Beta-Cell Insulin Secretory Response to Glucose in Odd-Carbon Number Fatty Acid-enriched Rats

Preservation During Starvation

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SUMMARY

The adipose tissue of fifty male Sprague-Dawley rats was enriched with 26.3 per cent odd-carbon number fatty acids (OCE) by feeding a complete diet containing a 7:3 mixture of triundecanoin and corn oil for six weeks. An equal number of control animals (C) was fed a similar diet except that the fat was exclusively corn oil. After six weeks, the animals were given intravenous glucose (500 mg.) either in the fed state or after forty-eight- or ninety-six-hour fasts. Fasting plasma glucose and immunoreactive insulin levels were higher in OCE than in C during starvation. K values of glucose disappearance after an intravenous glucose load were similar in OCE and C in the fed state; with starvation, K values were less impaired in OCE than in C. The areas under the insulin response curve on days 2 and 4 of starvation were reduced to 24 per cent and 19 per cent of the fed value in C and 73 per cent and 47 per cent in OCE. Insulin-to-glucose ratio diminished significantly with starvation in C, whereas no significant change occurred in OCE. Total pancreatic insulin was not different in fed or two- and four-day starved OCE and C animals. Thus, fasting in C appears to impair the insulin secretory response of beta cells to glucose, whereas OCE maintain significantly greater responsiveness. The sustained beta-cell sensitivity to glucose of the starved OCE animal probably is related to preservation in the beta cells of a glucose receptor or an intracellular enzymatic system. DIABETES *23*:605-09, **July, 1974.**

The adipose tissue of mammals can be substantially enriched by a diet containing odd-carbon number fatty acids.[1-3] Beta oxidation of the odd-carbon number fatty acids yields propionate residues which are potentially glucogenic.[4-6] Weanling rats fed a diet rich in triundecanoin, a C-11 triglyceride, for four to six weeks and subsequently starved are able to maintain blood glucose and liver glycogen at significantly higher concentrations than conventionally fed controls.[7,8] Rats previously enriched with conventional even-carbon number fatty acids and starved for forty-eight hours show a markedly impaired insulin secretory response to glucose.[9] This impairment of glucose-stimulated insulin secretion during starvation has been postulated to result from a reduced activity of a glucose-inducible enzyme system in the pancreatic beta cell.[9] The purpose of the present study was to investigate whether rats previously enriched with odd-carbon number fatty acids would exhibit an insulin secretory response to glucose during starvation different from that of controls.

MATERIALS AND METHODS

Sprague-Dawley male rats weighing approximately 100 gm. were used in this study. The rats were divided into two equal groups. Each group was fed one of two nutritionally complete diets with 50 per cent of calories as carbohydrate (sucrose), 20 per cent as protein (casein), and 30 per cent as fat. The fat for the experimental group of animals consisted of a seven to three mixture of triundecanoin and corn oil. In the group of control animals, corn oil was used exclusively as the fat source. All the animals were weighed weekly. After six weeks of ad libitum diet, fifty-four animals were used for glucose tolerance testing and forty-six animals for pancreatic insulin extraction studies. In both experimental and control groups, animals were subdivided into three groups, to be studied in the fed state, and after forty-eight and ninety-six hours of starvation.

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Intravenous glucose tolerance testing was performed as follows: rats were anesthetized with sodium pentobarbital intraperitoneally (4 mg. per 100 gm. of body weight) and a PE 50 catheter was introduced through the right jugular vein into the right atrium for subsequent glucose injection and sampling. After an equilibration period, a blood sample was drawn into a heparinized tube and then 1 ml. of 50 per cent glucose was injected rapidly. Blood samples then were drawn into heparinized tubes at 10, 20, 30, 45 and 60 minutes after the glucose load. The plasma was separated and frozen for subsequent analysis for glucose and insulin. Plasma glucose was determined by enzymatic analysis utilizing hexokinase.[10] Insulin was measured by radioimmunoassay using a double antibody system[11,12] with a rat insulin standard.*

For the pancreatic insulin extraction studies, rats were sacrificed by decapitation and the whole pancreas was immediately removed and frozen in dry ice and acetone. Pancreases were then homogenized in a Potter-Elvehjem homogenizer and insulin was extracted with acid-alcohol by a micromodification of the method of Scott and Fisher.[13]

Perirenal adipose tissue samples were taken from a representative number of animals. The fatty acid composition of the lipid extracts was determined by temperature-programmed gas liquid chromatography.

