Rats Enriched with Odd-carbon Fatty Acids

Effect of Prolonged Starvation on Liver Glycogen and Serum Lipids, Glucose and Insulin

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SUMMARY

Male rats were fed a complete diet containing 30 per cent of calories as fat, with corn oil exclusively in the controls and a 7:3 mixture of triundecanoin (C11) and corn oil in the experimental group. After six weeks, rats were sacrificed in the fed state and at 24, 48, 96 and 144 hours of starvation. In the experimental group, adipose tissue fatty acids were enriched with undecanoate to an average of 28 per cent of total fatty acids. Serum glucose was found to be significantly higher in the experimental group at all periods of starvation, and liver glycogen in the experimental rats was significantly higher at 48, 96 and 144 hours of starvation. Planimetry of the area circumscribed by values plotted from 24 to 144 hours of starvation gave mean values of immunoreactive insulin of 22.37 mU. hours per ml. for the experimental versus 13.55 mU. hours per ml. for the control group ($P < .005$), and mean values of free fatty acids of 967 mEq. hours/L. for the experimental versus 1,128 mEq. hours/L. for the control group ($P < .05$). Weight loss and nitrogen excretion were not significantly different among the two groups, nor were levels of serum cholesterol and triglycerides.

The data suggest that during prolonged starvation, the odd chain, fatty acid-enriched animals are capable of maintaining liver glycogen and serum glucose better than even-chain-enriched control animals. These changes are reflected in concurrently higher serum immunoreactive insulin and lower free fatty acid levels. The odd-chain fatty-acid-enriched rat appears to be endowed with potentially glucogenic fatty acids. DIABETES *20*:200-05, April, 1971.

The adipose tissue of animals can be substantially enriched with odd-carbon fatty acids by feeding the C11 triglyceride triundecanoin.[1-3] Beta oxidation of the odd-carbon fatty acids yields propionate residues which are potentially glucogenic.[4-6] Recently it has been shown that rats fed triundecanoin and subsequently starved for periods up to six days are able to maintain normal blood glucose and higher levels of liver glycogen than corn-oil-fed controls.[7] The suggestion has been made that the terminal three-carbon units provide available carbohydrate to the animal in the fasted state in quantities sufficient to counteract both the depletion of glycogen in the liver and the drop in plasma glucose concentrations which are characteristic of the starvation state in the even-carbon fatty acid-enriched animal.[7] The purpose of this paper is to investigate in rats enriched with odd-chain fatty acids the rates of glycogen diminution in liver and muscle and the rate of nitrogen loss, together with certain plasma parameters of carbohydrate and lipid metabolism, in response to starvation of varying duration.

METHODS

Ninety Charles River male rats (C-D strain) were divided at weaning into two equal groups. Each group was fed one of two nutritionally complete diets differing only in the quality of the fat that was present. The diet for the first group of animals (experimental) consisted of a "fat-free" test diet* supplemented with adequate vitamins and minerals and with a 7:3 mixture of triundecanoin† and corn oil. The final concentration of nutrients by calories was carbohydrate (50 per cent), protein (20 per cent) and fat (30 per cent). The second group of animals (control) was fed an identical diet except that the fat was exclusively corn oil. At the end of this period, the rats in each of the two groups were weighed and divided into five subgroups each consisting of nine animals. The first subgroup was sacrificed during the fed state. The other four sub-

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groups were starved with access to water only and sacrificed at 24, 48, 96 and 144 hours of starvation respectively. The animals were given sodium pentobarbital anesthesia (6 mg. intraperitoneally per 100 gm. weight) and blood was obtained by aortic puncture.

Immediately after bleeding, pieces of liver and quadriceps muscle were excised for determination of their glycogen content. Glycogen was determined by the method of Good et al.[8] Serum glucose was determined by an automated procedure based on the method of Hoffman.[9] Serum FFA were determined by a single phase titration procedure[10] utilizing the extraction technic of Dole.[11] It was found that approximately 60 per cent of undecanoate free fatty acid in plasma was extracted by this procedure. Since undecanoate constitutes between 7.5 and 13.5 per cent of the plasma FFA during starvation in the C11-enriched animals[7] however, the net loss factor in the total FFA values due to incomplete extraction is a mean of 4 per cent. The FFA values in the experimental group were corrected upward by this amount. Triglycerides,[12,13] cholesterol,[14,15] and immunoreactive insulin[16,17] were also determined.

