Biochemical Pharmacology, Vol. 34, No. 24, pp. 4305–4310, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

EFFECTS OF DIABETES MELLITUS ON RENAL FATTY ACID ACTIVATION AND DESATURATION

DANIEL L. CLARK and SHERRY F. QUEENER*
Department of Pharmacology, Indiana University School of Medicine, Indianapolis, IN 46223, U.S.A.

(*Received* 26 *November* 1984; *accepted* 28 *May* 1985)

Abstract—We report the first direct measurement of delta-6 desaturase and delta-9 desaturase (EC 1.3.99.3, acyl-CoA dehydrogenase) activities in the rat kidney. Crude renal cortical homogenates from alloxan-diabetic and from normal rats were assayed for delta-6 and delta-9 desaturase activities. The delta-6 desaturation pathway activity measured with 9,12-octadecadienoic acid (linoleic acid) as substrate was increased, while the delta-9 desaturation pathway measured with hexadecanoic acid (palmitic acid) as substrate was unchanged in diabetic renal cortex, suggesting that the two enzymes are regulated independently in this tissue. In contrast to the kidney, delta-6 desaturase pathway activity was unchanged and the delta-9 desaturase pathway activity was greatly depressed in diabetic liver. When exogenous long-chain acyl-CoA synthetase (EC 6.2.1.3; acid:CoA ligase, AMP-forming) was added to the delta-6 desaturase assay system, the rate of delta-6 desaturation in normal kidney increased to a rate similar to that found in diabetic kidney; rates in diabetic extracts were unchanged. These results suggest that the rate of fatty acid substrate activation to the coenzyme A ester limits the rate of delta-6 desaturation in normal renal cortex. These results also suggest that the rate of fatty acid activation by long-chain acyl-CoA synthetase activity is increased in diabetic renal cortex. Direct measurement of the activity of long-chain acyl-CoA synthetase demonstrated that its activity was indeed increased significantly in the renal cortex of diabetic rats.

Diabetes mellitus alters lipid composition and lipid metabolism in several tissues. Most of the work in this area has focused on the liver [1–3] and on vascular tissues, including platelets [4, 5]. Reported effects of diabetes mellitus on renal lipid metabolism include lowered prostacyclin synthesis in renal cortex [4], elevated thromboxane and lipoxygenase pathway products in glomeruli [6], and increased fatty acid synthesis [7].

In several tissues, including kidney, linoleic acid increases and arachidonic acid (5,8,11,14-eicosatetraenoic acid) decreases in the phospholipid fraction during diabetes [8]. The largest fatty acid changes in the diabetic renal cortex are found in PC† and PE [9]. An observed defect in the biochemical pathway converting linoleic acid to arachidonic acid, specifically at the delta-6 desaturation step, correlates with these fatty acid changes in diabetic liver [2, 10]. The delta-9 desaturation of saturated fatty acids has also been shown to be depressed in diabetic liver [2, 11].

Very little work has been done on the regulation of lipid composition and lipid metabolism in the kidney. The factors that alter renal lipid composition in diabetes are unknown. Because delta-6 and delta-9 desaturase activities have not been directly measured in the kidney, the first purpose of this study was to determine if they are present in the kidney. Second, the effects of diabetes on the activity of delta-6 and delta-9 desaturases in kidney and in liver were investigated to compare the regulation of fatty acid metabolism in the two tissues and to see if alterations in these enzyme activities correlate with the observed fatty acid changes. Third, since activation of fatty acid substrate is a prerequisite to desaturation, the effect of diabetes on the ability of the kidney to activate fatty acids to the coenzyme A ester was examined.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats (180–210 g) were injected via the tail vein with a saline solution of alloxan (30–45 mg/kg). Plasma glucose values (monitored with a Beckman glucose analyzer) exceeded 500 mg/dl for all diabetics used in our experiments; normals ranged from 110 to 120 mg/dl. All diabetic animals used in this study were non-ketotic, were not losing weight, and were diabetic for 2–5 weeks before being used.

