α-Oxoglutarate Carboxylation in Liver Mitochondria Isolated from Rats Fed Diets of Varied Fat Concentration[1] (36625)

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Several reports have suggested that the fixation of CO_2 by NADP-linked isocitrate dehydrogenase may represent an important metabolic step in the synthesis of tricarboxylic acid cycle intermediates (1–4). Wagle (3) has shown that the "α-oxoglutarate carboxylase" activity of liver mitochondria from alloxan diabetic rats was greatly increased when assayed by the method of Ochoa (2). This finding led to the suggestion that α-oxoglutarate carboxylation is an important pathway for supporting the increased rate of gluconeogenesis present in alloxan diabetes (4). In a subsequent paper, Mackerer, Mehlman and Tobin (5) have shown that α-oxoglutarate carboxylation by intact rat liver mitochondria is stimulated by both alloxan and streptozotocin diabetes. Bowman (6), utilizing perfused rat hearts, has also presented evidence suggesting that diabetes can stimulate α-oxoglutarate carboxylation. However, increased rates of α-oxoglutarate carboxylation also appeared to be produced in normal hearts by addition of octanoate to the perfusion medium. This finding raises the possibility that α-oxoglutarate carboxylation might be increased by environmental or pathological conditions which cause increased rates of lipid catabolism. Diabetes represents one condition in which lipid catabolism is markedly increased.

In the present study, rats were fed artificially prepared high-fat diets, rather than the usual high-carbohydrate chow diet, so that lipid catabolism would be the dominant energy generating system. It was found that raising the dietary fat level increased the rate of α-oxoglutarate carboxylation by liver mitochondria *in vitro*.

Methods. Diets. Male albino rats (Sasco, Inc., Omaha, NB) of approximately 150 g weight were randomly divided into three groups of 6 rats each. These groups were fed *ad libitum* artificially prepared diets containing 10, 30 or 70% fat for 12 weeks. The diet compositions are shown in Table I. On the final day, the rats were killed by decapitation between 11 and 12 a.m. The rats were not fasted prior to sacrifice.

Mitochondria. Liver mitochondria were isolated by the method of Johnson and Lardy (7) except that the 0.25 *M* sucrose used in the homogenizing step contained 5 mg/ml of bovine serum albumin (fatty acid poor). The protein content of the mitochondrial suspen-

TABLE I. Diet Compositions.

	Diets (%)		
	1(10% fat)	2(30% fat)	3(70% fat)
Casein (purified-vitamin free)[a]	22.0	22.0	22.0
Glucose	20.0	13.0	—
Sucrose	20.0	13.0	—
Dextrin	20.0	13.0	—
Lard	7.5	22.5	52.5
Corn oil	2.5	7.5	17.5
Cellulose	2.6	3.6	2.6
Salt mix[b]	4.0	4.0	4.0
Vitamin mix[c]	1.4	1.4	1.4

[a] The protein levels for diets 1–3 represented 21, 17, and 13% of the dietary calories.

[b] Roger's-Harper salt mixture (General Biochemicals).

[c] Vitamin diet fortification mixture (Nutritional Biochemicals).

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TABLE II. α-Oxoglutarate Consumption and Metabolite Formation by Liver Mitochondria from Rats Fed a Purina Chow Diet.[a]

Incubation time (min)	Metabolite changes (μmoles/9 mg protein)			
	α-Oxoglutarate consumed	Found		
		Citrate	Succinate	Malate
5	0.84	0.35	0.2	<0.01
10	1.34	0.61	0.4	<0.01
15	2.19	0.88	1.0	<0.01
30	4.15	1.61	2.0	0.01
60	7.67	2.74	4.3	0.03

[a] The 3 ml reaction mixtures (pH 7.4) contained 4 m*M* ATP, 10 m*M* $MgCl_2$, 6.7 m*M* P_i, 13.3 m*M* TEA, 6.7 m*M* α-oxoglutarate, 2 m*M* EDTA, 20 m*M* malonate, 53 m*M* KCl, 42 m*M* sucrose, and 1 μg of antimycin A. Mitochondria, equal to 9 mg of protein, were added at time 0; and the incubation times were varied as indicated. The incubation vessels were gassed for 1 min with a mixture of 95% N_2 and 5% CO_2. The values represent the means of three replicate experiments.

sion was determined, by the biuret procedure (8), and was adjusted to 20 mg/ml with 0.25 *M* sucrose. Incubations were carried out in stoppered 25 ml Erlenmeyer flasks which were shaken in a water bath at 37°. Reactions were started by addition of 0.5 ml of mitochondrial suspension to 2.5 ml of media and were stopped by addition of 3 ml of 0.66 *M* perchloric acid. Processing of samples for analysis was done by the method of Walter, Paetkau and Lardy (9). The compositions of the incubation media are given in the footnotes to the tables.

