J. A. Med. Assoc. 168, 377 (1958).
5. Fleisher, D. S., J. Pediat 70, 54 (1967).
6. Bilka, P. J., Arthritis Rheumat. 10, 267 (Abstr.) (1967).
7. Anderson, R. A., Bottomly, D. R., Bachman, D. M., Arthritis Rheumat. 7, 294 (Abstr.) (1964).
8. Dubois, E. L., "Lupus erythematosis," p. 370. McGraw-Hill, New York (1966).
9. Fried, J. and Borman, A., Vitamins Hormones 16, 303 (1963).
10. Travis, R. H. and Sayers, G. *in*, "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), p. 1608. Macmillan, New York (1965).
11. Billingham, R. E., Krohn, P. L., Medawar, P. B., Brit. Med. J. 1, 1157.
12. Medawar, P. B., J. Anat. 78, 176 (1944).
13. Snedecor, G. W., "Statistical Methods," 5th ed. The Iowa State Univ. Press, Ames, Iowa (1956).
14. Ref. (13), 320.
15. Dougherty, T. F., Brown, H. E., Berliner, D. L., Endocrinology 62, 455 (1958).
16. Ward, P. A. and Johnson, A. G., J. Immunol. 82, 428 (1959).
17. Favour, C. B., *in* "Inflammation and Diseases of Connective Tissue" (L. C. Mills and J. H. Moyer, eds.), p. 486. Saunders, Philadelphia, Pennsylvania (1961).
18. Yates, F. E., *in* "The adrenal cortex" (A. B. Eisenstein, eds.), p. 133. Little, Brown, Boston, Massachusetts (1967).

Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 131.

Acetyl CoA Carboxylase and Fatty Acid Synthetase Activities in Liver and Adipose Tissue of Meal-fed Rats* (34038)

KRISHNA CHAKRABARTY AND GILBERT A. LEVEILLE

Division of Nutritional Biochemistry, Department of Animal Science, University of Illinois, Urbana, Illinois 61801

The cytoplasmic malonyl-CoA pathway, catalyzed by acetyl CoA carboxylase and fatty acid synthetase multienzyme complex, is recognized as the major pathway of fatty acid biosynthesis (1, 2). The activity of fatty acid synthetase has been reported to be many times in excess of acetyl CoA carboxylase activity thereby suggesting that the carboxylation of acetyl CoA to malonyl CoA is the rate-limiting step in fatty acid biosynthesis (3, 4). More recently, however, Chang *et al.* (5) have reported the activity of both enzymes to be similar in liver of mice, chickens, and rats.

Meal-feeding, the limitation of access to food to a single, daily 2-hr meal, markedly stimulates lipogenesis in adipose tissue of the rat (6–8). This increased lipogenic capacity is accompanied by an enhanced activity of several enzymes related to fatty acid synthesis, including acetyl CoA carboxylase (9). However, the influence of meal-feeding on the activity of fatty acid synthetase has not been investigated. Consequently, the present experiment was conducted in which the activities of acetyl CoA carboxylase and fatty acid synthetase were assayed in liver and adipose tissue of meal-fed and nibbling (*ad libitum*-fed) rats.

The results presented show that the specific activities of these two enzymes are similar in adipose tissue and liver of the *ad libitum*-fed rat. Also, the activity of both enzymes is significantly higher in adipose tissue, but not liver, of meal-fed as compared to nibbling animals.

Experimental. Male rats of the Sprague-Dawley strain weighing 294 ± 10/(AU ± SEM) were used. The "meal-fed" animals had access to food from 8 AM to 10 AM only whereas the "nibblers" were fed *ad libitum*. Water was available at all times. The composition of the diet has been previously described (10). The rats were maintained on their respective feeding schedules for at least 3 weeks, a period adequate to induce the

* This study was supported in part by U. S. Public Health Service Grant AM-10774 from the Institute of Arthritis and Metabolic Diseases.