RESULTS

The mean ± standard error of the mean (S.E.) weight of the rats at the beginning of the study was 99.1 ± 1.3 gm. for the odd-carbon number fatty acid-enriched animals (OCE) and 99.5 ± 1.0 gm. for the controls (C). At the end of the six-week feeding period, the weights of the two groups were similar, being 269.7 ± 5.6 gm. for OCE and 266.8 ± 4.5 gm. for C ($p > .5$).

The fatty acid patterns of adipose tissue of OCE and C rats after six weeks of feeding from the two diets are shown in table 1. The adipose tissue of the OCE animals contained 24.4 per cent C11 fatty acids and 1.9 per cent odd-carbon number fatty acids longer than C11. The adipose tissue enrichment with odd-carbon number fatty acids occurred at the expense of linoleate (C18:2) and to a lesser extent oleate (C18:1). There were small increases in the proportions of the palmitate series (C16:0, C16:1). No odd-carbon number fatty acids were found in the adipose tissue of C.

During starvation the OCE and C rats behaved similarly with regard to weight loss. In those fasted forty-eight hours, OCE lost 26.8 ± 0.7 gm., and C lost 27.2 ± 1.0 gm. ($p > .5$). In those fasted ninety-six hours, OCE lost 43.0 ± 3.0 gm. and C lost 39.9 ± 1.0 gm. ($p > .2$). The effect of varying degrees of starvation (zero to four days) on plasma glucose and immunoreactive insulin concentrations are shown in table 2. In the fed state plasma glucose was not statistically different in the two groups. After starvation there was a striking fall of glucose at days 2 and 4 in C; these values were significantly lower in C than in the OCE animals ($p < .005$, $p < .05$). Expressed in per cent of the baseline fed glucose value, glucose fell to 41 per cent of fed values on day 2 and 59 per cent of fed values on day 4 in C, whereas in OCE animals glucose only fell to 67 per cent of fed values on day 2 and 82 per cent of fed values on day 4. In the fed state

TABLE 1

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

Adipose Tissue Fatty Acids	OCE Mean ± S.E. (n = 27)	C Mean ± S.E. (n = 15)
11:0	24.4 ± 0.6	———
12:0	0.9 ± 0.1	0.4 ± 0.1
13:0	1.0 ± 0.1	———
14:0	1.2 ± 0.1	0.9 ± 0.0
15:0	0.8 ± 0.1	———
16:0	21.7 ± 0.7	18.4 ± 0.5
16:1	3.5 ± 0.3	2.8 ± 0.2
17:0	0.1 ± 0.0	———
18:0	3.3 ± 0.3	2.9 ± 0.3
18:1	24.1 ± 0.6	28.9 ± 0.4
18:2	20.3 ± 0.7	46.0 ± 1.0

TABLE 2

Effect of varying degrees of starvation (zero to four days) on plasma glucose and plasma immunoreactive insulin concentration in rats previously enriched with odd-carbon number fatty acids by feeding triundecanoin (OCE) and in corn oil-fed controls (C).

Groups	Days of Starvation 0	2	4
	Plasma Glucose (mg./100 ml.)		
OCE	125 ± 8.2	84 ± 9.5	103 ± 10.1
C	119 ± 4.3	49 ± 4.7	69 ± 9.6
	Plasma Immunoreactive Insulin (μU./ml.)		
OCE	59 ± 8.2	56 ± 12.1	30 ± 9.4
C	72 ± 18.3	34 ± 6.2	17 ± 4.6

Nine rats for each group, mean ± S.E.

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plasma immunoreactive insulin levels were not significantly different between OCE and C rats. However, on days 2 and 4 of fasting, insulin values in C declined to significantly lower levels than in the fed state ($p < .05$, $p < .01$), whereas values in OCE were not significantly different from the fed state on day 2 and fell to significantly lower values only on day 4 ($p < .05$).

Results of intravenous glucose tolerance tests are shown in figure 1. K values of glucose disappearance[14] were calculated from the ten- to forty-five-minute values. On days 0, 2 and 4, mean ± S.E. values were 4.44 ± 0.69, 2.55 ± 0.22 and 2.52 ± 0.71 in OCE, and 4.24 ± 0.38, 1.78 ± 0.46, and 1.98 ± 0.35 in C. The K value thus was significantly impaired on day 2 in both groups as compared to the K during the fed state (in C: $p < .001$; in OCE: $p < .025$). The K value during starvation was more impaired in C than in OCE.

