Lipogenic activation after nibbling and gorging in mice

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Abstract Lipogenic activation was studied in mice that had been restricted to a single large meal once a day rather than being allowed to eat at frequent intervals throughout the night. Mice were injected intravenously with [U-^{14}C]glucose, and the flux of glucose C to total lipid fatty acids (TLFA) and to all "end products" was estimated from serial plasma glucose specific activities and measurements of incorporation of ^{14}C into TLFA of hepatic and extrahepatic tissues. Tracer studies were carried out in mice fasted for 1 day and at various times after the mice ate one or two small test meals or a single large test meal. Test meals consisted of a fat-free, 58% glucose diet. The flux of glucose C to TLFA increased by an order of magnitude within an hour after mice nibbled a test meal for several minutes. After ingestion of two small test meals or a single large test meal, the flux of glucose C to TLFA increased from a fasting rate of 0.5 to 35 and 87 μg of glucose C/min/20 g body wt, respectively. Although trained meal eaters are thought to have abnormally increased lipogenesis, their lipogenic response to a single test meal was the same as that previously reported for untrained nibbling mice. Most of the newly synthesized fatty acids were found in extrahepatic tissues. Ingestion of a first test meal completely prevented the expected hyperglycemic response following ingestion of a second test meal even though the latter contained over 10 times more glucose than that in the total body glucose pool.

Supplementary key words [U-^{14}C]glucose · liver · extrahepatic tissue · fatty acid synthesis · glucose irreversible disposal · semicompartmental analysis · glucose tolerance · absorption · non-steady state · dietary control

THE FEEDING of a low fat, high carbohydrate meal is known to increase the lipogenic capacities of various tissues (1–3). This response appears to be especially pronounced in animals that have been trained to eat all of their daily intake in a single meal (4). The latter animals have been called "meal eaters" (4, 5) or "gorgers" (6). However, lipogenesis in tissues of animals such as rodents that eat many small meals throughout the night ("nibblers") is also known to be greatly increased following ingestion of a high carbohydrate meal (1, 7–10).

As yet there have been no quantitative, comparative studies of the rates at which fatty acids are synthesized from glucose C in vivo in nibblers and gorgers before and after the ingestion of a test meal. There have been two major obstacles to such a study. One problem, previously emphasized by Tepperman and Tepperman (11), is that, given a large meal, the nibbler won't eat the same amount of food that the trained gorger will eat in a given period of time. The second obstacle has been the interpretation of tracer experiments in nonsteady hyperglycemic states, such as those that accompany the ingestion of carbohydrate. In the present study we have overcome these problems, first, by offering a *small* test meal which each type of animal ingested in a period of several minutes and, second, by waiting until the plasma glucose concentration reached a near steady state following the ingestion of a test meal (10).

We have previously (10) estimated the flux of glucose C to fatty acids in fasted nibbling mice before and after ingestion of a small test meal. This flux increased at least sevenfold within 2 hr after the mice ate their food (10). We have now carried out a comparable study in mice that have been converted from nibblers to gorgers. We have also compared the effects of repetitive ingestion of small meals and of a single large meal upon the flux of glucose C to fatty acids. Our experiments show that lipogenesis from glucose C in gorgers is rapidly activated after ingestion of a single test meal and is greatly augmented when mice nibble consecutive test meals. The degree of lipogenic activation in gorgers after ingestion

Abbreviations: TLFA, total lipid fatty acids.

of a single small test meal did not differ quantitatively from that previously found in nibblers.

MATERIALS AND METHODS

Animals

Male mice (strain 129/J, Jackson Laboratory, Bar Harbor, Maine) weighing 15–26 g were housed individually in metabolism cages (Acme Metal Products, Chicago, Ill.) for training and adaptation to a controlled diet (see below). Water was available ad lib.

Training procedure

The only food given was a fat-free, 58% glucose diet (see below), and this was fed between the hours of 8 and 10 AM each day for 4–11 days prior to the experiment.

Tracer administration

[U-^{14}C]Glucose (New England Nuclear, 5.4 mCi/mmole) in water was injected i.v. in 1-μCi amounts as described previously (10). Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.5 μCi of sodium [1-^{14}C]-acetate (New England Nuclear, 2 mCi/mmole) was used as the incubation medium in the study to estimate lipogenesis, in vitro, in specific tissues.

