Adaptive changes in enzyme activity and metabolic pathways in adipose tissue from meal-fed rats

GILBERT A. LEVEILLE and RICHARD W. HANSON

U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado

ABSTRACT A number of metabolic factors and the activity of a number of enzymes were determined in meal-fed (animals fed a single daily 2 hr meal) and nibbling (ad libitum-fed) rats. The dependency of the observed adaptive changes on the ingestion of carbohydrate was studied by feeding diets high in carbohydrate or fat.

Glucose-6-phosphate dehydrogenase and NADP-malic dehydrogenase were more active in adipose tissue from high carbohydrate meal-fed rats than in tissue from ad libitum-fed rats. The activity in adipose tissue of isocitric dehydrogenase, 6-phosphogluconate dehydrogenase, and NAD-malic dehydrogenase did not increase significantly in response to meal-feeding the high carbohydrate diet. No increase in lipogenesis or enzyme activity could be demonstrated in adipose tissue from rats meal-fed a high fat diet. Lipase activity of adipose tissue was increased by high carbohydrate meal-feeding and decreased by feeding a high fat diet. The in vitro uptake of palmitate-1-^{14}C by adipose tissue was depressed by a high fat diet and enhanced in rats meal-fed a high carbohydrate diet. Diaphragm or slices of liver from high fat-fed rats oxidized palmitate-1-^{14}C more rapidly than did tissue from ad libitum-fed animals. Evidence is presented for the quantitative importance of citrate as a source of extramitochondrial acetyl CoA in adipose tissue of meal-eating and ad libitum-fed rats. The relationship of extramitochondrially formed citrate to the NAD-malic dehydrogenase–malic enzyme system in adipose tissue is discussed.

KEY WORDS lipogenesis · enzymes · activity · adaptations · meal-feeding · nibbling · high fat · high carbohydrate · diet · rat · citrate · pathways · adipose tissue · metabolism · lipase activity · palmitate utilization

Fatty acid synthesis from acetate has been shown to be enhanced in liver and adipose tissue of rats fed a single daily meal as compared to animals fed ad lib. (1–4). Recently, we demonstrated that the oxidation of glucose to CO_2 and the incorporation of glucose into fatty acids, glyceride-glycerol, glycogen, and nonsaponifiable lipids by adipose tissue are increased by meal-feeding (5). Adipose tissue from meal-fed rats also had higher NAD-NADP levels.[1] That report, as well as a number of others showing increased enzyme activities as a consequence of meal-feeding (4, 6), suggests an enhancement of lipogenesis induced by the adaptation of key enzymes. Conceivably, these metabolic alterations are dependent upon carbohydrate in a manner analogous to the induction of urea cycle enzymes in rat liver by protein catabolism (7). In this study, we have attempted to measure the relative effect of diets high in lipid or in carbohydrate on the activity of enzymes involved in lipogenesis.

Because of the rapid rate of lipid synthesis in adipose tissue from meal-eating rats, such animals are well suited for the study of specific pathways of fatty acid formation. Recent reports (8, 9) have stressed the importance of mitochondrial permeability as a factor influencing the reaction sequence involved in lipogenesis from acetate. The data presented herein favorably support the contention that citrate and the citrate cleavage enzyme are of significance in the supply of acetate for extramitochondrial fatty acid synthesis. This work also lends support to the recent suggestion of Young, Shrago, and Lardy (10) that NAD-malic dehydrogenase and NADP-malic dehydrogenase play important roles in the formation of NADPH for reductive synthesis.

[1] *Convention for coenzyme nomenclature.* NAD^+ and $NADP^+$ refer to the oxidized, NADH and NADPH to the reduced forms of the dinucleotide coenzymes. NAD and NADP are used for the coenzymes in general, without reference to their state of oxidation.

Downloaded from www.jlr.org by guest, on June 19, 2012

MATERIALS AND METHODS

Treatment of Animals

Male Holtzman rats weighing 180–250 g were used for all studies. The animals were fed Purina rat chow or the following synthetic diets: a high fat diet containing 30% casein, 57.5% hydrogenated fat, 6% mineral mix (USP XIV), 5% non-nutritive fiber, 1% vitamin mix,[2] and 0.5% L-cystine; or a normal high carbohydrate diet (hereafter referred to as a "high carbohydrate" diet) containing 18% casein, 33% glucose, 34% cornstarch, 4% mineral mix (USP XIV), 5% non-nutritive fiber, 5% corn oil, 0.5% vitamin mix,[2] and 0.5% L-cystine. The animals were fed ad libitum (nibblers) or were allowed access to food from 8:00 to 10:00 AM (meal eaters). They were maintained on these feeding regimes for at least 3 weeks prior to sacrifice. Food consumption and body weight were determined weekly. Initially, the meal-fed rats consumed less food and gained less weight than did the ad libitum-fed animals. However, following an initial 4–5 day adjustment to the feeding regimen, the rate of weight gain was similar for both groups of rats (5).

Materials

Uniformly labeled glucose-^{14}C, citrate-1,5-^{14}C, and palmitate-1-^{14}C (Nuclear-Chicago Corp., Des Plaines, Ill.), acetate-1-^{14}C and DL-glutamate-5-^{14}C (New England Nuclear Corp., Boston, Mass.), DL-aspartate-4-^{14}C, DL-glutamate-3,4-^{14}C, and DL-glutamate-2-^{14}C (California Corp. for Biochemical Research, Los Angeles, Calif.), DL-aspartate-3-^{14}C and DL-glutamate-1-^{14}C (Volk Radiochemical Co., Chicago, Ill.), glucose (Mann Research Lab., New York City) and glucagon-free porcine insulin (gift of Dr. W. Bromer, Eli Lilly & Co., Indianapolis, Ind.) were used for the metabolic studies. For the determination of enzyme activity, the following compounds were used: NAD^+, NADH, NADPH, Coenzyme A, ATP (all Sigma Chemical Co., St. Louis, Mo., highest purity); sodium DL-β-hydroxybutyrate, isocitrate, oxaloacetate, DL-aspartate, DL-glutamate, potassium citrate, glycylglycine (California Corp. for Biochemical Research, Los Angeles, Calif.); L-malate, glucose-6-phosphate, 6-phosphogluconate, and pig heart malic dehydrogenase (Sigma Chemical Co., St. Louis, Mo.).

