# Dietary fat and cholesterol and serum cholesterol in the gerbil

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ABSTRACT Groups of gerbils were fed purified diets containing either 10 or 20% of safflower, olive, or coconut oil. Each diet was fed without cholesterol and with 0.1 and 0.2% of added cholesterol. The animals were bled after 2, 4, and 8 wk for the determination of the level of serum cholesterol. The major factors affecting the level of serum cholesterol were the kind of dietary oil, the amount of dietary cholesterol, and the length of time the diet was fed. The level of safflower oil had a statistically significant effect but the level of olive or coconut oil had no significant effect. Various other statistically significant interactions were observed which make simple interpretations of the data difficult.

The levels of serum cholesterol achieved in the gerbils fed the different oils with no or very low levels of dietary cholesterol were similar to those seen in men fed the same oils. Although the gerbil is apparently resistant to the development of atherosclerosis, it may be a useful model for studying the effect of dietary fats upon cholesterol metabolism.

KEY WORDS gerbil · serum cholesterol · dietary fat · cholesterol · hypercholesterolemia

Studies in the general field of experimental atherosclerosis have been dominated, for obvious reasons, by the development of diets or other conditions which will produce atherosclerotic lesions in the experimental animals used. An elevated level of the serum cholesterol produces lesions in nearly all species that have been studied. Such data plus the clear evidence that hypercholesterolemia in man is an important risk factor in the development of atherosclerotic heart disease leaves little doubt that an elevated level of serum cholesterol is a causal factor in the development of the atherosclerotic lesion.

From the dietary point of view the most important object of study may be, therefore, the mechanisms by which diet influences the serum cholesterol level. This study does not necessarily require conditions that produce atheroslcerotic lesions, but does require experimental models in which diet affects the serum cholesterol. It seems to us that the great concern with the production of lesions has led to the use of experimental models that may be inappropriate and unlikely to be useful in understanding the effects of diet upon cholesterol metabolism in man.

The studies on man have led to a reasonable degree of agreement, but not complete accord, upon the general effects of dietary cholesterol and dietary fat on the level of serum cholesterol. Thus, practically all recent studies (1–4) agree that dietary cholesterol is a factor governing the serum cholesterol level in man but that under the usual conditions it is not as important as the kind or amount of dietary fat. Although agreement has not yet been reached about the effect of specific fatty acids on the level of serum cholesterol (4–6), it seems clear that highly unsaturated fats containing substantial proportions of polyunsaturated fatty acids lower the serum cholesterol level; highly saturated fats raise it; and fats high in monounsaturated fatty acids fall in between and have relatively little effect (4, 7, 8). These conclusions depend somewhat upon the base line from which one makes the calculations. In contrast, there is no agreement upon the mechanism by which the level of serum cholesterol is controlled (9). We may assume that it must act by affecting cholesterol synthesis, catabolism, exchange with tissues, or excretion, since dietary effects persist for extended periods of time.

If control of cholesterol synthesis is a possible mechanism, most of the animal experiments may have been done under inappropriate conditions, since the experiments usually include the incorporation of substantial amounts of cholesterol in the diet which may of itself inhibit cholesterol synthesis. An ideal animal model

would be one that shows pronounced changes in the level of serum cholesterol with change of dietary fats when the diet contains little or no cholesterol, since this occurs in man, and one in which the response to fats of different fatty acid compositions is similar to that seen in man. In addition, small, readily available, and easily handled animals would be advantageous.

The rat, which has been most often used in recent years, appears to be a rather poor model for studies of the effect of diet on cholesterol metabolism. This animal shows relatively little change in serum cholesterol on cholesterol feeding; only when cholic acid is added to the diet can pronounced hypercholesterolemia and atherosclerotic lesions be produced (10). We have shown (11, 12) that in rats under these conditions marked changes in the level of serum cholesterol result from changes in the dietary fat but that the responses to various fats are markedly different from those seen in man. Such animals also develop severe fatty livers, as do most species when fed high cholesterol diets.

