Equally rapid activation of lipogenesis in nibbling and gorging mice

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Abstract Minimal average rates of exogenous glucose-C conversion to whole body, total lipid fatty acids were measured in nibbling and gorging mice. Gorgers trained to eat 1 meal/day (8–10 AM) were fasted 22–24 hr and given [^{14}C]glucose with pure glucose, 30% glucose in water, or a 58% glucose, fat-free diet. Conversion of glucose-C to total lipid fatty acids increased from 0.6 (fasted) to approximately 20 μg/min/20 g body weight during 40 min after glucose feeding using each test meal. Dietary amino acids were not required for activation of lipogenesis in gorgers. Exogenous glucose-C was incorporated into fatty acids as fast in nibbling mice as in gorgers. This was true after varying all of the following conditions: training period, number of meals gorged, previous fasting time, and diet composition. The total rate of fatty acid synthesis from body glucose-C during absorption of a glucose load was also estimated in previously fasted nibbling and gorging mice. These estimates were based on composite, serial measurement of both plasma glucose specific activities and ^{14}C-labeled fatty acids. The total rate of fatty acid synthesis from both exogenous and endogenous glucose-C was only 15% higher than the rate from exogenous glucose-C between 10 and 40 min. No significant differences between nibblers and gorgers were found.

Supplementary key words glucose · fatty acid synthesis · meal eaters · obesity · body fat · carbohydrate · glucose absorption · blood glucose

Many workers have studied lipogenesis in gorging and in nibbling animals under a variety of experimental conditions. Almost without exception the conclusion has been reached that lipogenesis is much more rapid in gorging than in nibbling rats (1–4). Recently, we presented evidence that the flux of glucose-C into total lipid fatty acids (TLFA), as measured after intravenous injection of tracer [U-^{14}C]glucose in mice, was increased by about an order of magnitude in both nibblers (5) and in gorgers (6) within 2 hr after the previously fasted animals ingested a small, 58% glucose test meal.

Another technique for measuring lipogenic activation is to compare the flux of endogenous glucose-C to TLFA in starved mice with the average minimum flux of exogenous (fed) glucose-C to TLFA during a brief period after ingestion of a [U-^{14}C]glucose-labeled test meal (7). We have recently used this approach to study fatty acid synthesis in nibbling mice (7) and found that lipogenesis (average flux of glucose-C to TLFA) was activated about 50- to 60-fold during the first 40 min after ingestion of a glucose-containing test meal. We have now carried out a parallel study in gorging mice. Our studies show that activation of lipogenesis in previously fasted mice occurs to approximately the same extent in nibbling and gorging mice after the ingestion of a small glucose test meal. Measurements of plasma glucose specific activity after ingestion of the labeled glucose test meals establish that differential dilution of the tracer by endogenous glucose is not responsible for our failure to observe marked hyperlipogenesis from dietary glucose-C in the gorging mice.

MATERIALS AND METHODS

Animals

Male mice (strain 129/J, Jackson Laboratory, Bar Harbor, Maine) that had been eating Purina Lab Chow ad libitum and housed 10/cage were placed in individual metabolism cages for training and adaptation to a controlled diet (see below). They were given a 58% glucose, fat-free diet ad libitum for 2 days prior to the training period. Water was available ad libitum. Mean body weights and food intake of the experimental animals are summarized in **Table 1.**

Abbreviation: TLFA, total lipid fatty acids.

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TABLE 1. Mean body weights and food intakes of nibbling (N) and gorging (G) mice

Experiment	Dietary Regime	No. of Mice	Body Weight		Food[a] Intake
			Initial (fed)	Final (fasted)	
			g		
I	G	12	14.7 ± 0.26[b]	12.0 ± 0.23	1.4 ± 0.11
II	G	12	18.4 ± 0.58	15.7 ± 0.35	1.6 ± 0.090
	N	10	20.2 ± 0.77	19.5 ± 0.44	4.0 ± 0.26
IIIA	G	6	18.4 ± 0.72	16.0 ± 0.36	3.1 ± 0.082
	N	6	18.4 ± 0.84	17.7 ± 0.74	3.8 ± 0.11
IIIB	G	6	16.6 ± 0.71	16.4 ± 0.94	3.1 ± 0.20
	N	6	17.0 ± 0.11	20.4 ± 0.34	3.6 ± 0.12

[a] Food consumed during last 24-hr period before fasting.
[b] Mean ± standard error.