The total insulin response to the intravenous glucose load is shown in figure 2. Total insulin response represents the area circumscribed by the plasma insulin curve above basal values and is expressed as microunit minutes $ml.^{-1}$. On day 0, total insulin was not significantly different in C than OCE ($p > .5$). In C, total insulin dropped significantly at day 2 ($p < .05$) and day 4 ($p < .05$) of fasting as compared to the fed state. The drop in total insulin response was markedly less in OCE, with no significant change from the fed state to either day 2 or day 4 of starvation.

The insulin response to glucose administration also was evaluated utilizing the ratio of the area of total insulin response above basal values to the area circumscribed by the corresponding plasma glucose curve above the basal values. The data are shown in figure 2. In the fed state, the insulin-to-glucose ratio was not significantly different in C than in OCE ($p > .2$). When compared to the fed state, the insulin-to-glucose ratio in C dropped significantly at day 2 ($p < .025$) and day 4 ($p < .01$) of fasting. In OCE the drop in insulin-to-glucose ratio is much less than in C and is not significantly different at day 2 ($p > .2$) or day 4 ($p > .05$) from the value in the fed state.

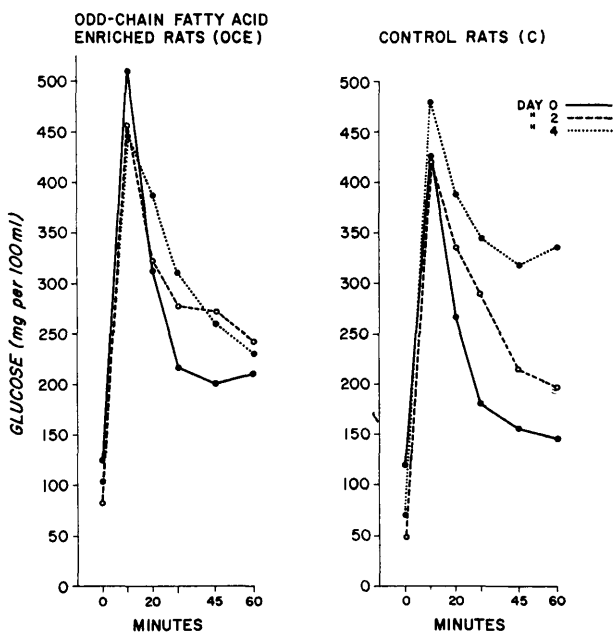

FIG. 1. Effect of varying degrees of starvation (zero to four days) on tolerance to rapidly administered (one minute) intravenous glucose (500 mg.) in rats previously enriched with odd-carbon number fatty acids by feeding triundecanoin (OCE) and in corn oil-fed controls (C). (n = 9 for each group).

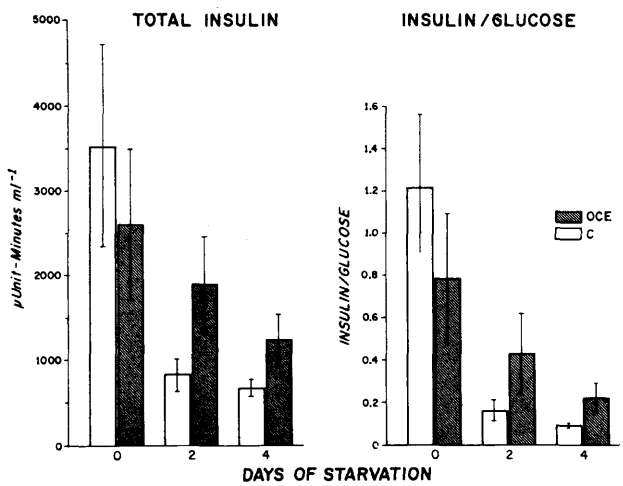

FIG. 2. Effect of varying degrees of starvation (zero to four days) on mean ± S.E. total insulin response and insulin-to-glucose ratio in rats previously enriched with odd-carbon fatty acids by feeding triundecanoin (OCE) and in corn oil-fed controls (C). To calculate total insulin response, planimetry was done of the areas circumscribed by the plasma insulin curve above basal values after intravenous glucose. Total glucose was measured similarly to calculate the insulin-to-glucose ratio.

If the above data on total insulin secretion and insulin-to-glucose ratio are plotted on a per cent basis using fed state values as a baseline, OCE total insulin dropped to 73 per cent of fed values on day 2 and 47 per cent on day 4, while the total insulin in C showed a significantly greater drop, falling to 24 per cent of the fed value on day 2 and 19 per cent on day 4. Similarly, the insulin-to-glucose ratio was maintained

at levels much closer to the fed baseline in the OCE animals than in C. While OCE ratio dropped to 54 per cent of fed values on day 2 and 28 per cent on day 4, the ratio in C dropped to 14 per cent of fed values on day 2 and 7 per cent on day 4.