Although in cholesterol-fed chicks the response to different fats appeared to be more similar to that seen in man (13), the necessity of feeding cholesterol to obtain responses in serum cholesterol reduces the appropriateness of the experimental design. Some studies with chicks fed different fats in cholesterol-free diets (14, 15) indicate that the serum cholesterol of the chick is not responsive to changes in dietary fat alone.

The number of papers in the literature in which changes in serum cholesterol in various species have been related to changes in dietary fat are too numerous to review. If one rejects the papers in which cholesterol feeding was employed, the number is greatly reduced, but in few, if any, have fats been fed in sufficient variety to enable one to reach any substantial conclusion as to the similarity between the response of the animal and that of man. Some of the studies on monkeys (16–19), swine (20), rabbits (21–23), and dogs (6, 24) indicate that serum cholesterol may be raised by saturated fats with little or no dietary cholesterol, but the effects of relatively few fats have been studied.

We report in this paper the first of a series of experiments with the Mongolian gerbil (*Meriones unguiculatus*) which indicate that this species may be useful for the purposes discussed above. This species is clearly more sensitive to cholesterol feeding than is man, but the response to three typical oils given in a cholesterol-free diet is at least grossly similar to that seen in man. The animal is of convenient size and is easily handled.

## METHODS

Young adult male gerbils (Tumblebrook Farm, Brant Lake, N.Y.)[1] were fed, ad lib., purified diets[2] containing either 10 or 20% (by weight) dietary fat. Safflower oil, olive oil, and coconut oil were used. These oils provide a wide range in the degree of unsaturation and fatty acid composition and we have had substantial experience with their effects upon serum cholesterol in man (4). Each of the diets was fed without cholesterol and with 0.1 and 0.2% cholesterol. About 0.2 ml. of blood was obtained from the tail of the animals after 2, 4, and 8 wk and serum cholesterol was measured by the microfluorometric method (28). There were six animals in each group.

Complete variance analysis (29) of the data obtained with each oil was performed to evaluate the effects of the level of dietary fat and cholesterol and the length of time for which the diet was fed. The data were then combined and analyses of variance performed to evaluate the differences between oils and the possible significance of interactions between the different oils and the other variables: dietary level of oil, presence and level of cholesterol, and time.

## RESULTS

The average serum cholesterol values for each group at each bleeding period are shown in Table 1. It is apparent that when the cholesterol-free diet was fed, the kind of dietary fat had a marked effect upon the level of serum cholesterol. Animals fed safflower oil maintained substantially lower levels than those fed the other two oils, and animals fed coconut oil appeared to be more hypercholesterolemic than those fed olive oil. The addition of small amounts of dietary cholesterol raised the serum cholesterol level markedly but the effects of the different oils were still apparent in the animals receiving dietary cholesterol.

The data from animals fed each oil were subjected to analysis of variance as shown in Table 2. As was apparent from Table 1, time, dietary cholesterol, and the time–cholesterol interaction were the most significant variables. Whereas the level of serum cholesterol rose little up to the 4th wk in the animals fed the cholesterol-free diets, it continued to rise in those that received cholesterol, especially at the 0.2% level. The significant

[1] Although the animals were purchased as "young adults," the weight of all of them (average on arrival, 52 g) increased during the experimental period (to an average of 85 g). There was no significant difference between the weight gains for different experimental groups.

[2] The diet containing 20% fat had the following composition (in per cent): purified casein, 15; salt mix (25), 5; celluflour, 5; choline chloride, 0.3; fat-soluble vitamins in corn oil (26), 0.1; vitamin mix (27), 0.5; glucose, 54.1; appropriate fat, 20. In the preparation of the diets containing 10% fat, one-half of the fat was replaced by an equal weight of glucose. When cholesterol was added the crystalline cholesterol was simply mixed into the appropriate diet.

