Regulation of Mammalian Fatty-Acid Synthetase. The Roles of Carbohydrate and Insulin

(liver/adipose tissue/brain/fructose/diabetes)

JOSEPH J. VOLPE* AND P. ROY VAGELOS†

Departments of †Biological Chemistry, *Pediatrics, and *Neurology, Washington University School of Medicine, St. Louis, Missouri 63110

ABSTRACT The regulation of fatty-acid synthetase has been studied in liver, adipose tissue, and brain of diabetic and normal rats in relation to insulin administration and glucose or fructose feeding. The data indicate that: (*1*) insulin is not necessary for regulation of synthetase activity in liver but may be necessary in adipose tissue; (*2*) synthetase of liver can be regulated by carbohydrate, which can enter the glycolytic scheme in the absence of insulin (e.g., fructose), suggesting the possibility that regulation of synthetase may depend on the concentration of certain intermediates of the glycolytic pathway or beyond; (*3*) fructose feeding affects the synthetase by causing an increase in the rate of synthesis of fatty-acid synthetase; and (*4*) unlike fatty-acid synthetase of liver and adipose tissue, the enzyme of brain is unaffected by the diabetic state or by glucose or fructose feeding.

The regulation of fatty-acid synthetase, the multi-enzyme complex that catalyzes the synthesis of palmitate from malonyl-CoA and acetyl-CoA, has been studied primarily in relation to nutritional changes or hormones (see ref. 1 for review). The best studied of the hormone-dependent changes are the effects of the diabetic state on the enzyme of rat liver. It has been shown clearly that in diabetic animals the hepatic fatty-acid synthetase is markedly decreased (2), and that this decrease is caused by a diminution in synthesis of the enzyme (3). Insulin administration corrects these deficits (2, 3). Similar observations have been made for other lipogenic enzymes—e.g., acetyl-CoA carboxylase (4), malic enzyme (5), citrate-cleavage enzyme (6), and glucose 6-phosphate dehydrogenase (7). These data amplified the original observation of Stetten and Boxer (8) that fatty-acid synthesis in liver of diabetic rats is reduced to 5% of the synthesis in normal animals.

Porter and coworkers have demonstrated that the synthesis of fatty-acid synthetase in rat liver is stimulated by insulin and inhibited by glucagon and cyclic AMP, and they have suggested that the insulin effect may be mediated by changes in the intracellular concentration of cyclic AMP (3). Nevertheless, several questions concerning the regulation of the synthetase in states of insulin deprivation and/or excess remain unanswered: namely, (*1*) is the regulation of fatty-acid synthetase absolutely dependent on insulin, (*2*) could the regulation of the synthetase by insulin be mediated by a mechanism other than direct interaction of the hormone (or cyclic AMP) with the protein biosynthetic system for this enzyme, and (*3*) can the defect in fatty-acid synthetase in the diabetic state be corrected by means other than insulin administration, e.g., fructose feeding? To clarify these points we have studied the regulation of the synthetase by insulin and/or the feeding of glucose or fructose in liver, adipose tissue, and brain of diabetic as well as normal rats. Liver and adipose tissue are the two tissues of major importance for lipogenesis in the rat and are tissues exquisitely sensitive to metabolic regulation by insulin. We have studied regulation of the synthetase of brain under similar conditions, since this tissue is not notably insulin-sensitive and since we have shown previously other differences in the regulation of brain fatty-acid synthetase compared to the liver and adipose tissue enzymes (9–11).

MATERIALS AND METHODS

Materials. All materials obtained from commercial sources were of the highest possible grade. Acetyl-CoA was synthesized by the method of Simon and Shemin (12). L-[1-^{14}C]-leucine was obtained from New England Nuclear Corp. Insulin (N.P.H.) was purchased from Eli Lilly Co. Streptozotocin was a generous gift of Dr. W. E. Dulin of the Upjohn Co.

