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Differences in individual responsiveness of serum cholesterol to fat-modified diets in man

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Abstract. In the period 1963–1974, 82 monks and 48 nuns from five Dutch and Belgian Trappist monasteries each participated in two or more out of nine different trials designed to test the effect of 58 different fat-modified diets on serum cholesterol. We analysed these data to quantify the extent to which healthy, normolipaemic subjects differ in the responsiveness of their serum cholesterol to a change in dietary fatty acid composition. Statistically significant between-person variance components (SD_p^2) were found in the serum cholesterol responses for the whole group ($SD_p^2=[0{\cdot}20\ \text{mmol l}^{-1}]^2$), for the men ($SD_p^2=[0{\cdot}24\ \text{mmol l}^{-1}]^2$) and for those women who participated in three or more trials ($SD_p^2=[0{\cdot}14\ \text{mmol l}^{-1}]^2$). The between-person variation (expressed as SD) was on average only half as large as the within-person variation in response when the same subject was challenged repeatedly. It is concluded that medically significant differences in responsiveness to fat-modified diets exist in both men and women. However, few subjects fail entirely to respond to a change in dietary fatty acid composition. In addition, the large within-subject variability makes it difficult to identify hypo- and hyperresponders.

Keywords. Cholesterol, unsaturated fatty acids, essential fatty acids, individuality.

Introduction

In several animal species individuals or inbred strains may differ in the responsiveness of their serum cholesterol to hypercholesterolaemic diets [1–4]. These differences appear to have a genetic basis. Reliable data on the existence of hypo- and hyperresponders in man have been hard to come by, because prolonged observations of subjects, preferably spanning multiple experiments, are needed. However, variability in the responsiveness of serum cholesterol to dietary cholesterol and fatty acid composition has been described in man [5–8]. The differences in response to dietary cholesterol between humans are at least partly reproducible and stable over a prolonged period [9]. Less information is available on the response to dietary fatty acids.

Here we describe the extent of variability in innate responsiveness to dietary fatty acid composition in 130 men and women who had each participated in multiple, thoroughly controlled trials.

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Patients and methods

Design

From 1963 to 1974 the Unilever Research Laboratory in Vlaardingen, The Netherlands, carried out nine dietary trials designed to test the cholesterol-lowering effect of various unsaturated fatty acids [10–16]. Each trial lasted 4–6 weeks. The time between trials varied from almost 1 to almost 3 years; during that time all subjects consumed the habitual lacto-vegetarian monastery fare. Each participant reported on here served in several trials, but each received only one experimental diet per trial. In each trial, between three diets and ten diets were compared; the number of persons receiving the same diet was about eight (range: 1–20). In each experiment the diet groups were matched for age, sex, monastery and for baseline serum cholesterol level when on the habitual diet. The participants of the trials, which were mostly carried out during Lent, were Trappist monks from three monasteries in Tilburg, Achelse Kluis and Zundert (The Netherlands), and nuns from two monasteries in Berkel Enschot (The Netherlands) and Brecht (Belgium).

Before and during the experiment one to four blood samples were drawn, and one to three blood samples were taken several weeks after completing the trial (see Fig. 1). Cholesterol values during the experiment were usually obtained at least 2 weeks after the start of the liquid formula feeding; by that time, levels had stabilized [12, 13]. Serum cholesterol was estimated according to Abell *et al.* [17].

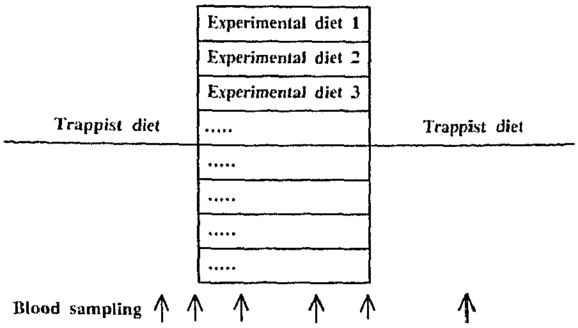

Figure 1. Design and blood sampling in a typical trial. Blood was drawn before, during and after the trial. One to four blood samples were obtained before, one to four during and one to three after the diet period. One trial was performed each year from 1963 to 1974, except for 1965, 1966 and 1969.

Subjects

The subjects involved in the studies were healthy Trappist monks and nuns mainly living on a lacto-vegetarian diet. In 1969 mean ages were $53{\cdot}1 \pm 12{\cdot}8$ years for the monks and $43{\cdot}4 \pm 18{\cdot}4$ years for the nuns. Mean body height was 170 ± 8 cm in the men and 163 ± 8 cm in the women; mean body mass index was $27{\cdot}1 \pm 3{\cdot}7$ kg m^2 in the men and $23{\cdot}6 \pm 2{\cdot}5$ kg m^{-2} in the women. Most subjects lost about 2–3 kg of weight during the first week of each trial. The investigators [12] ascribed this to a loss of body fluid caused by the low salt content of the formula diets.

