Studies on serum lipids, insulin, and glucagon and on muscle triglyceride in rats adapted to high-fat and high-carbohydrate diets

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Abstract A comparison was made of lipid circulation, storage, and mobilization in rats adapted to lard or glucose diets. In the morning, lard-fed rats had higher blood triglyceride (TG) and free fatty acid (FFA) levels. In the evening TG was higher, but FFA was significantly lower in the lard vs. the glucose group. Fasting did not produce the characteristic increase in blood FFA in the lard-fed rats but was associated with a severe drop in their serum TG. Circulating glucose and insulin were not affected, while glucagon levels were increased by lard feeding. Nicotinic acid decreased fasting FFA levels to a greater extent in the glucose-fed rats. It was concluded that lard feeding depresses mobilization of fat from adipose tissue; on the other hand, it was found to increase storage and utilization of muscle TG. Fat feeding increased diaphragm TG concentrations threefold, as well as the number and size of intracellular fat droplets at the light and electron microscopic levels. Fasting decreased diaphragm TG in both groups, but the amount lost was greater in the fat-fed rats. Also, in vitro basal isoproterenol-stimulated (1 μg/ml) FFA release by the incubated diaphragm was higher in the lard-fed group.

Supplementary key words blood glucose · free fatty acids · triglycerides · adipose tissue lipolysis · muscle lipolysis

In a recent study from this laboratory (1) it was found that adipose tissue of lard-fed rats was less responsive to lipolytic stimuli (epinephrine, glucagon) than fat tissue of carbohydrate diet-adapted animals. In prior studies the feeding of a high-fat diet was found to be associated with an increased rate of fatty acid oxidation in muscle and liver and a decreased rate of glucose utilization by those tissues (2).

These observations raised the question of the source of supply of fatty acids to the muscle of rats on a high-fat diet, especially during the part of the day when little food is in the gastrointestinal tract. In the fed state, animals fed a high-fat diet have more lipoprotein lipase activity in their muscles than do carbohydrate-fed controls (3). Since this enzyme regulates the rate of intravascular lipolysis locally, it is reasonable to suppose that fatty acids could be supplied to the muscles of such animals from chylomicron triglycerides when they are present after a meal. It has been previously suggested that the muscles of animals fed a high-fat diet play an important role in the clearance of dietary chylomicron triglycerides from the plasma (3, 4).

The experiment from our laboratory that showed decreased hormone-stimulated adipose tissue lipolysis in fat-fed rats (1) was an in vitro study and did not test the capacity of fat-fed rats to mobilize adipose tissue fatty acids in response to fasting. Moreover circulating free fatty acids released by the adipose tissue may not be the only source of fatty acids for the muscle of the fasted rat adapted to glucose or fat diets; local stores of triglycerides within muscle fibers could be another important source. Lipids have been identified in normal skeletal muscle by different histochemical techniques (5) and recently Dagenais, Taneredi, and Zierler (6) hypothesized a cycling of plasma free fatty acids into these muscle triglycerides before their mitochondrial oxidation. Therefore, a contribution of intramuscular triglycerides to muscle metabolism in the fasted state could be significant.

The initial studies to be described in this report consisted of measurements of serum levels of glucose, triglycerides (TG) and free fatty acids (FFA), as well as those of insulin and glucagon. Values were obtained at two different times of day and after an overnight fast. In the second part of this report, experiments to be described were designed to study the effect of different nutritional conditions on intramuscular fat, and also to compare the relative contributions of adipose tissue and muscle TG to metabolism of fasted rats previously adapted to lard or glucose feeding.

Abbreviations: TG, triglyceride; FFA, free fatty acid; EM, electron microscopy; VLDL, very low density lipoprotein; LPL, lipoprotein lipase.

TABLE 1. Weight gain and caloric intake of rats maintained on glucose and lard diets for 5 days

Diet	Initial Body Wt	Weight Gain	Calories
	g	*g/day*	*cal/day*
Glucose	119	5.6 ± 0.3[a]	61.3 ± 4.4
Lard	123	7.7 ± 0.5	61.7 ± 3
P		<0.05	>0.05

[a] Each value is the average of six observations ± SEM.

Morphological study by light and electron microscopy was carried out in order to localize intramuscular TG, and to complement the chemical tissue analysis.

MATERIALS AND METHODS

Animals and diets

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 80–90 g were fed chow for one day upon arrival and then were divided into two groups: one group was given a G diet which, on a caloric basis, consisted of 33% casein and 67% glucose, and the other group was given an L diet in which a calorically equivalent amount of lard was substituted for glucose. Adequate quantities of vitamins (4% by wt), salt mixture (USP XIV, 5% by wt), and cellulose (5% by wt), purchased from Nutritional Biochemicals Corp., Cleveland, OH, were added to each diet. Food was available ad libitum and the rats were maintained on their respective diets for a period of 10–11 days. The caloric intake and weight gain of animals maintained on both diets were measured in a separate experiment (**Table 1**). Similar numbers for weight gain were obtained in these studies (6.4 ± 1.6 g/day for glucose-fed and 7.6 ± 1.9 g/day for lard-fed rats). Weights before death were 163 ± 2 g and 174 ± 2 g, respectively, for glucose- and lard-fed rats.