Samples of perirenal fat were obtained from six experimental and three control animals at the end of six weeks of maintenance on their respective diets. The fatty acid composition of the lipid extracts was determined by temperature-programmed gas-liquid chromatography. Complete twenty-four-hour collections of urine were obtained from rats placed in metabolic cages, and urinary nitrogen was determined on nine rats from each of the experimental and control groups. The quantitative determination of total nitrogen was based on a modification of the Kjeldahl technic adapted for an automated system.[18,19] Urinary creatinine was also determined[20,21] and nitrogen excretion was calculated as mg. per gm. of creatinine.

RESULTS

The rates of weight gain of the rats are shown in table 1. After the first two weeks of adaptation to the diet, there was no significant difference between the control and the experimental groups, and the weights of the two groups did not differ significantly at the beginning and at the end of the feeding periods.

The fatty acid pattern of adipose tissue of rats after six weeks of diet in experimental and control rats is shown in table 2. An average enrichment with undecanoate of 28.3 per cent of total adipose tissue fatty acids was achieved in the experimental group, with traces of higher odd-carbon fatty acids. It is evident that no odd-carbon fatty acids were found in the control group.

The results of other biochemical determinations are shown in table 3. Serum glucose was found to be significantly higher in the experimental group at all intervals of starvation. Liver glycogen was significantly higher in the experimental rats after 48, 96 and 144 hours of starvation. Muscle glycogen was not significantly different between the two groups. Serum immunoreactive insulin levels were significantly higher in the experimental rats at twenty-four and ninety-six hours of starvation while serum free fatty acids were significantly lower at forty-eight hours. Since insulin was consistently higher in the experimental group and free fatty acids were consistently lower, calculations were made by planimetry to compare the area circumscribed under the curves of the values plotted from 24 to 144 hours of starvation. These were expressed in mU. hours/ml. and mEq. hours/L. For the purpose of calculating these values, a diurnal constancy of serum FFA and insulin levels was assumed to be present. It is noteworthy that the animals in both groups were subjected to identical starvation procedures. The values so calculated are shown in figure 1. The immunoreactive insulin concentration of the experimental animals during starvation was significantly higher ($P < .005$) than that of the corn-oil-fed control animals (23.37 vs. 13.55 mU. hours/ml.), and the free fatty acid levels were significantly lower (967 vs. 1,128 mEq. hours/L.—$P < .05$). Serum cholesterol and triglycerides were determined on two thirds of both the control and experimental groups. The values for fed rats for serum cholesterol ± S.E. were 83 ± 8 mg. per 100 ml. for the controls and 71 ± 3 for the experimental; those for triglycerides were 296 ± 20 mg. per 100 ml. for the controls, and 287 ± 35 for the experimental group. There was no significant difference between the groups in either cholesterol or triglycerides and there was no significant difference during the various periods of starvation.

Rates of weight loss of the rats during starvation are shown in table 4. There was no significant difference in weight loss between the control and experimental groups at any starvation-time interval. Total nitrogen excretion measured in the nine rats in each group carried to 144 hours of starvation showed no significant difference between the control and experimental groups. Values expressed as milligrams of nitrogen per gram of creatinine and given in sequence for the control versus the experimental as the mean ± S.E. were: at 24 hours,

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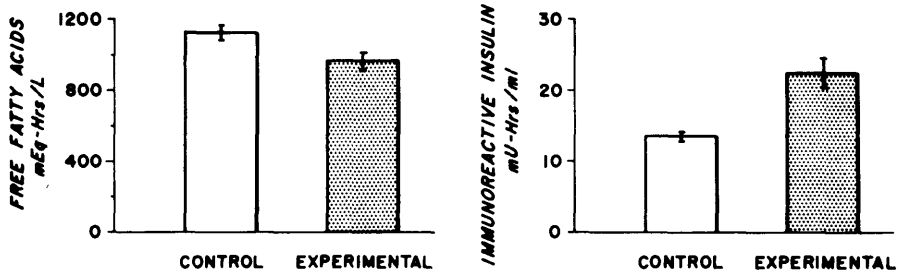

FIG. 1. Planimetry of the areas under the curves of values of serum free fatty acids and of immunoreactive insulin during 24 to 144 hours of starvation. Values were plotted by ranking rats 1 through 9 at each of the 24, 48, 96 and 144 hours and considering each rank as the component of one curve.