Animals rendered diabetic with streptozotocin (35–90 mg/kg) were used in several preliminary studies and were found to be similar in all respects to the alloxan-diabetic rats. Although these animals were not included in the final experiments reported herein, the patterns of enzymatic change were the same as for the alloxan-diabetic rats.

Chemicals. Reference lipids and reagents came from the Sigma Chemical Co. (St. Louis, MO) or Nu-Check Prep, Inc. (Elysian, MN). Thin-layer plates came from Supelco, Inc. (Bellefonte, PA). Radio-

* Address all correspondence to: Prof. Sherry F. Queener, Department of Pharmacology, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46223.

† Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; BSA, bovine serum albumin; 18:2, linoleic acid, or 9,12-octadecadienoic acid; and 16:0, hexadecanoic acid, or palmitic acid.

active compounds were from New England Nuclear (Boston, MA).

Sample preparation. Kidneys were removed after cervical dislocation and exsanguination of the rats. Crude renal cortical homogenates and partially purified renal plasma membranes were prepared by the method of Marx *et al.* [12], summarized in Fig. 1.

A weighed portion of fresh rat liver was homogenized in 10 vol. of 10 mM phosphate buffer (pH 7.4) in a Dounce Vitro homogenizer. The homogenate was centrifuged for 15 min at 36,940 *g*. The supernatant fraction was discarded, and the pellet was resuspended in a 10 mM Tris/0.25 M sucrose/1 mM EDTA buffer (pH 7.45).

Biochemical assays. Delta-6 desaturation of linoleic acid (18:2) and delta-9 desaturation of palmitic acid (16:0) were assayed in renal cortex and in liver by a previously published method [13]. For the delta-6 desaturase assay, incubations were carried out in a 0.15 M KCl/0.25 M sucrose solution buffered by 0.1 M phosphate (pH 7.4) containing 5 mM ATP, 0.1 mM CoA, 0.9 mM NADH, and 5 mM $MgCl_2$. A mixture of [1-^{14}C]-labeled and unlabeled 18:2 (100 nmoles, 0.1 μCi/tube) was added to the incubation mixture as the bovine serum albumin–fatty acid complex. Renal protein (0.75 mg) or hepatic protein (1.0 mg) was incubated in a reaction volume of 1 ml at 37° for 20 or 6 min respectively. The reaction was terminated with 6 N HCl. Lipids were extracted with chloroform–methanol [2:1 (v/v) containing 1 μg/ml of butylated hydroxytoluene (BHT)] and dried under nitrogen. Lipids were transesterified with 14% boron trifluoride in methanol. The fatty acid methyl esters were extracted, concentrated, and separated by argentation thin-layer chromatography. Fatty acid methyl esters of the delta-6 desaturase assay were separated in a solvent system of chloroform–methanol [100:3 (v/v) containing BHT as an antioxidant]. Lipids were visualized under u.v. light after spraying the plates with 1,6-diphenylhexatriene in hexane (10 mg/100 ml). Bands corresponding to dienoic and trienoic methyl esters were scraped and counted by liquid scintillation.

The same buffer used for the delta-6 assay was used in the assay of delta-9 desaturase activity, but the cofactor concentrations were: 7.5 mM ATP, 0.5 mM CoA, 2.6 mM NADH, and 7.5 mM $MgCl_2$. The BSA complex of [1-^{14}C]-labeled and unlabeled 16:0 (100 nmoles, 0.1 μCi/tube) was used for the assay. Renal protein (0.75 mg) or hepatic protein (1.0 mg) was incubated for 20 min at 37°. After extraction and methylation of the fatty acids as described above, the substrate and product fatty acid methyl esters were separated by argentation TLC in a solvent system of petroleum ether–diethyl ether (95:5, v/v). The bands corresponding to saturated and monounsaturated fatty acid methyl esters were quantitated by liquid scintillation. The conditions for the measurement of delta-6 and delta-9 desaturation in the kidney and in the liver were optimized with respect to time and protein concentration. In the kidney, the assays were also standardized with respect to substrate concentration.