Baseline serum total cholesterol was $5{\cdot}0 \pm 0{\cdot}8$ mmol l^{-1} in the monks and $5{\cdot}1 \pm 0{\cdot}8$ mmol l^{-1} in the nuns. Usual dietary intake of the Trappists has been relatively constant for many years [18–20], and in an earlier study [20] it was estimated that it contains on average 26% energy as fat, 14% as protein and 60% as carbohydrates. Saturated fat provided 9%, monounsaturated fat 12% and polyunsaturated fat 5% of total energy intake.

One hundred and thirty subjects participated in two or more, 94 in three or more and 61 in at least four trials.

Diets

Fifty-eight different liquid formula diets were tested with respect to their effect on serum cholesterol. The diets contained 20–50% energy as fat, provided by vegetable fats and oils plus synthetic triglycerides, 15–20% energy as protein, and the remainder as carbohydrates (Table 1). Within one trial, all experimental diets usually provided the same percentage of energy as fat, with a total fat content of 40 or 50% of energy being the most common. In one trial, three diets provided 20%, another three diets 35% and the last three diets 50% of total calories as fat, with the fatty acid composition being varied systematically at each level of fat intake. Altogether, the 58 diets studied provided a very wide range of fatty acid composition,

Table 1. Example of the composition of the powder used in the preparation of liquid diets for studies on the effect of different fatty acids on serum cholesterol in Trappist monks and nuns in The Netherlands (the present formula provided 40% of energy as fat)

Ingredient	Percentage of weight
Ca-caseinate	14·0
Skimmed milk powder	30·0
Sucrose	9·0
Amylopectin G	9·5
Maltodextrin	2·2
Precooked cereals	10·0
Cellulose	3·0
Experimental fat*	21·3
Vitamins/minerals supplement†	1·0

* The amount and composition of the experimental fat varied between experiments: 200 mg butylated hydroxytoluene (BHT) antioxidant, 200 mg artifical flavourings and 10 g soy lecithin were added to the fat per kg.

† The mixture contained the vitamins A, B_1, B_6, biotin, folic acid and C and also vitamin E if the dietary fat contained less than 15 mg vitamin E kg^{-1}. Iron and iodine were also added.

from almost purely saturated to almost purely polyunsaturated plus all shades of intermediate compositions. The diets were prepared in powdered form by the Unilever Research Laboratory (Vlaardingen, The Netherlands) and diluted with hot water before consumption by the subjects. The liquid formula diets were provided *ad libitum* for a period of 4–6 weeks. The amount and fatty acid composition of the fat was the sole dietary variable; oleic and linoleic acid were used in varying proportions in the different dietary fats and combined with either lauric and myristic acid, palmitic and stearic acid or the *trans* isomer of oleic acid.

In addition to the formula diets, all subjects were allowed each day 50 g bread, tea and coffee without milk or sugar, two apples or other pieces of fresh fruit, and small amounts of fresh vegetables (carrots, radishes etc.).

Statistical analysis

The hypothesis to be tested was that a subject's response was determined entirely by the diet fed plus a random error term, and that no systematic differences in responsiveness existed between subjects. The response was defined as the difference between a subject's serum cholesterol on the experimental diet minus that on the habitual Trappist diet. In order to make it possible to compare responses to cholesterol-elevating diets with responses to cholesterol-lowering diets, the sign of the responses to cholesterol-lowering diets was reversed. A hyperresponder to the dietary fatty acid composition was thus defined as someone whose serum cholesterol was raised more than average by saturated or lowered more than average by (poly)unsaturated fatty acids. Keys' formula [21] was used to calculate whether a diet should be designated as cholesterol-lowering or -elevating.

The data were analysed by analysis of variance [22] using the following model:

$$X_{ij}=\mu+\beta_j+P_i+e_{ij}\ (\text{mmol l}^{-1}).$$

Here i denotes a particular subject ($i=1, 2, \ldots 130$), j denotes a particular diet ($j=1, 2, \ldots 58$), X_{ij} denotes the serum cholesterol response of subject i to diet j, and μ is the mean response of all subjects averaged over all diets; our best estimate of μ equalled 0·57 mmol l^{-1}, which was the mean of all responses observed. The mean fixed effect of a particular diet j is represented by β_j. The diet-independent responsiveness of subject i is accounted for by the term P_i. This term is positive for hyperresponders, and negative for hyporesponders. It has an expected value of 0 and variance σ_p^2. The random error term e_{ij} can be interpreted as the within-subject variation, i.e. the variability of the response when a particular subject is challenged repeatedly with the same diet. It has an expected value of 0 and a variance σ_e^2. All P_i and e_{ij} are assumed to be mutually independent and normally distributed.

