Effect of fasting on the concentrations of cholesterol, total fatty acids and polyenoic acids in plasma and heart of the rat

By JENS G. NØRBY

Department of Biochemistry, University of Århus, Århus, Denmark

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In studies concerning the effect of diet on the fatty acid pattern of animals it is a common procedure to starve the animals before blood sampling or autopsy. The influence of the last meal is thereby reduced and the results chiefly reflect the long-term effect of the diet.

It has been shown recently (Feigenbaum & Fisher, 1963) that fasting for 1–3 days leads to changes in the liver polyenoic acids in adult male rats, but little is known about the effect on the lipids of other tissues.

The effect of fasting for 0–26 h on the concentration of cholesterol, total fatty acids and polyenoic acids in plasma and heart of the adult rat is reported here.

In long-term dietary experiments illustrating the effect of diet on the polyenoic acids in animals, these tissues are of special interest. The changes during the experiment may be followed by repeated sampling of blood. Analysis of heart tissue can only be performed at the termination of the experiment, but Widmer & Holman (1950) and others have shown that the heart tissue responds more drastically than other tissues to changes in the polyenoic fatty acid content of the diet.

EXPERIMENTAL

Procedure. Twenty-six male albino rats, 6–10 months old, were housed individually in cages with raised wire screen bottoms. From weaning they had been fed on a commercial chick diet (Karatgryn; Karensmølle, Århus, Denmark) providing per kg about 3500 kcal and 25 g of fatty acids containing 41 % dienoic, 0·9 % trienoic and 1·5 % tetraenoic acids. Water was given *ad lib.* throughout the experiment. Before they were killed, the rats were starved for different periods, the fasting period beginning with the removal of the food cup at about 9 am (rats are nocturnal eaters) and ending with the killing of the animal with a parenteral injection of 5 mg Nembutal (Abbott Laboratories Ltd; 6 % solution)/100 g body-weight. The thorax was opened and blood was collected from the heart with a heparinized syringe and centrifuged at 1700 g for 20 min. The heart was removed and freed from adhering fat and blood, and plasma and heart were stored at $-20°$ until analysed.

Analytical methods. Extraction of lipid, isolation and titration of total fatty acids (TFA), as well as the determination of polyenoic acids (by the method of Herb & Riemenschneider, 1953) were performed as described by Nørby (1961).

Cholesterol was determined by the Tschugaeff reaction on a sample of the

Table 1. *Concentrations of cholesterol, total fatty acids (TFA) and polyenoic acids in plasma (A) and heart (B) of rats starved for various periods*