In one set of experiments, the fate of linoleic acid in renal cortex under the conditions for the delta-6 desaturase pathway assay was determined (Table 2). The production of $^{14}CO_2$ was measured, and [^{14}C]-linoleic acid incorporation into neutral lipids and phospholipids (separated by silicic acid chromatography [14]) was determined.

Long-chain acyl-CoA synthetase was assayed by a published method [15]. The enzyme was solubilized in 2 mM Triton X-100 and 5 mM 2-mercaptoethanol. The assay mixture contained in micromoles, unless otherwise specified, Tris–HCl buffer, pH 8.0, 20; dithiothreitol, 1; KCl, 30; $MgCl_2$, 3; ATP, 2; CoA, 0.2; Triton X-100, 0.32; [^{14}C]-labeled + unlabeled linoleic acid (0.35 Ci/mole) or [^{3}H]-labeled + unlabeled arachidonic acid (0.46 Ci/mole), 0.2; and enzyme, 0.4 mg. Substrate was added to the mixture as a 10 mM solution in 16 mM Triton X-100. The mixture (0.19 ml), containing all ingredients except CoA, was preincubated for 1 min at 37°. The reaction was initiated by adding 10 μl of 20 mM CoA, and terminated after 3 or 20 min by adding 2.5 ml of a solution of isopropanol–*n*-heptane–1 M H_2SO_4 (40:10:1, by vol.). Then, 1.5 ml of *n*-heptane and 1 ml of water were added, and the mixture was shaken. After allowing the phases to separate, the upper phase was removed and the lower phase was washed twice with 2 ml of *n*-heptane containing 4 mg/ml of linoleic or arachidonic acid. A 1-ml aliquot of the lower phase, containing the fatty acid-CoA ester, was assayed for radioactivity.

Protein was measured by the biuret method [16].

Statistical analysis was performed using the unpaired Student's *t*-test.

RESULTS

We initially attempted to assay delta-6 desaturase in microsomal fractions (fractions 5a and 6a, Fig. 1) but found little or no activity in samples from several purifications. This result led us to evaluate earlier, less purified fractions for activity. The distribution of delta-6 desaturase pathway activity in the purification fractions (Fig. 1) for normal and diabetic renal cortex is shown in Table 1. Very low delta-6 desaturase activity was present in the membrane-enriched fractions (fractions 5, 5a, 6, and 6a) from the renal cortex of normal or diabetic rats. Recovery of delta-6 desaturase activity beyond the crude homogenate was greatly reduced. Although fraction 7 appears to have much desaturase activity, the protein yields were too low to make this fraction useful. Because fraction 3 contained the most consistent and the highest desaturase activity of the renal purification fractions from rats, this fraction was used for the studies of renal cortical delta-6 desaturation.

Similar purification fractions from normal and diabetic renal cortex were assayed for delta-9 desaturase (data not shown). Although activity could be measured in some membrane fractions, fraction 3 was also used for the studies of renal delta-9 desaturation to simplify comparisons of delta-9 and delta-6 desaturase activities in the renal cortex.

The pool of linoleic acid available for delta-6 desaturation may be altered in diabetes. To examine whether pathways for the utilization of linoleic acid other than desaturation may affect this pool, the incorporation of [^{14}C]linoleic acid in the renal cortex