Circulating substrates and hormones measurements

The data on circulating fuels and hormones were collected at two different daytime periods, morning and evening, and after an overnight fast. In the morning rats were killed 1 hr after the end of the dark period, and in the evening 1 hr before its onset, i.e., at 9 AM and 7 PM since the dark period extended from 8 PM to 8 AM. It has been shown that rats eat most of their food in the dark (7). Blood was collected from the vena cava. For the glucagon assay, serum separation was done in the cold. Glucose was measured by the glucose oxidase method (8) using a Glucostat reagent purchased from Worthington, San Francisco. FFA was determined according to the procedure of Falholt, Lund, and Falholt (9) and TG by the Oxford trichol technique (Oxford Laoratories, Foster City, CA). Insulin was assayed according to the procedure of Hales and Randle (10) with a kit obtained from Amersham Searle Corporation, Arlington Heights, IL; glucagon was measured by the radioimmunoassay of Faloona and Unger (11). Their antiserum 30 k was used; the radioiodinated glucagon was purchased from Nuclear Medical Lab., Dallas TX, and glucagon standard from the Eli Lilly Company, Indianapolis, IN.

Nicotinic acid administration

The nicotinic acid effect on blood FFA was studied in rats fed the high-carbohydrate or high-fat diet. Rats fasted for 18 hr were given an intraperitoneal injection of a long-acting thiobarbiturate, Inactin (Promonta, Hamburg). Tracheostomy was routinely performed. A catheter was inserted in the portal vein for blood collection and for injection of nicotinic acid (30 mg/kg). A small amount of saline was then added to insure passage of all the drug into the bloodstream. The abdomen was covered with saline-moistened gauze and the rats were kept under a warm lamp. The second blood collection was obtained from the vena cava 60 min after injection. It has been previously reported that the maximal effect of nicotinic acid on blood FFA occurred approximately 1 hr after the administration of the drug (12); we confirmed this finding in a preliminary trial. The experimental manipulations were found not to affect blood FFA, which did not change in controls receiving saline injections instead of nicotinic acid. In vitro experiments carried out in our laboratory showed that nicotinic acid was effective in inhibiting adipose tissue lipolysis when fat pads from lard or glucose fed rats were tested.

Diaphragm TG analysis

For determination of diaphragm triglycerides the rats, fed or 42-hr fasted, were killed by decapitation. The diaphragm was excised rapidly from the rib cage, and hemidiaphragms were weighed and homogenized in chloroform–methanol 2:1. The next day aliquots of the chloroform layer were evaporated under a stream of nitrogen. Isopropyl alcohol was added and the TG was extracted and assayed according to the trichol procedure.

Morphological studies

For light microscopy, diaphragm tissue was quickly frozen in liquid nitrogen; frozen sections were formalin-fixed and stained. An oil red O stain (13) was used to visualize fat droplets and nuclei were stained with hematoxylin.

For electron microscopy (EM), portions of the dia-

phragm were removed from control and experimental animals. They were immediately cut into small pieces and placed in a solution of 1% osmium tetroxide buffered at pH 7.4 with veronal acetate. After fixation, the tissues were dehydrated with increasing concentrations of ethanol and embedded in Araldite 502 by a standard procedure (14). The blocks were sectioned on a Sorvall Porter-Blum MT2-B ultramicrotome and thin sections were stained with 4% uranyl acetate followed by 0.4% lead citrate and then examined with an RCA EMU 3-F electron microscope.

In vitro diaphragm lipolysis

Diaphragm lipolysis was studied in vitro using the intact diaphragm preparation according to Kipnis and Cori (15) in which all the insertions to the rib cage and spine are kept intact. This preparation is thought to be more physiological than the cut diaphragm (15). Hemidiaphragms were obtained by cutting carefully along the midline tendinous part. From each rat one hemidiaphragm was used to measure basal lipolysis and the other for lipolysis stimulated by 1 μg/ml isoproterenol. The incubation was carried out at 37°C, 110 oscillations/min in a bicarbonate buffer medium with 3% albumin (bovine albumin, Fraction V, FFA poor, Pentex Miles Lab., Kankakee, IL). At the end of the incubation period (1 hr), vials were transferred to an ice bath and aliquots of the medium were stored at −20°C for FFA analysis. The hemidiaphragms were then dissected and weighed. Results were expressed as μmol of FFA released per hr per wet weight of the isolated hemidiaphragm.

Statistics

Differences between means were tested for statistical significance using the Student's *t* test.