Metabolic Studies

All animals were fasted for 22 hr and re-fed for 2 hr prior to sacrifice. The rats were stunned with a sharp blow to the head and decapitated, and the tissues to be used in the metabolic studies and for enzyme assays were quickly removed. Pieces of epididymal fat pads weighing approximately 100 mg were weighed on a torsion balance and incubated as indicated below. The left lateral lobe of the liver was sliced with a Stadie-Riggs microtome and the slices were weighed. The slices were transferred to 25-ml Erlenmeyer flasks, constructed with 1.5 × 3 cm glass center wells, containing 3.0 ml of Krebs-Ringer phosphate buffer (12), pH 7.4, with the appropriate substrates. The center well held a 2 × 2 cm piece of Whatman No. 1 filter paper saturated with 0.1 ml of 25% KOH. Incubation was carried out in air at 38° in a metabolic shaker at 90 strokes/min. At the end of the incubation period, 0.5 ml of 1 N H_2SO_4 was added to the main compartment of the flask to insure the complete liberation of CO_2 from the medium.

After the incubation, $^{14}CO_2$, fatty acid-^{14}C, glyceride-glycerol-^{14}C, nonsaponifiable lipid-^{14}C, and glycogen-^{14}C were determined as outlined in a previous report (13). Palmitate-1-^{14}C incorporation into triglycerides was measured as described previously (13). Samples, with the exception of glycogen, were prepared for the determination of radioactivity by dissolving in 10 ml of toluene containing, per liter, 4 g of 2,5-diphenyloxazole (PPO), 0.015 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene, and 230 ml of absolute ethanol. Glycogen was dissolved in 10 ml of Bray's scintillation medium (15). Radioactivity was measured at 25° in a liquid scintillation spectrometer designed for use at ambient temperature (Nuclear-Chicago Model 722). Free fatty acid (FFA) release was measured by the method of Dole and Meinertz (14).

Enzyme Studies

Epididymal fat pads were homogenized in 0.15 M KCl with a Virtis homogenizer and centrifuged for 15 min at 1,000 × *g* at 5°. The homogenate separated into an upper lipid cake, a middle liquid layer containing the soluble cell components, and a sediment consisting of fibrous stroma and cell debris. The middle layer was removed by puncturing the bottom of the plastic centrifuge tube with a hypodermic needle. When enzyme assays were performed on a "mitochondria-free" supernatant fraction, the middle layer of the homogenate was recentrifuged at 22,000 × *g* for 30 min to sediment the mitochondria.

In experiments designed to distinguish between extra- and intramitochondrial activity of NAD-malic dehydrogenase and the citrate cleavage enzyme, the following methods of homogenization were used to minimize mitochondrial destruction. The epididymal fat pads from three animals were incubated in phosphate buffer (0.15 M) pH 7.4, containing collagenase (gift of Dr. M. Tanzer) as described by Rodbell (16). The fat cells were collected by centrifugation, washed twice with 0.25 M sucrose, and ruptured by shaking on a Vortex Jr. oscillating shaker in tubes containing glass beads. The soluble cell components were separated by centrifugation at

[2] For composition, *see* Leveille, Sauberlich, and Shockley (11).

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 1 In Vitro Utilization of Glucose-^{14}C and Acetate-^{14}C by Adipose Tissue from Meal-Eating or Nibbling Rats Fed a High Fat or a High Carbohydrate Diet

Substrate and Conversion Product	Dietary Treatment					
	High Carbohydrate			High Fat		
	Meal Eaters	Nibblers	P	Meal Eaters	Nibblers	P
	mμmoles converted per 100 mg of tissue in 3 hr					
Glucose-U-^{14}C						
Carbon dioxide	603 ± 469* (39.8)†	145 ± 49 (43.6)	<0.05‡	81 ± 12 (41.0)	88 ± 22 (38.2)	N.S.
Fatty acids	581 ± 380 (38.4)	52 ± 43 (15.6)	<0.05	5.0 ± 2.9 (2.5)	2.3 ± 1.0 (1.0)	N.S.
Glyceride-glycerol	268 ± 63 (17.7)	128 ± 42 (38.5)	<0.01	108 ± 36 (54.6)	137 ± 36 (59.5)	N.S.
Nonsaponifiable lipids	3.8 ± 2.9 (0.25)	0.4 ± 0.2 (0.12)	<0.05	0.7 ± 1.1 (0.35)	0.7 ± 1.1 (0.3)	N.S.
Glycogen	57.4 ± 23.2 (3.8)	7.1 ± 3.9 (2.1)	<0.005	3.0 ± 0.8 (1.5)	2.4 ± 1.1 (1.0)	N.S.
Acetate-1-^{14}C						
Fatty acids	697 ± 413	155 ± 78	<0.025	28.5 ± 13.4	12.6 ± 4.5	<0.05
Nonsaponifiable lipids	4.4 ± 3.7	1.2 ± 0.6	N.S.	0.6 ± 0.3	0.3 ± 0.1	N.S.

Approximately 100 mg of adipose tissue was incubated in phosphate buffer (pH 7.4) for 3 hr. Glucose utilization: tissue incubated in 3.0 ml of buffer containing 30 μmoles of D-glucose, 0.5 μc of glucose-U-^{14}C, and 0.3 unit of insulin in air. Acetate utilization: tissue incubated in 5.0 ml buffer containing 28 μmoles of glucose, 0.5 unit of insulin, 10 μmoles of sodium acetate, and 0.5 μc of acetate-1-^{14}C in an atmosphere of oxygen.

* Mean ± SD for six animals.

† Values in parentheses represent percentage distribution of ^{14}C in the various metabolites.

‡ Probability of differences being significant; N.S. = not significant.

1,000 × *g* for 20 min at 5° and the mitochondria sedimented by recentrifugation at 22,000 × *g* for 30 min. The supernatant fraction and the mitochondria were assayed separately for enzyme activity.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were measured separately by the method of Horecker and Smyrniotis (18). NADP-malic dehydrogenase (EC 1.1.1.40), NAD-malic dehydrogenase (EC 1.1.1.37), and isocitric dehydrogenase (EC 1.1.1.42) were assayed as described by Ochoa (19), and citrate cleavage enzyme as outlined by Srere (20). β-Hydroxybutyrate dehydrogenase (EC 1.1.1.30) activity in liver was measured in sonicated mitochondria by the method of Lehninger, Sudduth, and Wise (17). Lipase activity was determined at pH 8.5 and 6.8 as described by Björntorp and Furman (21) using as a substrate the 50% coconut oil emulsion, Ediol (gift of Riker Laboratories, Inc., Northridge, Calif.). Tissue lipase activity was determined on homogenates of adipose tissue prepared as described above for the assay of the enzymes. Lipase activity is expressed as micromoles of fatty acid produced per gram of tissue per hour. All other enzyme activities are expressed as units per milligram of N, where a unit is that amount of enzyme which will catalyze the transformation of 1 micromole of substrate per min at 30°. A Beckman DK2A recording spectrophotometer equipped with a constant temperature cell holder was used for all enzyme assays with the exception of lipase. Nitrogen was determined by micro-Kjeldahl digestion followed by nesslerization (22).