Animals and Diets. Animals used were of the Sprague–Dawley strain (National Laboratory Animals, St. Louis, Mo.). Diets utilized for feeding at will included Purina Laboratory Chow and two fat-free diets (Nutritional Biochemicals), formulated according to Wooley and Sebrell (13), except that the carbohydrate was either glucose or fructose. Tube-feeding diets were formulated similarly and were supplied by General Biochemicals. Animals were tube fed at the same time for each experiment, i.e., 8:30 a.m., 4:30 p.m., and 12:30 a.m. Tube feeding was carried out while animals were narcotized by 1-min exposure to a gaseous mixture of 50% O_2–50% CO_2.

Diabetes was produced by injection into the tail vein of streptozotocin, 65 mg/kg, after animals were fasted for 24 hr. Animals were given a 10% w/v glucose solution for drinking during the first 24 hr after injection and thereafter maintained on Purina Laboratory chow. Rats were not considered diabetic nor used for further experiments if blood sugar was not >250 mg/100 ml and urine sugar 1 g/100 ml or greater. All diabetic animals also exhibited the characteristic clinical features of marked polyphagia, polydipsia, and polyuria.

Purification of Fatty-Acid Synthetase and Assays. Preparation of tissue extracts of liver, adipose tissue, and brain, purification of hepatic fatty-acid synthetase, and assays were carried out as described (9, 10). One unit of enzyme activity is defined as the amount required to catalyze the oxidation of 1 nmol of NADPH per min at 25°.
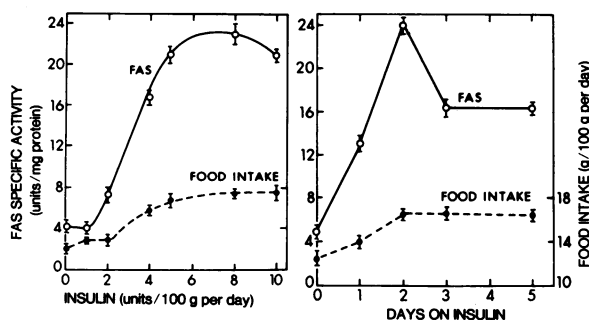
FIG. 1. (*Left*) Hepatic fatty-acid synthetase (*FAS*) specific activity as a function of dose of insulin. Pairs of 150-g rats were injected with the indicated daily dose of insulin (N.P.H.), administered subcutaneously every 12 hr for 2 days. Animals were allowed to freely eat Purina Laboratory chow, and food consumption of each animal was carefully recorded. Tissue extracts were 105,000 × *g* supernatant solutions. Each point represents the mean (± range) of separate determinations for each liver of a pair. Duplicate determinations of two levels of enzyme were performed for each liver. Average blood sugars (Dextrostix) in mg/100 ml for each pair of animals were as follows: 0 unit/100 g per day, 90; 1 and 2 units/100 g per day, 45–90; 4 units/100 g per day, 45; 5 units/100 g per day, 25–45; and 8 and 10 units/100 g per day, 0–25.

FIG. 2. (*Right*) Hepatic fatty-acid synthetase (*FAS*) specific activity as a function of duration of insulin administration. Pairs of 150-g rats were injected with insulin (N.P.H.), 5 units/100 g per day, for the times indicated. See legend to Fig. 1.

Immunological Procedures. The hepatic synthetase was used for production of antibodies, and immunological identity of synthetase of rat liver, adipose tissue, and brain was demonstrated as reported (9, 10). Quantitative precipitin analyses were carried out as described by Kabat and Meyer (14). The isotopic-immunochemical technique for measuring synthesis of fatty-acid synthetase and the trichloroacetic-acid precipitation method for measuring synthesis of total protein were performed after a 60-min pulse of L-[1-^{14}C]leucine, administered intraperitoneally in a dose of 0.2 μCi/g of body weight, as described (9).

