Dietary protein and the control of fatty acid synthesis in rat adipose tissue

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ABSTRACT Fatty acid synthesis in adipose tissue normally proceeds at a high rate when fasted animals are refed a diet containing carbohydrate, protein, and low levels of fat. This study investigated the effect of omitting protein from the refeeding diet.

Rats were fasted for 48 hr and refed either a protein-free diet or a balanced diet, and the rate of fatty acid synthesis from glucose, pyruvate, lactate, and aspartate was measured. Refeeding the animals a diet devoid of protein resulted in a low rate of fatty acid synthesis from each of these substrates as well as a reduction in carbon flow over the citrate cleavage pathway. The activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NADP-malate dehydrogenase, and ATP-citrate lyase were also reduced in epididymal fat pads from these rats. On the other hand, adipose tissue phosphoenolpyruvate carboxykinase activity was five times as great as that in tissue from animals refed a balanced diet. This difference could be eliminated if actinomycin D was injected coincident with refeeding.

Refeeding rats diets high in carbohydrate is not, therefore, capable of inducing high rates of fatty acid synthesis in adipose tissue in the absence of dietary proteins. Thus, liver and adipose tissue respond differently to dietary protein.

SUPPLEMENTARY KEY WORDS fasting–refeeding · lipogenesis · lactate · pyruvate · pentose pathway · citrate cleavage pathway · phosphoenolpyruvate carboxykinase

REFEEDING RATS a balanced diet after a prolonged period of starvation causes an increase in lipogenesis in both adipose tissue and liver (1–5). The biochemical basis for this hyperlipogenesis has been intensively studied and has offered considerable insight into the control of fatty acid synthesis in mammalian tissues. Several enzymes related to lipogenesis, including glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, NADP-malate dehydrogenase, and ATP-citrate lyase all increase in activity after fasting-refeeding (1–5). Carbon flow over the pathways in which these enzymes participate has also been shown to increase (6, 7). The nature of the diet markedly influences the response to refeeding after fasting. A diet high in fat reduced the increase in lipogenesis in both adipose tissue and liver (4, 7), and this is accomplished by a similar reduction in the adaptive changes in the activities of various enzymes related to lipogenesis (4, 7–9). Recently, Tepperman, de la Garza, and Tepperman (10) reported that diets containing high levels of carbohydrate, but no protein, when refed to fasted rats caused a decrease in hexose monophosphate dehydrogenases, ATP-citrate lyase, and NADP-malate dehydrogenase of rat liver with no effect on the high capacity for lipogenesis from either acetate or glucose. This effectively separates the activity of the two important NADPH-generating enzyme systems and the over-all process of lipogenesis.

Lipogenesis in adipose tissue is remarkably sensitive to alterations in diet (4, 5, 7), to the age of the animal (11–13), and to dietary pattern (5, 7, 14). An important factor in the regulation of fatty acid synthesis in this tissue is the generation of NADPH for reductive synthesis. Two important sources of NADPH in adipose tissue are the initial dehydrogenase reactions of the pentose pathway (15, 16) and the transhydrogenation of NADH to NADPH, catalyzed by the coupling of cytosol NAD and NADP-malate dehydrogenase (4, 7, 17, 18). In view of the reported decrease in the dehydrogenases of the pentose pathway and of malic enzyme in rat liver from fasted animals refed a protein-free diet (10), the effect of

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dietary protein on fatty acid synthesis in adipose tissue was investigated.

MATERIALS AND METHODS

Chemicals

L-lactate, sodium pyruvate, L-aspartate, glucose-6-phosphate, 6-phosphogluconate, citrate, isocitrate, and GSH were purchased from Sigma Chemical Co., St. Louis, Mo. CoA, ATP, NAD, IDP, NADH, and NADP were from P-L Laboratories, Milwaukee, Wis. NAD-malate dehydrogenase (EC 1.1.1.37) and potassium phosphoenolypyruvate were from Boehringer Mannheim Corp., New York. $NaH^{14}CO_3$, sodium pyruvate-3-^{14}C, sodium L-lactate-3-^{14}C, and D-glucose-U-^{14}C were purchased from New England Nuclear Corp., Boston, Mass., and DL-aspartate-1-^{14}C and DL-aspartate-3-^{14}C were from Mallinkrodt Nuclear Corporation, Orlando, Florida. The highly purified glucagon-free insulin used in this study was a gift of Dr. O. K. Behrens, Eli Lilly Laboratories, Indianapolis, Ind. Actinomycin D (Lyovac Cosmogen) from Merck, Sharp & Dohme, West Point, Pa., was dissolved in 0.9% NaCl and injected intraperitoneally at a concentration of 40 μg/100 g body weight (13).