Differences between groups were tested for statistical significance using the "*t*" test.

RESULTS AND DISCUSSION

There is considerable evidence demonstrating alterations in enzyme activity in response to changes in diet (23–26), re-feeding after a 48 hr fast (6), and meal-feeding (4). Fitch and Chaikoff have reported the effect of diets high in glucose or fructose on the levels of 17 enzymes in rat liver (23). They conclude that the level of an enzyme's activity is related to the activity of the metabolic pathway in which it participates and that any change in the level of activity reflects alterations in the usage of that pathway.

Recent work in this laboratory (5) demonstrated an increased lipogenesis from glucose and acetate by adipose tissue as a result of meal-feeding. The adaptive response appeared to be the result of the relatively large inflow of carbohydrate over the short, 2-hr daily feeding period. An increased activity of enzymes in pathways supporting lipogenesis in adipose tissue was therefore expected. The extent to which the specific substrate influenced the alteration of enzyme activity was also tested by feeding diets high in fat or high in carbohydrate (Tables 1 and 2). Glucose-U-^{14}C utilization and lipogenesis from acetate-^{14}C by adipose tissue were increased markedly as a result of meal-feeding a diet high in carbohydrate, whereas these processes were depressed sharply by the feeding of high fat diets. Of the five adipose tissue enzymes studied, only glucose-6-phosphate dehydrogenase and NADP-

Downloaded from www.jlr.org by guest, on June 19, 2012

malic dehydrogenase (malic enzyme) were increased significantly by meal-feeding a high carbohydrate diet. Enzyme activity was depressed by the high fat diet and no differences were noted between ad libitum- and meal-fed rats.

Tepperman and Tepperman (6) have reported a parallel increase in malic enzyme and glucose-6-phosphate dehydrogenase levels in rat livers rapidly synthesizing fatty acid. The activity of isocitric dehydrogenase was not affected by meal-feeding either of the diets (Table 2). This enzyme has been studied in liver and adipose tissue by Young et al. (10) and in liver by Fitch and Chaikoff (23), and its activity was found to be unchanged under conditions favoring lipogenesis. NAD-malic dehydrogenase activity was not increased significantly by meal-feeding, but the level of activity was far higher than that of other enzymes studied.

The increased activity of glucose-6-phosphate dehydrogenase might stimulate lipogenesis in adipose tissue by increasing the level of NADPH for reductive synthesis of fatty acids. However, Flatt and Ball (27) have calculated that NADPH generation via the hexose monophosphate pathway in adipose tissue cannot totally support lipogenesis. The concerted action of NAD-malic dehydrogenase and malic enzyme could supply additional NADPH. The adaptability of malic enzyme, coupled with the extremely high levels of NAD-malic dehydrogenase in adipose tissue, strongly supports the suggestion of Young et al. (10) that transhydrogenation of NADH to NADP via the two malic dehydrogenase enzymes is an important source of NADPH.

The relative amounts of glucose-U^{14}C oxidized to CO_2 and incorporated into glyceride-glycerol, fatty acid, nonsaponifiable lipid, and glycogen were increased markedly by meal-feeding a high carbohydrate diet (Table 1). Meal-feeding the high fat diet did not stimulate the conversion of glucose to any of these intermediates, but severely depressed the total glucose utilized. These findings agree with the enzyme data (Table 2) showing a depression of enzyme activity (malic enzyme and glucose-6-phosphate dehydrogenase) in tissues of meal eaters consuming the high fat diet.

The effects of meal-feeding and dietary composition on the pattern of glucose utilization by adipose tissue are of interest. The percentage of total glucose incorporated into fatty acids was greater in high carbohydrate meal-fed rats, but lower in nibbling rats, while the percentage converted to glyceride-glycerol was greater in the nibblers. This shift is similar to that noted by Cahill, Leboeuf, and Renold in response to insulin (28). In the high fat-fed animals, there was a greater percentage conversion of glucose to glyceride-glycerol than in the high carbohydrate-fed rats. This is interpreted to mean that the small amounts of glucose available in the high fat diet are shunted into the production of α-glycerophosphate required for triglyceride formation. In animals consuming a carbohydrate-free diet, the rate of α-glycerophosphate synthesis may limit triglyceride formation since, in adipose tissue, the glycerol formed from lipolysis is not reutilized for glyceride synthesis (29).

The differences in glucose conversion to α-glycerophosphate induced by meal-feeding and variations in dietary composition are closely reflected in the uptake of palmitate-1-^{14}C by adipose tissue (Table 3). Uptake was greater in high carbohydrate-fed meal eaters and was depressed significantly by feeding the high fat diet; this suggests in the latter case a lowered triglyceride synthesis. We are presently measuring the activity of α-glycerophosphate dehydrogenase in adipose tissue to determine if changes in fatty acid uptake and (or) α-glycerophosphate formation from glucose are related to the activity of this enzyme.

TABLE 2 Activity of Various Enzymes in Adipose Tissue of Meal-Eating or Nibbling Rats Fed a High Carbohydrate or a High Fat Diet

	Nibblers	Meal Eaters	Per Cent Change	P
	Units/mg of tissue N			
High carbohydrate diet				
Glucose-6-phosphate dehydrogenase	0.255 ± 0.110*	0.812 ± 0.293	+218	<0.005
NADP-malic dehydrogenase	0.235 ± 0.101	1.244 ± 0.354	+409	<0.001
NAD-malic dehydrogenase	13.21 ± 8.25	19.28 ± 9.42	+ 46	N.S.
Isocitric dehydrogenase	0.268 ± 0.096	0.353 ± 0.072	+ 32	N.S.
6-Phosphogluconate dehydrogenase	0.146 ± 0.049	0.241 ± 0.062	+ 65	N.S.
High fat diet				
Glucose-6-phosphate dehydrogenase	0.185 ± 0.043	0.173 ± 0.045	− 6	N.S.
NADP-malic dehydrogenase	0.065 ± 0.006	0.062 ± 0.012	− 5	N.S.
NAD-malic dehydrogenase	12.11 ± 5.36	12.78 ± 4.92	+ 6	N.S.
Isocitric dehydrogenase	0.287 ± 0.013	0.319 ± 0.031	+ 11	N.S.
6-Phosphogluconate dehydrogenase	0.162 ± 0.030	0.170 ± 0.022	+ 5	N.S.

