we present evidence that human hypo- and hyperresponders do exist and we describe this phenomenon in both animals and man.

HYP0- AND HYPERRESPONDERS AMONG ANIMALS

The feeding of cholesterol-rich diets to random-bred animals results in marked inter-individual differences in the response of serum cholesterol. Clarkson and coworkers (8) observed this phenomenon in squirrel monkeys (*Saimiri sciureus*) and showed that it was at least partly genetically determined. Eggen (10) reported individual differences in the response of serum cholesterol to a high-fat, high-cholesterol diet in rhesus monkeys (*Macaca mulatta*).

In studies with inbred strains hypo- and hyperresponsiveness to dietary cholesterol has been clearly demonstrated. Wagner and Clarkson (25) performed studies with a hypo- and hyperresponsive strain of Show Racer pigeons. Van Zutphen and Fox (24) have tested six inbred strains of rabbits (Table 1) and found a pronounced between-strain difference in the response to dietary cholesterol. Such differences were also observed when inbred strains of rats were challenged with a high-cholesterol, high-cholelate diet (1, 23).

Table 1

Effect of dietary cholesterol on plasma cholesterol levels in inbred strains of rabbits

Strain	Plasma cholesterol, mmol/l	
	day 0	day 21
III VO/J	0,7 ± 0,1	8,0 ± 1,9
WH/J	0,3 ± 0,0	8,0 ± 0,8
X/J	0,9 ± 0,3	17,3 ± 4,2
ACEP/J	0,7 ± 0,0	18,9 ± 3,5
OS/J	0,8 ± 0,1	29,4 ± 4,8
AX/J	0,6 ± 0,1	33,6 ± 3,1

Results are expressed as means ± SE for five male animals per strain. Up until day 0 of the experiment all rabbits received a cholesterol-free, commercial diet; then, 0,5% (ww.) cholesterol was added to the diet. Data taken from Van Zutphen and Fox (24).

Hyper- and hyporesponsiveness also extends to other dietary components known to affect serum cholesterol levels. 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.

In a study with random-bred rabbits we found that animals hypo- and hyperresponsive to dietary cholesterol are also hypo- and hyper-responsive, respectively, to dietary casein (3). This observation has recently been extended to inbred strains (Table 2). A rabbit strain hyperresponsive to dietary cholesterol also showed a significantly higher response of plasma cholesterol to casein than the hyporesponsive strain. In this study the rabbits were fed for periods of 4 weeks on cholesterol-free, semipurified diets which differed in the source of protein only (4).

Table 2

Plasma cholesterol responses to dietary variables in inbred rabbits

	Change in plasma cholesterol, mmol/l	
	Hyporesponder n=4	Hyperresponder n=4
Cholesterol vs no cholesterol	4,39 ± 2,25	15,20 ± 9,73*
Casein vs soy protein	-0,36 ± 0,008	1,28 ± 0,96*
Coconut fat vs corn oil	0,64 ± 0,77	4,93 ± 3,70*

Results are expressed as means ± SD. Change in plasma cholesterol is the difference between values at the end of the dietary periods indicated.

* Significantly different from hyperresponders: $P < 0.05$ (Student's test *t*). After Beynen et al. (4).

In the two inbred strains of rabbits we have also 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. Table 2 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 hyperresponsiveness to dietary cholesterol and to the type of fatty acids coincided (4). A similar association, although weaker, was found in random-bred rabbits (5).

HUMAN HYPO- AND HYPERRESPONDERS

In the numerous studies which have dealt with the effect of dietary cholesterol on serum cholesterol levels in man, a striking variability in the response between subjects was generally seen. However, the response of serum cholesterol to the dietary challenge in a given subject was usually measured in one study only. Studies with up to six subjects tested twice (12, 18), showed that a subject hyperresponsive to the addition of egg yolk to the diet in one experiment may appear hyporesponsive in the second experiment and vice versa (Table 3). Thus, the observed difference in response could be due to random fluctuations. So-called "spontaneous" fluctuations in serum cholesterol concentrations can be as high as 20% in both directions, and these fluctuations are of the same order of magnitude as the generally observed response to dietary cholesterol loads. Thus, observed differences in serum cholesterol response between individuals are not necessarily due to stable, true differences in sensitivity to dietary cholesterol.

Table 3

Changes in serum cholesterol levels in six human volunteers after daily consumption of six egg yolks for 10 days

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

Twelve months elapsed between Expt 1 and Expt 2; the design was otherwise identical. The pre-experimental and experimental serum cholesterol values were both based on two blood samples obtained on successive days. After Katan and Beynen (12).