TABLE 1 MEAN SERUM CHOLESTEROL LEVELS AT DIFFERENT TIMES IN GERBILS FED DIFFERENT DIETS

| Diet | Mean Serum Cholesterol | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 10% Oil Diet | | | | 20% Oil Diet | | | |
| | 2 Wk | 4 Wk | 8 Wk | Mean | 2 Wk | 4 Wk | 8 Wk | Mean |
| | *mg/100 ml* | | | | | | | |
| Safflower oil | 133 | 136 | 161 | 144 | 107 | 78 | 123 | 103 |
| Safflower oil + 0.1% chol. | 145 | 193 | 277 | 206 | 166 | 178 | 298 | 215 |
| Safflower oil + 0.2% chol. | 174 | 252 | 493 | 308 | 165 | 265 | 327 | 254 |
| Olive oil | 209 | 292 | 237 | 247 | 167 | 260 | 327 | 252 |
| Olive oil + 0.1% chol. | 197 | 260 | 459 | 305 | 219 | 327 | 517 | 355 |
| Olive oil + 0.2% chol. | 248 | 355 | 701 | 436 | 324 | 439 | 740 | 501 |
| Coconut oil | 252 | 297 | 318 | 289 | 253 | 317 | 289 | 287 |
| Coconut oil + 0.1% chol. | 308 | 473 | 479 | 420 | 249 | 353 | 602 | 402 |
| Coconut oil + 0.2% chol. | 287 | 436 | 1135 | 620 | 202 | 471 | 988 | 549 |
| Mean values (all oils) | | | | | | | | |
| No cholesterol | 198 | 242 | 239 | 227 | 176 | 218 | 246 | 214 |
| 0.1% cholesterol | 217 | 309 | 405 | 310 | 211 | 286 | 472 | 324 |
| 0.2% cholesterol | 236 | 348 | 776 | 455 | 230 | 392 | 683 | 435 |

TABLE 2 ANALYSIS OF VARIANCE OF SERUM CHOLESTEROL DATA IN GERBILS FED DIFFERENT OILS

| Source of Variation | d.f. | Safflower Oil | | Olive Oil | | Coconut Oil | |
|---|---|---|---|---|---|---|---|
| | | Mean Square | *F** | Mean Square | *F** | Mean Square | *F** |
| Level of oil | 1 | 22,044 | 8.76 | 41,890 | — | 25,607 | — |
| Time | 2 | 168,050 | 66.8 | 671,403 | 37.7 | 1,332,321 | 53.0 |
| Cholesterol level | 2 | 222,831 | 88.6 | 443,585 | 24.9 | 795,642 | 31.7 |
| Level × time | 2 | 7,564 | 3.0† | 4,483 | — | 3,387 | — |
| Level × cholesterol | 2 | 9,979 | 3.97† | 8,463 | — | 11,606 | — |
| Time × cholesterol | 4 | 36,153 | 14.4 | 108,287 | 6.08 | 540,353 | 21.5 |
| Level × time × cholesterol | 4 | 11,465 | 4.55 | 7,553 | — | 36,556 | — |
| Within groups | 90 | 2,515 | — | 17,788 | — | 25,086 | — |

d.f., degrees of freedom.
* All *F* values shown indicate significance at the 1% level or below, with the two exceptions marked †.
† $P < 0.05$.

interaction means that the time trends were different at different levels of cholesterol. These, however, were the only significant variables in the animals fed olive and coconut oil; the level of oil had no significant effect. On the contrary, all variables and interactions were significant in the data from the groups fed safflower oil. The 20% level of safflower oil was more effective in reducing the level of the serum cholesterol than the 10% level, but the extent of this reduction was variable and depended upon the time the diet was fed and the level of dietary cholesterol.

Complete analysis of variance of the combined data was done in order to evaluate the effects of the different oils and the interactions of oils with the other variables under study (Table 3). As expected, the kind of oil, time, and the level of dietary cholesterol produced highly significant effects. The level of oil did not produce significant effects but, as would be expected, the interaction between type of oil and its dietary level was now significant; this again indicates that the level of oil was important only with some oils. Oil–time interaction was also significant, which indicates that the time trend was different for the various oils. Other significant interactions are those to be expected from consideration of Table 2.