TABLE I. Activities of Acetyl CoA Carboxylase and Fatty Acid Synthetase in Liver and Adipose Tissue of Meal-Fed Nibbling Rats.

		Dietary treatment		
		Meal-fed	Nibbling	p^a
Body weight, g		292 ± 16[b]	349 ± 9	<.01
Acetyl CoA carboxylase[c]	Adipose tissue	15.7 ± 1.9	6.9 ± 1.2	<.01
	Liver	4.1 ± 1.0	4.4 ± 1.3	ns
Fatty acid synthetase[d]	Adipose tissue	7.6 ± 0.9	3.0 ± 0.4	<.01
	Liver	3.4 ± 0.2	3.7 ± 0.4	ns
Ratio: Fatty acid synthetase /Acetyl CoA carboxylase	Adipose tissue	0.50 ± 0.05	0.48 ± 0.06	ns
	Liver	0.98 ± 0.15	1.26 ± 0.36	ns

[a] Probability of the difference between values for meal-fed and nibbling rats being significant; ns = not significant.

[b] Mean ± SEM for 6 rats.

[c] Values are mμmoles of $H^{14}CO_3^-$ incorporated/min/mg protein.

[d] Values are mμmoles of malonyl CoA incorporated/min/mg protein.

lipogenic and enzymatic responses to meal-feeding (11).

Meal-fed rats were killed at the end of the daily 2-hr meal and the nibbling animals had access to food until the time of killing. The animals were decapitated, exsanguinated, and the liver and epididymal adipose tissue were rapidly removed; separate pieces of tissue were homogenized for enzyme assay. The tissues were homogenized in a buffer (pH 7.2) containing 0.05 *M* tris, 0.001 *M* EDTA, and 0.15 *M* KCl for the assay of acetyl CoA carboxylase activity and in a 0.25 *M* sucrose solution containing 0.001 *M* EDTA and 0.01 *M* dithioerythrytol (pH 7.0) for fatty acid synthetase assay. The homogenates were centrifuged at 100,000*g* for 1 hr at 5° and the resulting supernatant fraction was used.

Acetyl CoA carboxylase was assayed by the method of Chang *et al.* (5). $MnCl_2$ (0.008 *M*) was added to the reaction mixture for the assay of the adipose tissue enzyme since Mn^{2+} was found to stimulate acetyl CoA carboxylase activity in homogenates of adipose tissue but not liver. Fatty acid synthetase activity was determined by a modification of the spectrophotometric method of Martin *et al.* (12). The assay mixture contained (in μmoles): malonyl CoA, 0.2; acetyl CoA, 0.1; NADPH, 0.5; phosphate buffer (pH 6.8), 20, and enzyme in a total volume of 1.0 ml. The rate of decrease of absorbancy at 340 mμ was determined. For each assay a blank cell was included from which malonyl CoA was omitted. The malonyl CoA converted to fatty acids was calculated assuming 2 moles of NADPH to be oxidized per mole of malonyl CoA incorporated.

Malonyl CoA was prepared by the method of Trams and Brady (13) and acetyl CoA by a modification of the method of Stadtman (14) as previously described (9). The protein content of the homogenates was determined by the method of Lowry *et al.* (15).

Results. The results of this investigation are summarized in Table I. The final body weight of the meal-fed rats was less than that of the nibbling animals, a finding in accord with previous investigations (8).

The activities of acetyl CoA carboxylase and fatty acid synthetase were significantly higher in adipose tissue of meal-fed as compared to nibbling animals. However, the activity of these enzymes in liver was not significantly altered by meal-feeding. The ratios of fatty acid synthetase to acetyl CoA carboxylase (Table I) in adipose tissue and liver were virtually identical for meal-fed and nibbling animals although the ratio for liver was greater than that for adipose tissue.

Discussion. The activity of acetyl CoA carboxylase was previously reported to be greater in adipose tissue but not liver of meal-fed as compared to nibbling rats (9).