RESULTS

This study was concerned with two main goals. *1*) Measurement of circulating substrates (glucose, TG, FFA), as well as serum insulin and glucagon concentrations, in rats adapted to glucose or lard diets under different nutritional conditions (morning, evening, 18-hr fast). *2*) Assessment of the relative importance of triglyceride stores in adipose tissue and skeletal muscle during fasting in rats adapted to the two diets.

Blood studies

Circulating substrates. Serum glucose levels (**Fig. 1*a***) were found to be similar in the glucose- and lard-fed rats. A slight drop in blood glucose in the evening (7 PM) and in the fasted state occurred and was significant only in the group fed glucose (G group).

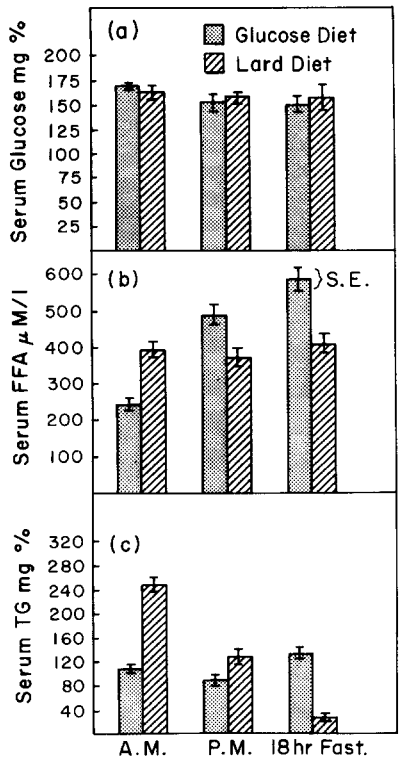

Fig. 1. Serum, glucose (*a*), FFA (*b*), and TG (*c*) of glucose- and lard-fed rats. Means ± SE of eight observations are represented. The table below gives the levels of significance ($P < 0.05$ represents statistical significance). No significant differences in serum glucose were found. AM (9 AM) and PM (7 PM) refer to times of killing. The animals were in the dark from 8 PM to 8 AM.

	Comparison	FFA	TG
Glucose vs. Lard	AM	$P < 0.01$	$P < 0.001$
	PM	$P < 0.05$	$P < 0.05$
	18 hr fast	$P < 0.05$	$P < 0.001$
Glucose	AM—PM	$P < 0.01$	$P > 0.05$
	AM-fasted	$P < 0.01$	$P > 0.05$
Lard	AM—PM	$P > 0.05$	$P < 0.001$
	AM-fasted	$P > 0.05$	$P < 0.001$

Fig. 1*b* shows that a different pattern for morning (9 AM), evening (7 PM), and fasting serum FFA is observed within each diet group. Fed G rats had low serum FFA which increased significantly in the evening ($P < 0.05$) and in the fasted state ($P < 0.01$). Lard-fed rats maintained the same serum FFA throughout the three time periods. When levels were compared in G and L groups, morning serum FFA was higher in the L rats; however, in the evening, as well as in the fasted state, G rats had significantly higher serum FFA ($P < 0.05$ in both cases).

Morning serum TG (Fig. 1*c*), was increased by fat feeding and was more than twice the level found in

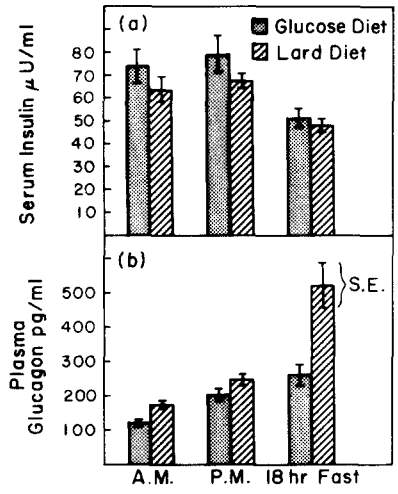

Fig. 2. Circulating insulin (*a*) and glucagon (*b*) levels in rats fed glucose or lard. Means ± SE of eight observations are represented. The table below gives the levels of significance ($P < 0.05$ represents statistical significance).

	Comparison	Insulin	Glucagon
Glucose vs. Lard	AM	$P > 0.05$	$P < 0.01$
	PM	$P > 0.05$	$P > 0.05$
	18 hr. fast	$P > 0.05$	$P < 0.01$
Glucose	AM—PM	$P > 0.05$	$P < 0.01$
	AM-fasted	$P < 0.05$	$P < 0.001$
Lard	AM—PM	$P > 0.05$	$P < 0.01$
	AM-fasted	$P < 0.05$	$P < 0.001$

rats fed the glucose diet ($P < 0.001$). Afternoon serum TG was still significantly higher in the lard-fed group, but the difference was much less pronounced ($P < 0.05$). When the fasting serum TG was compared in both groups it was observed that fat-fed rats had markedly lower values (134 ± 12 mg% in the G group vs. 25 ± 7 mg% in the L group).

