ON THE PROPOSED RELATION OF CITRATE ENZYMES TO FATTY ACID SYNTHESIS AND KETOSIS IN STARVATION

Paul A. Srere and Daniel W. Foster

Veterans Administration Hospital and the Departments of Biochemistry and Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas

Received January 27, 1967

Citrate cleavage enzyme and citrate synthase activities have been widely assumed to be regulatory in fatty acid synthesis (Kornacker and Lowenstein, 1964; Kornacker and Lowenstein, 1965; Kornacker and Ball, 1965) and ketogenesis (Wieland, et al, 1964). The observation by Srere (1959) that citrate cleavage enzyme is located in the soluble fraction of the cell, together with the demonstration that citrate is an effective substrate for fatty acid synthesis (Srere and Bhaduri, 1962; Spencer and Lowenstein, 1962; Formica, 1962) led to the postulate that this enzyme played an important role in the generation of acetyl CoA in the cytoplasm of the cell (Srere and Bhaduri, 1962; Spencer and Lowenstein, 1962; Srere, 1965). Since hepatic cleavage enzyme activity was reduced after a 48 hour fast and increased on re-feeding, Kornacker and Lowenstein (1965) concluded that changes in fatty acid synthesis and citrate cleavage enzyme activity occur in parallel and implied that the two were causally related; e.g., that decreases in citrate cleavage enzyme activity occur in starvation and diabetes and result in the failure of cytoplasmic generation of acetyl CoA with consequent impairment of fatty acid synthesis. Studies of citrate cleavage activity under varying conditions of nutrition and hormone imbalance (Brown and McLean, 1965; Brown et al., 1966; Howanitz and Levy, 1965) were interpreted as supporting this conclusion despite previously noted objections (Srere, 1965).

Citrate synthase, which catalyzes the condensation of acetyl CoA with oxalacetate, has been postulated to play a regulatory role in ketosis (Wieland, et al., 1964). Its involvement is based on the observations that hepatic fatty acyl CoA concentrations are increased in fasting (Bortz and Lynen, 1963; Tubbs and Garland, 1964) and that citrate synthase is inhibited by palmityl CoA (Tubbs, 1963; Wieland and Weiss, 1963). The inhibition of citrate synthase is believed to contribute to an increased hepatic acetyl CoA concentration which has been assumed to cause an accelerated ketone body synthesis.

In this communication we wish to report non-parallel behaviour of citrate cleavage enzyme activity and fatty acid synthesis in liver from fasted rats, a situation where parallel behaviour has been claimed. In addition, we have measured citrate synthase activity during the onset of fasting ketosis and found no decrease in its total activity.

Experimental Procedure

Two-hundred gram rats maintained on a high carbohydrate, low fat diet were tube fed 7.5 G of the diet in suspension at the start of the experiment. Groups of six rats were killed at 0, 6, 12, and 24 hours after the final tube feeding. Blood was collected for determination of acetoacetate concentration by the method of Walker (1954). Fatty acid synthesis was measured in slices and 100,000 X G supernatant fractions as described by Siperstein and Fagan (1958) utilizing acetate-2-^{14}C as substrate. Citrate cleavage enzyme and citrate synthase were measured in the supernatant fraction and alcohol-KCl extracts of aliquots of the same liver as described previously (Srere, 1959; Srere, et al., 1963; Srere, in press).

Results and Discussion

The sequential changes occurring in fatty acid synthesis and citrate cleavage enzyme activity with the onset of fasting are shown in Fig. 1. Fatty acid synthesis is given for liver slices though qualitatively similar changes were observed in the high speed supernatant fractions. Data for the citrate cleav-

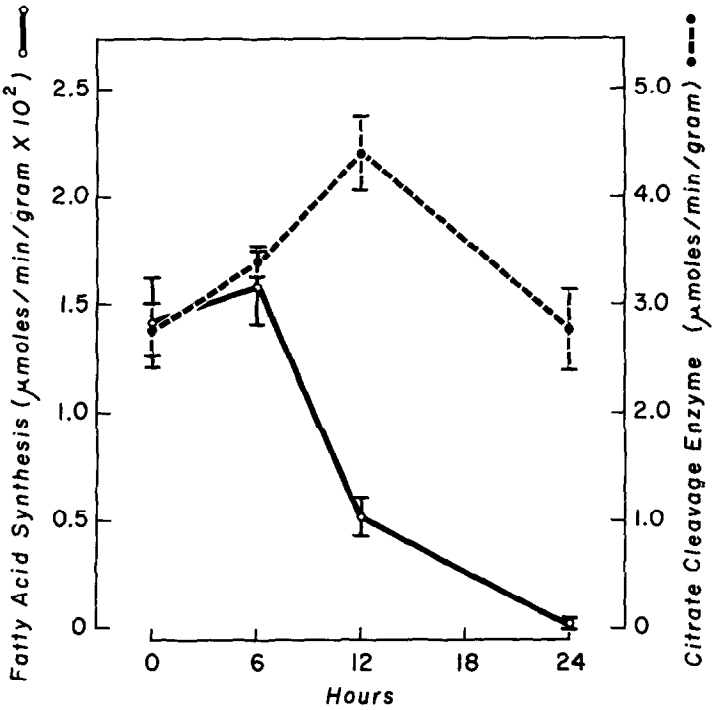

Fig. 1 Citrate Cleavage Enzyme and Fatty Acid Synthesis in Starvation

age enzyme are shown for alcohol-KCl extracts. Previous experiments have shown that identical results are obtained whether this activity is measured in 100,000 X G supernatant fractions or KCl-alcohol extracts of the same whole liver. Six hours after the last food intake fatty acid synthesis remained at control levels while cleavage enzyme activity was slightly increased. At twelve hours a sharp dissociation occurred. Fatty acid synthesis decreased 67% from 15.1 to 5.0 mμmoles per minute while citrate cleavage activity increased from 3.4 to 4.4 μmoles per minute. By twenty-four hours fatty acid synthesis fell to near zero while citrate cleavage activity was still equal to that found in the zero time controls. It is known that prolonged fasting does result in decreased hepatic citrate cleavage activity. The kinetics of this change measured here concomitantly with fatty acid biosynthesis, however, indicate that changing citrate cleavage enzyme levels cannot be the cause of the altered fatty acid metabolism of starvation.