Test meals

A commercial, synthetic, fat-free, 58% glucose diet was offered as the test meal in 0.21- or 1.5-g amounts. The quantity of diet actually ingested is reported in the text. The composition of the diet was as follows: 58% glucose, 22% casein, 6% Hawk-Oser salt mixture, 11.8% nonnutritive cellulose, 2% liver VioBin, and 0.2% vitamin mixture (10).

In vivo study

After ingestion of a test meal, serial blood samples (35 μl each) were drawn at various times from an ophthalmic venous capillary sinus of each mouse (12, 13). Tracer glucose was injected into a tail vein at various times after ingestion of a test meal. Mice were decapitated 15–60 min after tracer injections; in selected groups, livers and alimentary tracts (stomachs through large intestines) were quickly dissected and immersed in liquid nitrogen. Carcasses were frozen in the same manner, and all specimens were stored at −16°C for subsequent analysis. The methods used to estimate plasma glucose concentrations and specific activities have been described earlier (14, 15). Plasma was deproteinized and glucose was measured enzymatically (14). Plasma glucose specific activities were estimated after separation of the glucose by thin-layer chromatography (15). Liver and extrahepatic tissues were saponified, the unsaponified lipids were extracted and discarded, and the fatty acids were extracted and assayed for radioactivity using methods described previously (10). Glucose was extracted from the alimentary tracts and contents by homogenization in cold 70% ethanol followed by brief heating at 80°C. Supernatant liquid was deproteinized (16) and glucose was estimated enzymatically (14).

In vitro study

Over a 1–2 hr period, 1.5-g test meals were ingested by four mice, and the mice were killed 30–60 min after removal of the test meals. Other mice were killed in the 24-hr-fasted condition to provide control tissues. Epididymal adipose tissue and liver specimens were removed after the mice were decapitated. Paired fat pads were weighed and placed in the center wells of modified Erlenmeyer flasks containing 0.5 ml of buffer and tracer; liver slices were weighed and placed in the outer wells of the respective flasks, which contained 2 ml of buffer and tracer. After gassing with 5% CO_2–95% O_2, each flask was stoppered and incubated in a Dubnoff shaker at 120 cycles/min for 60 min at 37°C; then the tissues plus media were transferred with 10% KOH in 50% methanol to stoppered 15-ml centrifuge tubes for overnight digestion at 60°C. Unsaponifiable lipids in the alkaline digests were extracted with petroleum ether (30–60°C boiling range) and discarded. The digests were then cooled to 0–4°C prior to acidification with concentrated HCl. Fatty acids were extracted with petroleum ether (30–60°C boiling range) and aspirated into separate tubes. The fatty acids were dried under N_2 and redissolved with 0.5 ml of chloroform. Aliquots were then dried in counting vials for radioassay.

Radioactivity

^{14}C was measured by liquid scintillation counting as described earlier (17).

Calculation of glucose C flux to fatty acids

The method of calculation, theoretical limitations, basic assumptions, and validation of our data analysis have been presented in earlier studies (10, 17). The data required for this estimate are plasma glucose specific activity–time curves and ^{14}C-labeled TLFA at one point in time, t, after i.v. injection of [U-^{14}C]glucose, ($q_{fa}[t]$). From these data, one may calculate the irreversible disposal rate of glucose C to all "end products" (R_1), the total ^{14}C incorporation into all "end products" at time t, ($q_n[t]$), and the fraction of irreversibly metabolized glucose C that is converted to TLFA. The flux of glucose C to TLFA (R_2) may be estimated simply by the following equation (10, 17, 18): $R_2 = R_1 q_{fa}(t)/q_n(t)$.