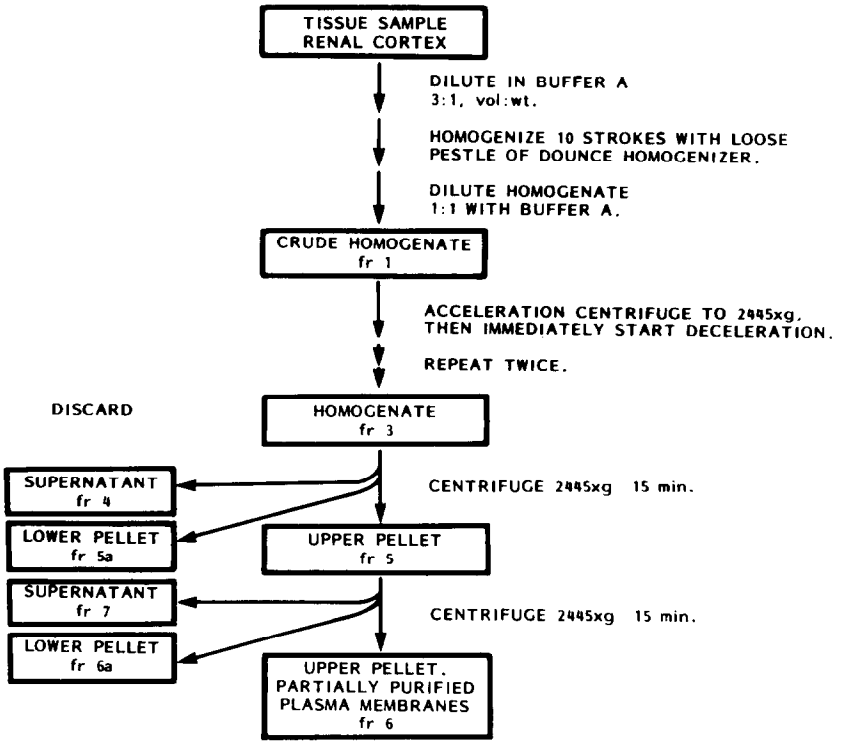

Fig. 1. Scheme for preparation of renal cortical homogenates and partially purified plasma membranes.

was determined (Table 2). No significant changes in the metabolic fate of linoleic acid were observed in diabetes.

Because the free fatty acid was used as the substrate in these studies, the delta-6 and delta-9 desaturase activities measured include the enzymatic activation of the fatty acid substrates by long-chain acyl-CoA synthetase and desaturation of the fatty acid-CoA ester by the desaturase. The activities are therefore reported as delta-6 and delta-9 "pathway" activities. Results of these assays in tissues from normal and diabetic animals are presented in Table 3. As a control, a crude liver homogenate was assayed for delta-6 and delta-9 desaturase pathway activities using the same method employed for the kidney studies.

Table 1. Distribution of delta-6 desaturase activity in renal cortical purification fractions

	Activity			
Fraction	Normal	%	Diabetic	%
3	1,449	100	13,956	100
4	293	20	2,298	16.5
5	64	4.4	1,116	8
5a	26	1.8	705	5.1
6	27	1.9	112	0.8
6a	19	1.3	13	0.1
7	118	8.1	537	3.8

Activity is expressed as nmoles product formed/hr and as percent of fraction 3. The values represent duplicate determinations for one experiment. Renal tissue was pooled from eight normal and five alloxan-diabetic rats for the purification.

Diabetes had no effect on the renal delta-9 desaturase pathway (N = 15), whereas the activity of the same pathway was greatly reduced in liver (Table 3) in the two samples assayed. In contrast to the delta-9 desaturase pathway, delta-6 desaturase pathway activity was increased significantly in diabetic kidney. The activity of the delta-6 desaturase pathway in liver was unchanged by diabetes (Fig. 2).

To determine if activation of fatty acid substrate to the CoA ester limits the rate of delta-6 desaturation in renal cortex, exogenous long-chain acyl-CoA synthetase was added to the incubation mixture. Addition of exogenous fatty acid activating enzyme significantly increased delta-6 desaturase pathway activity in normal kidney, whereas exogenous enzyme had no effect on the same pathway in diabetic renal cortex (Table 4). The activity of the delta-6 desaturase pathway was the same in normal and diabetic kidney when exogenous long-chain acyl-CoA synthetase was present.

The previous result suggested that long-chain acyl-CoA synthetase activity may be increased in diabetic renal cortex. The measured activity of the enzyme was increased significantly in diabetic kidney using either linoleic acid or arachidonic acid as substrate (Table 5). A similar result was also obtained when the incubation was allowed to proceed for 20 min: linoleic acid as substrate—normal, 5.76 ± 0.10, and diabetic, 7.91 ± 0.24 ($N = 5$, $P < 0.001$); arachidonic acid as substrate—normal, 6.51 ± 0.20, and diabetic, 7.50 ± 0.23 ($N = 5$, $P < 0.01$).