Training procedure

In Series I and II, and IV, gorging mice were given a fat-free, 58% glucose diet at 8–10 AM each day for 4 days prior to the experiment. Food was not provided during the 22-hr interim, whereas nibbling animals were fed the same diet ad libitum. The semipurified diet consisted of 58% glucose, 22% casein, 6% Hawk-Oser salt mixture, 11.8% non-nutritive cellulose, 2% liver VioBin, and 0.2% vitamin mixture (5). No food was available for the 25 hr immediately preceding the feeding of the labeled test meals (see below).

In Series III-A and -B, half of the mice were trained to gorge their food twice daily, 9–11 AM and 3–5 PM, for 2 weeks. The remaining mice (the nibblers) were trained to consume most of their food between 9 AM and 5 PM, and at a nearly constant rate. This training of nibblers allowed us to control the fasting period for nibblers and gorgers immediately prior to the experiment (5 PM–9 AM) and to insure that the nibblers consumed approximately the same amount of food as the gorgers during the 8-hr period preceding the preexperimental fast. The training of nibblers was carried out as follows. One week before the experimental day, food was removed from the nibblers for 3 days during the light period (5 PM–9 AM). The nibbling mice were again allowed access to food ad libitum (24 hr/day) during the 4 days preceding the experiment. The effect of this regimen was to shift the pattern of ad libitum food intake from 20% of the total daily food between 9 AM and 5 PM to 50–60% during this 8-hr dark period. When food was not removed during the light period for 3 days, the shift in eating pattern was more subtle, with a constant rate of intake over both light and dark periods. We did not study whether reversal of the light cycle was required to reverse the eating pattern.

In Expt. III-A, animals were fed a chow diet (Purina rat chow, pulverized) the entire training period; whereas in Expt. III-B, they were fed chow the first week and the semipurified, 58% glucose diet the second week.

[U-^{14}C]glucose

Uniformly labeled tracer (New England Nuclear, 15.5 mCi/mmole) in 90% ethanol was dried under nitrogen and redissolved in water.

Test meals

In Series I, 150 mg of glucose/20 g body wt was given as one meal in one of three forms: (*1*) 30% aqueous glucose solution; (*2*) solid, neat glucose; or (*3*) the fat-free 58% glucose diet. [U-^{14}C]Glucose was incorporated into all test meals. Shortly before scheduled feedings, 50 μl of the labeled glucose (1 μCi) dissolved in water, was added to dry, weighed test meals, in food cups, and the water was allowed to evaporate partially. Gentle mechanical mixing of the air-dried, but still moist, test meals yielded a rather homogenous mixture and helped insure proportional ingestion of both labeled and unlabeled glucose. The 30% glucose was given by gastric intubation via an animal feeding needle.

Since Series I showed that glucose feeding by gastric intubation gave a high rate of lipogenic activation (Fig. 2), and since this method of feeding labeled glucose test meals is the simplest and most reproducible method for feeding untrained nibblers, this dosing procedure was used in Series II, III, and IV. The test meal (120 mg glucose/20 g body wt containing 5 μCi of [U-^{14}C]glucose) was given as a 30% aqueous glucose solution.

In vivo studies

Series I. After ingestion of a test meal, serial blood samples were drawn at various times from an ophthalmic venous capillary sinus of each mouse (8,9). Mice were decapitated 40 min after ingestion of test meals. Alimentary tracts (stomachs through large intestines) were quickly dissected and immersed in liquid nitrogen. Immediately thereafter, carcasses were immersed in liquid nitrogen. All frozen specimens were stored at −16°C for subsequent analysis. Urine was collected on Parafilm at the time of decapitation, drawn into capillaries, sealed, frozen, and later analyzed for total glucose content and radioactivity (10, 11). Plasma was deproteinized and glucose was measured enzymatically (10, 11). All carcasses were saponified, the unsaponifiable lipids extracted and discarded, and the fatty acids extracted and assayed for radioactivity using methods described previously (5). Glucose was extracted from the alimentary tracts and contents by homogenization in cold 70% ethanol followed by brief heating at 80°C. The supernatant liquid was deproteinized and glucose was estimated enzymatically (6).

Series II and III. Mice were decapitated 15 min after gastric intubation of the test meals. The stomach was washed out and the carcass was immediately placed in 40 ml of 30% aqueous KOH and digested for 90–120 min at 90–100°C. Total body fatty acids were extracted with petroleum ether as previously described (5). Varying the ratio of petroleum ether: aqueous ethanol from 1:1 to 3:1 in the final extraction of TLFA did not significantly affect the radioactivity found. Random checks showed 10–20% of the ^{14}C was in nonsaponifiable lipids, additional assurance that label was not lost through incomplete saponification. Addition of ethanol (50% final concentration) to the KOH digest and additional refluxing at 90°C for 4 hr increased recovery of labeled fatty acids by less than 10%.