RESULTS

A dose–response curve (Fig. 1) of the effect on hepatic fatty-acid synthetase of insulin administration to normal animals feeding freely on Purina Laboratory chow exhibits maximum increase in specific activity, approximately 5- to 6- fold over control values, after doses of 5 units/100 g of body weight per day or more. Such doses are 2-fold or more higher than the amounts of insulin secreted by normal rats, i.e., 2–3 units/100 g per day (15). More interesting, the increase of food intake, which correlated with the hypoglycemia produced by the administered insulin, paralleled the increase in fatty-acid synthetase activity. The time course (Fig. 2) of the effect of insulin administered in a dose of 5 units/100 g per day shows that a maximum increase in synthetase activity of 5- to 6-fold occurs after 48 hr. The shape of the curve is similar to that obtained with starvation-refeeding (16), and the increase in fatty-acid synthetase activity was in fact closely paralleled by the increase in food consumption associated with insulin-induced hypoglycemia. These data are similar to those obtained previously with hepatic glucose-6-phosphate dehydro-

genase activity (17) and suggest that the stimulatory effect of insulin on fatty-acid synthetase activity is secondary to increased consumption of carbohydrate. Quantitative precipitin analyses of liver extracts from insulin-injected (fatty-acid synthetase specific activity, 16.3) and control animals (fatty-acid synthetase specific activity, 4.1) resulted in identical equivalence points. This result indicates that there were equal amounts of immunoprecipitable enzyme per unit of activity in each extract; thus, the differences in enzyme activity are related to differences in enzyme content, not in catalytic efficiency. Isotopic-immunochemical analysis of control animals and those given insulin (5 units/100 g per day) for 5 days indicated that the differences in content were caused by increase in synthesis of enzyme. Thus, hepatic fatty-acid synthetase specific activity was 15.2 in injected animals and 3.7 in control animals, and relative rates of synthesis (fatty-acid synthetase radioactivity, in dpm per mg of protein/protein radioactivity, in dpm per mg of protein, × 100) were 11.6 and 2.1, respectively. These results are similar to those we have observed when normal chow-fed animals are placed on a high-carbohydrate, fat-free diet (9).

In order to dissociate the effect on fatty-acid synthetase of insulin from that of increased food consumption, two experimental approaches were attempted, i.e., pair feeding and tube feeding. In pair-feeding experiments, insulin-injected animals were fed only the amount of food consumed by paired controls; in the tube-feeding experiments, both control and insulin-injected animals were tube-fed equal but supranormal quantities of a glucose diet. In both types of experiments insulin-injected animals developed severe hypoglycemia and either died or were moribund at the time of killing. Thus, meaningful assays of enzyme activity were not obtained. The etiology of the severe hypoglycemia in the pair-fed, insulin-injected animals was related to the inadequate carbohydrate intake, and in the tube-fed, insulin-injected animals, to the additional secretion of endogenous insulin stimulated by the high glucose diet.

The regulation of fatty-acid synthetase in diabetic animals was studied next. Although diabetic rats feeding freely con-

TABLE 1. *Effect of glucose feeding and insulin on fatty-acid synthetase of liver and adipose tissue in diabetic rats*

Condition	Time* (hr)	FAS† specific activity‡ (units/mg of protein)	
		Liver	Adipose tissue
Control	0	6.82	4.07
Control	24	21.9	9.00
Diabetic	0	0.75	2.24
Diabetic	24	0.85	2.10
Diabetic + insulin§	24	18.4	14.4

* Zero time for diabetic animals was 7–10 days after streptozotocin injection. Control and untreated and treated diabetic animals were then tube-fed three meals of the glucose diet every 8 hr and killed after 24 hr.

† Fatty-acid synthetase.

‡ Organs were pooled from three animals for each point. Tissue extracts were 105,000 × *g* supernatant solutions.

§ Dose of insulin (N.P.H.) was 1.5 units/100 g, given subcutaneously at zero time and 12 hr.

TABLE 2. *Effect of fructose feeding on fatty-acid synthetase of liver and adipose tissue in diabetic rats*

Condition	Time* (hr)	FAS† specific activity‡ (units mg of protein)	
		Liver	Adipose tissue
Control	0	5.12	4.74
Control	24	20.6	11.0
Diabetic	0	0.35	1.63
Diabetic	24	4.00	1.64

* See legend to Table 1. Animals were tube-fed the fructose diet.
† Fatty-acid synthetase.
‡ See legend to Table 1.