TABLE 1

Growth of two groups of rats maintained on two diets which differed only in quality of fat contents. (Growth is expressed in gm. per day ± S.E.*)

Week	Control Diet (Corn oil fed)	Experimental Diet (Triundecanoin fed)	P
1	5.0 ± 0.16	4.6 ± 0.55	< .05
2	5.1 ± 0.15	4.4 ± 0.16	< .01
3	6.8 ± 0.44	6.6 ± 0.38	NS
4	7.4 ± 0.09	7.4 ± 0.26	NS
5	5.8 ± 1.35	6.3 ± 0.30	NS
6	5.6 ± 0.55	5.9 ± 0.35	NS
Average weight in gm. ± S.E. at start of experiment	57 ± 0.9	58 ± 1.0	NS
Average weight in gm. ± S.E. at start of fast	293 ± 5.0	281 ± 4.8	NS

* 9 rats for each group mean ± S.E.

TABLE 2

Fatty acid pattern (per cent of total) of adipose tissue of rats after six weeks of diet containing triundecanoin (experimental) as compared to that of rats after six weeks of diet containing corn oil (control).

Adipose Tissue Fatty Acid	Experimental Mean ± S.E. (n = 6)	Control Mean ± S.E. (n = 3)
11:0	28.3 ± 1.0	
12:0	1.1 ± 0.0	0.07 ± 0.03
13:0	0.5 ± 0.2	
14:0	1.5 ± 0.1	0.8 ± 0.1
15:0	1.1 ± 0.1	
16:0	23.4 ± 0.5	18.6 ± 0.7
16:1	3.7 ± 0.2	2.3 ± 0.2
17:0	0.1 ± 0.1	
18:0	2.5 ± 0.2	2.4 ± 0.2
18:1	19.6 ± 0.7	27.6 ± 1.4
18:2	17.5 ± 0.5	48.2 ± 1.0

TABLE 3

Effect of starvation on certain biochemical parameters of corn oil-fed control (C) and undecanoate-enriched experimental (E) rats*

Groups		Hours of Starvation 0	24	48	96	144
			Liver Glycogen (gm./100 g.)			
C		6.12 ± 0.42	0.27 ± 0.08	0.09 ± 0.02	0.91 ± 0.18	0.51 ± 0.08
E		5.87 ± 0.46	0.49 ± 0.12	0.95 ± 0.14	1.86 ± 0.20	1.98 ± 0.35
	P	(N.S.†)	(N.S.)	(< .001)	(< .01)	(< .01)
			Muscle Glycogen (gm./100 g.)			
C		0.48 ± 0.03	0.31 ± 0.03	0.29 ± 0.03	0.32 ± 0.02	0.39 ± 0.02
E		0.42 ± 0.02	0.26 ± 0.02	0.35 ± 0.02	0.37 ± 0.02	0.45 ± 0.03
	P	(N.S.)	(N.S.)	(N.S.)	(N.S.)	(N.S.)
			Serum Glucose (mg./100 ml.)			
C		174 ± 5.7	93 ± 1.0	81 ± 6.0	88 ± 9.2	105 ± 5.5
E		180 ± 10.8	132 ± 7.8	133 ± 7.9	141 ± 5.5	149 ± 4.2
	P	(N.S.)	(< .01)	(< .001)	(< .001)	(< .001)
			Serum Insulin (μU./ml.)			
C		86 ± 11.5	16.8 ±2.9	9.8 ± 1.5	12.1 ± 2.2	12.8 ± 2.7
E		84 ± 10.5	27.6 ± 4.1	15.1 ± 3.5	19.0 ± 2.0	13.6 ± 3.6
	P	(N.S.)	(p = .05)	(N.S.)	(< .05)	(N.S.)
			Serum FFA (μEq./L.)			
C		732 ± 103	1040 ± 95	1012 ± 52	910 ± 45	853 ± 76
E		706 ± 228	905 ± 33	779 ± 44	855 ± 76	729 ± 53
	P	(N.S.)	(N.S.)	(< .005)	(N.S.)	(N.S.)

* Each mean ± S.E. is calculated for 9 rats.
† N.S. = not significant.