Diets

A protein-free diet containing 84% dextrin, 9% corn oil, 4% salt mixture, 2% agar, 1% cod liver oil, and a complete vitamin supplement was purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio. This diet was modified to give the balanced diet by reducing the content of dextrin to 64% and substituting 20% casein. Male Sprague–Dawley rats, each weighing 140 g, were fed the balanced diet for 2 wk prior to the commencement of these studies, at which time the weight of each animal was 180–200 g.

Incubation Studies

Pieces of epididymal adipose tissue were incubated in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4. Substrates were dissolved in the buffer at the following concentrations: 0.25 mM sodium pyruvate-3-^{14}C (0.5 μc), 25 mM sodium pyruvate-3-^{14}C (2.5 μc), 5 mM glucose-U-^{14}C (0.5 μc), 5 mM DL-aspartate-1-^{14}C (0.5 μc), 5 mM DL-aspartate-3-^{14}C (0.5 μc), 0.25 mM sodium L-lactate-3-^{14}C (0.5 μc), and 25 mM sodium L-lactate-3-^{14}C (2.5 μc). When glucose was present in the buffer, 0.1 unit of insulin was added. The incubation was carried out for 3 hr at 37°C in a 25 ml Erlenmeyer flask equipped with a rubber stopper which served both to seal the flask and to hold a plastic bucket suspended above the buffer. At the end of the incubation 0.3 ml of Hyamine hydroxide was injected through the stopper into the plastic bucket and 0.5 ml of 1 N H_2SO_4 into the buffer to insure the complete liberation of CO_2. After 45 min of shaking the tissue was removed, rinsed in 0.9% NaCl, and placed in 10 ml of chloroform–methanol 2:1. The lipid was extracted, and the radioactivity measurements were performed as described previously (19). The plastic buckets were immersed in vials containing 10 ml of toluene-based liquid scintillation fluid to remove the Hyamine. All radioactivity measurements were performed on a Nuclear-Chicago liquid scintillation spectrometer.

Enzyme Determination

Glucose-6-phosphate dehydrogenase was assayed as outlined by Kornberg and Horecker (20), 6-phosphogluconate dehydrogenase by the method of Horecker and Smyrniotis (21), and NADP-malate dehydrogenase as outlined by Ochoa (22), with final concentration of reagents as reported by Ballard and Hanson (23). ATP citrate lyase was assayed by a method that was slightly modified (23) from the procedure of Srere (24). P-enolpyruvate carboxykinase was assayed by our modification (25) of the method of Chang and Lane (26).

Epididymal adipose tissue was homogenized with a coaxial homogenizer in buffered isotonic sucrose (27). The homogenates were centrifuged at 100,000 *g* for 30 min in a Spinco model L ultracentrifuge. The supernatant was used for the assay of all enzymes. A portion of this supernatant was used for the determination of nitrogen (28).

RESULTS

Refeeding a diet devoid of protein to rats fasted for 48 hr markedly affects glucose utilization by epididymal adipose tissue in vitro (Table 1). The normal increase in fatty acid synthesis by adipose tissue which accompanies the refeeding of a balanced diet for either 48 or 72 hr did not occur when the rats were refed a protein-free diet. Refeeding fasted rats a protein-free diet also reduced CO_2 output below levels found for adipose tissue from normal fed or fasted-refed animals. Glyceride-glycerol synthesis from glucose by fat pads was not affected by refeeding the protein-free diet as compared with pads from normal fed animals.