Table 2. Utilization of [^{14}C]linoleic acid in renal cortex

	^{14}C Incorporated [dpm · (mg protein)$^{-1}$ · (20 min)$^{-1}$]		
	CO_2	Phospholipid	Neutral lipid
Normal	1,670 ± 150	20,670 ± 3,960	198,650 ± 12,370
Diabetic	2,370 ± 340	17,340 ± 1,510	191,730 ± 4,370

Values are means ± S.E.M. of two experiments; there were four samples in each group. All samples were run in duplicate. Conditions of incubation were as described in Materials and Methods for delta-6 desaturase assays. The samples were fraction 3 from normal or alloxan-diabetic rats. The product was extracted, purified, and quantitated as described in Materials and Methods.

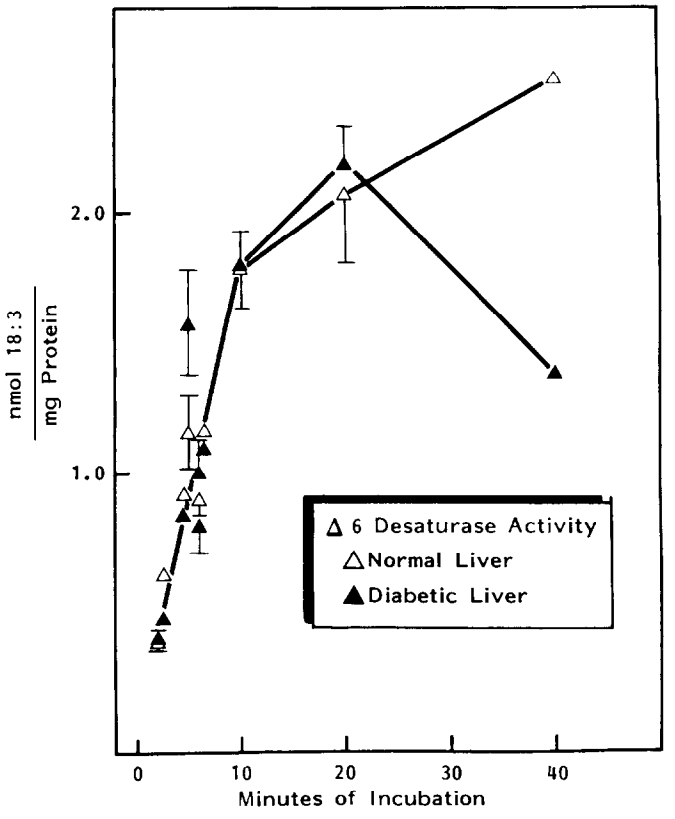

Fig. 2. Time course of delta-6 desaturase pathway activity in liver of normal and diabetic rats. Delta-6 desaturase activity was determined in whole homogenate of liver using linoleic acid as substrate, as described in Materials and Methods. Values at 2, 5, 6, 10, and 20 min are mean ± S.E.M. of three to five experiments. Values at 2.5, 4.5, 6.5, and 40 min are from one experiment.

Table 3. Desaturase pathway activity

	Activity [nmoles product · (mg protein)$^{-1}$ · hr^{-1}]			
Desaturase pathway	Kidney		Liver	
	Normal	Diabetic	Normal	Diabetic
Δ9	0.80 ± 0.05 (15)	0.95 ± 0.07 (15)	0.85 ± 0.43 (1)	0.27 ± 0.07 (2)
Δ6	2.28 ± 0.31 (10)	5.12 ± 0.83* (10)	9.82 ± 0.63 (6)	10.56 ± 0.55 (7)

Kidney (0.75 mg) or liver (1 mg) protein was incubated at 37° in 1 ml of 0.15 M KCl/0.25 M sucrose/100 mM phosphate buffer containing either 100 nmoles total (labeled + unlabeled) palmitic acid (delta-9 desaturase) or 100 nmoles total (labeled + unlabeled) linoleic acid (delta-6 desaturase). All incubations were carried out for 20 min, except for the hepatic delta-6 desaturase incubation, which was for 6 min. Other experimental details are given in Materials and Methods. Values are means ± S.E.M.; the numbers in parentheses indicate the number of preparations.