Serum insulin and glucagon. Serum insulin levels were found to be similar in both diet groups (Fig. 2*a*). A consistent, slight tendency for lower values was associated with fat feeding although it never reached statistical significance.

Serum glucagon (**Fig. 2*b***) was increased by fat feeding, the difference between the G and L groups being significant in the morning and after an 18-hr fast.

Studies on the sources of fuel

The potential contribution of adipose tissue triglycerides and muscle triglycerides to the supply of muscle FFA during food deprivation was assessed in rats adapted to the lard and glucose diets.

Adipose tissue lipolysis. It was noted before (Fig. 1*b*) that fat-fed rats had significantly lower serum FFA than the glucose-fed animals in the evening and after an overnight fast. This observation as well as the decreased epinephrine-stimulated lipolysis in fat pads (1), were the reasons we decided to compare the respective contributions of adipose tissue lipolysis to blood FFA in the two diet groups.

The following experiment was designed for this purpose. Nicotinic acid was given intravenously to 18-hr-fasted rats and the resulting depression in blood FFA was measured. According to our hypothesis, nicotinic acid would be expected to depress blood FFA by inhibiting adipose tissue lipolysis (16). If this effect were more pronounced in one of the two groups it would suggest a more important contribution of adipose tissue lipolysis to blood FFA in that particular group.

The data plotted in **Fig. 3** show that the decrease in serum FFA in the glucose-fed rats after nicotinic acid administration was almost three times as large as that seen in the lard-fed group. This suggested that the concentration of circulating FFA was more dependent on adipose tissue lipolysis in the former group than in the latter.

It is also apparent in Fig. 3 that the G rats, as expected, started with a significantly higher baseline FFA level. The absolute decrease (Δ) was larger in these rats, but also the percentage decrease (Δ/baseline × 100) was found to be higher in glucose-fed rats (72% vs. 43%).

Intramuscular triglycerides as a source of fuel. Since adipose tissue lipolysis appeared to be depressed in lard-fed rats, it seemed probable that such animals were more dependent on triglycerides stored in skeletal muscle than were carbohydrate-fed rats. In order to

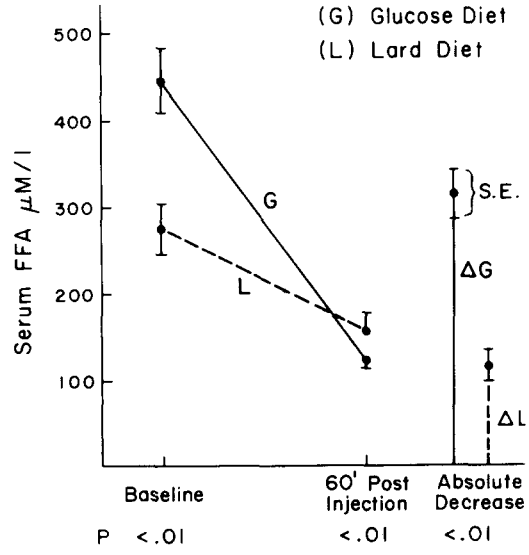

Fig. 3. Nicotinic acid effect on blood FFA of rats fed the G and L diets. Each point represents the mean ± SE of eight observations. Rats were fasted for 18 hr prior to the intravenous injection of nicotinic acid.

test this hypothesis, the following experiments were performed; *1*) measurement of TG concentration of muscle in lard and carbohydrate diet-adapted rats and estimation of changes in muscle TG that occurred on fasting; *2*) histochemical and EM localization of muscle TG in both groups; and *3*) a test of hormone-induced lipolysis in skeletal muscle preparations from animals on both diets.

1) Diaphragm TG analysis. It was found (**Fig. 4**) that feeding lard to rats resulted in an almost three-fold increase in diaphragm TG. Fasting the rats for 42 hr produced a significant decrease in TG levels in both groups. The extent of the decrease was greater in the lard-fed group.

2) Morphological studies. Microscopic findings were found to be consistent with the chemical data. At the light microscopic level (**Fig. 5**), diaphragms of lard-fed rats contained impressively more fat than the ones from rats fed glucose. It is also interesting to note that fat droplets were not distributed homogeneously among all the fibers. In this connection it has been shown previously that one type of muscle fibers (red fibers) characteristically store more fat (17). Electron microscopy (**Fig. 6**) showed more numerous fat droplets in the muscle cells of the lard-fed group. These droplets were seen to cluster around the mitochondria, which suggests a functional relationship between the lipid droplets and the organelles in which fatty acid oxidation occurs.

3) FFA release by diaphragm in vitro. **Fig. 7** shows the in vitro release of FFA obtained with the intact hemidiaphragm in the presence or absence of isoproterenol. Both basal and stimulated lypolysis were found to be higher in the lard-fed group. It can be noted that the absolute increase in FFA released into the medium when isoproterenol was added was greater in the case of the diaphragms from lard-fed rats.