The present results corroborate those findings and extend them by showing that the activity of fatty acid synthetase in these two tissues responds to meal-feeding in the same way as acetyl CoA carboxylase activity. Higher values for acetyl CoA carboxylase activity of adipose tissue were observed in the present investigation as compared to those reported earlier, particularly in tissue of the nibbling animals (9). The reason(s) for this difference is not obvious. However, older animals were used in the previous study and this may account for the observed difference. The lipogenic capacity of rat adipose tissue is known to decrease with age (16) and we have noted that adipose tissue acetyl CoA carboxylase activity also decreases with age (unpublished observation).

Adipose tissue is recognized as the major site of fatty acid biosynthesis in the rat (17, 18) and mouse (19). It is logical, therefore, that this tissue should be considered as the major site of the lipogenic adaptive changes induced by meal-feeding in the rat. The finding that the activity of the lipogenic enzymes studied is increased in adipose tissue but not liver of the meal-fed as compared to the nibbling rat, supports this concept. The greater adaptability to meal-feeding of other enzymes related to lipogenesis in rat adipose tissue as compared to liver (9) also supports the role of adipose tissue as the major lipogenic organ.

The approximately equal activity of fatty acid synthetase and acetyl CoA carboxylase in rat liver observed in this study is in accord with the recent report of Chang *et al.* (5) but is in contrast to earlier publications in which the fatty acid synthetase activity was found to be many times greater than that of acetyl CoA carboxylase (3, 4). The lower activity of acetyl CoA carboxylase observed in these early studies can undoubtedly be attributed to suboptimal assay conditions as Chang *et al.* (5) have suggested. The results of the present study extend the observations of Chang *et al.* (5) by demonstrating that in adipose tissue, as in liver, the activity of both enzymes is of the same order of magnitude and in fact, the carboxylase activity may be slightly greater than that of the synthetase.

Our results and those of Chang *et al.* (5) do not necessarily detract from the generally accepted concept that the carboxylation of acetyl CoA is the rate-limiting step of fatty acid biosynthesis. The numerous properties of acetyl CoA carboxylase which are generally considered characteristic of regulatory enzymes strongly support the control function assigned to this enzyme. Thus, it has been shown that acetyl CoA carboxylase is activated by di- and tricarboxylic acids, particularly citrate (20–23) and that this activation involves an equilibrium between an active polymeric form and an inactive protomeric form of the enzyme (23–26). Evidence has also been presented suggesting that acetyl CoA carboxylase is under feedback control, being inhibited by acyl CoA derivatives of long chain fatty acids (27, 28). The dissociation of the active polymeric form of acetyl CoA carboxylase is favored by malonyl CoA (23) which thereby inhibits the enzyme. The similarity in acetyl CoA carboxylase and fatty acid synthetase activity implies that malonyl CoA may play a significant role in regulating acetyl CoA carboxylase and hence the flow of acetyl CoA to fatty acids as suggested by Chang *et al.* (5). This proposed regulatory role of malonyl CoA appears even more important when viewed in the light of recent implications that the inhibitory effect of fatty acyl CoA derivatives on various enzyme systems if due to their detergent action and therefore is not of physiological significance (29–31).

Summary. The activities of acetyl CoA carboxylase and fatty acid synthetase in liver and adipose tissue of meal-eating (limited to a single, daily, 2-hr meal) and nibbling (*ad libitum*-fed) rats were determined. The activities of both enzymes were significantly higher in adipose tissue but not liver of meal-eating as compared to nibbling animals. This observation is in accord with the concept that adipose tissue is the primary site of fatty acid biosynthesis and the major site of the lipogenic adaptive changes induced by meal-feeding.

The activities of both enzymes were found to be of the same order of magnitude in liver and adipose tissue. In adipose tissue the ac-

tivity of acetyl CoA carboxylase was actually greater than that of fatty acid synthetase. The significance of this observation with regard to the regulatory role of acetyl CoA carboxylase in fatty acid biosynthesis is discussed.

The authors thank Mr. L. Nash for care of the animals.

