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Starvation-Induced Ketosis: Reduction in Dogs Enriched with Odd-Carbon Fatty Acids[1] (37895)

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(Introduced by T. B. Van Itallie)

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It is possible to enrich the adipose tissue of healthy dogs with appreciable quantities of odd-numbered medium-chain fatty acids by incorporation into the diet of a synthetic triglyceride, triundecanoin (1). Beta oxidation of subsequently mobilized odd-carbon fatty acids yields propionate residues which are potentially glucogenic (2–4). Rats similarly enriched and subsequently starved are able to maintain higher concentrations of blood glucose and liver glycogen than normally fed controls (5, 6).

This paper describes the ketonemic response to starvation in odd-carbon fatty acid-enriched dogs.

Materials and Methods. One litter of mongrel dogs consisting of seven animals was used. The animals were born in the laboratory and, at weaning, they were divided into two groups: experimental (four dogs) and control (three dogs). Each group was fed one of two nutritionally complete diets differing only in the type of fat present. The diet for the experimental group consisted of a fat-free test diet[3] supplemented with adequate vitamins and minerals to which was added equal amounts of triundecanoin and cottonseed oil. The control group was fed an identical diet except that the fat was exclusively cottonseed oil. Both diets contained 43% of calories as carbohydrate, 17% as protein, and 40% as fat. All animals were fed ad lib. for 13 weeks.

At the end of this period the dogs were weighed and subcutaneous fat was sampled under local anesthetic by surgical biopsy from the hindquarter of each animal. Blood also was obtained during the fed state by venipuncture. The animals then were starved with access to water only. Additional samples of blood were obtained on Days 2, 4, and 8 of starvation. In an attempt to test the glycogenolytic response of the liver *in vivo*, on the eighth day of fasting the animals were given 1 mg of glucagon intravenously, and a blood sample was drawn 15 min afterwards.

Serum glucose was determined by autoanalyzer (7). Blood acetoacetate and beta-hydroxybutyrate were determined spectrophotometrically (8) on protein-free filtrates. The fatty acid composition of subcutaneous fat was determined by temperature-programmed gas–liquid chromatography after transmethylation of the extracted lipid with sodium methoxide.

Results. The mean ± standard error of the mean (SE) weight of the animals at weaning was 1.28 ± 0.62 kg. The three puppies comprising the control group were picked at random and weighed 1.25 ± 0.05 kg; the four puppies making up the experimental group weighed 1.30 ± 0.11 kg. At the end of 13 weeks of feeding of their respective diets, the control group weighed 18.16 ± 2.03 kg and the experimental group 16.43 ± 1.51 kg ($P > 0.5$). There

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TABLE I. Fatty Acid Pattern (% of Total) of Adipose Tissue of Dogs after 13 Weeks of Diet Containing Triundecanoin (Experimental) as Compared to That of Dogs After 13 Weeks of Diet Containing Cottonseed Oil (Control).

Adipose tissue fatty acid	Experimental Mean ± SE ($n = 4$)	Control Mean + SE ($n = 3$)
11:0	16.1 ± 0.4	—
12:0	1.7 ± 0.1	0.1 ± 0
13:0	2.9 ± 0.3	—
14:0	4.9 ± 0.6	4.2 ± 0.1
15:0	2.9 ± 0.1	—
16:0	20.7 ± 0.8	21.5 ± 0.5
16:1	5.4 ± 0.5	4.1 ± 0.4
17:0	0.8 ± 0.2	—
18:0	2.3 ± 0.4	4.4 ± 0.4
18:1	18.6 ± 0.5	25.2 ± 0.7
18:2	22.3 ± 0.6	39.9 ± 0.8

was no significant difference in the rate of weight loss between the control and experimental animals during starvation. After 8 days of fasting, the control animals had lost 23.1 ± 1.4% of their weight, whereas the experimental group had lost 25.7 ± 1.2% ($P > 0.2$).

The adipose tissue fatty acid patterns of the dogs at the end of the experimental feeding period are shown in Table I. Undecanoate comprised 16.1% of total adipose tissue fatty acids in the experimental group. Smaller amounts of C13, C15, and C17 fatty acids also were present, making a total of 23% odd-carbon fatty acids. Adipose tissue in the controls contained only even-chain fatty acids, with a relatively high level of linoleate (C18:2), reflecting their cottonseed oil intake.