Rat no.	Age (months)	Weight (g)	Period of fast (h)	Cholesterol (mg/100 g)		TFA (m-equiv./kg)		Polyenoic acids (m-equiv./kg)									
								Dienoic		Trienoic		Tetraenoic		Pentaenoic		Hexaenoic	
				A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	8	380	0	63	146	11·4	74	2·91	14·3	0·25	−1·2	1·20	11·7	0·36	1·4	0·39	9·0
2	8	430	0	60	141	13·8	72	3·36	15·8	0·33	−0·5	1·19	11·8	0·33	1·4	0·50	8·8
3	10	450	0	69	124	16·8	71	4·00	14·8	0·35	−0·5	1·34	10·6	0·29	1·3	0·50	7·7
4	10	530	0	70	—	17·9	79	3·54	21·9	0·30	1·4	1·25	9·7	0·21	1·2	0·57	8·8
5	8	460	3	64	133	9·0	72	1·94	15·6	0·13	−0·1	1·14	12·5	0·23	1·1	0·42	9·1
6	8	330	4	59	145	6·8	67	1·65	14·2	0·10	−0·7	1·05	11·7	0·25	1·1	0·28	10·4
7*	6	375	4	—	122	—	87	—	17·6	—	−0·5	—	11·2	—	1·1	—	9·0
8	6	410	4	69	123	15·1	75	3·27	13·1	0·51	−1·1	1·48	12·3	0·18	1·5	0·33	8·4
9	6	370	5	55	128	9·3	76	1·95	16·3	0·34	0·8	1·02	12·2	0·09	1·1	0·25	8·1
10	8	455	5	58	—	13·5	73	2·78	14·5	0·19	−0·7	1·22	12·2	0·38	1·5	0·46	7·6
11	8	483	5	59	—	7·8	72	1·30	18·2	0·08	0·6	0·79	10·2	0·23	1·0	0·23	5·3
12	8	390	7	59	135	11·9	67	2·57	14·5	0·23	−0·3	1·21	11·5	0·39	1·2	0·46	7·8
13	8	530	7	73	132	13·9	85	2·70	14·6	0·19	0·7	1·67	13·3	0·33	1·4	0·67	8·9
14	6	375	9	49	129	8·1	73	1·83	16·1	0·14	−0·4	1·05	12·3	0·11	1·2	0·27	8·8
15	6	340	10	57	127	7·9	74	1·75	15·3	0·11	1·4	1·11	12·7	0·16	1·4	0·23	7·9
16	6	330	13	45	136	6·3	82	1·48	17·6	−0·02	1·5	1·10	13·4	0·18	1·2	0·27	7·5
17	6	420	13	53	131	5·9	80	1·22	15·7	0·23	0·3	1·11	13·0	0·05	1·4	0·12	9·0
18	6	400	14	53	141	7·2	76	1·52	16·9	0·01	0·9	1·27	13·8	0·20	1·4	0·24	6·8
19	8	390	17	57	134	10·3	72	2·47	15·8	0·02	−0·9	1·61	12·3	0·28	1·7	0·39	6·8
20	8	430	17	58	130	10·3	65	2·47	14·2	0·04	−0·5	1·38	11·0	0·28	1·7	0·31	5·7
21	8	390	19	55	—	8·0	65	1·89	14·6	−0·01	−0·7	1·32	11·0	0·21	1·4	0·35	6·0
22	8	370	20	66	134	10·0	71	1·83	14·2	−0·02	−0·7	1·44	11·1	0·30	1·4	0·46	6·7
23	6	390	24	55	126	7·2	76	1·66	14·2	0·32	−1·1	1·09	12·3	0·04	1·6	0·15	9·1
24	6	320	26	56	140	7·2	70	1·71	14·3	0·09	−0·4	1·22	12·7	0·10	1·1	0·24	8·6
25	6	355	26	50	127	6·3	73	1·42	14·2	0·13	−1·1	1·21	12·8	0·05	1·3	0·21	8·8
26	6	390	26	58	—	7·2	82	1·55	17·4	0·21	−0·1	1·32	12·4	0·06	1·0	0·24	9·7

* Unsuccessful heart-puncture.

unsaponifiable extract. The method of Hauge & Nicolaysen (1958) was used, and values for all samples were corrected for background absorption as described by these authors. Preliminary experiments showed that the extinction of the developed colour was a rectilinear function of the cholesterol concentration in the extinction interval 0·1–0·8. However, the straight line always cut the extinction axis below 0. In all analyses, therefore, standard solutions of pure cholesterol in at least two different concentrations were analysed along with the samples. In agreement with Nicolaysen & Ragård (1961) the discrepancy from Beer's law was found to be related to the batch of acetylchloride used. Acetylchloride, *pro analysi*, from E. Merck AG, Darmstadt, Germany, gave the smallest deviation from Beer's law.

Duplicate determinations of all the above-mentioned lipids were made on plasma and heart from each rat individually.

RESULTS

In Table 1, in which the rats are listed according to the length of the fasting period, the concentrations of plasma and heart lipids are given.

Plasma. It appeared that the concentration of TFA decreased from about 15 m-equiv./kg to about 8 m-equiv./kg during the first 10 h, after which it remained relatively constant. The cholesterol concentration also declined somewhat.

The plasma concentration of dienoic acid (m-equiv./kg) decreased with increasing length of the fasting period and with decreasing TFA concentration. The proportion of dienoic acid in plasma TFA (expressed as equiv./100 equiv. TFA) was found to be independent of the TFA concentration, the mean value with its standard error being $22{\cdot}05 \pm 0{\cdot}41$.

Similarly the concentration of tetraenoic acid, m-equiv./kg plasma, was nearly independent of length of fasting period or TFA concentration.