* Mean for three rats ± SD.

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH

TABLE 3 Palmitate-1-^{14}C Uptake by Adipose Tissue and Oxidation by Diaphragm Muscle and Liver Slices from Meal-Eating or Nibbling Rats Fed a High Fat or a High Carbohydrate Diet

	Meal Eaters	Nibblers	*P*
	mμmoles converted/100 mg tissue in 3 hr		
High carbohydrate diet			
*Palmitate-1-^{14}C uptake**			
Adipose tissue	460 ± 116†	235 ± 26	<0.01
Palmitate-1-^{14}C oxidation‡			
Diaphragm	9.1 ± 2.4	10.5 ± 2.1	N.S.
Liver slices	3.0 ± 0.5	4.7 ± 1.3	<0.01
High fat diet			
Palmitate-1-^{14}C uptake			
Adipose tissue	195 ± 31	154 ± 30	<0.05
Palmitate-1-^{14}C oxidation			
Diaphragm	15.4 ± 4.0	17.1 ± 4.2	N.S.
Liver slices	11.2 ± 2.5	7.8 ± 1.2	<0.02

Tissues weighing approximately 100 mg were incubated in 3.0 ml of phosphate buffer (pH 7.4) containing 3% bovine serum albumin (Fraction V), 6.0 μmoles of palmitic acid, and 0.3 μc of palmitic acid-1-^{14}C at 38° in air.

* Into glycerides.

† Mean for eight rats ± SD.

‡ To $^{14}CO_2$.

The oxidation of palmitate-1-^{14}C by diaphragm was not changed by meal-feeding, while liver slices from meal-fed rats oxidized significantly greater amounts of the fatty acid (Table 3). Oxidation of palmitate-1-^{14}C to $^{14}CO_2$ by diaphragm was also enhanced by ingestion of the high fat diet. In a recent study (5), we were unable to demonstrate any statistically significant difference in the ability of diaphragm or liver to oxidize fatty acids as a consequence of meal-feeding; however, the differences noted were in the same direction as observed in this study. The findings of the present study are in line with the report of Eaton and Steinberg (30), which demonstrates that muscle has the capacity to oxidize large quantities of fatty acids. The increase in fatty acid oxidation by liver slices of high fat-fed rats may indicate an adaptation of one or more enzymes involved in the utilization of this substrate (31). Results similar to those observed in this study have recently been reported by Petrasek, Fabry, and Poledne (32).

The lipase activities studied include that of the enzyme which is active at pH 8.5 and which is released from adipose tissue when incubated with heparin. This enzyme is identical with that studied by Cherkes and Gordon (33) and Björntorp and Furman (21) and is generally considered to be the lipoprotein lipase described by Korn (34). The lipase active at pH 6.8 is similar to the epinephrine-sensitive enzyme studied by Rizack (35) and Björntorp and Furman (21). Although controversy exists concerning the significance of these enzymes, lipoprotein lipase is generally thought to hydrolyze glycerides prior to their uptake into adipose tissue, while the pH 6.8 enzyme is active in FFA release from adipose tissue. An increase in the pH 6.8 enzyme activity in the adipose tissue of meal-fed rats was expected since FFA release would provide the principal energy source during the period between meals. In animals meal-fed a high fat diet, and consequently depositing large quantities of preformed lipid, the activity of lipoprotein lipase might also be enhanced. Meal-eating did increase the activity of both lipases and the release of lipoprotein lipase by heparin (Table 4). However, feeding a high fat diet depressed the activity of both the pH 8.5 and 6.8 enzymes. The significance of these data is not clear particularly the lowered activity observed as a consequence of feeding the high fat diet. However, lipoprotein lipase may not be essential for the uptake of preformed lipid (36) or this enzyme may be present in excess and therefore may not be adaptive. Further study is necessary to elucidate the meaning of these results.

There has been considerable interest in the role of mitochondrial permeability as a factor in the control of lipogenesis. The synthesis of fatty acid from carbohydrate involves the extramitochondrial formation of pyruvate which would then be converted to acetyl CoA by oxidative decarboxylation within the mitochondria. Since fatty acid synthesis is thought to occur in the cell cytoplasm (37), acetyl CoA must be transferred out of the mitochondria. Spencer and Lowenstein (38) have reported that diffusion of acetyl CoA out of the mitochondria is too slow to meet the demands for rapid lipogenesis. Other possible mechanisms for circumventing the problem of mitochondrial impermeability would involve the cleavage of mitochondrial acetyl CoA to acetate and CoASH and the diffusion of the acetate into the cytoplasm to be reactivated to acetyl CoA by an acetate-activating enzyme (38).

There is considerable experimental evidence for a third pathway, involving the intramitochondrial formation of citrate. The citrate would leave the mitochondria and be cleaved to acetyl CoA and oxaloacetate by citrate cleavage enzyme (39). Recently, Young et al. (10) have presented evidence suggesting a conversion of the oxaloacetate formed by citrate cleavage to pyruvate. This would couple the NAD- and NADP-malic dehydrogenases extramitochondrially, with a resultant increase in cytoplasmic NADPH. The pyruvate formed would then enter the mitochondria for decarboxylation to acetyl CoA. This pathway would require cytoplasmic NAD-malic dehydrogenase and malic enzyme as well as citrate cleavage enzyme. Table 5 shows the activity and distribution of NAD-malic dehydrogenase and malic en-

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 4 Lipase Release, Tissue Lipase Activity, and FFA Release by Adipose Tissue of Meal-Eating or Nibbling Rats Fed a High Carbohydrate or High Fat Diet

	Dietary Treatment					
	High Carbohydrate			High Fat		
	Meal Eaters	Nibblers	*P*	Meal Eaters	Nibblers	*P*
	μmoles fatty acid produced/g tissue/hr					
Lipase release—pH 8.5	5.2 ± 3.6 (6)*	1.8 ± 0.9 (6)	N.S.	1.7 ± 1.1 (6)	1.0 ± 0.3 (6)	N.S.
Tissue lipase—pH 8.5	16.2 ± 9.0 (6)	5.7 ± 1.5 (6)	<0.05	5.1 ± 0.6 (6)	3.2 ± 1.1 (6)	<0.025
Tissue lipase—pH 6.8	11.4 ± 5.9 (4)	7.1 ± 4.5 (4)	N.S.	6.9 ± 2.1 (4)	3.0 ± 1.7 (4)	N.S.
	μmoles/100 mg tissue/hr					
Free fatty acid release	1.26 ± 0.76 (2)	1.55 ± 0.61 (2)	N.S.	1.19 ± 0.18 (3)	1.33 ± 0.20 (3)	N.S.