In order to determine whether the differing insulin responses of the OCE and C animals were reflected in their pancreatic insulin content, insulin extraction studies were carried out. Total pancreatic content for the OCE animals expressed in mean ± S.E. units per total pancreas were: 2.10 ± 0.2 on day 0, 2.15 ± 0.4 on day 2, and 2.60 ± 0.2 on day 4. In C animals, the values were: 2.79 ± 0.3 on day 0, 2.12 ± 0.2 on day 2, and 2.37 ± 0.1 on day 4. These six values are not significantly different from each other. Thus, the insulin content of the pancreas was not different between OCE and C groups in the fed state or at days 2 and 4 of starvation.

DISCUSSION

The adipose tissue of animals can be enriched with diet-derived undecanoate to concentrations of 22 to 32 per cent of total adipose tissue fatty acids[1-3,7,8] with no alteration in growth rate.[3,8] The present study confirms the previous observations as shown by the growth figures and the gas liquid chromatographic data.

In C, after six weeks, the adipose tissue fatty acid pattern approached that of corn oil, the sole fat source in the diet. This observation is consistent with previous studies showing that, after achievement of a steady state, the fatty acid composition of adipose tissue approximates that of the diet, particularly as regards the essential fatty acid, linoleate.[15-17] Thus, for a given amount of linoleate in the fat of the diet of C (50 per cent), the proportion of linoleate in the adipose tissue rose to 46 per cent. When, in the OCE group, 70 per cent of the corn oil in the diet was replaced with triundecanoin, the net dietary linoleate dropped to 16 per cent. Under these conditions, the linoleate content of the adipose tissue also dropped to 20 per cent, reflecting dietary input. During this time, odd-carbon number fatty acid enrichment was being achieved.

The higher plasma glucose and immunoreactive insulin levels in response to starvation in OCE as compared to C support data previously published from our laboratory.[7,8] The odd-carbon number fatty acids that were initially degraded by B-oxidation in the same manner as even-carbon number acids yield three-carbon units. These terminal units are potentially glucogenic insofar as propionyl CoA is converted to succinyl CoA via methylmalonyl CoA.[18,19] The succinate readily forms glucose and glycogen. This is the mechanism postulated whereby, during starvation, glycogen and blood glucose are maintained closer to fed values in the odd-carbon number fatty acid-enriched animal.

The progressive deterioration of glucose tolerance after two to four days of starvation in the normal control rats is consistent with previous reports.[9,20] The OCE animals, however, maintain a higher K value of glucose disappearance during starvation than do the control animals. This observation suggests that starved OCE animals have a greater ability to dispose of a glucose load.

The changes in total insulin and in insulin-to-glucose ratio during starvation in rats have been described previously by Grey et al.[9] These investigators showed that rats fasted for forty-eight hours had marked impairment in their insulin secretory response to glucose. The insulin secretion remained subnormal after starvation if the animals had been refed a carbohydrate-free diet. The insulin responsiveness could be restored with calorically insignificant intraperitoneal carbohydrate injections during the fast. Also, return of insulin responsiveness by refeeding carbohydrate could be blocked by actinomycin D. These authors therefore postulated a glucose-inducible enzyme system mediating glucose-stimulated insulin secretion in the pancreatic beta cell.

In the OCE animals, the total insulin and the insulin-to-glucose ratios after starvation were diminished far less than in C. Thus, given the same glucose load, the OCE animals after starvation were able to respond with a markedly less impaired insulin secretory response than the C animals. Since these OCE animals maintained their blood glucose and liver glycogen levels[7,8] closer to those found in the fed state, it is possible that during starvation the higher circulating glucose perfusing the islet cells maintained beta-cell sensitivity to glucose much closer to maximal fed levels than was the case in C animals whose blood glucose and liver glycogen levels fell much lower. The present study does not distinguish whether this enhanced sensitivity is at the level of an intracellular enzymatic system[9] or a glucose receptor molecule.[21]

Although the total insulin secretory response to a glucose stimulus was higher in the OCE rats, pancreatic insulin content did not differ between OCE and C either in the fed or starved state. The data in C would confirm reports of Malaisse et al.[20] and Grey et al.[9]

that during starvation, pancreatic insulin content is not depleted even though the insulin responsiveness to glucose is impaired. The data in OCE suggests that the enhanced insulin response to glucose in these animals is not related to a higher pancreatic insulin content but rather to a facilitated insulin release.

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