In a previous study from this laboratory (13), we reported that the normally lower rates of fatty acid synthesis in vitro from pyruvate by adipose tissue from fasted rats could be almost completely restored to fed-rat values by increasing the pyruvate concentration from 0.25 to 25 mM. It therefore seemed of interest to determine whether the capacity to synthesize fatty acids by adipose tissue, which was markedly reduced by refeeding a diet devoid of protein, would be affected by increasing the substrate

concentration. As seen in Table 2, refeeding fasted animals a protein-free diet resulted in a level of fatty acid synthesis from pyruvate that was lower than that of adipose tissue from the fasted rat. At a pyruvate concentration of 0.25 mM the extent of fatty acid synthesis in rats fasted as well as fasted-refed a protein-free diet was very low. Increasing the pyruvate concentration to 25 mM, however, resulted in a marked increase in fatty acid synthesis in adipose tissue from the fasted animals as well as in tissue from fasted rats refed a protein-free diet. Fatty acid synthesis by adipose tissue from both fed and fasted-refed (balanced diet) animals also increased with the higher concentration of pyruvate. In both of these groups the over-all extent of fatty acid synthesis by adipose tissue was the same. Glyceride-glycerol synthesis from pyruvate was highest in adipose tissue from fasted rats, but only at a pyruvate concentration of 25 mM.

Animals refed the protein-free diet after fasting for 48 hr consumed 60% less than comparable animals refed the balanced diet. Rats were therefore fasted for 48 hr and then refed the same amount of balanced diet as was consumed by fasted animals refed a protein-free diet. Adipose tissue from these pair-fed animals was then incubated; the amounts of fatty acid synthesized from pyruvate were 3.0, 7.2, 2.8, and 0.3 μmoles/g per 3 hr for animals that were fed, refed a balanced diet, pair-fed, or refed a protein-free diet, respectively. This indicates that although food intake was lower in rats refed a protein-free diet, the lowered food consumption does not account for the very low rates of lipogenesis in adipose tissue noted in these animals.

The lower incorporation of glucose into fatty acids in adipose tissue from rats fasted and refed the protein-free diet suggested a reduction in the over-all flow of carbon through the citrate cleavage pathway. This series of reactions involves citrate formation inside the mitochondria, its passage into the cytosol, and citrate cleavage via ATP-citrate lyase. The oxaloacetate formed from this cleavage is converted to malate by NAD-malate dehydrogenase, and the malate subsequently decarboxylated to pyruvate. This cycle has been shown to function in rat adipose tissue and is presumably important in supplying acetyl CoA in the cytosol as well as generating NADPH by coupling NAD and NADP malate dehydrogenases (4, 7, 18, 29). One method for determining carbon flow over the citrate cleavage pathway is a measurement of the rate of aspartate-3-^{14}C incorporated into fatty acids. The metabolic fate of the specific carbons of aspartate has been discussed in detail previously (7). The C-1 of aspartate cannot be incorporated into fatty acids, and as shown in Table 3, negligible aspartate-1-^{14}C radioactivity was found in lipid. However, the C-1 of aspartate was converted to $^{14}CO_2$ at appreciable rates by adipose tissue from rats on the four dietary treatments studied. The C-3 of aspartate is converted to fatty acids, but the amount of incorporation by adipose tissue of rats refed diets devoid of protein was only 25% of that in tissue from animals fasted and refed the balanced diet, and approximately half of that found in adipose tissue from fasted rats.

TABLE 1 EFFECT OF DIETARY TREATMENTS ON UTILIZATION OF GLUCOSE BY RAT ADIPOSE TISSUE

Dietary Treatment		CO_2	Fatty Acid	Glyceride-Glycerol
		μmoles glucose used/g tissue/3 hr		
Fed		5.82 ±0.65	5.74 ±0.55	1.76 ±0.09
Fasted (48 hr)		2.75 ±0.28	0.81 ±0.16	2.19 ±0.14
Fasted 48 hr, refed balanced diet	48 hr	5.17 ±0.53	6.97 ±0.81	1.98 ±0.19
	72 hr	4.93 ±0.22	12.90 ±1.45	2.49 ±0.49
Refed protein-free diet	48 hr	1.81 ±0.18	1.03 ±0.14	2.28 ±0.11
	72 hr	1.70 ±0.26	1.43 ±0.47	2.37 ±0.43

Values are the mean ± SEM for six animals. Tissue was incubated for 3 hr in 5.0 ml of Krebs–Ringer bicarbonate buffer, pH 7.4, containing 5 mM glucose (0.5 μc) and 0.1 unit of insulin. Metabolites were isolated as outlined in the Materials and Methods section.