TABLE 4

Body weight loss during starvation in corn oil-fed control (C) and undecanoate-enriched experimental (E) rats

Hours Fasted	Group	N	Weight Loss Gm.	Per cent of initial wt.	P
24	C	9	16 ± 1.7	6.0 ± 0.5	NS
	E	9	14 ± 2.7	5.0 ± 0.8	
48	C	9	24 ± 2.8	8.6 ± 0.8	NS
	E	9	24 ± 1.6	8.8 ± 0.4	
96	C	9	46 ± 2.7	15.3 ± 0.8	NS
	E	9	48 ± 1.2	16.6 ± 0.6	
144	C	9	63 ± 2.7	20.0 ± 0.5	NS
	E	9	58 ± 1.4	18.9 ± 0.5	

15.6 ± 0.7 vs. 17.2 ± 0.8; at 48 hours, 15.6 ± 0.6 vs. 15.2 ± 0.8; at 72 hours, 14.2 ± 0.2 vs. 14.4 ± 1.0; at 96 hours, 11.4 ± 0.5 vs. 14.0 ± 1.1; at 120 hours, 12.4 ± 0.6 vs. 13.9 ± 1.2; at 144 hours, 13.0 ± 0.5 vs. 14.4 ± 1.4.

DISCUSSION

Studies in our laboratory[1,2] have shown that the adipose tissue of rats can be enriched with undecanoate to proportions of 22 to 32 per cent of total adipose tissue fatty acids. A similar pattern of enrichment has been reported in intact dogs.[3] In the present study, the total adipose tissue fatty acid that was undecanoate averaged 28.3 per cent, which agrees well with the above-quoted levels. In the undecanoate-enriched animal, odd-chain fatty acids are mobilized readily from adipose tissue, and together with even-chain fatty acids, appear in the plasma FFA fraction.[3] During starvation, the proportion

of odd-chain fatty acids in adipose tissue does not change appreciably.[7]

Previous studies of the response of rat liver glycogen to starvation show that the glycogen is depleted rapidly in the first twenty-four to forty-eight hours followed by a subsequent gradual increase that never reaches the level of a fed animal.[22] This drop in glycogen is accompanied by a more gradual decline in blood glucose. However, in the undecanoate-enriched animals, blood glucose and liver glycogen is maintained during starvation at remarkably higher levels than those in rats fed "normal," even-chain fat. In a recent study, Van Itallie and Khachadurian[7] reported that during starvation, rats enriched with undecanoate maintained higher liver glycogen and serum glucose concentration than did corn-oil-fed controls. The data presented here are consistent with their findings.

The odd-chain fatty acids, initially degraded by B-oxidation in the same manner as even-chain acids, yield residual three-carbon units. These terminal units are potentially glucogenic insofar as propionyl CoA is converted to succinyl-CoA via methylmalonyl CoA.[23,24] The succinate readily forms glucose and glycogen. It is postulated that this is the mechanism whereby the glycogen and glucose are maintained during starvation in the odd-chain fatty acid-enriched animal, a mechanism unavailable to an animal fed even-chain fat, since in the latter situation a similar reservoir of carbohydrate precursor in the adipose tissue is nonexistent.

To ascertain whether this unique response to starvation was reflected in other biochemical parameters, studies of circulating hormonal and lipid constituents were done. The results show that the concentration of immunoreactive insulin in time is significantly higher in the experimental than in the control group, while free fatty acids in time are significantly diminished. With the higher blood glucose, more insulin presumably is secreted by the pancreatic islets, thereby dampening the rate of lipolysis. The end result would seem to be a lower rate of egress of free fatty acids and glycerol from adipose tissue triglyceride stores.

It appears that during starvation both groups of animals were conserving nitrogen at a comparable rate. It has been shown that certain control systems involved in fasting are set to limit nitrogen loss to the minimum to provide sufficient glucose for the central nervous system. Lipid derivatives, such as ketones, have been shown to be preferentially utilized by extrahepatic tissues during starvation.[25] This may be true even for the brain.[26] Thus, the hyperketonemia induced by starvation indirectly might have provided a dampening effect on gluconeogenesis from protein in the control animal by decreasing glucose need. The degree of hyperketonemia in the experimental group, although not measured, would be expected to be lower than in the control in view of the enhanced insulin secretion and the diminished lipolysis. Thus on this basis, the experimental group would be expected to lose more nitrogen if another mechanism for nitrogen conservation had not been present. Since nitrogen loss indeed was comparable in both groups, it is possible that the glucogenic component of the odd-chain fatty acid in the experimental group served to inhibit gluconeogenesis to the same extent as the hyperketonemia in the control group.

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