The concentrations of trienoic, pentaenoic and hexaenoic acids were low and all showed a tendency to fall with the TFA concentration. Some negative values were found for concentrations of trienoic acid in both plasma and heart tissue because values were calculated as the difference between a number of relatively large values (for method of calculation see Nørby, 1961).

Heart. The heart lipids were apparently unaffected by the fast as the concentrations were the same in all animals.

DISCUSSION

Sure, Kik & Church (1933) and Bragdon, Havel & Gordon (1957) have reported a decrease in plasma TFA in the rat as a result of 24 h fast. A similar finding has been published by Hølmer, Kristensen, Søndergaard & Dam (1960) who fed chickens on a diet containing 10% hydrogenated or unhydrogenated arachis oil. Starving the chicks for 20 h resulted in a TFA concentration lower than found for unstarved chicks. The observations here reported agree with this.

As mentioned, a slight fall in plasma cholesterol was observed. It should be noted that there was a positive correlation between TFA and cholesterol concentrations (Fig. 1), the regression coefficient (Snedecor, 1956) for cholesterol (mg/100 g) on TFA (m-equiv./kg) being $1{\cdot}55 \pm 0{\cdot}26$. This significant regression supports the observation

that both TFA and cholesterol concentrations decreased during fasting. Sure *et al.* (1933), Kohn, Ledford, Robertson & Swingley (1950) and Quackenbush & Pawlowski (1960) have all reported that fasting does not change the plasma concentration of cholesterol in rats fed before fasting on a diet containing 20% vegetable oil. According to Quackenbush & Pawlowski (1960) fasting increased serum cholesterol concentration in rats fed on a synthetic diet with 2% hydrogenated coconut oil, and an increase has also been found by Bragdon *et al.* (1957) and by Yacowitz, Kahn, Wind & Amrein

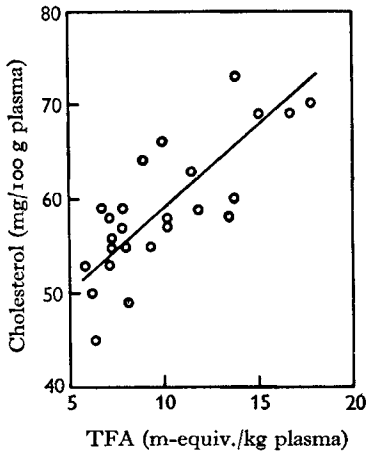

Fig. 1

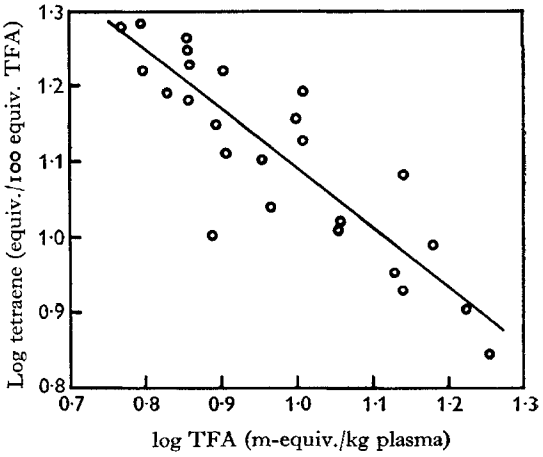

Fig. 2

Fig. 1. Relation between concentrations of cholesterol and total fatty acids in plasma of rats starved for 0–26 h.

Fig. 2. Double logarithmic relation between the proportion of tetraenoic acid in total fatty acids and the concentration of total fatty acids in plasma of rats starved for 0–26 h.

(1957). In better agreement with the results presented here are the observations by Mayfield & Roehm (1961) that the serum cholesterol concentration of male and female rats, fed before fasting on diets containing 35–40% fat, declined by about 30 mg/100 ml during 10–12 h of fast. In this connexion it should be mentioned that livers from starved rats incorporate acetate into fatty acids (Lyon, Masri & Chaikoff, 1952) and cholesterol (Tomkins & Chaikoff, 1952) much more slowly than livers from fed rats.