TABLE 2 EFFECT OF DIETARY TREATMENTS ON UTILIZATION OF PYRUVATE BY RAT ADIPOSE TISSUE

Conversion Products	Pyruvate Concentration (mM)	Dietary Treatment			
		Fed	Fasted	Fasted-Refed Balanced Diet	Fasted-Refed Protein-Free Diet
		μmoles pyruvate used/g tissue/3 hr			
CO_2	0.25	1.44 ± 0.17	1.38 ± 0.15	1.33 ± 0.03	3.86 ± 0.47
	25	12.66 ± 1.04	18.46 ± 2.57	8.68 ± 0.63	8.63 ± 1.03
Fatty acids	0.25	0.57 ± 0.10	0.03 ± 0.005	1.41 ± 0.36	0.004 ± 0.0009
	25	31.71 ± 2.25	14.0 ± 2.31	26.05 ± 2.74	11.1 ± 2.58
Glyceride-glycerol	0.25	0.32 ± 0.05	0.33 ± 0.09	0.55 ± 0.15	0.20 ± 0.06
	25	3.47 ± 0.29	9.72 ± 1.68	2.45 ± 0.23	4.46 ± 0.45

Values are the mean ± SEM for six animals. Incorporation was measured in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 0.25 mM pyruvate (0.5 μc) and 25 mM pyruvate (2.5 μc). Animals were fasted 48 hr and refed 48 hr as indicated.

Lactate utilization by adipose tissue offers several interesting contrasts to the metabolism of pyruvate in the same tissue. As is shown in Table 4, fatty acid synthesis from 0.25 mM lactate by adipose tissue from fed animals occurs at a low rate. Increasing the concentration of lactate to 25 mM increases the rate of lipogenesis 40-fold. In adipose tissue from the fasted animals, however, an increased lactate concentration did not result in a marked stimulation in fatty acid synthesis. Refeeding animals fasted for 48 hr a balanced diet resulted in a very marked increase in lipogenesis from lactate (25 mM) by adipose tissue, whereas refeeding the diet devoid of protein resulted in only a very small increase in fatty acid synthesis. It seems clear that lactate is being utilized by epididymal fat pads from animals on the four dietary treatments since the rate of CO_2 output is approximately the same despite the extreme variations in fatty acid synthesis noted. The addition of glucose plus insulin to the incubation medium at equimolar concentration with lactate caused an increase in fatty acid synthesis by adipose tissue from normal fed rats to levels as great as those found in fat pads from the animals fasted-refed the balanced diet. Adipose tissue from fasted rats or animals fasted and refed the protein-free diet had similar rates of fatty acid synthesis from lactate in the presence of glucose and insulin. These rates, although lower than noted for the fed animals and for rats fasted and refed the balanced diet, were markedly increased over those found with 25 mM lactate in the absence of glucose and insulin. The output of $^{14}CO_2$ by adipose tissue from lactate-^{14}C in the presence of glucose and insulin was greatest in tissue from the fasted rat and approximately equal in tissue from animals fed, fasted-refed the balanced diet, or refed the protein-free diet.

The activities of several enzymes involved in fatty acid synthesis in adipose tissue were measured under the dietary conditions being studied (Table 5). Fasting for 48 hr followed by refeeding the animals a balanced diet for 48 hr resulted in the well-established increase in the activity of glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase, 6-phosphogluconate dehydrogenase, and ATP-citrate lyase in adipose tissue as compared with tissue from fed or 48-hr-fasted rats. By con-

TABLE 3 Effect of Dietary Treatment on Utilization of Specifically Labeled Aspartate by Rat Adipose Tissue

Substrate	Products Isolated	Dietary Treatment: Fed	Fasted	Fasted-Refed Balanced Diet	Fasted-Refed Protein-Free Diet
		μmoles aspartate used/g tissue/3 hr			
Aspartate-1-^{14}C	CO_2	5.09 ± 0.55	3.35 ± 0.27	4.78 ± 0.070	3.38 ± 0.18
	Fatty acids	0.003 ± 0.001	<0.001	<0.001	<0.001
	Glyceride-glycerol	0.06 ± 0.007	0.08 ± 0.03	0.03 ± 0.005	<0.001
Aspartate-3-^{14}C	CO_2	0.38 ± 0.04	0.63 ± 0.04	0.27 ± 0.05	0.53 ± 0.10
	Fatty acids	2.86 ± 0.19	1.11 ± 0.16	2.75 ± 0.30	0.67 ± 0.10
	Glyceride-glycerol	0.15 ± 0.02	0.23 ± 0.03	0.13 ± 0.03	0.02 ± 0.006

Values are the mean ± SEM for six animals. Incorporation is measured in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 5 mM L-aspartate (0.5 μc DL-aspartate-1-^{14}C) and 5 mM L-aspartate (0.5 μc DL-aspartate-3-^{14}C). Animals were fasted 48 hr and refed 48 hr as indicated.