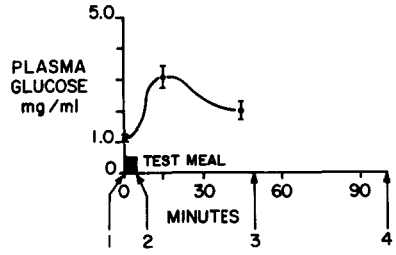
RESULTS

Response of mice to altered dietary regimen

Our mice did not fare well when provided a fat-free, 58% glucose diet 2 hr each day for 11 days.[1] The animals lost weight and the mortality was high (about 50%). Although most of the animals appeared to be trained within 4 days, there was great variability among animals with respect to the time at which they started eating their meals after a preliminary fast. In many instances, at the beginning of training, mice did not eat until their third meal was offered; as a result, they fasted for 72 hr. This variability in training pattern may have been responsible for the large variability (range, 14–118 mg) in the weights of epididymal fat pads isolated from trained meal-eating mice (Table 1). Despite the variation in fat pad weights, all trained mice responded similarly and in a well-known fashion (4, 19) with respect to the lipogenic capacity of the fat pads in vitro after ingestion of a large meal, as shown in Table 1. Thus, the mean percentage of [1-^{14}C]acetate incorporated into fatty acids of epididymal fat pads was 50-fold greater in tissues from refed mice than in those from fasted mice (Table 1); a 15-fold increase of incorporation was observed in the case of liver slices from refed compared with fasted mice.

Plasma glucose and lipogenic responses to 58% glucose test meals in trained meal-eating mice

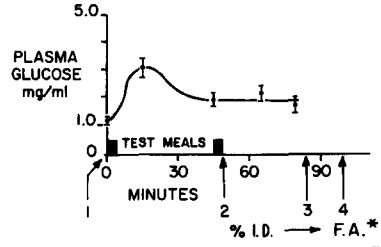
The plasma glucose levels tripled within 15 min after the mice were fed a single 210-mg test meal for 4 min. Although hyperglycemia decreased within 45 min after the test meal, plasma glucose levels were still elevated relative to the fasted state (Fig. 1). The incorporation of intravenously injected tracer [U-^{14}C]glucose into total lipid fatty acids of hepatic and extrahepatic tissues at various times before and after ingestion of the test meals is also indicated in Fig. 1. The incorporation of [U-^{14}C]-glucose into total body fatty acids was increased immediately after ingestion of the test meal (group 2 vs. group 1) despite the greatly increased dilution of the tracer by unlabeled glucose in the case of the refed mice. The rapid increase in the incorporation of [U-^{14}C]glucose into fatty acids was also observed at 50 and 100 min after ingestion of the test meal (Fig. 1), when plasma glucose levels were in a nearly steady state (10; see also Fig. 2).



GROUP	FED	% I.D. → F.A.* CARCASS	LIVER
1	NO	0.22 ± 0.023 (4)	0.15 ± 0.047 (4)
2	YES	0.57 ± 0.022 (3)	——
3	YES	1.2 ± 0.41 (3)	——
4	YES	0.70 ± 0.26 (4)	0.30 ± 0.058 (4)

* IN 30 min AFTER I.V. INJECTION

FIG. 1. Mean plasma glucose concentrations and incorporation (mean % of injected ^{14}C ± SE) of intravenously injected [U-^{14}C]-glucose into total lipid fatty acids of hepatic and extrahepatic tissues of fasted and refed mice. Each plasma glucose concentration is the mean (± SE, vertical bars) of determinations using six mice. Four groups of mice were fasted for 25 hr and injected with [U-^{14}C]glucose at various times (see arrows) before and after being refed a 210-mg 58% glucose test meal. Mice in group 1 (arrow *1*) were injected with tracer glucose after the 25-hr fasting period. Mice in groups 2–4 (arrows *2*, *3*, and *4*) were injected at 5, 50, and 100 min, respectively, after they began to eat their 4-min test meal. The number of mice injected with [U-^{14}C]glucose is shown in parentheses in the table. "Carcass" refers to extrahepatic and hepatic tissues in toto where liver data are not separately indicated. Mice were trained to gorge their meals for 4–5 days prior to the experiment. I.D., injected dose; F.A., fatty acids.