Using analysis of variance, the total sum of squares [23] was decomposed into a component due to diets, a component (SSP) for persons adjusted for diets (but not for within-subject variability), and the sum of squares of the residuals (SSR). Division of the latter by its number of degrees of freedom yielded the estimate SD_e^2 of the variance of the error term σ_e^2:

$$SD_e^2=SSR/(N-n-k+1).$$

Here N is the total number of response values (474), n the number of subjects (130), and k the number of diets (58). The variance σ_p^2 of the intrinsic subject-specific responsiveness P_i was estimated according to Searle [22] by:

$$SD_p^2=[SSP/(n-1)-SD_e^2]/[(N-k)/(n-1)].$$

The denominator $(N-k)/(n-1)$ represents a correction for non-orthogonality. The significance of the between-person variance was tested with Snedecor's F statistic [23]:

$$F=(SSP/(n-1))/SD_e^2.$$

Results

Mean effects of fatty acids on serum cholesterol

The mean responses per diet group in the various trials have largely been reported by the original investigators [10–15]. However, as some of these publications are not easily accessible we summarize the main findings [11] below.

(i) A total fat intake of 20% of energy provided entirely by high-linoleic acid safflower oil caused lower total serum cholesterol levels than equivalent amounts of either olive oil or glyceryltrilaurate in cholesterol-free liquid formula diets [10, 12, 13].

(ii) Increasing the amount of safflower oil from 20 to 35% of energy (and thus the amount of linoleic acid from 15 to 26 en%) at the expense of maltodextrose caused a significant fall in cholesterol [10, 12]. A further increase from 35 to 50% did not lower cholesterol any further.

(iii) At all levels of intake tested (20, 35 and 50 en%), safflower oil caused lower cholesterol levels than olive oil when either was provided as the sole source of fat in cholesterol-free formula diets [10, 12, 13].

(iv) In diets providing 35% of energy as linoleic acid and 12·5% as synthetic saturated triglycerides, lauric and myristic acid produced smaller effects on cholesterol levels than expected from literature data.

(v) Under the same conditions, palmitic acid caused a significant [11, 13] elevation of cholesterol, while stearic acid had only a negligible effect [11, 13].

(vi) In the presence of egg yolk cholesterol (115 mg 1000 kcal^{-1}), elaidic acid (C 18:1 Δ 9 *trans*) had a definite serum cholesterol increasing effect compared with oleic acid (C 18:1 Δ 9 *cis*) [14–16].

By and large these findings agree with those reported by other investigators at that time [18, 22].

Individual differences in responsiveness

The present analysis focussed on the reproducibility of the response of each Brother or Sister to dietary changes. Therefore, the data of subjects who had participated only once were eliminated. Of the others, each had on average participated in three to four experiments (Table 2). After application of the sign correction (see Patients and methods section) the average response to the experimental diets was estimated to be 0·57 mmol l^{-1}. There were no differences in average response between men (0·55±0·39 mmol l^{-1}) and women (0·60±0·25 mmol l^{-1}). Thus, men and women were on average equally responsive to dietary fatty acid modification. A similar result was found in our studies of responsiveness to dietary cholesterol [24].

The between- and within-person variance, and probability values for the existence of differences in response between subjects are given in Table 2. Although the within-person variance was about four times as large as the between-person variance, the between-person variance was still significantly larger

Table 2. Between- and within-person variance of the effect of different dietary fatty acids on serum cholesterol in Trappist monks and nuns participating in multiple controlled dietary trials

	All	Men	Women	Women*
No. subjects	130	82	48	36
No. diets	58	58	26	25
No. responses (N)	474	324	149	124
Between-persons variance SD_p^2 (mmol l^{-1})^2	$0{\cdot}20^2$	$0{\cdot}24^2$	$0{\cdot}14^2$	$0{\cdot}17^2$
Within-persons variance SD_e^2 (mmol l^{-1})^2	$0{\cdot}42^2$	$0{\cdot}47^2$	$0{\cdot}33^2$	$0{\cdot}33^2$
Probability value for no between-person effect	$P<0{\cdot}001$	$P<0{\cdot}001$	$P=0{\cdot}057$	$P=0{\cdot}032$

* Participating in three or more trials.

than zero in the men and in all subjects combined. It was of only borderline significance in the women. However, in the 36 women for whom data from three or more independent trials were available, there was a clearly significant between-subject component in the variance of the response.

Discussion

The average response of serum cholesterol to dietary fatty acid modification in this series of experiments was 0·57 mmol l^{-1}. This corresponds to about 11% of the baseline serum cholesterol value, and is typical of what has been found in other trials involving dietary fatty acid modification [21, 24–26].

A wide variation was seen in the individual responses to the different diets. After adjusting for differences in composition between diets, it was found that much of the apparent variation was probably due to spontaneous within-person fluctuations of serum cholesterol. However, there were also systematic differences in the responsiveness of subjects to changes in dietary fatty acids. The results of this study confirm and extend earlier findings that hypo- and hyperresponders to dietary modification do exist [5–9, 27–30]; they also confirm that, if enough data are collected, few or no subjects will prove entirely non-responsive [5, 9, 28, 29]. Wherever cases of apparent extreme susceptibility or resistance to fat-modified diets are encountered in clinical practice, these should first be ascribed to chance excursions of the serum cholesterol rather than to innate idiosyncrasies of lipid metabolism. Pure hyper- and non-responders are rare, if they exist at all.

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