TABLE 4 Effect of Dietary Treatment on Utilization of Lactate by Rat Adipose Tissue

Metabolites	Products Isolated	Dietary Treatment: Fed	Fasted	Fasted-Refed Balanced Diet	Fasted-Refed Protein-Free Diet
		μmoles lactate used/g tissue/3 hr			
0.25 mM lactate	CO_2	0.91 ± 0.05	0.21 ± 0.05	0.77 ± 0.10	0.67 ± 0.07
	Fatty acids	0.06 ± 0.01	0.0004	1.13 ± 0.26	0.001 ± 0.0003
	Glyceride-glycerol	0.25 ± 0.03	0.0001	0.18 ± 0.03	0.05 ± 0.002
25 mM lactate	CO_2	2.54 ± 0.15	1.27 ± 0.12	3.16 ± 0.28	2.02 ± 0.29
	Fatty acids	2.45 ± 0.83	0.008 ± 0.001	28.2 ± 4.2	0.02 ± 0.01
	Glyceride-glycerol	0.60 ± 0.06	0.13 ± 0.04	1.42 ± 0.31	0.11 ± 0.04
25 mM lactate + 25 mM glucose 0.1 unit insulin	CO_2	1.27 ± 0.12	2.87 ± 0.19	1.20 ± 0.08	1.57 ± 0.07
	Fatty acids	73.07 ± 8.4	28.17 ± 3.42	71.36 ± 10.42	21.71 ± 4.11
	Glyceride-glycerol	2.77 ± 0.59	2.08 ± 0.35	2.40 ± 0.64	1.22 ± 0.26

Values are the mean ± SEM for six animals. Tissues were incubated in 5.0 ml of Krebs-Ringer bicarbonate buffer pH 7.4, containing 0.25 mM L-lactate-3-^{14}C (0.5 μc), 25 mM L-lactate-3-^{14}C (2.5 μc), or 25 mM glucose and 0.1 unit of insulin as indicated. Animals were fasted for 48 hr and refed 48 hr where indicated.

TABLE 5 EFFECT OF DIETARY TREATMENT ON THE ACTIVITIES OF VARIOUS ENZYMES OF RAT ADIPOSE TISSUE

Enzymes Measured	Dietary Treatment					
	Fed	Fasted 48 hr	Fasted-Refed Balanced Diet		Fasted-Refed Protein-Free Diet	
			48 hr	72 hr	48 hr	72 hr
	units/g issue					
Glucose-6-phosphate dehydrogenase	1.08 ±0.07	1.09 ±0.11	1.56 ±0.13	1.34 ±0.03	0.83 ±0.04	0.83 ±0.03
6-Phosphogluconate dehydrogenase	0.39 ±0.04	0.37 ±0.04	0.51 ±0.04	0.53 ±0.03	0.26 ±0.03	0.45 ±0.01
NADP-malate dehydrogenase	1.65 ±0.14	1.36 ±0.18	2.56 ±0.25	1.57 ±0.07	1.40 ±0.08	1.08 ±0.06
ATP-citrate lyase	0.32 ±0.04	0.18 ±0.08	0.53 ±0.08	0.75 ±0.03	0.18 ±0.02	0.23 ±0.01

Values are the mean ± SEM for six animals. All activities are expressed as μmoles of product formed per min per g of adipose tissue at 37°C.

TABLE 6 EFFECT OF DIETARY TREATMENT AND ACTINOMYCIN D ON THE ACTIVITY OF PHOSPHOENOLPYRUVATE CARBOXYKINASE IN RAT ADIPOSE TISSUE

	Fed	Fasted 48 hr	Fasted 48 hr Refed Balanced Diet 48 hr	Fasted 48 hr Refed Protein-Free Diet 48 hr	Actinomycin D after 48 hr Fast Followed by:		
					24 hr Fast	Refed Balanced Diet 24 hr	Refed Protein-Free Diet 24 hr
Units/g	0.042* ±0.004	0.159 ±0.013	0.051 ±0.003	0.262 ±0.013	0.011 ±0.0005	0.013 ±0.002	0.008 ±0.001
Units/mg N	0.031 ±0.002	0.071 ±0.005	0.030 ±0.003	0.121 ±0.003	0.005 ±0.0002	0.007 ±0.001	0.003 ±0.0003

* Values are the mean ± SEM for six rats. Actinomycin D (40 μg/100 g body weight) was injected intraperitoneally after a 48 hr fast as indicated. All activities are expressed as μmoles of CO_2 fixed per min at 37°C.

trast, these same four enzymes were not increased by refeeding 48-hr-fasted animals the diet devoid of protein.