GROUP	FED	% I.D. → F.A.* CARCASS	LIVER
1	NO	0.22 ± 0.023 (4)	0.15 ± 0.047 (4)
2	YES	1.5 ± 0.099 (3)	——
3	YES	4.2 ± 0.69 (4)	0.94 ± 0.19 (4)
4**	YES	4.0 ± 0.57 (4)	0.84 ± 0.30 (4)

* IN 30 min AFTER I.V. INJECTION
** 15 min INCORPORATION X 2

FIG. 2. Mean plasma glucose concentrations and incorporation (mean % of injected ^{14}C ± SE) of intravenously injected [U-^{14}C]-glucose into total lipid fatty acids of hepatic and extrahepatic tissues of mice before or after ingestion of *two* successive 210-mg 58% glucose test meals. Each plasma glucose concentration is the mean (± SE, vertical bars) of determinations using six mice. Group numbers (see arrows and table below the figure) correspond to fasted and refed mice injected with tracer at the times indicated. Mice in group 1 (arrow *1*) were injected with tracer glucose after a 25-hr fasting period. Mice in groups 2–4 (arrows *2*, *3*, and *4*) were injected 50, 85, and 100 min, respectively, after they began to eat their first 4-min test meal. The number of mice injected with [U-^{14}C]-glucose is shown in parentheses in the table. "Carcass" refers to both hepatic and extrahepatic tissues where liver data are not separately indicated. Mice were trained to gorge their meals for 4–5 days prior to the experiment. I.D., injected dose; F.A., fatty acids.

[1] Our diet was deficient in essential fatty acids and in fat-soluble vitamins. However, we do not know whether these deficiencies were responsible for the high morbidity. The amount of food ingested during the 2-hr feeding period was only 2.5 g/mouse, which is about two-thirds the quantity of this diet ingested ad lib. by nibbling mice. Those animals that survived the training period maintained their weight at a nearly steady level after the first several days of weight loss.

TABLE 1. Effect of refeeding on the incorporation of [1-^{14}C]acetate into total lipid fatty acids in vitro

Tissue	^{14}C Incorporated into Total Lipid Fatty Acid		Relative Incorporation, Refed/Fasted
	Fasted	Refed	
	% of added ^{14}C		
Adipose[a]	0.49	46	
	1.0	61	
	1.7	64	
	1.8	68	
Mean ± SE	1.2 ± 0.83	60 ± 4.8	50
Liver[b]	0.28	2.1	
	0.40	6.2	
	0.43	7.3	
	0.70	12.0	
Mean ± SE	0.45 ± 0.088	6.9 ± 2.0	15

All mice were trained meal eaters and had been fasted 22–24 hr. The refed mice ate 1.5 g in 1–2 hr and were killed 30–60 min after refeeding. Tissues were incubated with the labeled acetate for 60 min.

[a] Paired epididymal fat pads from individual mice; tissue wet weights averaged (± SE) 59 ± 22 mg (fasted) and 85 ± 24 mg (refed). Tissue weights ranged from 14 to 118 mg/mouse in the two groups of mice.

[b] Liver slices from individual mice; tissue wet weights averaged (± SE) 399 ± 25 mg (fasted) and 522 ± 38 mg (refed).

Both hepatic and extrahepatic tissue fatty acids were more highly labeled in the refed mice after injection of the tracer glucose; however, the response in extrahepatic tissues exceeded that in hepatic tissues.

The feeding of a second 58% glucose test meal (210 mg) 45 min after a first test meal (210 mg) did not cause alimentary hyperglycemia the second time (Fig. 2). As shown below, glucose in the second test meal was rapidly absorbed; therefore, the suppression of hyperglycemia after the second meal was due to an increased capacity of tissues to remove the newly absorbed glucose from the circulation. Thus, glucose was "utilized" as fast as it was absorbed after the second test meal, and the body glucose was in a steady state during all four studies of fatty acid synthesis from tracer [U-^{14}C]glucose (groups 1–4, Fig. 2). As shown in Fig. 2, the incorporation of tracer [U-^{14}C]glucose into both hepatic and extrahepatic tissues was increased markedly after ingestion of the two test meals. The incorporation of [U-^{14}C]glucose into extrahepatic tissue fatty acids in groups 3 and 4 (Fig. 2) after ingestion of two meals was about 20-fold greater than in fasted mice. The animals in group 4 of Fig. 2 and in group 4 of Fig. 1 were injected 100 min after they began to eat their first or only test meal, respectively; yet the incorporation of [U-^{14}C]glucose into extrahepatic fatty acids was about sixfold greater in the mice fed two test meals (Fig. 2) than in those (Fig. 1) fed only one test meal (4.0 vs. 0.70% of the injected ^{14}C).