The results of other biochemical determinations are shown in Table II. Although both groups of animals had similar serum glucose concentration during the fed state, the experimental group maintained significantly higher glucose levels at all sampling times during starvation. Similar levels of blood acetoacetate and beta-hydroxybutyrate were present in both groups in the fed state. During starvation, although acetoacetate concentration rose in both groups, it was consistently higher in the control group, the difference reaching statistical significance on Day 4. During starvation, beta-hydroxybutyrate rose in both groups but to levels 3-fold higher in the control group than in the experimental group. Since total ketones were consistently lower in the experimental group, calculations were made by planimetry to compare the areas circumscribed by the curves of the total ketone values plotted from 24 to 192 hr of starvation. Calculated by this method, the values ± SE were 0.168 ± 0.005 mmole-hr/liter in the experimental group and 0.409 ± 0.062 in the controls. Thus, total ketones were significantly higher in the con-

TABLE II. Effect of Starvation on Serum Glucose and Blood Ketone Bodies of Triundecanoin-Fed Experimental (E) and Cottonseed Oil-Fed Control (C) Dogs.[a]

Groups	Days of starvation 0	2	4	8
Serum glucose (mg/100 ml)				
E	110 ± 6.1	91 ± 4.4[b]	99 ± 0.9[b]	110 ± 6.0[b]
C	112 ± 9.3	76 ± 6.3	80 ± 8.4	85 ± 8.4
Blood acetoacetate (mmoles/liter)				
E	.036 ± .014	.175 ± .017	.216 ± .014[b]	.341 ± .030
C	.036 ± .012	.241 ± .036	.682 ± .195	.603 ± .175
Blood beta-hydroxybutyrate (mmoles/liter)				
E	.049 ± .012	.158 ± .052[b]	.222 ± .026[b]	.354 ± .030[b]
C	.071 ± .017	.552 ± .040	.699 ± .125	.830 ± .184

[a] Each mean ± SE is calculated for four dogs in E and three dogs in C.
[b] $P < 0.05$.

trols than in the experimental animals ($P < 0.005$).

Intravenous glucagon was administered on the eighth day of starvation. In the control animals, the serum glucose level did not change significantly after glucagon, the mean ± SE being 85 ± 8.4 mg/100 ml before the glucagon injection and 91 ± 8.9 mg/100 ml 15 min after glucagon administration. In contrast, the experimental animals had a significant elevation of glucose after glucagon, the serum level being 110 ± 5.9 mg per 100 ml prior to and 151 ± 7.6 mg per 100 ml 15 min after glucagon ($P < 0.01$).

Discussion. During fasting, free fatty acids are mobilized in increasing quantities from adipose tissue stores and are utilized by the liver and other tissues. In the undecanoate-enriched animal, odd-carbon fatty acids are mobilized readily but not preferentially from adipose tissue (5), and, together with even chain fatty acids undergo β-oxidation in a manner similar to that of the even-numbered ones, except that the final thiolytic cleavage can yield propionyl CoA rather than acetyl CoA residues. These terminal 3-carbon units are potentially glucogenic, propionyl Coa being convertible to succinyl CoA via methyl malonyl CoA (9, 10). The succinate readily forms glucose and glycogen. Van Itallie and Khachadurian (5) reported that during starvation rats enriched with undecanoate maintained higher liver glycogen and serum glucose concentration than did corn-oil fed controls. It was suggested that the terminal 3-carbon units provide enough available carbohydrate to animals in the fasted state to counteract both the depletion of glycogen in the liver and the drop in blood glucose characteristic of starvation. Subsequent studies in our laboratory have shown, furthermore, that odd-carbon-enriched rats have significantly higher circulating insulin levels and significantly lower serum FFA levels than controls (6). The higher blood glucose in the odd-carbon-enriched animals presumably enhances insulin secretion and dampens lipolysis.

The present study shows that odd-carbon fatty acid-enriched dogs maintain significantly higher blood glucose levels during starvation than do control animals. Also, the significantly greater blood glucose response to intravenous glucagon in the undecanoate-enriched dog after 8 days of starvation implies a greater reserve of mobilizable liver glycogen. These observations are consistent with similar studies reported in rats (5, 6).

The degree of ketosis in the undecanoate-enriched dogs was strikingly less than that in control animals. The regulation of ketogenesis is probably mediated through the action of insulin. In ketotic states there is a relative or absolute deficiency of this hormone, and both the ketosis of starvation (11) and of diabetes (12) can be rapidly reversed with insulin. The higher ambient blood glucose in the odd-carbon-enriched animal may cause enhanced insulin secretion (6), thereby resulting in diminished ketogenesis.

Summary. For 13 weeks, a litter of weanling dogs was fed a nutritionally complete diet containing 40% of its calories as fat: cottonseed oil exclusively in the control group (three dogs) (C) and triundecanoin substituted for 50% of the fat calories in the experimental group (four dogs) (E). In E, 23% of the total adipose tissue fatty acids were odd numbered. Throughout an 8-day fast, serum glucose concentration was maintained significantly higher in E than in C. Blood acetoacetate and betahydroxybutyrate were significantly lower in E than in C. On the eighth day of fasting, a greater mobilizable liver glycogen reserve was found in E. Thus, ketogenesis during fasting is strikingly inhibited in the odd-carbon-enriched animal. This inhibition is thought to relate directly to maintenance during starvation of blood glucose and insulin concentrations at appreciably higher values than is usual.

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