LIVER ACETYL CoA CARBOXYLASE AND FATTY ACID SYNTHETASE: RELATIVE ACTIVITIES IN THE NORMAL STATE AND IN HEREDITARY OBESITY*

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Received July 17, 1967

The reactions of fatty acid biosynthesis from acetyl CoA in higher organisms are catalyzed by acetyl CoA carboxylase and a fatty acid synthetase multienzyme complex (Wakil, 1963; Vagelos, 1964). Evidence from many laboratories (Numa et al., 1961; Martin and Vagelos, 1962; Waite and Wakil, 1962; Kallen and Lowenstein, 1962) indicates that regulation of fatty acid synthesis occurs at the level of acetyl CoA carboxylase. The carboxylase is activated by citrate and even when fully activated is alleged to be rate-limiting in fatty acid synthesis (Ganguly, 1960; Numa et al., 1961). However, no condition has been reported in which changes in carboxylase level per se account fully for changes in the rate of fatty acid synthesis. Hepatic fatty acid synthesis is 6-8 times higher in the genetically obese hyperglycemic mouse than in normal mice (Jansen et al., 1967).

The initial objective of this investigation was to determine whether hepatic carboxylase and/or synthetase levels are increased commensurate with the elevated rate of fatty acid biosynthesis in obese mice. Both hepatic acetyl CoA carboxylase and fatty acid synthetase levels were found to be elevated to about

*Supported by research grants AM-09116 from the National Institutes of Health, U.S. Public Health Service and GB-5699 from the National Science Foundation.
**Career Scientist of the Health Research Council of the City of New York (I-275)
+Career Development Awardee of the U.S. Public Health Service (Research Career Program Award K3-AM-18487).

the same extent. It was found that in livers of obese and normal mice, as well as in the chicken and rat, the capacity of the carboxylase to generate malonyl CoA approximately equals the capacity of the synthetase to incorporate malonyl CoA into long chain fatty acids. In view of the latter finding, the possible role of malonyl CoA as an inhibitor of fatty acid synthesis is discussed.

Experimental Procedure. Male obese-hyperglycemic mice (C576J/ob) 10-14 weeks of age and lean littermates were obtained from the Jackson Laboratory and fed Wayne Lab Blox ad libitum. Laying hens were obtained locally. Livers were homogenized in 3 volumes of a medium (pH 7.2 at 25°) containing 0.15 M KCl, 0.05 M Tris (Cl^-) and 0.1 mM EDTA. The 105,000 x g supernatant solution (cytosol) was either used directly for synthetase assays or subjected to gel filtration on Sephadex G-25 in the homogenization medium prior to carboxylase assays. Carboxylase assays involved preincubation of the gel-filtered cytosol at 37° for 30 minutes in a medium (final pH 7.0 at 37°) containing 60 mM Tris (Cl^-) buffer, 3 mM GSH, 8 mM $MgCl_2$, 0.1 mM EDTA, bovine serum albumin (0.6 mg per ml) in the presence or absence of 5 mM citrate (K^+). Carboxylation was initiated by addition of the following components (final pH 7.0 at 37°) to produce final concentrations of: 60 mM Tris (Cl^-) buffer, 2 mM ATP, 8 mM $MgCl_2$, 10 mM $KH^{14}CO_3$ (specific activity, 0.2 μc per μmole), 0.2 mM acetyl CoA, 3 mM GSH, 0.1 mM EDTA, 0.6 mg per ml bovine serum albumin and 5 mM citrate (K^+) as indicated. Reaction was for 5 minutes at 37°. Incorporation of ^{14}C-activity into malonyl CoA was determined as described by Gregolin et al. (1966a). Synthetase assay reaction mixtures (final pH 7 at 37°) containing 100 mM phosphate (K^+) buffer, 0.025 mM acetyl CoA, 0.10 mM 2-^{14}C-malonyl CoA (chromatographically purified; 0.2 μc per μmole), 1.2 mM NADPH, 2 mM dithiothreitol, 3 mM EDTA and high-speed supernatant solution were incubated for 10 minutes at 37°.

Incorporation of ^{14}C-activity into long chain fatty acids was determined as described by Guchhait et al. (1966).

Results and Discussion. The correlation of the levels of acetyl CoA carboxylase and fatty acid synthetase, with the hepatic hyperlipogenesis characteristic of the obese-hyperglycemic mouse was investigated. Both carboxylase and synthetase activities (Table I) were markedly elevated in the obese mouse. The specific activities (milliunits per mg of cytosol protein) of both enzymes were about 3-fold higher in livers from the obese mice compared to lean siblings. Total liver cytosol activities were 6- to 8-fold higher. A nearly absolute citrate requirement for carboxylase activity was observed in all cases (Table I, values in parentheses).