On the whole, the concentrations of dienoic, trienoic, pentaenoic and hexaenoic acids, expressed as m-equiv./kg plasma, declined with the TFA concentration (Table 1), their proportions in TFA being nearly constant. It was not so with tetraenoic acid, the concentration (m-equiv./kg plasma) of which was nearly constant. The regression coefficient of tetraenoic acid concentration on TFA concentration, both in m-equiv./kg, was found to be only $0{\cdot}025 \pm 0{\cdot}010$ ($0{\cdot}01 < P < 0{\cdot}05$). The proportion of tetraenoic acid in TFA increased from about 8 to about 16 equiv./100 equiv. TFA during the first 10–15 h and then remained constant, and a rectilinear relationship between log tetraenoic acid (equiv./100 equiv. TFA) and log TFA (m-equiv./kg) with the coefficient of regression $= -0{\cdot}78 \pm 0{\cdot}09$ (Fig. 2) could be demonstrated.

These findings indicate that, as the concentration of plasma total fatty acids decreases during fasting, they become increasingly unsaturated owing to an increase in the relative amount of tetraenoic acid. This finding is in agreement with that of Hølmer *et al.* (1960) who found that the concentration of tetraenoic acid (as a percentage of that of TFA) was higher in plasma of starved (20 h) than in plasma of unstarved chicks.

During alimentary lipaemia in man, Dole, James, Webb, Rizack & Sturman (1959) have found the fatty acid pattern of plasma to be remarkably constant and not to reflect the composition of the fat ingested. It deserves to be mentioned that, in agreement with the results reported here, Table 5 of the paper of Dole *et al.* (1959) clearly shows a decrease in the percentage of tetraenoic acid in all fractions, corresponding to an increase in total lipids after ingestion of 100 g maize oil. Dole *et al.* assume hypothetically that the constant composition of plasma fat is due to a rapid equilibration of the ingested fatty acids with tissue fatty acids, primarily from depot fat. The depot fat of rats contains about 10 % of dienoic acid but little or no tetraenoic acid (Dam & Engel, 1958; Ostwald, Okey, Shannon & Tinoco, 1962) and the dietary fatty acids in the experiment reported here contained about 40 % dienoic and 1·5 % tetraenoic acids.

In this experiment, the concentration of dienoic acid in equiv./100 equiv. TFA was independent of the time after the last meal, whereas the concentration of tetraenoic acid in equiv./100 equiv. TFA was considerably lower in unstarved than in starved rats. This finding seems to support the theory of Dole *et al.* (1959), since the addition of dietary fatty acids and depot fatty acids to plasma fatty acids from starved rats would result in a change in fatty acid pattern similar to that described above.

The constant fatty acid composition of the heart lipids may indicate that fasting does not change the metabolism of polyenoic acids in this tissue.

In the light of the above-mentioned observations a fasting period of at least 12 h is recommended for long-term dietary experiments. Further, in studies of plasma polyenoic acids, it seems to be important to record the concentration of total fatty acids so that changes in the relative as well as in the absolute concentrations of the individual polyenes can be measured.

SUMMARY

1. Food was withheld from twenty-six adult male rats for various periods. The rats were killed and the concentrations of cholesterol, total fatty acids (TFA) and polyenoic fatty acids were measured in their plasma and hearts.

2. Fasting for 0–26 h had no influence on the concentrations of cholesterol, TFA or polyenoic acids in the heart.

3. TFA concentration in plasma fell from about 16 to about 8 m-equiv./kg and cholesterol concentration fell slightly during the first 10 h of fasting.

4. The proportion of dienoic acid (equiv./100 equiv. TFA) was independent of the length of the fasting period, and the same seemed to be true for trienoic, pentaenoic and hexaenoic acids.

5. The proportion of tetraenoic acid (equiv./100 equiv. TFA) increased with

decreasing TFA concentration, the coefficient of regression for log tetraenoic acid (equiv./100 equiv. TFA) on log TFA (m-equiv./kg) being $-0{\cdot}78 \pm 0{\cdot}09$. The concentration of tetraenoic acid in m-equiv./kg was nearly constant.

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