* Mean ±SD for number of animals shown in parentheses.

zyme in tissues of rats fed a high carbohydrate diet. The levels of activity of both enzymes were much higher in the cytoplasmic fraction of adipose tissue cells. Meal-feeding a high carbohydrate diet greatly enhanced the levels of citrate cleavage enzyme. In addition, the greatest activity of NAD-malic dehydrogenase occurred in the soluble fraction of the fat cell. Malic enzyme has been demonstrated to be an extramitochondrial enzyme (10).

The level of enzyme activities and their distribution within the cell favor a pathway (Fig. 1) of the type originally suggested by Young et al. (10) which would, in turn, be stimulated by meal-feeding. The activity of this pathway and its significance in the adaptive response to meal-eating were studied by using specifically labeled substrates. The present studies demonstrate citrate incorporation into fatty acid by isolated epididymal fat pads and a reduction of this incorporation by the addition of unlabeled acetate (Fig. 2). Madsen, Abraham, and Chaikoff (40, 41), and D'Adamado and Haft (42) have shown the synthesis of fatty acid from α-ketoglutarate via a "reversal" of the Krebs cycle to citrate. In the present paper, the pathway of lipogenesis was investigated by using specifically labeled glutamate-^{14}C and aspartate-^{14}C. The former compound would enter the Krebs cycle as α-ketoglutarate and the latter as oxaloacetate. The metabolic fate of these two intermediates in adipose tissue from animals fed the high carbohydrate diet is shown in Fig. 3, while the actual incorporation data are shown in Tables 6 and 7.

TABLE 5 Enzyme Activity in Subcellular Fractions of Adipose Tissue and Liver of Rats Fed the High Carbohydrate Diet

Enzyme	Mitochondrial Fraction: Meal Eaters	Mitochondrial Fraction: Nibblers	Supernatant Fraction*: Meal Eaters	Supernatant Fraction*: Nibblers
Citrate cleavage enzyme (adipose tissue)	—	—	0.206	0.019
β-Hydroxybutyrate dehydrogenase† (liver)	0.543	0.235	—‡	—
NAD-malic dehydrogenase (adipose tissue)	—	0.950	—	9.705

* 22,000 × *g* for 30 min.
† Mitochondria were isolated and sonicated as described by Lehninger et al. (17).
‡ No appreciable activity could be demonstrated.
Each value is the mean for two determinations of enzyme activity.

Values for the incorporation of C-1, C-2, and C-5 of glutamate-^{14}C into fatty acids by adipose tissue are in accord with the reports of Madsen et al. (40, 41) and with the proposed pathway shown in Fig. 3. These data demonstrate a reversal of the glutamate-derived α-ketoglutarate to citrate and, subsequently, to acetyl CoA for fatty acid synthesis. This pathway is significantly more active in adipose tissue from meal-eating than from nibbling animals. The pattern of lipogenesis from C-3 and C-4 of aspartate-^{14}C also fits the pathway proposed. Aspartate-4-^{14}C is oxidized to $^{14}CO_2$, but virtually none of the activity is incorporated into lipid. The higher rate of incorporation of aspartate-3-^{14}C into fatty acids as compared to the C-2 or C-5 of glutamate-^{14}C suggests that the intramitochondrially formed citrate is a much more significant precursor of cytoplasmic acetyl CoA than is that formed from a "reversal" of α-ketoglutarate in the cytoplasm.

This suggestion was more fully studied by measuring lipogenesis from glutamate labeled in the C-2, C-5, or C-3 plus C-4 positions. The C-2 and C-5 or glutamate-^{14}C would be incorporated into fatty acid only by a "reversal" of α-ketoglutarate, while glutamate-3,4-^{14}C would be incorporated by both forward and backward reactions (Fig. 3). Therefore, the difference between the incorporation of C-2 and C-5 and the C-3 plus C-4 of glutamate would be a measure of the forward activity of the Krebs cycle. The results of such an experiment (Table 7) indicate a greater activity of the forward reaction of α-ketoglutarate, accounting for approximately 80% of

Downloaded from www.jlr.org by guest, on June 19, 2012

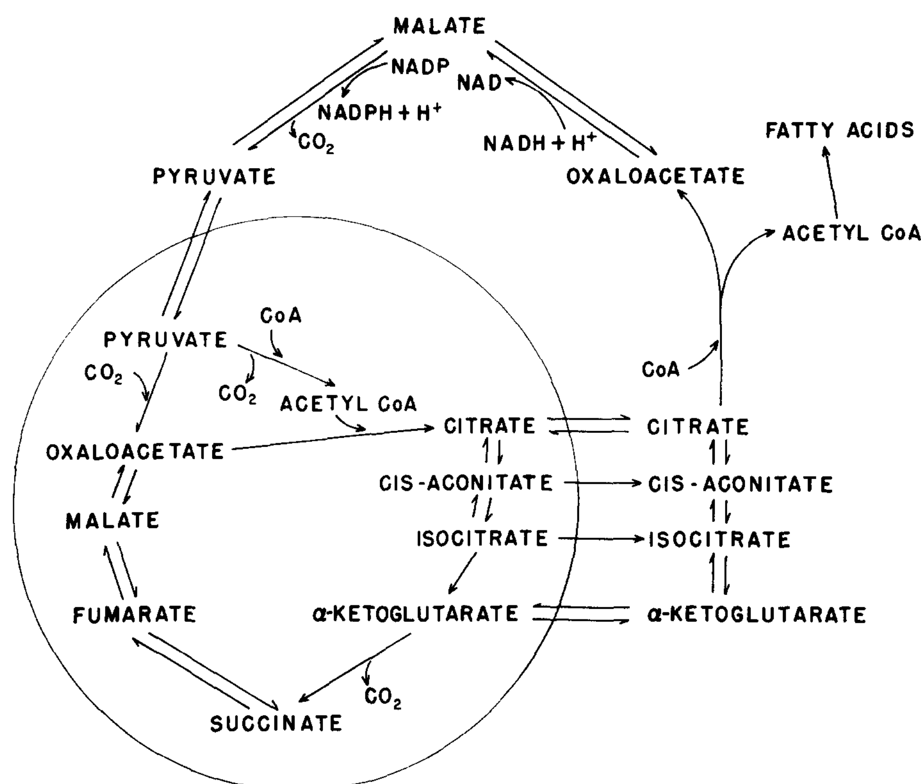

FIG. 1. Proposed pathway for the synthesis of fatty acids. Circle represents the mitochondrial membrane; pathways within the circle represent intramitochondrial metabolism as compared to extramitochondrial pathways outside the circle [after Young et al. (10)].

the total glutamate-^{14}C found in fatty acids. The magnitude of both pathways was increased by meal-feeding.

