Effect of the Mode of Feeding of Fats on Serum Cholesterol Levels and Plasma Fibrinolytic Activity of Monkeys

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SEVERAL investigators have studied the effect of different dietary fats on serum cholesterol concentrations. They have shown that both the quantity and quality of dietary fats influence serum cholesterol levels. The possible role of the *mode* of feeding of fats in influencing serum cholesterol concentrations, however, has received little attention. In the present study it has been shown that apart from the quantity and quality of fat, the manner of distribution of fat in the different meals during the day, may also determine serum cholesterol levels.

EXPERIMENTAL

Twelve adult male monkeys of the species Macacus radiata weighing between 3.2 and 6.6 kg. were used as experimental animals for this investigation. They were maintained on an adequate stock diet containing 8 per cent fat for a period of four weeks before they were taken up for the investigation. Their initial serum cholesterol levels were determined by the method of Abell et al.[1] They were then divided into two groups with respect to an equal distribution of body weights and initial values for serum cholesterol and were fed a diet high in hydrogenated vegetable fat (Table 1), the fat constituting 30 per cent of the diet and providing nearly 50 per cent of the total calories, as follows:

Group I (*continuously fed group*): Six monkeys were supplied the diet *ad libitum* and were allowed free and continuous access to the food for practically all the twenty-four hours.

Group II (*Intermittently Fed Group*): Six other animals were maintained on the same high fat diet as group I, but the mode of feeding was different. Part of the day's diet was given at 11 A.M. and the food was withdrawn exactly half an hour later. The other portion of the diet was supplied at 3 P.M. and, as before, the uneaten food was removed after exactly half an hour.

The animals were maintained on the high fat diet with their respective modes of feeding for a period of nine weeks. A record of total daily food intake of each animal in both groups was maintained. The animals were weighed weekly.

Serum cholesterol levels were estimated at the end of four weeks and again at eight weeks. Plasma fibrinogen and fibrinolytic activity were also determined at the end of eight weeks in the experimental animals and in a group of monkeys (control group) maintained on the low fat stock diet mentioned earlier. The blood samples for these estimations were drawn between 9 and 9:30 A.M. in all animals. The procedure adopted for the determination of plasma fibrinolytic activity was that described by Biggs and Macfarlane[2] with the modifications found to be necessary and reported earlier by Jagannathan and Gopalan.[3] At the end of the experimental period total body water was estimated in both groups using urea according to the method of McCance and Widdowson.[4]

RESULTS

Food Intake

The animals which were fed the high fat diet "intermittently" (group II) were found to consume nearly 70 per cent of their daily total intake of food within half an hour in the

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TABLE I
Composition of Feedings

High Fat Diet*:	
Wheat flour	53.0 parts
Casein	12.0 parts
Salt mixture	4.0 parts
Vitamin mixture	1.0 part
Hydrogenated vegetable fat	30.0 parts
Vitamin Mixture†:	
Thiamine	200 mg.
Riboflavin	400 mg.
Nicotinic acid	2,000 mg.
Pyridoxine	400 mg.
Calcium pentothenate	800 mg.
p-aminobenzoic acid	40 gm.
Inositol	40 gm.
Choline	40 gm.
Vitamin K	400 mg.
Salt Mixture:	
Calcium lactate	390 gm.
Calcium phosphate	162 gm.
Potassium phosphate	183 gm.
Sodium chloride	52 gm.
Magnesium sulfate	80 gm.
Sodium phosphate	104 gm.
Potassium iodide	5 gm.
Iron sulfate	35 gm.

* The fat supplies 50 per cent of the calories in the diet.
† Made up to 200 gm. with glucose.

forenoon, and the remaining 30 per cent in the afternoon within the same short duration of time. On the other hand, the monkeys which had continuous access to food for practically the whole day and night (group I) consumed their diet in a more leisurely manner throughout the day.

The mean daily intake of food was nearly similar in both groups being 85 gm. in the "continuously fed" group and 83 mg. in the "intermittently fed" group.