* $P < 0.01$.

Table 4. Effect of exogenous long-chain acyl-CoA synthetase on renal desaturation of linoleic acid

	Desaturation activity [nmoles product · (mg protein)$^{-1}$ · hr^{-1}]		
	Exogenous long-chain acyl-CoA synthetase		
	Omitted	Added	
Normal	5.24 ± 0.57	8.34 ± 0.65	$P < 0.01$
Diabetic	7.87 ± 0.56	8.33 ± 0.23	NS
	$P < 0.05$	NS	

Values are means ± S.E.M. (N = 4). Renal cortical protein (0.75 mg) was incubated for 20 min at 37° in 0.15 M KCl/0.25 M sucrose/100 mM phosphate buffer containing 100 nmoles total (labeled + unlabeled) linoleic acid. The rate of desaturation of linoleic acid was determined with or without exogenous long-chain acyl-CoA synthetase (0.033 to 0.1 units). NS = not significant.

DISCUSSION

Previous studies have provided indirect evidence for the presence of delta-6 desaturase activity in the renal cortex [17, 18], but no direct measurement of this enzyme has been made in the kidney. Our results show that whole renal cortical homogenate contained measurable delta-6 desaturase activity which was stimulated significantly during diabetes. The increased activity of the delta-6 desaturase pathway does not correlate with the observed changes in fatty acid composition of phospholipids in diabetic renal cortex [9].

In contrast to renal cortex, the hepatic delta-6 desaturase pathway activity was unchanged in diabetes. Most studies on the effect of diabetes on hepatic delta-6 desaturation have found a significant decrease in the activity of this enzyme [2, 19, 20], but Riisom *et al.*, using methods similar to ours, reported no statistically significant change of delta-6 desaturase activity in diabetic liver [13]. Differences in methodology may explain why discrepancies exist in the literature.

The assay incubation time used in the present study differs from those used in previous studies of delta-6 desaturation in the liver. Some early investigators of hepatic desaturation used a 4-min incubation to determine the initial rate of delta-6 desaturation in liver microsomes, while a 20-min incubation was used to measure the steady-state rate [21]. No prior studies examining the effect of diabetes on liver microsomal delta-6 desaturation have presented time courses for the assay. All of the studies used either a 15- or a 20-min incubation time, but they did not state whether they were measuring an initial rate or a rate at steady state. In the present study, product accumulation from delta-6 desaturation of linoleic acid by crude liver homogenate was no longer linear by 15 min of incubation (Fig. 2). From this result, we conclude that previous studies did not determine the effect of diabetes on the initial rate of hepatic delta-6 desaturation. Our results measuring initial rates in crude homogenates would not be expected necessarily to agree with measurements of some later time steady-state rate measured in microsomal preparations that may be influenced by many factors other than the enzyme concentration in the tissue.

Table 5. Activity of long-chain acyl-CoA synthetase in renal cortex

Substrate	Synthetase activity [nmoles CoA ester formed · (mg protein)$^{-1}$ · min^{-1}]	
	Normal	Diabetic
Linoleic acid	9.28 ± 0.28 (5)	13.68 ± 0.46* (5)
Arachidonic acid	10.86 ± 0.24 (8)	13.77 ± 0.65† (9)

Renal cortical protein (0.4 mg) was preincubated in 0.19 ml of 100 mM Tris–HCl buffer for 1 min at 37°. After adding 200 nmoles CoA, the mixture was incubated for 3 min at 37°. Other experimental details are given in Materials and Methods. Values are means ± S.E.M., with the number of preparations given in parentheses.

* $P < 0.001$.

† $P < 0.01$.

The work of Montgomery and Cinti [22] suggests that the renal cortex should contain delta-9 desaturase activity. Although they found no measurable delta-9 desaturase activity in renal cortical microsomes, adequate levels of required coenzymes and electron transport proteins were present. Moreover, they saw a significant stimulation of desaturation when renal microsomes were added to liver or lung microsomes.

