Effect of diet on linoleic acid desaturation and on some enzymes of carbohydrate metabolism

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ABSTRACT The effect of diet on the desaturation of linoleic acid to γ-linolenic acid by liver microsomal preparations, on blood glucose and insulin levels, and on activities of glucokinase, hexokinase, pyruvate kinase, and α-glycerophosphate dehydrogenase have been studied. The female rats used in these experiments were maintained on one of the following dietary regimes: (*a*) fasted, (*b*) fasted for 96 hr and refed glucose, (*c*) balanced diet, (*d*) carbohydrate-free diet, (*e*) lipid-free diet, or (*f*) protein-free diet. Fasting for 96 hr caused a decrease of both linoleic acid desaturation and glucokinase and pyruvate kinase activity together with a slight decrease of the blood insulin level. α-Glycerophosphate dehydrogenase activity was not modified. Refeeding of glucose for 50 hr increased the conversion of linoleic acid to linolenic acid as well as the activities of all the enzymes studied except α-glycerophosphate dehydrogenase. The increase in desaturation, however, was transient. The feeding of a lipid-free diet did not modify the tested parameters. Feeding a carbohydrate-free diet for 96 hr resulted in increased linoleic acid desaturation but decreased glucokinase and pyruvate kinase activity, thus apparently eliminating a putative correlation between the fatty acid desaturating activity and glycolytic activity or blood insulin levels under these experimental conditions. The findings suggest that dietary proteins may play an important role in determining the level of fatty acid desaturation.

SUPPLEMENTARY KEY WORDS pyruvate kinase · glucokinase · α-glycerophosphate dehydrogenase · insulin · liver microsomes · fasting · lipid-free diet · carbohydrate-free diet · protein-free diet

ALTHOUGH THE EFFECT of insulin on lipid metabolism has been studied for many years, only in the last 8 yr has it been shown that insulin and dietary glucose modify the fatty acid desaturating capacity in liver and other tissues (1–7) of diabetic and fasted rats, respectively.

These modifications of the enzymic activity consequently produce changes in the fatty acid composition of the tissues (7, 8). Rats made diabetic by administration of alloxan have a decreased capacity to desaturate fatty acids. The administration of insulin restores this desaturating activity and thereby, causes conversions of stearic acid to oleic acid (2, 9), linoleic acid to γ-linolenic acid, α-linolenic to octadeca-6,9,12,15-tetraenoic acid, and oleic acid to octadeca-6,9-dienoic acid (5, 7). It has been suggested that insulin probably regulates these reactions by means of enzyme induction (4, 7). In the fasted rat there is also a decrease in fatty acid desaturation (7, 10). Administration of glucose for 12 hr practically restores the desaturation to normal. This effect has been attributed to an increase in the secretion of insulin evoked by the glucose administration (7, 10). However, recent findings have shown that thyroxine (11) may replace insulin in the restoration of the fatty acid desaturating capacity of the alloxan-diabetic rat. Besides, although the concentration of blood glucose may not be directly responsible for inducing the desaturating activity as the level of glucose is high in diabetic rats and low in fasted animals, it is impossible to elucidate clearly from the aforementioned studies (7, 10) whether the effect of insulin is due to insulin per se or whether it is actually mediated via the utilization of glucose at the cellular level. The following experiments constitute an attempt to study simultaneously the effects of different diets on fatty acid desaturation and on the activity of some enzymes involved in the metabolism of glucose.

MATERIAL AND METHODS

Animals

Female albino rats of the Institute strain weighing

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90–140 g and maintained on a standard laboratory diet were used.

Treatment of Animals

In the first experiment the rats, in groups of five animals, were killed after 24, 48, 72, or 96 hr of fasting. Five groups of rats which were fasted for 96 hr were offered a 5% glucose solution and were killed after 4, 8, 16, 32 and 50 hr of glucose refeeding. A group of animals fed a balanced diet was used as control. The fasted rats were given water ad lib.