The incorporation of glutamate carbon into lipid from the forward reaction was assumed to be mediated through citrate cleavage, although our data on this point are equivocal. However, the citrate cleavage enzyme is both active and adaptive in adipose tissue and citrate-1,5-^{14}C and glutamate-2-^{14}C and -5-^{14}C are incorporated into fatty acid (Tables 6 and 7). This is strong evidence favoring lipogenesis via citrate formation and citrate cleavage. Spencer, Corman, and Lowenstein (43) have shown recently a greater rate of lipogenesis from citrate than from acetate in a high-speed supernatant fraction (105,000 × *g*) from rat liver. These data further underline the possible importance of citrate and the citrate cleavage enzyme in the supply of acetyl CoA for lipid synthesis.

TABLE 6 INCORPORATION OF SPECIFICALLY LABELED SUBSTRATES INTO FATTY ACIDS AND OXIDATION TO $^{14}CO_2$ BY ADIPOSE TISSUE FROM MEAL-EATING OR NIBBLING RATS FED THE HIGH CARBOHYDRATE DIET

Substrate	Incorporation into Fatty Acids			Oxidation to $^{14}CO_2$		
	Meal Eaters	Nibblers	*P*	Meal Eaters	Nibblers	*P*
	mμmoles utilized/100 mg tissue in 3 hr					
Acetate-1-^{14}C	348 ± 236*	46 ± 15	<0.025	149 ± 19	200 ± 23	<0.005
Citrate-1,5-^{14}C†	104 ± 40	50 ± 14	<0.01	93 ± 10	84 ± 10	N.S.
DL-Aspartate-4-^{14}C	0.5 ± 0.3	0.5 ± 0.2	N.S.	316 + 79	210 ± 20	<0.01
DL-Aspartate-3-^{14}C	189 ± 117	20 ± 11	<0.01	48 ± 8	78 ± 8	<0.001
DL-Glutamate-1-^{14}C	0.4 ± 0.2	0.4 ± 0.1	N.S.	289 ± 87	265 ± 34	N.S.
DL-Glutamate-2-^{14}C	24 ± 22	2.4 ± 1.4	<0.05	311 ± 57	228 ± 26	<0.01
DL-Glutamate-5-^{14}C	32 ± 21	6.8 ± 3.8	<0.025	241 ± 49	227 ± 16	N.S.

Incubations were carried out as indicated in Table 1, except that buffers contained 5 μmoles of specifically labeled substrate (10 μmoles in the case of citrate) per ml of buffer, 10 μmoles of glucose, and 0.1 unit of insulin per ml, and approximately 0.5 μc of radioactivity.
* Mean for six animals ± SD.
† Incorporation into fatty acids would represent radioactivity of citrate-1-^{14}C only; consequently, values are mμmoles × 2 to correct for the fact that citrate-5-^{14}C would not be incorporated (Spencer and Lowenstein, reference 8).

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 7 INCORPORATION OF SPECIFICALLY LABELED GLUTAMIC ACID BY ADIPOSE TISSUE OF MEAL-EATING OR NIBBLING RATS FED THE HIGH CARBOHYDRATE DIET

Substrate	Incorporation into Fatty Acids			Oxidation to $^{14}CO_2$		
	Meal Eaters	Nibblers	*P*	Meal Eaters	Nibblers	*P*
	mμmoles utilized/100 mg tissue in 3 hr					
DL-Glutamate-2-^{14}C	39 ± 5.2*	4.2 ± 0.3	<0.01	213 ± 43	133 ± 18	<0.02
DL-Glutamate-5-^{14}C	43 ± 11	9.4 ± 1.9	<0.01	186 ± 15	124 ± 11	<0.01
DL-Glutamate-3,4-^{14}C	216 ± 34	43 ± 6.0	<0.01	27 ± 5.1	32 ± 1.6	N.S.

Incubation conditions and substrate levels were as indicated under Table 6.
* Mean for five experiments ± SD.

The labeling pattern observed in this study indicates that during periods of rapid lipogenesis, i.e. meal feeding, substrates are shunted into lipid synthesis at the expense of the oxidative pathways. This is well demonstrated by the inverse relationship between the relative amounts of ^{14}C found in $^{14}CO_2$ and fatty acids (Tables 1, 6, and 7).

The pathway shown in Fig. 1 was originally proposed by Young et al. (10) and is based in part on the work of Srere and Bhaduri (9), Spencer and Lowenstein (8), and Madsen et al. (40). The incorporation of specifically labeled intermediates into lipids, shown in this report, offers quantitative evidence for the importance of this pathway in lipogenesis by adipose tissue. A consideration of enzyme activities, cellular distribution of NAD- and NADP-malic dehydrogenases, and citrate cleavage enzyme, as well as the data on the incorporation of labeled substrates into lipid, strongly supports the pathway of lipogenesis shown in Fig. 1. In such a pathway, the role of citrate in the transfer of mitochondrially formed acetyl CoA and the role of NAD- and NADP-malic dehydrogenase are of prime importance. Such a pathway is self-priming in that citrate can stimulate acetyl CoA carboxylase activity (44), which is thought to be the rate-limiting enzymatic step in fatty acid formation. Thus the precursor, citrate, stimulates lipogenesis