DISCUSSION

In our study fat feeding markedly affected circulating TG and FFA but was without effect on serum glucose levels. The similarity in blood glucose levels found in carbohydrate- and lard-fed rats is in agreement with reports of others (18, 19). Since our lard diet was devoid of glucose, this observation is consistent with numerous reports (19–21) of an increased hepatic gluconeogenesis as a part of the adaptation to a high-fat diet.

An increase in intravascular lipolysis of TG usually results in elevated serum FFA (4). The morning FFA (Fig. 1*b*) data we describe are consistent with this finding. Decreased adipose tissue lipolysis, suggested by

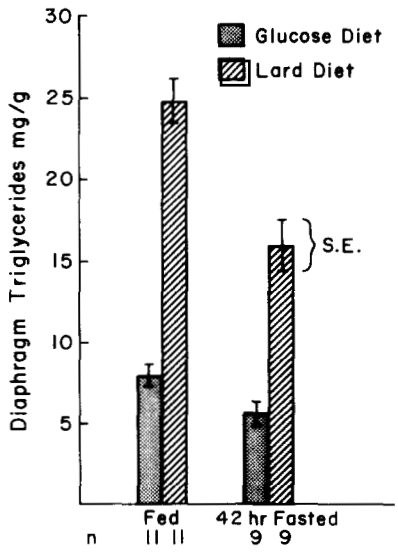

Fig. 4. Diaphragm TG (mg/g wet weight). The table below gives the levels of significance. G, glucose-fed rats; L, lard-fed rats.

Fed (G-L)	$P < 0.001$
Fasted (G-L)	$P < 0.001$
G (fed-fasted)	$P < 0.05$
L (fed-fasted)	$P < 0.01$

our nicotinic acid experiment and by the in vitro experiment of Gorman, Tepperman, and Tepperman (1), account for the observation that fat-fed rats had lower evening and fasting FFA levels than the glucose-fed animals.

Fat-feeding increased serum TG levels in nonfasted rats (Fig. 1*c*) as reported by others (22); however, this effect seems to be related to the presence of saturated fat in the diet (23). The large drop in serum TG levels that occurred with fasting in the lard-fed rats could result either from an efficient chylomicron and very low density lipoprotein removal mechanism, or from a low output of VLDL by the liver. Fat intake has been shown to stimulate endogenous plasma lipolytic activity (24), i.e., lipoprotein lipase which is known to clear both chylomicrons and VLDL triglycerides (25). However, it is also likely that a depression of VLDL output was produced by fat feeding. This process could be inhibited by the high circulating levels of glucagon found under these conditions (Fig. 2*b*). The hypolipemic effect of glucagon has been shown to result primarily from a generalized inhibition of hepatic protein synthesis and not from a decrease in FFA re-esterification (26). That such a mechanism could be operating in fat-fed rats is supported by the fact that liver TG content has been shown to be increased (19). Both suggested mechanisms may contribute to the low level of TG observed in fasted L rats.