In the second experiment the rats, in groups of 10 animals each, were kept in individual cages and fed different isocaloric diets (25 kcal/100 g body weight) for 96 hr. All the experimental diets contained the same amount of salts and vitamins (12). Water was given ad lib. Fat-free casein, sunflower seed oil, and dextrin were used as sources of protein, fat, and carbohydrate, respectively. In the balanced diet (CD) 50, 23, and 27% of the calories were supplied as carbohydrate, protein, and fat, respectively. The distribution of calories in the various deficient diets was as follows: carbohydrate-free diet (CF), 73% protein, 27% fat; fat-free diet (LF), 73% protein, 27% carbohydrate; protein-free diet (PF), 73% carbohydrate, 27% fat. An additional group (F) was fasted. The animals were killed after they had been on the respective diets (or fasting) for 96 hr.

Isolation of Microsomes

The rats were killed by decapitation without anesthesia. The blood was allowed to drain and was collected for glucose and insulin determinations.

The livers were excised, blotted on filter paper, and weighed. The whole liver was homogenized briefly in a cold solution (3:1 v/w) consisting of 0.15 M KCl, 0.005 M $MgCl_2$, 0.004 M EDTA, 0.004 *N*-acetylcysteine, 0.05 M phosphate buffer (pH7), and 0.25 M sucrose. Cell debris and mitochondria were removed by sedimentation at 12,000 *g* for 10 min at 0°C. The microsomal fraction was prepared by centrifugation at 140,000 *g* for 60 min in a Spinco Model L2 ultracentrifuge (13). The pellets were resuspended in the homogenizing solution (1:5 v/v).

Assay Procedures

The desaturation of linoleic acid to γ-linolenic acid by microsomes was measured by estimation of the percentage conversion of linoleic acid-1-^{14}C (51.9 mci/mmole; Radiochemical Centre, Amersham, England) to γ-linolenic acid. 5 nmoles of labeled linoleic acid and 5 nmoles of unlabeled linoleic acid were incubated with 2 mg of microsomal protein in a Dubnoff shaker at 25°C for 20 min in a total volume of 3 ml of a 0.15 M KCl, 0.25 M sucrose solution containing 4 μmole of ATP, 0.2 μmole of CoA, 2.5 μmole of NADH, 15 μmole of $MgCl_2$, 4.5 μmole of glutathione, 125 μmole NaF, 1 μmole nicotinamide, and 125 μmole of phosphate (pH 7). At the end of the incubation, the fatty acids were recovered by saponification of the incubation mixture and extraction with petroleum ether (bp 30°C). The acids were esterified with methanolic 3 M HCl (3 hr at 68°C), and the distribution of the radioactivity between linoleic and γ-linolenic acid was determined by gas–liquid radiochromatography in a Pye apparatus with a proportional counter. No measurable radioactivity was found in other fatty acid peaks. The recovery of the radioactivity from individual incubation mixtures was 80–100%. The methyl ester of γ-linolenic acid was identified by comparing its retention time to that of an authentic sample. The γ-linolenic acid which was synthetized by the microsomes, was also separated by preparative gas–liquid radiochromatography on a column of 10% diethylene glycol succinate on Chromosorb W (80–100 mesh) at 180°C. The structure and position of labeling was confirmed by reductive ozonolysis (14) followed by gas–liquid radiochromatography on the same column. Radioactivity was found only in the aldehyde-ester fraction with 6 carbon atoms.

The 140,000 *g* supernatant solution was used for the other enzyme assays. Glucokinase and hexokinase were measured by the methods of Walker and Parry (15). Pyruvate kinase was assayed according to the procedure of Bücher and Pfleiderer (16). α-Glycerophosphate dehydrogenase activity was measured by the method of Fitch and Chaikoff (17). The specific activities of these enzymes were expressed as μmoles of substrate transformed per minute. The values were expressed as units per mg of DNA in the total homogenate so that they might be related more directly to the number of cells.

Proteins in the microsomal and supernatant fractions were determined by the biuret method of Gornall, Bardawill, and David (18) using crystalline bovine serum albumin as standard. DNA in the homogenate was measured by the method of Burton (19). Immunoreactive insulin was determined by a radioimmunoassay procedure using beef insulin standards (20). Glucose was measured by the glucose oxidase method (21).