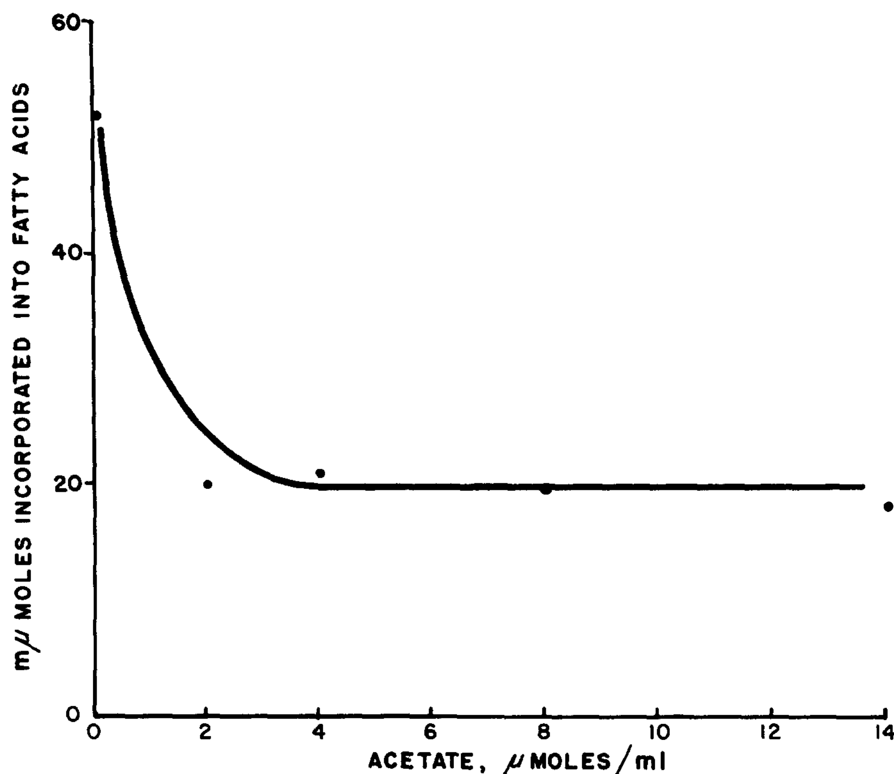

FIG. 2. Influence of acetate level on citrate incorporation. Incubations were carried out essentially as described in Table 1. All flasks contained 10 μmoles of potassium citrate, 0.5 μc of citrate-1,5-^{14}C, 10 μmoles of glucose, and 0.1 unit of insulin per ml; sodium acetate was added at the levels indicated. Each point is the average of six experiments.

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

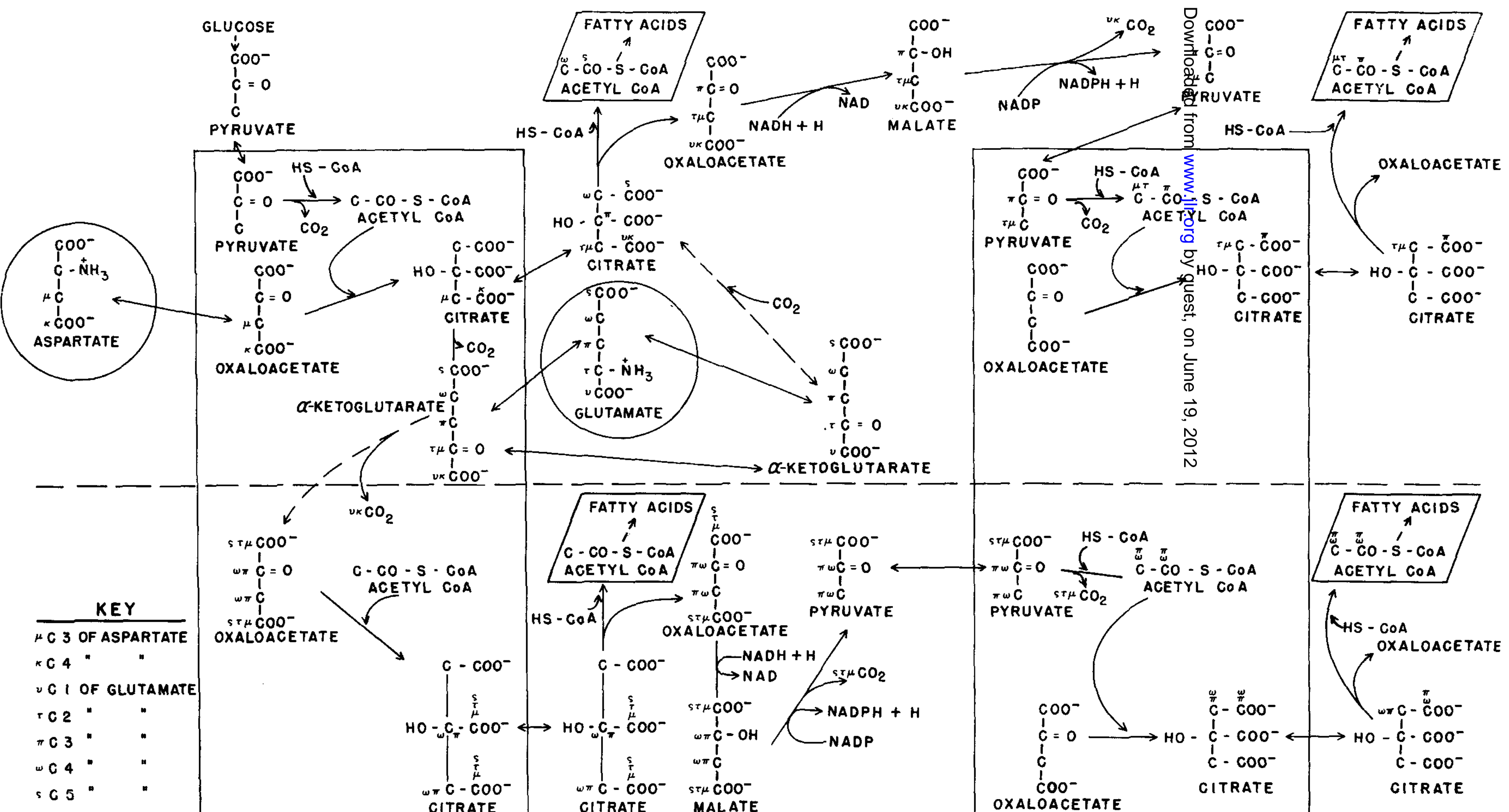

Fig. 3. Fate of specifically labeled glutamate and aspartate carbons. Pathways above the broken line show backward pathway of α-ketoglutarate and forward pathway for oxaloacetate; below the broken line the forward metabolism of α-ketoglutarate is illustrated. The broken arrows indicate the omission of intermediates. Glutamate and asparate are shown within the circles while the large rectangles represent the mitochondria. The intermediates in the pathways are represented in a partially abbreviated form.

Downloaded from www.jlr.org by guest, on June 19, 2012

by affecting a rate-limiting enzyme and the by-product of the cleavage of citrate is metabolized in a manner which provides essential reduced coenzymes.

Several other enzymes at key points in the metabolic pathways of lipid metabolism may also respond to meal-feeding. We have preliminary evidence to indicate that liver β-hydroxybutyrate dehydrogenase activity (Table 5) is increased in meal-eating rats and suggestive evidence that α-glycerophosphate dehydrogenase and acetyl CoA carboxylase activity may also be affected.