Our results show that low, but measurable, delta-9 desaturase pathway activity is present in the whole homogenate of renal cortex. The unchanged delta-9 desaturase pathway activity in diabetic kidney correlates with the unaltered levels of saturated and monounsaturated fatty acids in renal cortical phospholipids in diabetes [9].

Since diabetes had no effect on the activity of the delta-9 desaturase pathway in the renal cortex, whereas the delta-6 desaturase pathway was increased, it appears that the delta-9 and delta-6 desaturase pathways are regulated differently in the renal cortex. Studies of several desaturase enzymes in the liver have suggested previously that each enzyme is regulated uniquely [23, 24]. Thus, it is not surprising that an analogous situation also occurs in the renal cortex.

The delta-9 desaturase pathway activity was measured in liver of normal and diabetic rats as a control. Previous studies have established that delta-9 desaturation is greatly depressed in the liver microsomes of diabetic rats [11, 25]. Our results (Table 3) are in good agreement with the literature. Our observation of the dissimilar effects of diabetes on the activities of the delta-6 and delta-9 desaturase pathways in the liver agrees with previous studies [23, 24].

Because both the liver and the kidney contain delta-9 and delta-6 desaturase activities, both tissues apparently have similar biochemical pathways of saturated and unsaturated fatty acid metabolism. Since the delta-9 and delta-6 desaturase pathways in the two tissues are affected differently by diabetes, we suggest that the regulation of the individual desaturase enzymes may differ in the two tissues.

Two enzymatic steps, activation of the fatty acid

to the CoA ester by long-chain acyl-CoA synthetase and desaturation, were required to measure desaturation by the method we used [13]. In the liver, the rate of fatty acid activation is greater than the rate of desaturation, and, therefore, fatty acid activation does not limit measurement of the rate of desaturation [26]. It is not known if the same is true in the renal cortex, since desaturation has not been measured previously in this tissue. The increased delta-6 desaturase pathway activity after addition of exogenous long-chain acyl-CoA synthetase suggests that, in the normal state, fatty acid activation may limit the rate of delta-6 desaturation in the renal cortex (Table 4), even though the rate of fatty acid activation in the kidney is fairly high [27]. In contrast to the normal state, the lack of effect of exogenous long-chain acyl-CoA synthetase on the rate of delta-6 desaturation in diabetic renal cortex suggests that fatty acid activation does not limit the rate of delta-6 desaturation in diabetic renal cortex. Direct assay verified that long-chain acyl-CoA synthetase activity in renal cortex is increased in diabetes, using either linoleic acid or arachidonic acid as substrate.

No prior studies report the effects of diabetes on the activity of long-chain acyl-CoA synthetase in the kidney. A recent study on long-chain acyl-CoA synthetase in the liver reports no change of the enzyme activity in diabetes; however, long-chain acyl-CoA hydrolase was decreased in diabetes [28].

The studies reported herein and others suggest that correlating diabetes-induced changes in lipid composition with alterations of specific enzyme activities may not be simple. Two obvious problems must be considered. First, the enzymes of lipid metabolism with their relatively insoluble lipid substrates often do not follow simple Michaelis–Menten kinetics. Care must be taken to optimize assay conditions so that the activity measured is related to the amount of enzyme present. Measurements made at a steady state of product accumulation are influenced by the activities of any enzyme that might act on the product, and may not agree with measurements taken during the initial linear phase. Second, concentrations of fatty acids within various phospholipids must be recognized as being influenced by many factors and not strictly regulated by a single enzyme activity. For example, the elevated linoleic acid content of phosphatidylcholine and phosphatidylethanolamine may be influenced by dietary intake, rate of acyl-CoA formation, and by the rate of delta-6 desaturation. All of these processes are increased in diabetes. Although increased delta-6 desaturation, considered alone, might be expected to lower linoleic acid levels, the other metabolic changes may offset this effect. Likewise, arachidonic acid levels might be influenced not only by the activity of the pathway from linoeic acid but also by dietary intake and rates of arachidonate release from prostaglandin synthesis. Because of the complex interaction of these processes, and because of the differences in metabolism between the kidney and the liver, the kidney poses interesting problems in lipid metabolism deserving of further study.