TABLE I. Hepatic Acetyl CoA Carboxylase and Fatty Acid Synthetase Activities

	Carboxylase		Synthetase		Specific activity ratio
	Specific activity	Total activity	Specific activity	Total activity	
	m units* per mg protein	m units* per liver	m units** per mg protein	m units** per liver	synthetase/ carboxylase
Lean mouse	3.1 (0.2)+	474	4.7	700	1.5
	2.6 (0.1)	316	6.0	746	2.3
	4.8 (0.2)	648	7.3	980	1.5
	3.1 (0.2)	424	8.3	1030	2.7
Average	3.4 (0.2)	465	6.6	864	1.9
Obese mouse	10.1 (0.2)	3600	16.9	6100	1.7
	16.8 (0.4)	6100	18.5	6800	1.1
	9.1 (0.1)	2100	15.5	3600	1.7
	9.6 (0.2)	2940	19.5	5900	2.0
Average	11.4 (0.2)	3700	17.6	5600	1.5
Laying hen	7.3 (0.2)	-	10.5	-	1.4
	6.7 (0.1)	-	15.8	-	2.4
	10.0 (0.2)	-	13.2	-	1.3
	6.9 (0.2)	-	15.6	-	2.2
	8.7 (0.1)	-	18.1	-	2.1
	12.8 (0.2)	-	11.8	-	0.9
Average	8.7 (0.2)		14.2		1.6

Protein was determined by the biuret method (Layne, 1957).
*One milliunit = 1 mμmole of acetyl CoA carboxylated/minute. **One milliunit = 1 mμmole of 2-^{14}C-malonyl CoA incorporated into long chain fatty acids per minute.
+Values in parentheses are activities in the absence of citrate.

Kornacker and Lowenstein (1964) reported that the level of citrate cleavage enzyme (expressed per mg of liver cytosol protein) in obese mice is increased 3-fold compared to lean mice. Thus, it appears that the elevated rate of fatty acid synthesis in the obese-hyperglycemic mouse is associated with a coordinated rise in the levels of all principal enzymatic components of fatty acid synthesis.

An unexpected finding was the similarity in the activities of the carboxylase and synthetase. At pH 7 (37°) synthetase activity was only 1.5 to 1.9-fold greater than carboxylase activity in both obese and normal mice (Table I). To determine whether the similarity of these activities was unique to the mouse, hepatic carboxylase and synthetase activities of laying hens were also determined; the ratio of synthetase to carboxylase activity was found (Table I) to be similar to that observed in the mouse. Preliminary studies in the rat have yielded similar findings. It should be emphasized that carboxylase and synthetase were assayed at pH 7 (37°) which is below the optimum, pH 7.5 (37°), for the chicken liver carboxylase and slightly above the optimum, pH 6.8 (37°), for the chicken liver synthetase. Hence, at the more physiologic pH of 7.4 (37°) liver acetyl CoA carboxylase would be expected to exceed fatty acid synthetase activity by a small margin. Earlier investigations (Ganguly, 1960 and Numa et al., 1961) indicated that synthetase activity was 20-80 times greater than carboxylase activity; these low carboxylase activities can be attributed to suboptimal carboxylase assay conditions.

The fact that liver carboxylase and synthetase activities are nearly equal suggests that under certain circumstances the malonyl CoA concentration in vivo may be sufficient to depress carboxylase activity. Malonyl CoA is a potent inhibitor of chicken liver acetyl CoA carboxylase (Gregolin et al., 1966b) exhibiting a K_i of about 10^{-5}M. Furthermore, malonyl CoA inhibition is competitive with respect to both acetyl CoA and isocitrate (or citrate). In the case of acetyl

CoA (K_m = 1-4 x 10^{-5} M) malonyl CoA inhibition is of the classical competitive type. Evidence has been presented (Gregolin et al., 1966b) that the competitive kinetic relationship between malonyl CoA and isocitrate (or citrate) is the result of an equilibrium of an active polymeric form of the carboxylase promoted by isocitrate (or citrate) and a catalytically-inactive protomeric form promoted by malonyl CoA. Inhibition of acetyl CoA carboxylase by malonyl CoA could provide a safeguard against excessive acetyl CoA utilization for malonyl CoA production, thereby providing fine control over the committed step of fatty acid biosynthesis.

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