The citrate synthase contents of the same livers and simultaneous blood acetoacetate levels are shown in Table I. After a twenty-four hour fast there was no decrease in citrate synthase levels at a time when ketosis had clearly supervened. It appears that there is a slight increase in enzyme activity of about 25% during this period. That citrate synthase activity is not impaired in starvation is supported by the observation that acetate oxidation to CO_2 remains normal in liver slices from rats during a forty-eight hour fast (Foster, unpublished observations).

We have examined citrate cleavage and synthase activities in many other tissues (brain, lung, heart, kidney, intestine and testes) in rats starved for up to six days. Significant decreases were observed only in intestine and in that tissue only after a four day fast.

These results do not imply that citrate cleavage enzyme is uninvolved in cytoplasmic acetyl CoA production and fatty acid synthesis nor that citrate synthase does not play a role in acetyl CoA metabolism. They do suggest that the decreases in enzyme activity previously reported in starvation and diabetes are secondary rather than primary events. Similar conclusions have been drawn

Table I

Citrate Synthase Activity and Ketosis in Starvation

Duration of Fast (hours)	Citrate synthase (μmoles/min/G)	Acetoacetate (mg%)*
0	7.3 ± 0.39†	1.9 ± 0.19
6	7.9 ± 0.24	1.5 ± 0.07
12	8.7 ± 0.78	2.6 ± 0.17
24	9.3 ± 0.40	8.3 ± 1.7

* As Sodium acetoacetate † Mean ± S.E.M.

by Masoro (1962) and Kipnis and Kalkhoff (1965) in studies of the regulatory role of acetyl CoA carboxlyase activity on fatty acid synthesis in starvation and diabetes. In general, it is likely that alterations in metabolism are initiated by changes in concentrations of enzyme modifiers rather than changes in enzyme concentrations.

Summary

Sequence studies of citrate cleavage enzyme activity and fatty acid synthesis in rat liver during starvation demonstrated sharp dissociation in the two parameters and indicate that depressed lipogenesis in starvation is not due to decreased levels of citrate cleavage enzyme. Citrate synthase activity was not impaired by a twenty-four hour fast at a time when significant ketosis was observed.

Acknowledgements

The authors wish to express appreciation to Kathyrn Sims, Nancy Whissen and M. Joanne Guest for technical assistance. Supported in part by U.S.P.H.S. Grant # CA 08269. Daniel W. Foster is Research Career Development Awardee # 5-K3-AM 9968, U.S.P.H.S.

REFERENCES

Bortz, W. and Lynen, F., Biochem. Z. 339, 77 (1963).
Brown, J. and McLean, P., Nature 207, 407 (1965).
Brown, J., McLean, P. and Greenbaum, A. L., Biochem. J. 101, 197 (1966).
Formica, J. V., Biochim. Biophys. Acta 59, 739 (1962).
Foster, D. W., Unpublished observations.
Howanitz, T. J. and Levy, H. R., Biochim. Biophys. Acta 106, 430 (1965).
Kipnis, D. M. and Kalkhoff, R. J., J. Clin. Invest. 44, 1064 (1965).
Kornacker, M. S. and Lowenstein, J. M., Biochim. Biophys. Acta 84, 490 (1964).
Kornacker, M. S. and Lowenstein, J. M., Biochem. J. 94, 209 (1965).
Kornacker, M. S. and Ball, E. G., Proc. Natl. Acad. Sci. 54, 899 (1965).
Masoro, E. J., J. Lipid Res. 3, 149 (1962).
Siperstein, M. D. and Fagan, V. M., J. Clin. Invest. 37, 1185 (1958).
Spencer, A. F. and Lowenstein, J. M., J. Biol. Chem. 237, 3640 (1962).
Srere, P. A., J. Biol. Chem. 234, 2544 (1959).
Srere, P. A. and Bhaduri, A., Biochim. Biophys. Acta 59, 487 (1962).
Srere, P. A., Brazil, H., and Gonen, L., Acta Chem. Scand. 17, S 129 (1963).
Srere, P. A., Nature 207, 407 (1965).

Srere, P. A., in Methods in Enzymology, S. P. Colowick and N. O. Kaplan, editors, in press, Academic Press, New York.
Tubbs, P. K., Biochim. Biophys. Acta, 70, 608 (1963).
Tubbs, P. K. and Garland, P. B., Biochem. J. 93, 550 (1964).
Walker, P. G., Biochem. J. 58, 699 (1954).
Wieland, O. and Weiss, L., Biochem. Biophys. Res. Comm. 13, 26 (1963).
Wieland, O., Weiss, L. and Eger-Neufeldt, I., in Advances in Enzyme Regulation, G. Weber, editor, Vol. 2, Permagon Press, London, 1964, p. 85.