Acknowledgements—This work was supported by a grant-in-aid to Dr. Queener from the American Heart Association, Indiana Affiliate, and by the Project Development Program, Indiana University School of Medicine.

REFERENCES

1. P. H. Whiting, M. Bowley, R. G. Sturton, P. H. Pritchard, D. N. Brindley and J. N. Hawthorne, *Biochem. J.* **168**, 147 (1977).
2. F. H. Faas and W. J. Carter, *Lipids* **15**, 953 (1980).
3. J. A. Woods, T. E. Knauer and R. G. Lamb, *Biochim. biophys. Acta* **666**, 482 (1981).
4. H. E. Harrison, A. H. Reece and M. Johnson, *Diabetologia* **18**, 65 (1980).
5. J. M. Gerrad, M. J. Stuart, G. H. R. Rao, M. W. Steffes, S. M. Mauer, D. M. Brown and J. G. White, *J. Lab. clin. Med.* **95**, 950 (1980).
6. D. M. Brown, J. M. Gerrard, J. Peller, G. H. R. Rao and J. G. White, *Diabetes* **29**, 219 (1980).
7. S. S. Kang, R. Fears, S. Noirot, J. N. Mbanya and J. Yudkin, *Diabetologia* **22**, 285 (1982).
8. R. T. Holman, S. B. Johnson, J. M. Gerrard, S. M. Mauer, S. Kupcho-Sandberg and D. M. Brown, *Proc. natn. Acad. Sci. U.S.A.* **80**, 2375 (1983).
9. D. L. Clark, F. G. Hamel and S. F. Queener, *Lipids* **18**, 696 (1983).
10. O. Mercuri, R. O. Peluffo and R. R. Brenner, *Biochim. biophys. Acta* **166**, 409 (1966).
11. A. Gellhorn and W. Benjamin, *Biochim. biophys. Acta* **84**, 167 (1964).
12. S. J. Marx, S. A. Fedak and G. D. Aurbach, *J. biol. Chem.* **247**, 6913 (1972).
13. T. Riisom, S. Johnson, E. G. Hill and R. T. Holman, *J. Lab. clin. Med.* **98**, 764 (1981).
14. D. L. Clark, *Ph.D. Thesis*. Indiana University, Indianapolis (1984).
15. T. Tanaka, K. Hosaka and S. Numa, *Meth. Enzym.* **71**, 334 (1981).
16. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **193**, 751 (1949).
17. C. Widmer, Jr. and R. T. Holman, *Archs Biochem.* **25**, 1 (1950).
18. J. C. Chern and J. E. Kinsella, *Biochim. biophys. Acta* **750**, 465 (1983).
19. N. Friedmann, A. Gellhorn and W. Benjamin, *Israel J. med. Sci.* **2**, 677 (1966).
20. A. Gellhorn and W. Benjamin, *Science* **146**, 1166 (1964).
21. R. R. Brenner and R. O. Peluffo, *J. biol. Chem.* **241**, 5213 (1966).
22. M. R. Montgomery and D. L. Cinti, *Molec. Pharmac.* **13**, 60 (1977).
23. C. A. Inkpen, R. A. Harris and F. W. Quackenbush, *J. Lipid Res.* **10**, 277 (1969).
24. R. Jeffcoat, P. R. Brawn, R. Safford and A. T. James, *Biochem. J.* **161**, 431 (1977).
25. Y. Imai, *J. Biochem., Tokyo* **49**, 642 (1961).
26. Y. Marcel and G. Suzue, *J. biol. Chem.* **247**, 4433 (1972).
27. M. Aas, *Biochim. biophys. Acta* **231**, 32 (1971).
28. A. Q. Dang, F. H. Faas and W. J. Carter, *Lipids* **19**, 578 (1984).