Plasma glucose and lipogenic responses to the ingestion of a single 1-hr feeding of 58% glucose in trained meal-eating mice

The initial glycemic response to the feeding of a single large glucose-rich meal (Fig. 3) was similar to that seen after ingestion of a much smaller test meal (Fig. 1). However, a secondary hyperglycemic response was also observed between t = 60 and 120 min. The plasma glucose level was still declining between t = 120 and 180 min, but the rate of change was so slow that the body glucose pool could be considered to be in a near-steady, hyperglycemic state during this period. Incorporation of [U-^{14}C]glucose into fatty acids of extrahepatic tissues was 47-fold greater in refed mice than in fasted mice; incorporation into liver fatty acids was 17-fold greater in refed than in fasted mice (Fig. 3).

Absorption of glucose in trained mice fed 58% glucose test meals

The maximum rate of glucose absorption from the gastrointestinal tract in mice trained to eat a large 58% glucose meal was about 3.0 mg of glucose/min (Table 2, expts. 1–3). The average rate of absorption appeared to be somewhat slower when smaller test meals were fed (2.1 mg/min). Within 2 hr after the first of two small test meals was eaten, over 97% of the ingested glucose (244 mg) had been absorbed (Table 2, expt. 5). Data at earlier time intervals would be necessary to establish the exact rate of glucose absorption. Nevertheless, these data demonstrate that the glucose in the second of two test meals (more than 20 times the total body glucose pool of

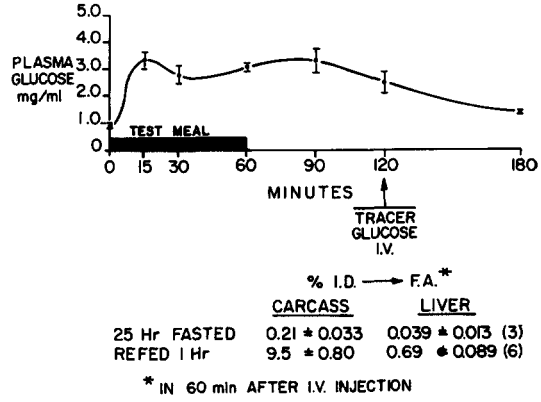


FIG. 3. Mean plasma glucose concentrations and incorporation (mean % of injected ^{14}C ± SE) of [U-^{14}C]glucose into total lipid fatty acids in hepatic and extrahepatic tissues of mice before or after ingestion of a large meal. Mice were fasted 25 hr or fasted 25 hr and refed a 58% glucose test meal (1.2 g for 1 hr), as shown, and were injected intravenously with [U-^{14}C]glucose in the fasted state or 1 hr after removal of the food cups. Each plasma glucose value is the mean (± SE, vertical bars) of 10 mice, except n = 7 at t = 15 min and n = 9 at t = 90 min. Mice were trained to gorge their meals for 10–11 days prior to the experiment. I.D., injected dose; F.A., fatty acids.

TABLE 2. Glucose absorption after ingestion of 58% glucose test meals by trained meal-eating mice

Expt. (No. of Mice)	Time Gastrointestinal Tract Removed	Glucose,[a] Ingested	Glucose, Residual, Gastrointestinal Tract	Rate of Absorption
	min[b]	*mg*[c]	*mg*[c]	*mg/min*[c]
60-min meal				
1 (3)	181	660 ± 79	277 ± 61.2	2.1 ± 0.084
2 (4)	184	930 ± 67	396 ± 50.2	2.9 ± 0.43
3 (6)	60	640 ± 67	426 ± 37.6	3.5 ± 0.56
1–3 (13)		730		3.0 ± 0.32
4-min nibble, once				
4 (4)	130	122	0.31	*d*
4-min nibble, twice				
5 (8)	115[e]	2 × 122[e]	6.24 ± 0.429	2.1 ± 0.18

[a] The actual amount of food ingested can be calculated by dividing these values by 0.58 since the test meal contained 58% glucose.
[b] After start of test meal.
[c] Mean ± SE.
[d] Animals were killed at too late a time to permit an accurate estimate of the glucose absorption rate. Based on expt. 5, all of the ingested glucose would have been absorbed within 58 min.
[e] These mice were given two test meals each; the second was offered 45 min after the start of the first. The gastrointestinal tract was removed 115 min after the first test meal was started.

fasted animals) was almost completely absorbed during the experiments described above. Despite the massive load of glucose which disappeared from the gastrointestinal tract, no appreciable alimentary hyperglycemia was observed after ingestion of the second test meal (Fig. 2).