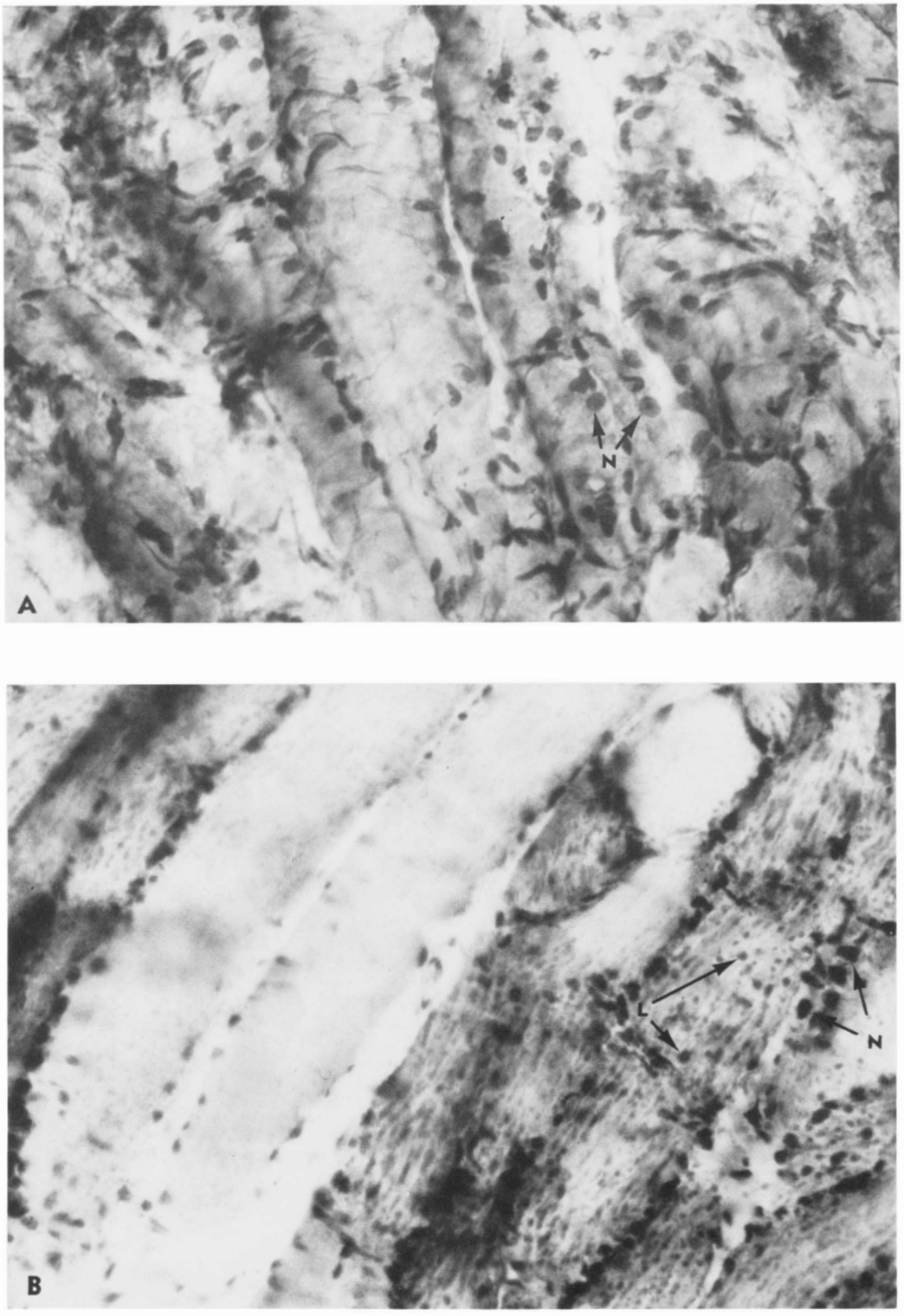

Fig. 5. Light microscopic examination of a section of a diaphragm from a rat fed glucose (panel *A*) or lard (panel *B*). Frozen sections were stained with the fat stain oil red O (*N* = nuclei, *L* = lipid droplets). Fat droplets, more abundant in rats fed lard (panel *B*), are seen to cluster in some fibers more than in others.