RESULTS AND DISCUSSION

As it was previously postulated and discussed in the introduction, insulin may induce the synthesis of the desaturating enzyme (2–10). The evidence for this was obtained by measuring the fatty acid desaturase activity and messenger RNA levels in tissues of diabetic or fasted animals previously injected with actinomycin D or puromycin before the administration of insulin or glucose, respectively. However the effect of insulin may

also be mediated by its effect on the utilization of glucose at the cellular level. Moreover, this hormone causes a nonspecific increase in protein synthesis (22). In this respect, there is evidence which supports the suggestion that insulin may also increase the generalized synthesis of proteins by a direct effect on the ribosomes (22, 23). This concept does not conflict with the hypothesis that the action of glucose in increasing the fatty acid desaturation activity in the fasted rat is mediated by an enhanced secretion of insulin.

Effect of Fasting and Subsequent Refeeding of Glucose on Hepatic Microsomal Desaturation of Linoleic Acid

The level of desaturation activity was studied in relation to blood glucose and insulin levels and hepatic activities of phosphorylation of glucose to glucose-6-phosphate ("glucokinase"), pyruvate kinase, and α-glycerophosphate dehydrogenase. Glucokinase (24) and pyruvate kinase are two insulin-dependent enzymes that may be responsible for the regulation of glycolysis (24). The level of α-glycerophosphate dehydrogenase activity may be related to glycerophosphate synthesis (17). Although the total glucose phosphorylation to glucose-6-phosphate includes both hexokinase and glucokinase activity, the hexokinase enzyme is not dependent on insulin. Thus, a measurement of total glucose phosphorylation can be substituted (25) for a direct assay of glucokinase activity in order to measure insulin-dependent variations of glucose metabolism.

Fig. 1 illustrates how the conversion of linoleic-1-^{14}C to γ-linolenic acid decreases during a 96 hr period of fasting. This decrease is coincident with significant declines in both glucokinase and pyruvate kinase activities. This latter effect has already been reported by several other laboratories (26–28). The decrease in glucokinase activity suggests a diminished utilization of glucose via the Emden–Meyerhoff pathway. The concomitant decrease in pyruvate kinase activity may also indicate a reduction in the flow of glucose metabolites into the tricarboxylic acid cycle. Fitch and Chaikoff (17) have found that the feeding of a carbohydrate-rich diet may modify the activity of α-glycerophosphate dehydrogenase. However, the level of this enzyme was not significantly changed in our experiment. If its activity is a measurement of the extent of the diversion of glucose metabolites toward lipid synthesis, the present results would imply that the fall of linoleic acid desaturating activity could not be ascribed to an increase of the direct incorporation of glucose metabolites into lipids. This observation is relevant since an increase in phospholipid synthesis could modify the microsomal fatty acid desaturating activity (29, 30). A 96 hr fast caused only a slight decrease in blood insulin levels (Fig. 2). However, the decrease in the activities of the two insulin-dependent enzymes, glucokinase and pyruvate kinase, was on the order of the decline of desaturase activity; this might be explained by the aforementioned postulate that all three of these enzymes are insulin-dependent. Moreover, the immunological estimation of insulin may not necessarily show real variations of biologically active insulin, since other forms of insulin such as proinsulin may be in the blood (31, 32). On the other hand, the level of desaturase activity may be related to the rate of glucose metabolism.

In the same experiment (Fig. 1), the administration of glucose in the drinking water caused an enhancement of linoleic acid desaturation activity, glucokinase and pyruvate kinase activity, and a significant increase in blood insulin levels (Fig. 2). These results would agree with the aforementioned hypothesis of insulin action. However after 16 hr, although the activities of the two glycolytic enzymes and the blood insulin levels had continued to increase, the fatty acid desaturating activity decreased abruptly (Fig. 1). The effect of insulin on fatty acid desaturation activity has already been thoroughly demonstrated in a number of experiments (1–9). The observed lack of correlation between circulating insulin levels and the desaturase activity suggests that another factor(s) may influence the desaturation.

Effect of Different Dietary Components on Linoleic Acid Desaturation

In the second experiment the effects of various dietary regimes on the desaturation of linoleic acid-1-^{14}C to γ-linolenic acid by liver microsomes, and on the glucokinase, pyruvate kinase, and α-glycerophosphate dehydrogenase activities of liver supernatant fractions were examined. The results are shown in Figs. 3–6. In the first place, these results confirm that fasting for 96 hr (F) causes significant decreases in linoleic acid desaturation (Fig. 3) and in activities of glucokinase (Fig. 4) and pyruvate kinase (Fig. 5). The hexokinase activity (Fig. 4) was not affected, and blood insulin levels (Table 1) and α-glycerophosphate dehydrogenase activity (Fig. 6) decreased slightly although these values were not significantly different from values obtained for animals on the balanced diet (CD).