1. Wakil, S. J., Ann. Rev. Biochem. **31**, 369 (1962).

2. Vagelos, P. R., Ann. Rev. Biochem. **33**, 139 (1964).

3. Ganguly, J., Biochem. Biophys. Acta **40**, 110 (1960).

4. Numa, S., Matsuhashi, M., and Lynen, F., Biochem. Z., **334**, 203 (1961).

5. Chang, H., Seidman, I., Teebor, G., and Lane, M. D., Biochem. Biophys. Res. Commun. **28**, 682 (1967).

6. Hollifield, G., and Parson, W., J. Clin. Invest. **41**, 245 (1962).

7. Stevenson, J. A. F., Feleki, V., Szlavko, A., and Beaton, J. R., Proc. Soc. Exptl. Biol. Med. **116**, 178 (1964).

8. Leveille, G. A., and Hanson, R. W., Can. J. Physiol. Pharmacol., **43**, 857 (1965).

9. Chakrabarty, K. and Leveille, G. A., J. Nutrition **96**, 76 (1968).

10. Leveille, G. A., and O'Hea, E. K., J. Nutrition **93**, 541 (1967).

11. Leveille, G. A., J. Nutrition **90**, 449 (1966).

12. Martin, D. B., Horning, M. G., and Vagelos, P. R., J. Biol. Chem. **236**, 663 (1961).

13. Trams, E. G., and Brady, R. O., J. Am. Chem. Soc. **82**, 2972 (1960).

14. Stadtman, E. R., *in* "Methods in Enzymology," (S. P. Calowick, N. O. Kaplan, eds.) Vol. 3, p. 931. Academic Press, New York.

15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, (1951).

16. Gellhorn, A. and Benjamin, W., "Handbook of Physiology" (A. E. Renold, G. F. Cahill, Jr., eds.), Sect. 5, Am. Physiol. Soc., Washington, D. C. (1965).

17. Jansen, G. R., Hutchison, G. F., and Zanetti, M. E., Biochem. J. **99**, 323 (1966).

18. Leveille, G. A., Proc. Soc. Exptl. Biol. Med. **125**, 85 (1967).

19. Favarger, P., *in* "Handbook of Physiology," p. 19. (A. E. Renold, and G. F. Cahill, Jr., eds.) Sect. 5, Am. Physiol. Soc., Washington, D. C. (1965).

20. Martin, D. B. and Vagelos, P. R., J. Biol. Chem. **237**, 1787 (1962).

21. Vagelos, P. R., Alberts, A. W., and Martin, D. B., J. Biol. Chem. **238**, 533 (1963).

22. Matsuhashi, M., Matsuhashi, S., and Lynen, F., Biochem. Z. **340**, 263 (1964).

23. Gregolin, C., Ryder, E., and Lane, M. D., J. Biol. Chem. **243**, 4227 (1968).

24. Gregolin, C., Ryder, E., Warner, R. C., Kleinschmidt, A. K., and Lane, M. D., Proc. Natl. Acad. Sci. U. S. **56**, 1751 (1966).

25. Numa, S., Ringelmann, E., Riedel, B., Biochem. Biophys. Res. Commun. **24**, 750 (1966).

26. Gregolin, C., Ryder, E., Warner, R. C., Kleinschmidt, A. K., Chang, H., and Lane, M. D., J. Biol. Chem. **243**, 4236 (1968).

27. Bortz, W. M. and Lynen, F., Biochem. Z. **337**, 505 (1963).

28. Numa, S., Ringelman, E., and Lynen, F., Biochem. Z. **343**, 243 (1965).

29. Srere, P. A., Biochim. Biophys. Acta **106**, 445 (1965).

30. Taketa, K. and Pogell, B. M., J. Biol. Chem. **241**, 720 (1966).

31. Dorsey, J. A., and Porter, J. W., J. Biol. Chem. **243**, 3512 1968).

Received Sept. 25, 1968. P.S.E.B.M., 1969, Vol. 131.