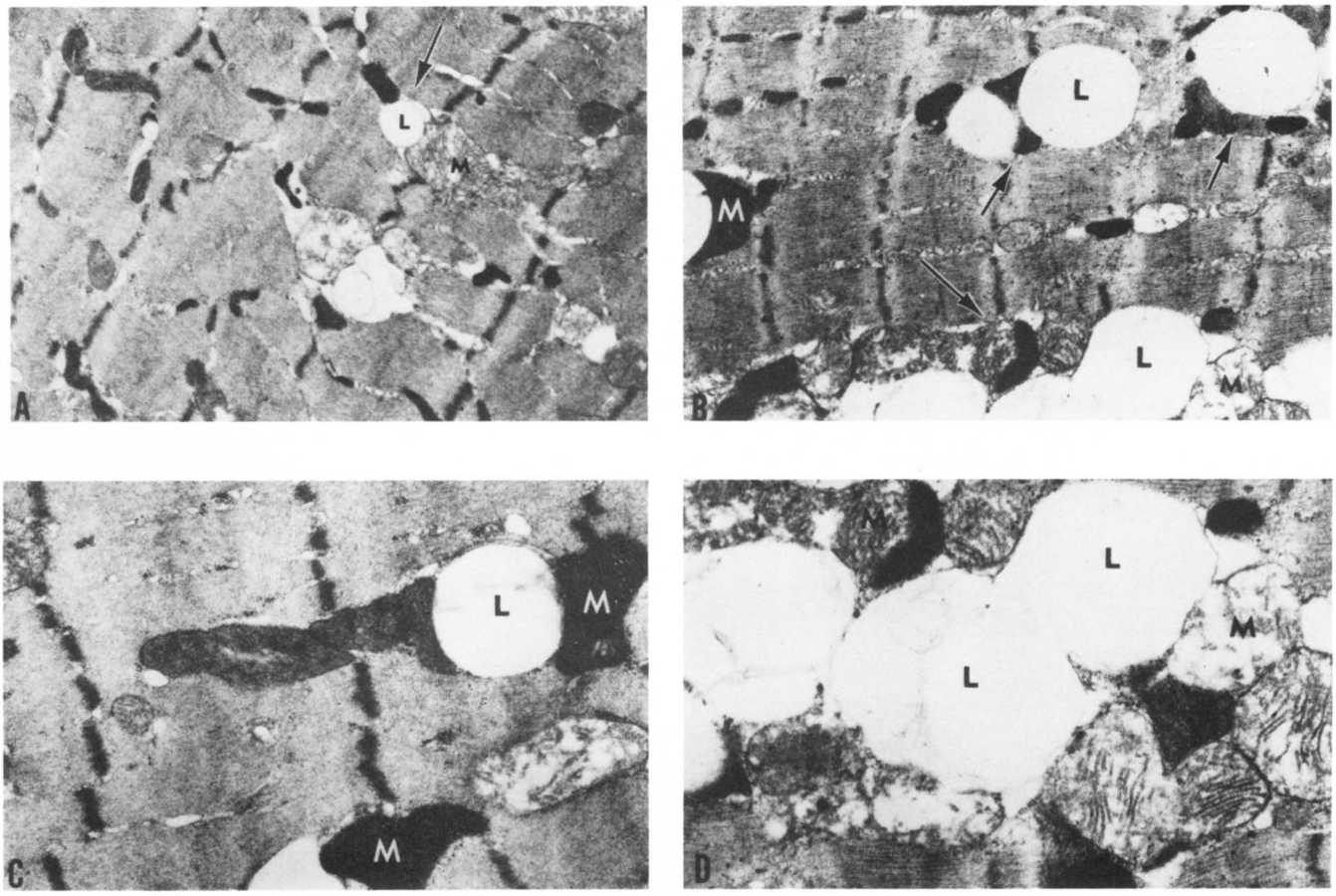

Fig. 6. *A*. Representative section of diaphragm tissue from a glucose-fed rat shows a lipid droplet (*L*) associated with a mitochondrion (*M*), ×9700. *B*. Extensive lipid deposition is evident in the diaphragm of a lard-fed rat. Lipid droplets (*L*) are seen in close proximity to mitochondria (*M*) (arrows), ×9700. *C*. Higher magnification of a diaphragm of a glucose-fed animal showing a typical lipid droplet (*L*) next to the mitochondria (*M*), ×17900. *D*. Larger and more numerous lipid droplets from a diaphragm of a fat-fed rat are seen associated with an array of mitochondria (*M*), ×17900.

Serum insulin and glucagon

Although our measurements showed only a trend toward lower insulin levels in fat-fed rats, previous work in our laboratory (27) showed lard-fed rats to have significantly lower insulin levels than the glucose-fed animals. Published data concerning the effect of fat feeding on serum insulin levels remain conflicting. A high-fat diet has been reported to produce no effect (18), a decrease (22, 28), or an increase in serum insulin levels (29). In the case of a lard diet, the effect on serum insulin was absent (Fig. 2*a*) (18) or small when observed (27). Differences in experimental conditions make it impossible to explain these conflicting results.

We reported an increase in serum glucagon with fat feeding. A comparable change was observed previously by Dobbs, Faloona, and Unger (30) in dogs fed a fat meal. Our results disagree with those of Eisenstein, Strack, and Steiner (19) who observed no changes in glucagon levels in rats fed a carbohydrate-free diet rich in cottonseed oil and butter. The discrepancy in results could be related to the diets used. It is noteworthy that blood FFA was not affected by the cottonseed–butter diet while blood TG of non-fasted rats was lowered. Both these observations are in disagreement with those we described in rats fed a lard diet.

In our study the increase in serum glucagon, with little change noted in serum insulin levels, would be expected to result in lower insulin to glucagon molar ratios (I/G). This hormonal milieu could be involved in many of the changes associated with fat feeding, including increased hepatic gluconeogenesis (20), low peripheral carbohydrate utilization (2, 22), and depressed lipogenic capacity of adipose (31, 22) and liver (31) tissues. However hormones other than insulin and glucagon could be involved in the adaptation to fat feeding. For example, an increase in the weight of the adrenals has been observed with a high fat intake (20).

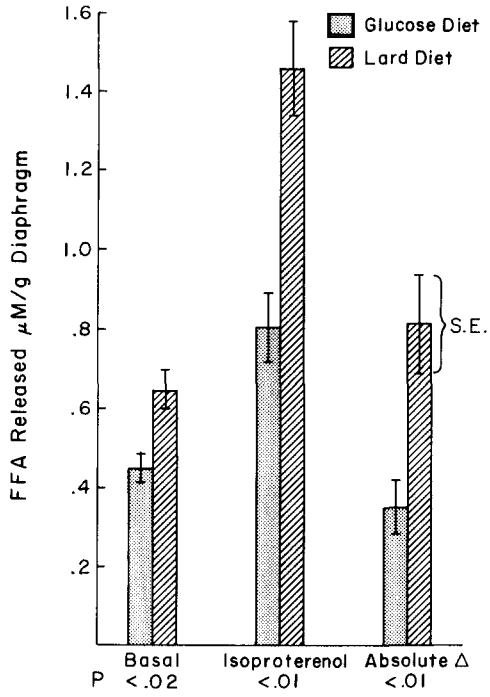

Fig. 7. FFA release by incubated diaphragm (μM FFA/g wet weight). Isoproterenol concentration was 1 μg/ml of incubation medium. Incubation was carried out for 1 hr at 37°C. Means ± SE of eight observations are represented.