Results and Discussion. As shown in Table II, rat liver mitochondria, incubated under reduced conditions in a medium containing $HCO_3^- + CO_2$ and malonate, converted α-oxoglutarate almost entirely to succinate and citrate which accumulated with time. Oxidation through succinate dehydrogenase was inhibited by the anaerobiosis and the presence of malonate and, therefore, malate did not accumulate. Under aerobic conditions, in the absence of malonate, malate, rather than succinate, accumulates (5). Previous work has shown that the citrate is synthesized by a reversal of the tricarboxylic acid cycle (10–14), which involves reductive carboxylation of α-oxoglutarate followed by equilibration of citrate and isocitrate through aconitase. In accordance with this, the accumulation of citrate was found to be absolutely dependent upon the presence of $HCO_3^- + CO_2$ in the medium (Table III). Since the citrate, synthesized by carboxylation, is not further metabolized (14) total citrate ac-

TABLE III. The Effects of $HCO_3^- + CO_2$ on α-Oxoglutarate Consumption and Metabolite Formation by Liver Mitochondria from Rats Fed a Purina Chow Diet.[a]

Added HCO_3^- (m*M*)	Composition of gas phase (%)	Metabolite changes (μmoles/hr/12.5 mg of protein)		
		α-Oxoglutarate consumed	Found	
			Citrate	Malate
0	100 N_2	1.79	0.06	0.01
20	95 N_2 5 CO_2	10.5	4.31	<0.01

[a] The basic reaction mixtures were the same as described for Table II. In the medium without HCO_3^-, an additional amount of KCl (16 m*M*) was added. The incubation vessels were gassed for 1 min. The values represent the means of three replicate experiments.

TABLE IV. The Effects of Varied Dietary Fat Levels on α-Oxoglutarate Consumption and Metabolite Formation by Rat Liver Mitochondria.[a]

Dietary fat (%)	Metabolite changes (nmoles/min/mg of protein)	
	α-Oxoglutarate consumed	Citrate found
10	12.8 ± 0.7	4.88 ± 0.33
30	17.0 ± 0.8[b]	6.20 ± 0.30[c]
70	19.1 ± 0.3[d]	7.40 ± 0.29[b]

[a] The reaction mixtures were the same as described for Table II except that antimycin A was not included. The incubation vessels were gassed for 1 min with a mixture of 95% N_2 and 5% CO_2. The values represent the mean ± SEM ($n = 6$).

[b] $p < .005$.

[c] $p < .05$.

[d] $p < .001$.

cumulation was representative of α-oxoglutarate carboxylation.

The effects of feeding high-fat diets on α-oxoglutarate metabolism are shown in Table IV. Mitochondria from both the 30% and 70% fat groups showed significant increases in both α-oxoglutarate consumption and citrate accumulation. α-Oxoglutarate consumption increased at a rate twice that of citrate accumulation in accord with the dismutation:

2 α-oxoglutarate → succinate + citrate.

The increased α-oxoglutarate carboxylation caused by the diets can be correlated with increased fatty acid oxidation in the rats fed the 30 and 70% fat diets. Ketone bodies are produced as a result of lipid catabolism and the rats fed the 30 and 70% fat diets had increased levels of blood ketone bodies (Fig. 1).

Thus, two conditions, diabetes and high-fat feeding, which have high rates of lipid catabolism associated with them, produce markedly increased rates of α-oxoglutarate carboxylation in liver mitochondria. Preliminary observations also indicate that fasting produces similar increases and that these are reversed by refeeding.

At the $HCO_3^- + CO_2$ concentrations used in the experiments of Tables II and IV, α-oxoglutarate carboxylation is driven at maximal rate with α-oxoglutarate as the sole hydrogen donor (14). Therefore, expression of the data on a per gram of tissue basis gives an estimation of the maximum contribution of mitochondrial α-oxoglutarate carboxylation to liver metabolism in the rats fed the varied diets. Assuming that liver contains 8.3 mg of mitochondrial nitrogen per gram of tissue (7), which is approximately 51.9 mg of mitochondrial protein/g, maximum citrate synthesis via α-oxoglutarate carboxylation corresponds to 0.25 ± 0.02 in the rats fed 30% fat; and 0.38 ± 0.02 in the rats fed 70% fat.

Summary. Rat liver mitochondria, incubated under anaerobic conditions, in media containing α-oxoglutarate and $HCO_3^- + CO_2$, oxidized α-oxoglutarate through the tricarboxylic acid cycle and utilized the reducing equivalents produced by this oxidation to drive α-oxoglutarate to citrate via reductive carboxylation. Feeding rats artificially prepared diets high in fat (30 and 70%) for 12 weeks prior to sacrifice increased the rates

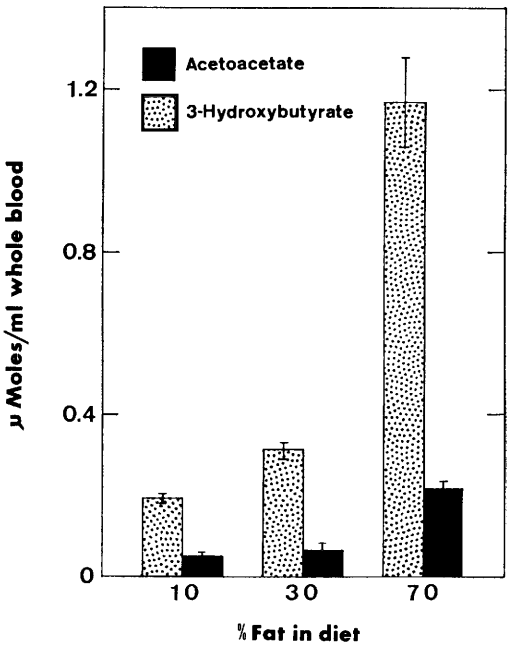

FIG. 1. Acetoacetate and 3-hydroxybutyrate concentrations in whole blood of rats fed 10, 30, and 70% fat diets. The values represent the mean ± SEM ($n = 6$).

of α-oxoglutarate oxidation and citrate accumulation by the isolated mitrochondria.

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