Plasma glucose specific activity–time curves following injection of [U-^{14}C]glucose in fasted and refed meal-eating mice

Plasma glucose specific activity–time curves for fasted and refed mice are shown in Fig. 4 (single 210-mg test meal), Fig. 5 (two 210-mg test meals), and Fig. 6 (one 1.2-g test meal). These correspond to the experiments shown in Figs. 1, 2, and 3, respectively. The curves tended to be lower and steeper in all refed mice, which indicates that the body glucose pool sizes of the hyperglycemic mice were elevated and turning over at a greater fractional rate. A more quantitative summary of body glucose irreversible disposal rates (18, 20, 21) based upon the data in Figs. 4–6 is given in Table 3. The irreversible disposal rates of glucose C in fasted mice (130–160 μg C/min/20 g body wt) were increased three- to sixfold following ingestion of the test meals. However, these rates were considerably below those expected from estimates which were based upon the maximal rates of glucose absorption from the gastrointestinal tract (1200 μg C/min).

Flux of glucose C into total body fatty acids in fasted and refed trained meal-eating mice

The data of Figs. 1–6 were used to calculate (10, 17) the incorporation of [U-^{14}C]glucose into all "end products" (18) and to estimate the flux of glucose C into total fatty acids. The results of these calculations are shown in Table 3. As can be seen from the right-hand column, the flux of glucose C into fatty acids increased more than 10-fold within an hour after one small test meal, 60-fold after two small test meals, and 200-fold after one large test meal. The much greater value after one large test meal is consistent with those values obtained after one or

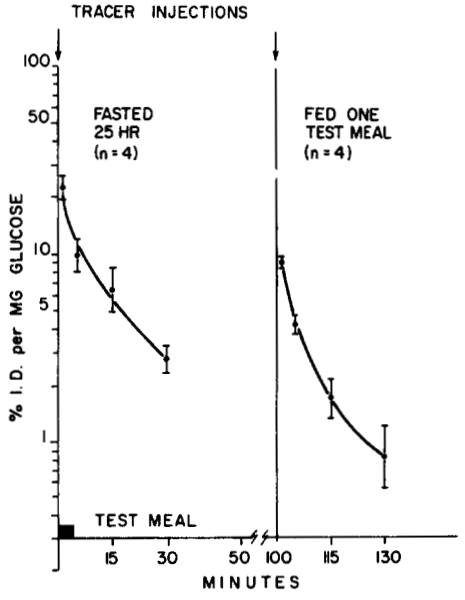


FIG. 4. Mean plasma glucose specific activity–time curves in mice after 25-hr fasting or after 25-hr fasting and refeeding of a test meal. Each value is the mean (± SE, vertical bars) observed at 1, 5, 15, and 30 min after injection. Specific activities have been adjusted to 20 g body wt. (See Fig. 1 for other details.) I.D., injected dose of [U-^{14}C]glucose.

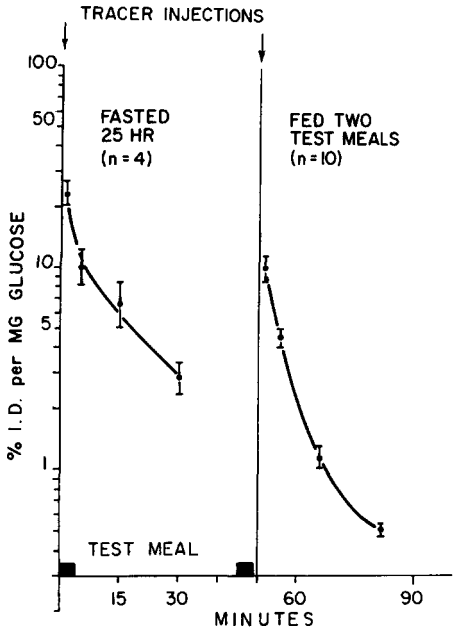


FIG. 5. Mean plasma glucose specific activity–time curves in mice after 25-hr fasting or after 25-hr fasting and refeeding two test meals. Each value is the mean (± SE, vertical bars) observed at 1, 5, 15, and 30 min after injection. Specific activities have been adjusted to 20 g body wt. (See Fig. 2 for other details.) I.D., injected dose of [U-^{14}C]glucose.