A lipid-free diet (LF), containing only proteins and carbohydrates, did not cause a modification of the desaturation activity of the microsomes (Fig. 3). However, carbohydrate-free diet (CF) caused an unexpected and significant increase in the desaturation of linoleic to γ-linolenic acid compared with the balanced diet. In contrast, the protein-free diet (PF), produced a small decrease in desaturation activity without affecting glucokinase activity (Fig. 4) or blood insulin levels (Table 1). Consequently, these results suggest that dietary lipids do not modify the desaturase activity of

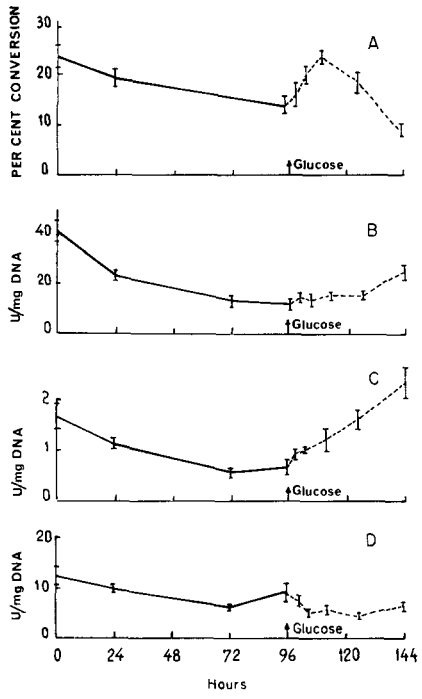

FIG. 1. Effect of fasting and glucose refeeding on the conversion of linoleic acid to γ-linolenic (*A*), and on activities of pyruvate kinase (*B*), "glucokinase" (*C*), and α-glycerophosphate dehydrogenase (*D*). The results are the means of duplicate analysis in studies of five animals. The vertical lines represent SEM.

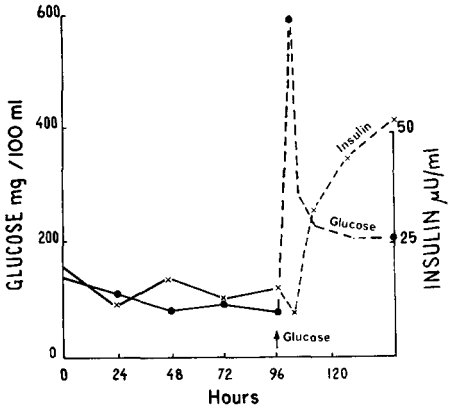

FIG. 2. Effect of fasting and glucose refeeding on the level of blood glucose (—●—) and immunoreactive insulin (—X—).

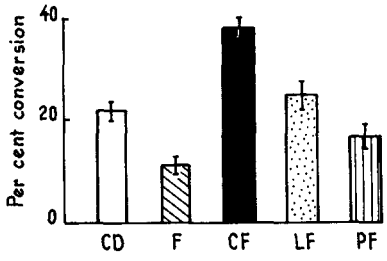

FIG. 3. Effect of different diets on the conversion of linoleic-1-^{14}C to γ-linolenic acid. Rats were fed 25 kcal/100 g body weight for 96 hr. CD, balanced diet; F, 96 hr fast; CF, carbohydrate free; PF, protein free. The results are the means of analysis of 10 animals (each analysis was performed in triplicate). The vertical lines represent SEM.

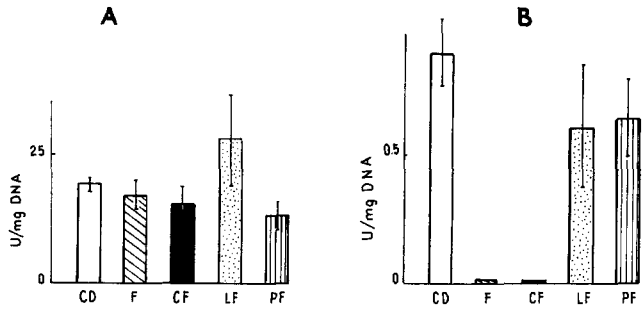

FIG. 4. Effect of different diets on hexokinase (*A*) and glucokinase (*B*) activity: See legend of Fig. 3 for details.