TABLE 3 ANALYSIS OF VARIANCE OF THE COMBINED DATA

| Source of Variation | d.f. | Mean Square | *F** |
|---|---|---|---|
| Oil | 2 | 1,328,539 | 74 |
| Time | 2 | 1,891,358 | 106 |
| Cholesterol level | 2 | 1,364,628 | 76 |
| Level of dietary oil | 1 | 3,593 | — |
| *Interactions* | | | |
| Oil × time | 4 | 191,717 | 10 |
| Oil × cholesterol | 4 | 73,714 | 4 |
| Oil × level of oil | 2 | 92,973 | 5 |
| Cholesterol × time | 4 | 500,699 | 28 |
| Cholesterol × level of oil | 2 | 8,501 | — |
| Time × level of oil | 2 | 883 | — |
| More complex interactions | 28 | 711 | — |
| Error | 270 | 17,734 | |

* All *F* values shown are significant at the 1% level or below.

## DISCUSSION

As indicated in the introduction, one of the major interests of comparative nutrition is to discover species and dietary conditions that may have relevance to human nutrition. It seems important, therefore, that with the cholesterol-free diet the serum cholesterol levels observed in the gerbil fed these three oils are of the same order of magnitude as those we have seen in human subjects (4). Since the human subjects were fed a complex diet of natural foods and many factors, such as the nature of the dietary carbohydrates, undoubtedly influence serum cholesterol (30–32), complete similarity is probably not to be expected. Nevertheless with diets containing about 100 mg of cholesterol per 2500 calories, the levels of serum cholesterol observed in man were 170, 220, and 265 mg/100 ml with safflower oil, olive oil, and coconut oil respectively. These values may be compared with those shown in Table 1.

Although the levels of dietary cholesterol we have used in these studies, 0.1 and 0.2% of the diet, appear to be low by comparison to diets often employed in studies with experimental animals, the 0.2% level supplied about 0.4 mg. of cholesterol per dietary calorie (the exact figure depending upon the amount of fat in the diet). A daily intake of 2500 calories from such a diet would provide 1.0 g of cholesterol. Thus, this diet is relatively high in cholesterol compared to the usual intakes of man. The levels of serum cholesterol observed in the gerbils on this diet are substantially above those seen in our human subjects fed diets that supplied 700 mg of cholesterol per day (4). It is clear, therefore, that the gerbil is more sensitive to cholesterol feeding than is man, but the effects of the oils relative to each other are similar in the two species.

Clarkson, King, and Warnock (33) observed a moderate rise in serum cholesterol in gerbils upon the addition of 4% peanut oil to laboratory chow and a much greater rise when 1% cholesterol was also added to the diet. No gross evidence of atherosclerosis was seen in the animals. Similarly, Gordon, Cekleniak, Stolzenberg, Benitz, and Moraski (34) reported that this species was resistant to atherosclerosis when diets containing 1% cholesterol were fed, even though the diets induced a marked hypercholesterolemia. In further studies, Gordon and Cekleniak (35) found that the feeding of rabbit pellets containing 1% cholesterol produced a marked rise in the low density lipoproteins. During the first few weeks the $S_f$ 35–400 lipoproteins were greatly elevated, but with continued feeding the higher density lipoproteins ($S_f$ 0–100) were most elevated. However, with such high levels of cholesterol feeding the animals developed greatly enlarged livers, heavily infiltrated with lipid, and the authors suggest that the shift in lipoprotein pattern with time might be due to liver damage Whether the pattern of lipoprotein response under the conditions we have used is similar to that seen in man and whether or not the pattern of lipoprotein response may provide an explanation of the resistance of the gerbil to atherosclerosis remains to be studied. In any event, the gerbil appears to be a species that is not suited to the study of the development of atherosclerosis but may be useful for studies of the effect of dietary fats upon cholesterol metabolism.

The major differences in the level of serum cholesterol observed in the gerbils in this study are explained by the kind of oil fed, the level of dietary cholesterol, and the time for which the diet was fed. These are, of course, the major factors influencing the level of serum cholesterol that have been identified in studies with man. However, the observations that the level of safflower oil was important whereas the level of olive or coconut oil was insignificant and that various other interactions may play a lesser but significant role in determining the level of serum cholesterol may be important. If such factors are also operative in human nutrition, minor discrepancies between the results obtained in different laboratories or in different experiments might be explained.

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