two test meals, for between the time mice began to eat the large test meal and the midpoint of the tracer study, the amount of glucose actually absorbed was equivalent to that contained in four small test meals. Thus, the lipogenic capacities of both hepatic and extrahepatic tissues, especially the latter, increase dramatically with each successive nibble. Although we observed, in vitro (Table 1), a very large increase in the lipogenic capacity of epididymal fat pads from mice that had ingested a 1-hr test meal, almost negligible quantities of ^{14}C-labeled fatty acids were found in this tissue after intravenous injection of [U-^{14}C]glucose into mice that had eaten a 1-hr test meal[2] and that showed a 200-fold increase in glucose C flux to total lipid fatty acids compared with the fasted controls. This observation is consistent with the earlier findings of Patkin and Masoro (22) and of De Freitas and Depocas (23) in rats. These authors concluded that epididymal fat was not an important site of fatty acid synthesis from glucose in fed rats. Our findings indicate that in mice, too, the epididymal fat pad is not capable of synthesizing large quantities of fatty acids from glucose, even when mice are trained meal eaters and their meals consist of a high carbohydrate, fat-free diet known to promote lipogenic activity. These observations do not rule out the possibility that the large incorporation of [^{14}C]glucose into fatty acids of extrahepatic tissues of refed mice represents lipogenic activity in white adipose tissue other than epididymal fat.

[2] Baker, N., and R. J. Huebotter. Unpublished observations.

DISCUSSION

Earlier in vivo studies of lipogenesis from glucose C have been largely restricted to postabsorptive or fasted animals in order to avoid the mathematical complications associated with tracer studies in non-steady states such as those expected during alimentary hyperglycemia. Yet, in order to study the possible immediate consequences of ingested carbohydrate, quantitative measurements are required immediately before and at various times after ingestion of one or more test meals. In the present study we have carried out such measurements of lipogenic rates in fasted and refed mice by restricting our isotopic experiments to time intervals in which a nearly constant level of body glucose existed, even during periods in which glucose was being absorbed rapidly from the gastrointestinal tract.

Our study shows that, although there is an extremely rapid and large increase in the flux of glucose C to fatty acids shortly after a trained meal-eating mouse nibbles a small test meal of 58% glucose, the response is comparable to that previously reported (10) in untrained fasted mice of the same strain. This observation indicates that the previously published enzymic adaptations, which require days of training during the conversion of nibblers to meal eaters (4, 24), are not required in order to increase the flux of glucose C into fatty acids. Rather, the ingestion of glucose (7, 25) appears to bring about changes in the flux of glucose C to fatty acids by control mecha-

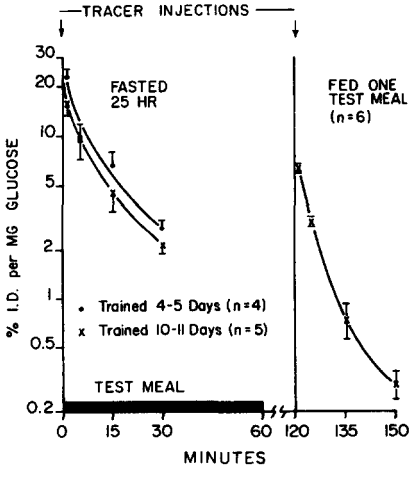


FIG. 6. Mean plasma glucose specific activity–time curves in mice after 25-hr fasting or after 25-hr fasting and refeeding of a 1.2-g test meal. Each value is the mean (± SE, vertical bars) observed at 1, 5, 15, and 30 min after injection. Specific activities have been adjusted to 20 g body wt. (See Fig. 3 for other details.) I.D., injected dose of [U-^{14}C]glucose.

TABLE 3. Rates of total body fatty acid synthesis from glucose in trained meal-eating mice.