We have carried out three controlled dietary trials (12, 13) 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- (about 120 mg of cholesterol/day) and a high-cholesterol diet (about 650 mg/day in the first and second experiment and about 1000 mg/day in the third trial), the cholesterol component of the diets (provided by egg yolk) being the only variable. Putative hyper- ($n = 17$) and hypo-responding subjects ($n = 15$) with mean serum cholesterol increases

of 0 and 19% were selected in the first trial and participated in the second and third experiment. Although the response in each subjects was only partly reproducible, the selected hyper-responders showed significantly higher serum cholesterol responses in the second and third trial than did the hypo-responders (Table 4).

Table 4

Effect of egg-yolk cholesterol on serum cholesterol in three controlled trials with the same subjects

	Change in serum cholesterol (mmol/l)	
	Hyperresponders n=15	Hypo-responders 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**

Results are expressed as means ± SD. Change significantly different from that in the hypo-responders (one-tailed Student's t test);

* P < 0.05; ** P < 0.005. Based on Katan et al. (13).

Standardized regression coefficients for individual responses in two experiments ranged from 0.34 to 0.53 (n = 32).

We have found that the results of these laboratory experiments can be extrapolated to a field situation. In 1976 the serum cholesterol response to cessation of egg consumption was studied in subjects who habitually consumed at least one egg/day (7). Daily cholesterol intake decreased from about 800 mg to 300 mg. Mean serum cholesterol fell slightly (by 3%), but the individual responses varied from -20% to +8%. In 1982, 34 of the subjects were re-investigated and on our request they again eliminated eggs and egg-containing products from their diet. It was found that the differences in serum cholesterol response between individuals were still partly reproducible (12). Thus it appears from our work that at least part of the cholesterolemic response to dietary cholesterol in man is individually determined.

Jacobs et al. (11) recently re-analysed data from dietary trials performed from 1963 to 1966 in Minnesota, (USA). In these experiments the amount of cholesterol and the type of fat were the dietary variables. Forty eight subjects had participated in two or more diet experiments and at least two serum cholesterol values per dietary period were known. It was found that 40 of these subjects showed a consistent response of serum cholesterol from one experiment to another. In these subjects the range of responsiveness across experiments did not exceed 70 to 130% of the value predicted by the empirical formula of Keys and coworkers (14-16).

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 had participated in three controlled trials on the effect of dietary cholesterol (13) 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.

From these studies with humans however, it is also clear that one always finds subjects who appear hyperresponsive in one experiment and hyporesponsive in another. This is caused by diet-independent within-person variability of serum cholesterol. From our studies (13) in which dietary cholesterol was the only variable, we calculated that the within-person variation (expressed as standard deviation) while on a constant diet was of the same order of magnitude as the true between-person variation in serum cholesterol response to dietary cholesterol. Thus the response determined in an individual in one experiment contains a large error term. This term can only be reduced by reduced by increasing the number of serum cholesterol measurements, and even then the calculated response should be interpreted with caution.

DISTRIBUTION OF RESPONSIVENESS WITHIN THE POPULATION

How many persons among the population are truly hypo- or hyper-responder? 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 observed true between-person variation of the response, we calculated from our controlled trials (13) that 10% of subjects will have a response of less than half of the mean response. Another 10% may have a responsiveness of more than 150% of the mean. The distribution of response is thus quite narrow. These figures are very similar to those presented by Jacobs et al. (11) for diets that differed in several components. Thus, it can be suggested that most patients will show some response to a cholesterol-lowering diet if adherence is good and the number of serum cholesterol measurements are sufficient.

MECHANISMS UNDERLYING HYPO- AND HYPERRESPONSIVENESS TO DIETARY CHOLESTEROL

The individual variability in cholesterolemic response must be due to differences in absorption and/or the efficiency of compensatory mechanism. 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 (21). There are at least two competing theories to explain hypo- and hyperresponsiveness. One theory proposes that hyperresponders more efficiently absorb cholesterol than hyporesponders. The other theory proposes that after cholesterol feeding hyperresponders less efficiently suppress cholesterol synthesis than hyporesponders.

Studies with rhesus monkeys (10), squirrel monkeys (17) and African green monkeys (22) have shown that hyperresponders absorb a higher percentage of intestinal luminal cholesterol than hyporesponders. This is in agreement with the observation that hyporesponsive monkeys (6, 17), as well as humans (2), have higher rates of cholesterol synthesis on low-cholesterol diets than hyperresponders. It would be anticipated that in order to maintain a steady-state of cholesterol metabolism high rates of cholesterol absorption (either dietary cholesterol or cholesterol excreted with bile) are associated with low rates of de novo cholesterol synthesis.