Body Weight and Body Composition

Despite the nearly identical food intake there was some difference in the mean increase in body weight of the two groups. At the end of eight weeks, the mean body weight of the animals fed intermittently (group II) was about 10 per cent higher than that of animals fed continuously. This difference, however, was not statistically significant. There were no significant differences between the two groups with regard to the content of total body water and body fat.

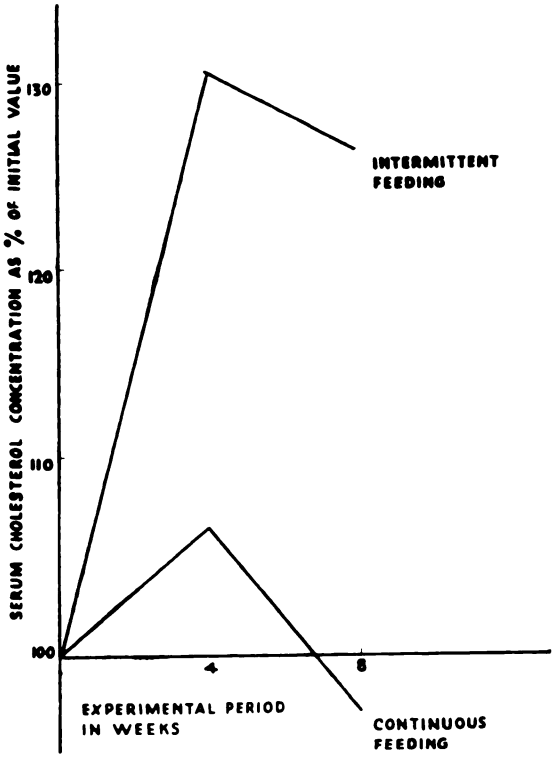

FIG. 1. Effect of the mode of feeding of a diet high in hydrogenated vegetable fat on serum cholesterol levels in monkeys.

Serum Cholesterol Levels

The pattern of changes in serum cholesterol levels was markedly different in the two groups as can be seen in Figure 1. In group I in which the animals had continuous access to food, the increase in serum cholesterol after four weeks of high fat feeding was only 11.0 ± 6.83 mg. per 100 ml.; whereas in group II, in which "intermittent" feeding was adopted, the increase in serum cholesterol was nearly four times higher *viz*, 41.3 + 8.36 mg. per 100 ml. At the end of eight weeks the serum cholesterol values practically returned to the basal level in the continuously fed group whereas the elevated level was sustained in the intermittently fed group although at a slightly lower level.

Statistical treatment of the results showed that the serum cholesterol level did not significantly change from the basal value in the continuously fed group. On the other hand,

TABLE II
Effect of the Mode of Feeding of a High Fat Diet* on Serum Cholesterol Levels in Monkeys

Group	Mode of Feeding	No of Monkeys	Initial Body Weight (kg.)	Body Weight at the End of 8 Weeks (kg.)	Initial Level of Serum Cholesterol (mg./100 ml.)	Serum Cholesterol Concentration at the End of	
						4 Weeks (mg./100 ml.)	8 Weeks (mg./100 ml.)
I	Continuous access to food	6	4.48 ± 0.46†	4.14 ± 0.45	139.2 ± 8.28	150.2 ± 13.81	135.2 ± 9.85
II	Intermittent feeding	6	4.39 ± 0.42	4.61 ± 0.49	136.7 ± 9.42	178.0 ± 13.39	172.5 ± 18.67

* Both fed hydrogenated vegetable fat supplying 50 per cent of the total calories.
† Standard error of the mean.

in the group fed intermittently, the increase in serum cholesterol from the basal value was found to be statistically significant with P values <0.01 at the end of four weeks and <0.05 at the end of eight weeks. Comparing the increases between the groups, the values were significantly higher at $P < 0.02$ level in the group fed intermittently than in the group fed continuously in both periods. Detailed results are given in Table II.