TABLE 3. *Effect of long-term fructose or glucose feeding on fatty-acid synthetase of liver and adipose tissue in diabetic rats*

Condition	Diet*	FAS† specific activity‡ (units/mg of protein)	
		Liver	Adipose tissue
Control	Glucose	17.3	37.4
Control	Fructose	27.3	17.7
Diabetic	Glucose	2.11	7.20
Diabetic	Fructose	25.8	3.36

* Animals were allowed to eat freely the pelleted fat-free diets containing either glucose or fructose as sole carbohydrate for 2 weeks.
† Fatty-acid synthetase.
‡ See legend to Table 1.

sumed approximately 25% more Purina Laboratory chow than did controls, the specific activity of synthetase in diabetic liver was only approximately 10% of that in controls, and in adipose tissue approximately 30–50% (see zero time values in Tables 1 and 2), confirming previous reports (2, 3, 18). To avoid the problems of unequal food consumption in the experiments summarized in Tables 1 and 2, all animals were fed by gastric intubation. The effect of glucose feeding, with and without insulin administration, on fatty-acid synthetase activity in liver and adipose tissue is shown in Table 1. Animals were fed meals of the glucose diet every 8 hr (at zero time, 8 and 16 hr) and killed 8 hr after the last meal. Total caloric consumption was 50 calories/100 g of body weight. In liver, although specific activity of fatty-acid synthetase of control animals increased approximately 3-fold, activity in diabetics did not change at all, confirming the observations of Porter and coworkers (3). In adipose tissue, the response of synthetase was similar to the hepatic enzyme. Thus, specific activity of the synthetase of control rats increased 2-fold and no change was observed in that of diabetic animals. When insulin (1.5 units/100 g, given at zero time and 12 hr) was administered during the 24 hr of glucose feeding, specific activities of fatty-acid synthetase of both diabetic liver and adipose tissue were similar to control values. When data are expressed as total activity (i.e., units per organ), differences between diabetic and control or insulin-treated diabetic animals are accentuated by approximately an additional 20%, since weights of liver and adipose tissue are smaller in the untreated diabetic animals. In brain, there were no differences among synthetase specific or total activities in control or diabetic animals before or after glucose feeding, with or without insulin administration (all showed specific activities 0.86–0.91). Essentially identical results were obtained in three separate experiments. Quantitative precipitin analyses and isotopic-immunochemical experiments confirmed previous findings obtained by other techniques (3), indicating that the differences in fatty-acid synthetase activity in liver reflect differences in enzyme content and that the content differences are caused by changes in synthesis of enzyme.

To determine whether insulin was absolutely necessary for regulation of fatty-acid synthetase activity in liver and adipose tissue, we evaluated the effect of fructose feeding in diabetic animals. These experiments were stimulated by a report 20 years ago that the defective synthesis of fatty acids from [14C]acetate by diabetic liver slices was corrected by 4

days of fructose feeding (19). The experiments were carried out in an identical manner to those just described for glucose feeding, except that the animals were tube-fed a fat-free diet containing 75% fructose. In liver (Table 2), synthetase specific activity in normal animals increased 4-fold, comparable to the 3-fold increase with glucose feeding. However, in marked contrast to the finding with glucose feeding, hepatic synthetase activity in diabetic animals fed fructose for only 24 hr increased approximately 12-fold. In adipose tissue, synthetase specific activity in controls increased approximately 2.5-fold, but in contrast to diabetic liver, synthetase of diabetic adipose tissue did not change with fructose feeding, just as it did not change with glucose feeding. When data are expressed as total activity per epididymal fat pad or liver, differences between diabetic and control animals are accentuated, as noted above. In brain, no differences in specific or total activity of fatty-acid synthetase were observed in controls or diabetics before or after fructose feeding (all showed specific activites 0.86–1.16). Essentially identical results were obtained in two experiments. Quantitative precipitin analyses and isotopic-immunochemical analyses indicated that the differences in hepatic synthetase activity between control and diabetic animals fed fructose reflected differences in enzyme content (equivalence points were equal) and that these content differences are caused by changes in enzyme synthesis (relative rate of synthesis was 6-fold higher in controls fed fructose than in diabetics fed fructose).