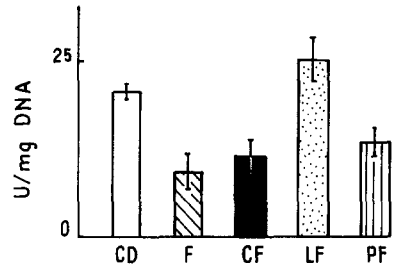

FIG. 5. Effect of different diets on pyruvate kinase activity. See legend of Fig. 3 for details.

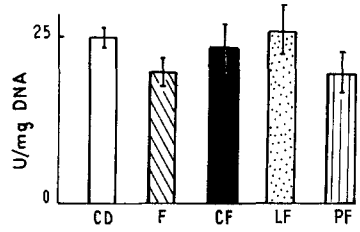

FIG. 6. Effect of different diets on α-glycerophosphate dehydrogenase activity. See legend of Fig. 3 for details.

the microsomes but that the level of dietary protein exerts an effect on the desaturation reaction.

We arrive at this conclusion as a result of the following reasoning. Previous experiments (7) have demonstrated that there is a nearly complete recovery of the linoleic acid desaturation activity in 48-hr fasted rats given glucose for 12 hr, but that this effect is blocked by prior administration of actinomycin D. Similar results are also illustrated in Fig. 1. The carbohydrate-free, protein-rich diet which was fed to the animals caused an increase, rather than a decrease, in the desaturation activity. Hence, since dietary lipids appear to have no effect, the excessively high intake of proteins in these experiments is probably the factor that causes the increase in desaturation activity. The small decrease in desaturation seen in the rats fed the protein-free diet, which contained carbohydrates and lipids, would also agree with this hypothesis. The activities of the two hepatic glycolytic enzymes, glucokinase (Fig. 4) and pyruvate kinase (Fig. 5), were not modified by the lipid-free diet (LF), but were significantly and similarly decreased in fasted animals and in animals fed the carbohydrate-free diet (CF). Hence, the glucokinase and pyruvate kinase activities showed no correlation with

TABLE 1 EFFECT OF DIFFERENT DIETS ON THE LEVELS OF BLOOD GLUCOSE AND IMMUNOREACTIVE INSULIN

	CD	F	CF	LF	PF
Glucose (mg/100 ml)	136 ± 0.54	85 ± 0.03	140 ± 0.04	133 ± 0.15	117 ± 0.03
Insulin (μU/ml)	36.9 ± 3.4	29.1 ± 7.4	30.5 ± 8.2	21.3 ± 4.3	38.5 ± 5.5

Diets: CD, balanced diet; F, fasted; CF, carbohydrate-free; LF, lipid-free; PF, protein-free.
Analysis were performed in duplicate on each of the 10 animals in each dietary group. The values are the means of ± SEM.

the fatty acid desaturating activity when a carbohydrate-free diet, rich in proteins and lipids, was administered. Therefore, these results appear to eliminate the possibility that the increase in linoleic acid desaturating activity under these experimental conditions is related to an increase in glucose metabolism. In fact, the desaturation activity is apparently not related to the blood insulin levels (Table 1), since when a carbohydrate-free diet was fed, desaturation activity increased, whereas the blood insulin levels were not significantly modified and the activity of the two insulin-dependent enzymes were reduced. The desaturation activity also appears to be independent of lipid synthesis since the α-glycerophosphate dehydrogenase activity is not significantly altered by diet (Fig. 6). Thus, in light of the aforementioned results, it becomes necessary to acknowledge the existence of a factor related to protein metabolism that may cause an increase in the microsomal desaturation of linoleic acid to γ-linolenic acid under certain experimental circumstances. This factor may play a special role in the biosynthesis of polyunsaturated fatty acids. In support of this is the recent work of Inkpen, Harris, and Quackenbush (33) who have published the results of a series of experiments which show that dietary proteins may modify the desaturation of α-linolenic acid.

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