Expt.[a]	Irreversible Disposal Rate of Glucose C[b]	^{14}C Incorporated into: "End Products"[c]	^{14}C Incorporated into: TLFA[d]	Flux of Glucose C into TLFA[e]
Fasted				
Expt. I	132	78 (30)	0.37 (30)	0.63
Expt. II	156	98 (60)	0.25 (60)	0.40
Refed one 210-mg meal[f]				
Expt. I 5 min			0.57 (30)	
50 min			1.2 (30)	7.3[g]
100 min	440	72 (30)	1.0 (30)	6.1
Refed two 210-mg meals[f]				
Expt. I 50 min	520[h]	75 (30)[h]	1.5 (30)	10
85 min	520	75 (30)	5.1 (30)	35
100 min	520	75 (30)	2.4 (15)	27 (max.)
Refed one 1260-mg meal[f]				
Expt. II 120 min	840	97 (60)	10.2 (60)	87

All mice were fasted 25 hr; when refed they were given a fat-free, 58% glucose diet.

[a] Dietary state, experiment number, and time interval between start of first meal and [U-^{14}C]glucose injection (i.v.).

[b] R_1, μg of glucose C/min/20 g.

[c] $q_n(t)$, % of injected ^{14}C incorporated as calculated at the times (min) indicated in parentheses.

[d] $q_{fa}(t)$, % of injected ^{14}C incorporated at times (min) indicated in parentheses.

[e] R_2, μg of glucose C/min/20 g.

[f] 210-mg meals were ingested within 4 min; when two meals were given, the second meal was offered 45 min after the first. The 1260-mg meal was eaten within 60 min.

[g] Assumes R_1 and $q_n(t)$ are equal to those of the experiment carried out at 100 min after refeeding.

[h] Valid plasma glucose specific activity data were obtained from 10 of the 11 mice fed two meals. Similar curves were obtained at all three times studied. Mean values for R_1 and $q_n(t)$, based on the composite data, are shown here.

nisms which must operate so rapidly that they influence the lipogenic rate during the time that dietary glucose is actually being absorbed from the gastrointestinal tract. The flux of glucose C to fatty acids appears to increase as long as dietary carbohydrate and perhaps other substances are being ingested and absorbed. Thus, when trained fasted mice absorbed glucose from two test meals or from the equivalent of four test meals, the flux of glucose C into fatty acids was increased 60-fold and 200-fold, respectively, over the fasting levels; in contrast, the increase was only 10-fold after one test meal. Although we are suggesting here that the lipogenic response of trained meal eaters after ingestion of a test meal does not differ markedly from that seen in untrained nibbling mice, there are distinct advantages to working with the trained animals. For example, they ingest their test meals in a shorter period of time and with greater reliability than do untrained animals, even when the latter have been previously fasted. Moreover, the trained meal eater will eat multiple test meals much more consistently and at shorter intervals than will untrained mice, at least in our laboratory, which is a well-lit, somewhat noisy room.

The study of the effects of multiple test meals on glucose metabolism, which heretofore has been restricted largely to glucose tolerance tests (26), is of considerable practical importance. The nibbling mouse is, in fact, a multiple *meal eater* as we have noted previously (10). Throughout the night he may ingest at least 10 times as much glucose as is present in his total body glucose pool each time he nibbles food for several minutes. The manner in which the mouse disposes of each of these large loads of glucose is a function of the time interval between "nibbling" and of the nonglucose constituents in the diet (2, 25, 27). In the present study one small test meal of 58% glucose was found to suppress completely the hyperglycemic response expected from a second equal test meal ingested 40 min later. This is especially remarkable if one considers that the feeding of comparable loads of solid glucose or of aqueous glucose solutions will cause the plasma glucose to rise to about 1000 mg/100 ml in both trained meal eating mice and in untrained nibbling mice.[2] The suppression of the hyperglycemic response was shown not to result from malabsorption of glucose. Rather, glucose was being removed from the extracellular fluid as rapidly as it was being absorbed from the gastrointestinal tract after the ingestion of the second test meal. Clearly, the ingestion of one meal produces biochemical changes which profoundly influence the rates of glucose metabolism after ingestion of subsequent meals. The tissues involved, the hormonal dependence, and the molecular basis of the control mechanisms remain to be elucidated.

Although enzymatic studies will be required in order to answer many of these questions, the techniques which we have employed in this study should prove useful in the study of dietary and hormonal factors that regulate the biochemical pathways of glucose metabolism in the living, unanesthetized animal.

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