On the basis of the fact that hyporesponders have higher rates of cholesterol synthesis than hyperresponders it could be suggested that the former should be able to suppress cholesterol synthesis over a wider range than hyperresponders. Indeed, Nestel and Poyser (20) have presented evidence that human subjects capable of reducing whole-body cholesterol synthesis (calculated from sterol balance data) most markedly, showed the smallest increase in plasma cholesterol upon cholesterol feeding. In addition, Mistry et al. (19) showed that upon cholesterol consumption the percentage decrease in 3-hydroxy-3-methylglutaryl CoA reductase activity in freshly isolated blood mononuclear cells was inversely related ($r = 0,49$, $n = 37$, $P < 0,01$) to the percentage increase in plasma cholesterol concentration; the reductase is the rate-limiting enzyme in de novo cholesterol synthesis.

PRACTICAL CONSIDERATIONS

The phenomenon of hyper- and hyporesponsiveness to diet 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 hyperresponders to egg-yolk cholesterol in our studies (13) had consistently higher mean serum cholesterol values than their hyporesponders counterparts while on their habitual 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 polyunsaturated fats at the expense of saturated fats. However, such dietary advice would probably only be effective in the hyperresponding segment of the population. A simple test for the identification of hyper- and hyporesponders would therefore be desirable. Up until now, however, no simple test is available which discriminates hyper- from hyporesponders. An improved understanding of the mechanism and genetics of hyperresponsiveness would help in developing such a test.

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MIĘDZYOSOBNICZA ZMIENNOŚĆ ODPOWIEDZI CHOLESTEROLU SUROWICY NA ZMIANY DIETY

W pracy przedstawiono w oparciu o wyniki wieloletnich badań własnych poglądy na temat odpowiedzi stężenia cholesterolu na takie składniki diety jak: cholesterol pokarmowy, białko i kwasy tłuszczowe. Przeprowadzone testy dietetyczne

позволили на выделение гипер- и гипореспондентов в отношении к холестерину кормовому, казеину и жиру кокосовому. Оказалось, что на общие люди реагирующие значительным ростом холестерина в сыворотке на холестерин кормовый являются также гиперреспондентами в отношении к остальным wymienionym składnikom pokarmowym (табела 2). Особнически надмиерно чувствительные выказывают вышнее положение холестерина перед введением в диету тестового компонента и наоборот, гипореспонденты имеют вышнее содержание холестерина на обычной диете. Авторы считают, что это может быть результатом различия в чувствительности к холестерину и жиру. Хотя у каждого лица ответ был только частично восстановлен, но у избранных гиперреспондентов обнаружилось характерно вышнее содержание холестерина во время второго и третьего исследования, чем у гипореспондентов (табл. 4).

Явление вышнего и вышнего ответа на пищевые компоненты может иметь практическое значение для консултации у лиц, предпринимающих усилия понизить содержание холестерина при помощи диеты. Все-таки идентификация настоящих гипер- и гипореспондентов мешает самопроизвольные, независимые от диеты внутрииндивидуальные изменения уровня холестерина в сыворотке. Оценка индивидуального ответа на диету в настоящее время должна основываться на относительно большом количестве определений концентрации холестерина.

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МЕЖИНДИВИДУАЛЬНАЯ ИЗМЕНЧИВОСТЬ ОТВЕТА ХОЛЕСТЕРИНА СЫВОРОТКИ НА ИЗМЕНЕНИЕ ДИЕТЫ

В труде на основании результатов многолетних исследований представили собственное мнение на тему ответа концентрации холестерина на следующие компоненты диеты: пищевой холестерин, белок, жирные кислоты. Проведенные диететические тесты позволили выделить гипер- и гипореспондентов по отношению к пищевому холестерину, казеину и кокосовому маслу. Оказалось, что люди, обычно реагирующие значительным повышением концентрации холестерина в сыворотке на пищевой холестерин, являются также гиперреспондентами по отношению к остальным вышеупомянутым пищевым компонентам (табл. 2). У чрезмерночувствительных индивидуумов обнаружили более вышнее содержание холестерина до введения в диету наблюдаемого компонента, и наоборот — у гипореспондентов более низкая концентрация холестерина в обычной диете. Авторы считают, что этот момент может быть именно результатом дифференцированной межиндивидуальной чувствительности к холестерину и пищевому жиру. Еще дважды оценивали у избранных гипер- и гипореспондентов ответ на пищевой холестерин. Хотя у каждого лица ответ был только частично восстановлен, но у избранных гиперреспондентов обнаружилось характерно более вышнее содержание холестерина во время второго и третьего исследования, чем у гипореспондентов (табл. 4).

Явление вышнего и вышнего ответа на пищевые компоненты может иметь практическое значение для консултации у лиц, предпринимающих усилия понизить концентрацию холестерина при помощи диеты. Все-таки идентификация настоящих гипер- и гипореспондентов мешает самопроизвольные, независимые от диеты внутрииндивидуальные изменения уровня холестерина в сыворотке. Оценка индивидуального ответа на диету в настоящее время должна основываться на относительно большом количестве определений концентрации холестерина.

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