Increased activity of an enzyme may be due to an increased protein synthesis (45). Recently, however, Schimke (46) has demonstrated adaptive enhancement of urea cycle enzyme activities due to a decrease in the rate of enzyme degradation. The balance between the rate of enzyme synthesis and enzyme degradation seems, therefore, to control the over-all adaptive response of the metabolic pathway involved. The importance of enzyme synthesis and degradation in adipose tissue of meal-fed rats is presently under consideration.

We wish to thank Drs. H. E. Sauberlich and Z. Z. Ziporin for comments and suggestions during the course of the work and for critically reading the manuscript. We also express our appreciation to Messrs. K. Sirinit, W. Goad, J. Taubr, and L. Schiff for technical assistance, to Mr. G. Isaac for the statistical analysis, to Mr. B. James for care of the animals, and to Mrs. B. Cathey for assistance in the preparation of the manuscript.

The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Manuscript received 2 April 1965; accepted 13 August 1965.

References

1. Tepperman, J., and H. M. Tepperman. *Am. J. Physiol.* **193:** 55, 1958.
2. Cohn, C. *Federation Proc.* **23:** 76, 1964.
3. Stevenson, J. A., F. V. Feleki, A. Szlavko, and J. R. Beaton. *Proc. Soc. Exptl. Biol. Med.* **116:** 178, 1964.
4. Hollifield, G., and W. Parson. *J. Clin. Invest.* **41:** 245, 1962.
5. Leveille, G. A., and R. W. Hanson. *Can. J. Physiol. Pharmacol.* (in press).
6. Tepperman, H. M., and J. Tepperman. *Federation Proc.* **23:** 73, 1964.
7. Schimke, R. T. *J. Biol. Chem.* **238:** 1012, 1963.
8. Spencer, A. F., and J. M. Lowenstein. *J. Biol. Chem.* **237:** 3640, 1962.
9. Srere, P. A., and A. Bhaduri. *Biochim. Biophys. Acta* **59:** 487, 1962.
10. Young, J. W., E. Shrago, and H. A. Lardy. *Biochemistry* **3:** 1687, 1964.
11. Leveille, G. A., H. E. Sauberlich, and J. W. Shockley. *J. Nutr.* **76:** 423, 1962.
12. Fritz, I. B., D. G. Davis, R. H. Holtrop, and H. Dundee. *Am. J. Physiol.* **194:** 379, 1958.
13. Hanson, R. W. *Arch. Biochem. Biophys.* **109:** 98, 1965.
14. Dole, V. P., and H. Meinertz. *J. Biol. Chem.* **235:** 2595, 1960.
15. Bray, G. A. *Anal. Biochem.* **1:** 279, 1960.
16. Rodbell, M. *J. Biol. Chem.* **239:** 375, 1964.
17. Lehninger, A. L., H. C. Sudduth, and J. B. Wise. *J. Biol. Chem.* **235:** 2450, 1960.
18. Horecker, B. L., and P. Z. Smyrniotis. *Methods Enzymol.* **1:** 323, 1955.
19. Ochoa, S. *Methods Enzymol.* **1:** 699, 1955.
20. Srere, P. A. *Methods Enzymol.* **5:** 641, 1962.
21. Björntorp, P., and R. H. Furman. *Am. J. Physiol.* **203:** 316, 1962.
22. Johnson, M. J. *J. Biol. Chem.* **137:** 575, 1941.
23. Fitch, W. M., and I. L. Chaikoff. *J. Biol. Chem.* **235:** 554, 1960.
24. Fitch, W. M., R. Hill, and I. L. Chaikoff. *J. Biol. Chem.* **234:** 1048, 1959.
25. Fitch, W. M., R. Hill, and I. L. Chaikoff. *J. Biol. Chem.* **234:** 2811, 1959.
26. Freedland, R. A., and A. E. Harper. *J. Biol. Chem.* **230:** 833, 1958.
27. Flatt, J. P., and E. G. Ball. *Biochem. Z.* **338:** 73, 1963.
28. Cahill, G. F., Jr., B. Leboeuf, and A. E. Renold. *J. Biol. Chem.* **234:** 2540, 1959.
29. Ball, E. G., and R. L. Jungas. *Recent Prog. Hormone Res.* **20:** 183, 1964.
30. Eaton, P., and D. Steinberg. *J. Lipid Res.* **2:** 376, 1961.
31. Tepperman, J., H. M. Tepperman, and M. P. Schulman. *Am. J. Physiol.* **184:** 80, 1956.
32. Petrasek, R., P. Fabry, and R. Poledne. *Experientia* **20:** 434, 1964.
33. Cherkes, A., and R. S. Gordon, Jr. *J. Lipid Res.* **1:** 97, 1959.
34. Korn, E. D. *J. Biol. Chem.* **215:** 1, 1955.
35. Rizack, M. A. *J. Biol. Chem.* **236:** 657, 1961.
36. Rodbell, M., and R. O. Scow. In *Fat as a Tissue*, edited by K. Rodahl and B. Issekutz, Jr. McGraw-Hill Co., New York, 1964, p. 110.
37. Green, D. E., and S. J. Wakil. In *Lipide Metabolism*, edited by K. Bloch. John Wiley and Sons, Inc., New York, 1960, p. 1.
38. Spencer, A. F., and J. M. Lowenstein. *J. Biol. Chem.* **237:** 3640, 1962.
39. Srere, P. A. *J. Biol. Chem.* **234:** 2544, 1959.
40. Madsen, J., S. Abraham, and I. L. Chaikoff. *J. Biol. Chem.* **239:** 1305, 1964.
41. Madsen, J., S. Abraham, and I. L. Chaikoff. *J. Lipid Res.* **5:** 548, 1964.
42. D'Adamado, A. F., Jr., and D. E. Haft. *J. Biol. Chem.* **240:** 613, 1965.
43. Spencer, A., L. Corman, and J. M. Lowenstein. *Biochem. J.* **93:** 378, 1964.
44. Vagelos, P. R., D. B. Martin, P. Goldman, and A. W. Alberts. In *Fat as a Tissue*, edited by K. Rodahl and B. Issekutz, Jr. McGraw-Hill Co., New York, 1964, p. 149.
45. Freedland, R. A., and A. E. Harper. *J. Biol. Chem.* **233:** 1041, 1958.
46. Schimke, R. T. *J. Biol. Chem.* **239:** 3808, 1964.

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012