P-enolpyruvate carboxykinase activity was also measured in adipose tissue (Table 6). This enzyme increased in activity after a 48 hr fast, and refeeding the animals the balanced diet reduced the level of activity to that of the fed rats. Refeeding 48 hr fasted rats the protein-free diet resulted in an increase in the activity of *P*-enolpyruvate carboxykinase. This increase could be due to a decrease in the synthesis of an enzyme that specifically degrades P-enolpyruvate carboxykinase, because of a lack of dietary protein. However, the injection of actinomycin D, which should also inhibit the synthesis of the postulated degradative enzyme, only reduced the activity of adipose tissue P-enolpyruvate carboxykinase to levels below those noted in fed animals. Refeeding the actinomycin D-treated animals either the balanced or the protein-free diet had no effect on the activity of P-enolpyruvate carboxykinase, which remained at the level of the fasted, actinomycin D-treated rat.

DISCUSSION

Our finding that refeeding a diet high in carbohydrate but devoid of protein to rats fasted for 48 hr will not cause an increase in fatty acid synthesis from glucose, pyruvate, lactate, or aspartate contrasts with the rise in lipogenesis from glucose and acetate reported in liver slices (10). It is possible that this difference reflects a failure in the adaptive response of a number of enzymes in adipose tissue that are normally increased in activity after fasting and refeeding a balanced diet. As noted in the present study, refeeding rats a protein-free diet does not cause the elevation of NADP-malate dehydrogenase or of ATP-citrate lyase activities that is characteristic of animals refed a balanced diet (4, 7). But it also seems probable that the activities of these two enzymes, normally thought to be involved in acetyl CoA translocation from the mitochondria to the cytosol and in NADPH generation (4, 7, 17, 29), do not limit the rate of fatty acid synthesis from pyruvate. This is evident from the fact that raising the concentration of pyruvate in the incubation medium from 0.25 to 25 mM results in an almost 400-fold increase in fatty acid synthesis in adipose tissue from fasted rats and a 3,000-fold increase in fat pads from animals fasted and refed a protein-free diet. It is most probable, as pointed out by Foster and Srere (30) in their study of rat liver ATP-citrate lyase, that the amount of activity of either ATP-citrate lyase or NADP-malate dehydrogenase does not limit the rate of fatty acid synthesis in adipose

tissue but rather that the activity of these enzymes in the cell is regulated by the concentration of substrates, activators, and other factors, as yet poorly understood.

The metabolism of pyruvate and lactate by adipose tissue provides a vaulable tool for the study of the control of fatty acid synthesis in this tissue. Since pyruvate enters the metabolic sequence at a point beyond the pentose pathway, the NADPH required to support fatty acid synthesis must be generated by reactions catalyzed by enzymes such as NADP-malate dehydrogenase of NADP-isocitric dehydrogenase. The rapid conversion of pyruvate to fatty acids by adipose tissue noted in the present study underlines the importance of pyruvate carboxylase in fatty acid synthesis in this tissue. A pathway for pyruvate conversion to fatty acids which would provide both acetyl CoA in the cytosol and the necessary NADPH to support the reductive synthesis of fatty acid has been suggested by Kneer and Ball (31). This pathway would involve the carboxylation of some fraction of the pyruvate to oxaloacetate in the mitochondria followed by the reduction of the oxaloacetate to malate with the subsequent diffusion of malate into the cytosol. Malate conversion to pyruvate via NADP-malate dehydrogenase would provide NADPH. This pathway, which might be termed the "malate cycle" would thus be a sequence for transhydrogenation of NADH formed in the mitochondria from pyruvate dehydrogenase to NADPH in the cytosol. Such a sequence is consistent with the observed activity of pyruvate carboxylase in adipose tissue mitochondria (18) and has been supported by Rognstad and Katz (32) using 2,4-dinitrophenol with epididymal fat pad segments. The latter workers suggested that the limiting step in pyruvate conversion to fatty acids may be the mitochondrial carboxylation of pyruvate to oxaloacetate.