To determine whether the defect in fatty-acid synthetase activity in diabetic liver could be completely corrected by fructose feeding and whether diabetic adipose tissue did in fact respond in a different manner than liver, a longer-term experiment with fructose and glucose feeding was carried out. Animals were maintained for 2 weeks on a fat-free diet containing either glucose or fructose as sole carbohydrate (Table 3). In liver, synthetase specific activity of controls was approximately 60% higher in animals fed fructose than those fed glucose. Of great interest, the diabetic animals fed fructose exhibited hepatic fatty-acid synthetase specific (and total) activity similar to the control value, whereas diabetic animals fed glucose continued to have very low synthetase specific activity. In adipose tissue, in contrast to liver, fatty-acid synthetase specific activity of controls was approximately 2-fold higher in animals fed glucose than those fed fructose, as noted previously (20). Diabetic animals fed *either* glucose or fructose
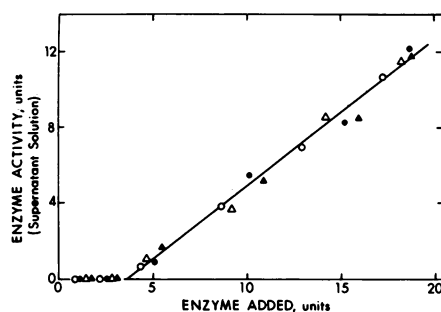
FIG. 3. Quantitative precipitin reactions of hepatic fatty-acid synthetase of control and diabetic rats consuming a fat-free diet with either glucose or fructose as the sole carbohydrate. The enzyme extracts were the 105,000 $\times$ g supernatant solutions described in Table 3. The precipitin reactions were carried out as described (9). Specific activities in units per mg were as follows: (O) control rats fed glucose (17.3); (●) control rats fed fructose (27.3); (△) diabetic rats fed glucose (2.11); (▲) diabetic rats fed fructose (25.8).

exhibited relatively low synthetase specific activities in adipose tissue, approximately 20% of control values. This failure of fatty-acid synthetase of diabetic adipose tissue to respond to fructose feeding is completely unlike the response of hepatic synthetase. Again, in brain no clear differences in fatty-acid synthetase specific activity could be delineated among controls or diabetics fed glucose or fructose (all showed specific activities 0.90–1.20).

Quantitative precipitin analyses (Fig. 3) demonstrated equal equivalence points for all liver extracts, thus indicating that the effects of the diets were on enzyme content. Data from isotopic-immunochemical experiments indicated that the differences in content were caused by changes in synthesis of enzyme (Table 4). Thus, in control animals hepatic specific activity in animals fed fructose was 60% higher than in those fed glucose, and the relative rate of synthesis was also 60% higher in fructose-fed animals. In diabetic animals fed fructose, hepatic specific activity was approximately 12-fold higher than in diabetic animals fed glucose, and the relative rate of synthetase synthesis was approximately 17-fold higher in the fructose-fed animals.

DISCUSSION

There are several conclusions to be drawn from these data. First, liver is not dependent on insulin for maintenance of normal synthetase activity, and, therefore, regulation of fatty-acid synthetase is unlike the regulation of an enzyme such as glucokinase (21). Thus, feeding of fructose led to a marked increase in synthesis of the synthetase in liver of diabetic rats, whereas such feeding does not correct the defect of hepatic glucokinase activity in diabetic animals (21). In contrast to the liver enzyme, fatty-acid synthetase of adipose tissue may be dependent on insulin for regulation of its activity, although this effect of the hormone may be secondary to effects on glycolysis and/or sugar transport (see below).

A second conclusion is that fatty-acid synthetase of liver can be regulated by carbohydrate, such as fructose, which can be metabolized in the absence of insulin. This conclusion may have important implications for the mechanism of regulation of fatty-acid synthetase by other compounds as well as by fructose. The mechanism whereby fructose leads to

TABLE 4. *Effect of long-term fructose or glucose feeding on synthesis of fatty-acid synthetase in liver of diabetic rats*

Condition	Diet*	FAS† radio-activity (A)‡ (dpm/mg of protein)	Protein radio-activity (B)§ (dpm/mg of protein)	Relative rates of synthesis A/B $\times$ 100
Control	Glucose	86	378	22.7
Control	Fructose	156	429	36.4
Diabetic	Glucose	7.1	526	1.35
Diabetic	Fructose	120	527	22.8

* Animals are the same as those described in legend to Table 3. Each was given L-[1-^{14}C]leucine (0.2 μCi/g of body weight) intraperitoneally 1 hr before it was killed.