Plasma Fibrinolytic Activity

This was measured both by the extent of lysis of the fibrin clot in a given time as well as by the time taken for the complete lysis of the clot. It can be seen from Table III that in animals on the stock diet containing eight per cent fat complete lysis of the clot took place within three to four hours. In monkeys of group I for which the high fat diet was available continuously, the plasma fibrinolytic activity was not significantly different from that of the control group on the stock diet. On the other hand, in animals of group II receiving the same high fat diet "intermittently" the fibrinolytic activity was significantly retarded.

While plasma fibrinogen content in the animals of the "continuously fed" group did not differ significantly from those receiving the stock diet, the value for fibrinogen was significantly lower in the "intermittently fed" group as compared to the "continuously fed" group or the control group.

COMMENTS

It was found in the present study that although the food intake in both groups of animals was nearly similar, animals fed intermittently showed greater weight gain than those fed continuously. The difference however, was not statistically significant. Levin[5] and Cohn and Joseph[6] demonstrated that force-fed rats showed significantly greater body fat than their pair-fed control animals. It has been suggested that force feeding "may alter the intermediary metabolic pathway in the direction of increased efficiency with resultant greater fat deposition."[6] Body composition studies carried out on monkeys used in the present study did not reveal gross difference in the amount of depot fat between the group which was continuously fed and the group fed intermittently. However, estimation of body fat in the present study was based on total body water determination and may not have been sensitive enough to demonstrate small differences.

It has been reported that fibrinolysis *in vitro* decreases with an increase in the amount of fibrinogen.[7] In the present study, however, a decrease in plasma fibrinolytic activity in the intermittently fed group occurred even without an increase in the plasma fibrinogen content, but this was associated with an actual decrease in the value. That the diminished fibrinolytic activity in the animals fed intermittently was not due to overnight fasting was shown by the fact that no reduction in plasma fibrinolytic

TABLE III
Effect of the Mode of Feeding of a High Fat Diet on Plasma Fibrinolytic Activity in Monkeys

Group	Diet	No.	Clot Lysis Time (hr.)	Lysis of Clot in 3 Hours (%)	Plasma Fibrinogen Nitrogen (mg./100 ml.)
Control	Stock (low fat)	1	3	100	65.8
		2	3	100	51.8
		3	4	48	71.4
		4	3	100	...
					Mean 63.0
I	High fat continuous feeding	1	1½	100	56.0
		2	3	100	63.0
		3	2½	100	49.0
		4	1½	100	...
		5	2	100	60.2
		6	3	100	72.8
					Mean 60.2
II	High fat intermittent feeding	1	≮26*	0	...
		2	6	7	50.4
		3	3	100	51.8
		4	≮ 8	0	54.6
		5	≮ 8	0	49.0
		6	≮25	0	...
					Mean 51.5

* ≮ Not less than.

activity could be found in monkeys on stock diet after overnight fasting.

The explanation for the striking difference in the serum cholesterol concentration and plasma fibrinolytic activity between the two groups in the present study requires elucidation. The observed difference between the two groups is of more than academic interest. It is well known that the composition of different meals eaten during the day and the fat content thereof vary widely especially in the diets of high income groups. The results of the present study indicate that apart from the quality and quantity of dietary fat, the mode of distribution of the fat in the daily diet is an important factor requiring consideration from the point of view of changes in serum cholesterol concentration and plasma fibrinolytic activity.

SUMMARY

The effects of "continuous" and "intermittent" feeding of a high fat diet to two groups of monkeys have been compared. Although the food intake was nearly the same in both groups, body weight was higher in animals fed intermittently that in those fed continuously at the end of the experimental period. This difference, however, was not statistically significant.

Serum cholesterol concentration was significantly higher and plasma fibrinolytic activity significantly lower in the intermittently fed group than in the continuously fed group. The results indicate the importance of the mode of distribution of fat in the daily diet.

ADDENDUM

During the preparation of this report the work of Cohn et al.[8] on the influence of the rate of ingestion of the diet on atherogenesis in chickens appeared. Our findings regarding the greater increase in serum cholesterol in animals fed intermittently are in line with their observation of a greater increase in serum cholesterol in the "meal eating" group of chicks.

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