Since lactate is more reduced than pyruvate, its entry into the metabolic pathway via lactic dehydrogenase should provide both carbon for fatty acid synthesis and cytosolic NADH. Thus, lactate should be an excellent precursor for fatty acid synthesis, provided the conditions for its oxidation to pyruvate are optimal. Our finding that rat adipose tissue from fasted-refed animals, but not fasted animals, can convert appreciable lactate to fatty acids is in agreement with a recent report by Schmidt and Katz (33). They suggested that during fasting the generation of NADH from lactate in the cytosol would greatly exceed the rate of NADH utilization for fatty acid synthesis and thereby limit lactate oxidation. Refeeding animals a diet high in carbohydrate will enhance fatty acid synthesis and stimulate lactate utilization by adipose tissue. In the present study we have shown that the addition of glucose and insulin to the incubation medium will also markedly enhance the incorporation of lactate carbon into fatty acids in adipose tissue from rats fasted and fasted-refed a protein-free diet. This is in accord with the findings of Schmidt and Katz (33), who attribute enhanced incorporation of ^{14}C of lactate into fatty acids in adipose tissue from fasted animals, to a pyruvate-lactate exchange. However, in our experiments with adipose tissue from rats fasted and refed the protein-free diet the over-all rate of fatty acid synthesis from 25 mM glucose was only 2.4 ± 0.6 μmoles/g tissue per 3 hr. This figure is far below the 21.7 ± 4.1 obtained with 25 mM lactate plus 25 mM glucose and insulin noted in Table 4 and suggests that glucose has a stimulatory effect on lactate utilization in excess of its own rate of conversion to fatty acids.

The activity of P-enolpyruvate carboxykinase has been shown to be markedly enhanced in rat adipose tissue by fasting (13). This increase in activity can be rapidly reduced by refeeding the animals a balanced diet for 24 hr. We have attributed the increase in P-enolpyruvate carboxykinase during fasting to its role in the dicarboxylic acid shuttle in adipose tissue (13, 34). This sequence results in an increase in glycerol 3-phosphate formation from compounds such as pyruvate, lactate, alanine, and aspartate and may support the normal turnover of triglycerides that takes place in adipose tissue (35–37). Shrago, Young, and Lardy (38) have reported that the depression in liver P-enolpyruvate carboxykinase after carbohydrate ingestion is initiated by the metabolism of carbohydrate in some extrahepatic site. They also noted an increase in the activity of this enzyme in liver if fasted rats were refed alanine by stomach tube (4). As shown in Table 4, refeeding animals fasted for 48 hr a diet free of protein increases P-enolpyruvate carboxykinase activity in adipose tissue. This effect is not due to an inhibition, by the lack of dietary protein, of a specific enzyme which degrades P-enolpyruvate carboxykinase, since actinomycin D injected after fasting reduced the activity of the enzyme to below that found in adipose tissue from fed rats. Our results do agree with those of Shrago, Young, and Lardy (4, 38) in that adipose tissue P-enolpyruvate carboxykinase activity is probably not regulated by the availablity of glucose. It is possible, however, that the absence of protein in the diet alters the synthesis of insulin thereby causing an increase in the activity of this enzyme, similar to that noted in liver (39) and adipose tissue (unpublished observations of Reshef, Ballard, and Hanson) of diabetic animals.

The role of dietary protein in the control of fatty acid synthesis in adipose tissue is not clear. Refeeding diets without protein but with a high content of carbohydrate may limit the de novo synthesis of enzyme protein required for the normal adaptive hyperlipogenesis characteristic of refeeding. As discussed previously, this does not seem to limit the rate of fatty acid synthesis from pyruvate at high substrate concentrations but could inhibit some step between glucose entry into the adipose tissue

cell and its conversion to pyruvate. However, carbon flow over the citrate cleavage pathway in adipose tissue, as measured by the incorporation of the C-3 of aspartate into fatty acids, is reduced by refeeding fasted animals the protein-free diet. It appears that refeeding animals diets high in carbohydrate is not, it itself, capable of inducing high rates of fatty acid synthesis in adipose tissue. In almost every parameter measured, adipose tissue from animals fasted and refed the protein-free diet was metabolically similar to tissue from fasted rats. Adipose tissue from the rat thus differs markedly from liver in the response of fatty acid synthesis to a lack of dietary protein.

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