† Fatty-acid synthetase.

‡ Fatty-acid synthetase was isolated by immunoprecipitation (9).

§ Total protein was isolated by precipitation with trichloroacetic acid (9).

increased synthetase in liver probably relates to the metabolism of this carbohydrate. Fructose is phosphorylated in liver by fructokinase to fructose 1-phosphate (22). This intermediate is converted by an aldolase to glyceraldehyde and the latter to glyceraldehyde 3-phosphate by a triokinase (23). This pathway is very active in liver (24), and fructose can be phosphorylated in liver at a much faster rate than glucose (25, 26). By this active pathway, fructose bypasses the first two slow steps in glycolysis, catalyzed by glucokinase and phosphofructokinase; in fact, fructose feeding results in 40–70% higher levels of pyruvate, acetyl-CoA, and malate in liver than does glucose feeding (26). These data have been invoked to explain the greater synthesis of fatty acids from glucose or fructose by liver slices from animals fed fructose compared to those fed glucose (26). It may be possible that an intermediate(s) at the triose phosphate step or beyond (e.g., acetyl-CoA, citrate), leads to the induction of the fatty-acid synthetase [as well as other lipogenic enzymes (27)]. This hypothesis would explain the higher concentration of synthetase in normal animals fed fructose rather than glucose. In addition, since fructokinase, aldolase, and triokinase are not affected by diabetes (28), whereas activity of glucokinase is decreased (29), the above hypothesis of induction by intermediates would be compatible with the decreased fatty-acid synthetase in diabetic liver, the failure of glucose to correct the defect if insulin (which corrects the glucokinase defect) is not also administered, and the increase in fatty-acid synthetase in diabetic liver after the feeding of fructose. [Also compatible with this hypothesis are previous findings that in diabetic animals the lowered hepatic activities of such enzymes as pyruvate kinase (27), ATP-citrate lyase (27), acetyl-CoA carboxylase (27) and Δ^9-desaturase (stearic $\rightarrow$ oleic) (30) are increased toward normal or above by the feeding of glycerol. Glycerol, like fructose, enters the glycolytic pathway at the triose phosphate step.] The difference in response to fructose feeding of fatty-acid synthetase in adipose tissue of both normal and diabetic animals also is consistent with this hypothesis. Thus, in adipose tissue, fructokinase is not present (31) and fructose must be phosphorylated to fructose 6-phosphate by hexokinase (type-II isoenzyme) (32). This latter enzyme has a 20-fold higher affinity for glucose than fructose,

and its activity is depressed in diabetic animals (33). Therefore, any potentially stimulatory intermediates would be lower in concentration in adipose tissue upon feeding of fructose rather than glucose and would be further depressed in diabetes. An equally plausible mechanism for these effects in adipose tissue involves the transport of glucose or fructose, since insulin has been shown to stimulate the transport of these two sugars into this tissue (34, 35). Finally, the isotopic-immunochemical data establish that the mechanism of regulation under discussion is mediated by synthesis of fatty-acid synthetase.

The alterations in glycolysis are the best established of the metabolic effects of insulin in liver. Thus, the effect of insulin (or cyclic AMP) on fatty-acid synthetase is probably not directly on the protein biosynthetic system for this enzyme. Our data, however, do not rule out the possibility that a direct effect of insulin, not secondary to a change in carbohydrate metabolism, contributes to the responses observed. This possibility is suggested by the fact that after 24 hr of glucose feeding and insulin administration, hepatic synthetase in diabetic rats returns completely to control levels, whereas after 24 hr of fructose feeding the hepatic activity in the diabetic is still only approximately 20% of that in the control animals (see Tables 1 and 2).

A major difference in the regulation of fatty-acid synthetase of brain relative to the enzymes of liver and adipose tissue is apparent from the data. Thus, synthetase of brain is unaffected by the diabetic state, the administration of insulin, or feeding of glucose or fructose. We have shown previously that synthesis, degradation, and prosthetic-group turnover of the fatty-acid synthetase of brain are not altered by various nutritional factors that profoundly affect the enzymes of liver and adipose tissue (9, 10).

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