Sources of fuel

The results of the nicotinic acid experiment suggest that adipocyte lipolysis contributes more FFA to the circulation in G rats than in L rats. This finding is consistent with the results of in vitro experiments of Gorman et al. (1) cited above.

Factors other than adipose tissue lipolysis could theoretically influence the concentration of circulating FFA. Peripheral utilization of FFA would lower its level. Since the tissues of fat-fed rats oxidize FFA more rapidly than those of glucose-fed animals (2), the differences observed in FFA lowering may represent an understatement of the actual decrease in adipose tissue lipolysis. Any contribution to the circulating fatty acid pool by nicotinic acid-activated lipoprotein lipase (32) would be expected to be smaller in the L rats, since their fasting TG levels were much lower than those of the G group. If this factor were eliminated, the actual difference between the two groups would then be magnified.

The finding of less efficient mobilization of adipose tissue FFA with fat feeding is consistent with the observation (Fig. 1*b* and baseline in Fig. 3) that fat-fed rats have significantly lower fasting serum FFA. The mechanism of the decreased adipose tissue lipolysis in fat-fed rats remains unclear.

The TG accumulation in muscle cells that we observed, by both chemical analysis and light microscopy (Fig. 4, and Fig. 5) in the diaphragms of fat-fed rats is not obviously related to the low circulating I/G ratios. Such ratios do not usually favor fat deposition (33). However, an increase in muscle TG has also been noted in alloxan diabetic rats, and accelerated lipolysis of TG was thought to result in the increased fatty acid oxidation observed in the muscle of diabetic animals (34).

Fat deposition in muscle tissues could probably be explained on the basis of studies of adipose tissue and muscle LPL activities. If it is assumed that a tissue LPL activity controls its uptake of TG-fatty acids (35, 36), an increased muscle LPL activity in circumstances where circulating TG is elevated (Fig. 1*c*) could result in an increase in intramuscular TG. This interpretation implies that, in such conditions, fat deposition may be controlled primarily by the capacity of the tissues to take up TG-fatty acids and to re-esterify them.

The finding that intramuscular TG was decreased with fasting suggests an active metabolic function of these stores; similar results were observed by Masoro (37) only in young rats and not in older animals. The effect of age on muscle lipid utilization requires further study. Our results in Fig. 4 are further supported by the EM observation (Fig. 6) of the fat droplets in close proximity to mitochondria, suggesting that this intracellular location may be importantly related to oxidative metabolism. The decrease in TG was more extensive in the diaphragms of fat-fed rats and could indicate that these rats are more dependent on their muscle TG in fasting. The observation that their adipose tissue lipolysis is depressed is consistent with this conclusion. Previous work by Carlson, Froberg, and Nye (38) has shown that inhibition of FFA mobilization from adipose tissue by nicotinic acid results in a decrease in heart and red muscle TG. Their experiment and ours suggest that reduced availability of exogenous FFA for the muscle could lead to an accelerated mobilization of its intracellular TG stores. Whether the intramuscular TG stores contribute to blood FFA, and more so in the fat-fed rats, remains to be answered.

The in vitro release of FFA by the incubated diaphragm and its response to stimulation by isoproterenol is further evidence that muscle TG can be mobilized. It is true that we cannot be certain that the preparation had absolutely no fat cells in it, although grossly visible adipose tissue was carefully removed and light microscopic examination did not show any fat cells among the fibers (Fig. 5). However, the lard diet would be expected to decrease mobilization of fat from adipose tissue (1); if there were any adipo-

cytes in our diaphragm preparation we have understated the degree of increased lipolysis in the diaphragm of fat-fed rats (Fig. 7). An increase in intracellular FFA of the incubated hemidiaphragm after addition of adrenaline has been shown previously by Garland and Randle (39). The same authors also showed that the glycerol output by the diaphragm preparation was not inhibited by insulin, although it was increased by alloxan diabetes. Similarly, insulin did not inhibit glycerol release from the perfused rat hindlimb (40); addition of isoproterenol to the perfusion medium stimulated lipolysis and increased tissue cAMP levels. These observations could mean that a cAMP system regulates lipolysis of skeletal muscle; however, more evidence is needed. Few studies have dealt with the control of lipolysis in the heart (41) and a system similar to the hormone-sensitive lipase of adipose tissue has already been suggested (42); whether isoproterenol initiates in the diaphragm the same sequence of events as those that lead to TG hydrolysis in the adipose tissue, and probably in the heart, remains to be elucidated.

This study shows that lard feeding results in the assumption by muscle of a greater role in storage and availability of triglycerides; whether this occurs with other types of fat diets is not known. The hypothesis is presented here that muscle tissues of lard-fed rats depend more on their local TG stores and less on FFA release from adipose tissue than those of the glucose-fed animals.

The control of fat deposition and mobilization in muscle and whether it is responsive to the same stimuli operating in adipose tissue deserves further investigation. It would be of interest to study the lipid metabolism of muscle under conditions in which hypertriglyceridemia coexists with low peripheral carbohydrate utilization.

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