Series IV. Previously fasted mice were fed their test meals by gastric intubation. Blood samples were taken at various times either from the ophthalmic venous sinus or after decapitation according to the following schedule: three nibblers and three gorgers, orbital sinus blood at 7.5 min, decapitated at 10 min; three nibblers and three gorgers, orbital sinus blood at 15 min, decapitated at 25 min; and three nibblers and three gorgers, orbital sinus blood at 35 min, decapitated at 45 min. Whole body fatty acids were obtained as in Series II and III, except that stomach contents were not washed out prior to saponification. Whole body ^{14}C-labeled fatty acid at 15 min (nine nibblers, nine gorgers), and at 45 min (six nibblers, six gorgers) was measured in separate studies in which blood samples were not obtained. Zero time plasma glucose concentration was determined as in Series I. Zero time values for plasma glucose concentration were determined using a separate group of three nibblers and three gorgers to minimize possible effects of excitement on lipogenic activation in the tracer studies. Plasma glucose specific activity was estimated as described earlier (10, 11); however, longer chromatogram strips were used and developed 18 cm to minimize contamination from lactate; a narrow band (1.2 cm) at the glucose R_f was used for analysis.

Radioactivity

Radioactivity measurements were performed in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill.). All ^{14}C samples were dissolved and counted in scintillator solution (footnote 3 in ref. 12). Appropriate C^{14} standards were simultaneously assayed in each case and quench corrections applied when necessary.

Calculations of glucose-C flux to total lipid fatty acids (TLFA)

Our theoretical approach was exactly the same as that described and used in a previously published study of lipogenic activation in nibbling mice (7). Briefly, the approach was as follows. Mice were fasted for 24 hr and then either injected with tracer [U-^{14}C]glucose i.v. or they were fed a glucose-rich test meal (above) labeled with [U-^{14}C]glucose. The endogenous flux of glucose-C to TLFA in the fasted gorging mice was taken from a previously published (6) semicompartmental analysis (12) of plasma glucose specific activity data and of the ^{14}C incorporated into TLFA (whole body) 30 min after tracer injection. The minimum, average flux of fed glucose-C to TLFA was estimated (7) during the 15- or 40-min interval between the time the mice started to eat (or were force-fed) and the time of decapitation. Data for this estimate were ^{14}C incorporated into TLFA and the specific activity of the fed glucose.

In Series IV additional data were obtained that permitted us to estimate the total rate of endogenous and exogenous glucose-C conversion to fatty acids between 10 and 45 min after feeding the labeled test meal (R_{21} = mg glucose-C converted to fatty acids/min/20 g body wt). The data needed for this calculation were a_{gluc}, plasma glucose specific activity (mean cpm/mg glucose-C, t = 10–45 min), and $\Delta q/\Delta t$, the rate of ^{14}C incorporation into

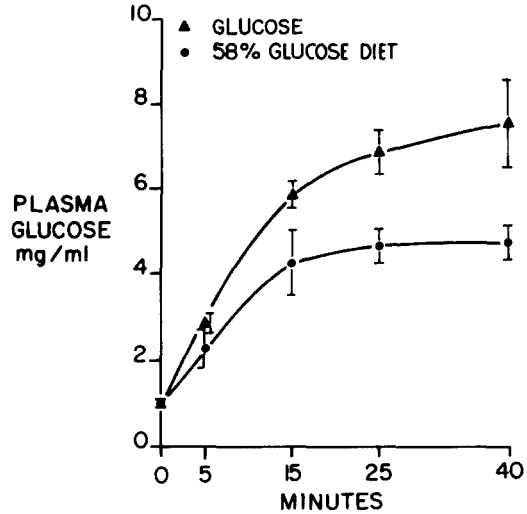

Fig. 1. Plasma glucose concentration after ingestion of a single, glucose-rich test meal. The mice had been maintained on a 2 hr/day feeding schedule for 4 days, fasted 25 hr and re-fed 150 mg of glucose/20 g body wt. as pure glucose (△), or as a 58% glucose, fat-free diet (●). Each value is the mean (± SE, vertical bars) of eight (△) or four (●) mice. Four of the mice fed pure glucose were offered solid glucose. The other four mice were intubated with a 30% aqueous glucose solution. No significant difference between the latter groups was observed; therefore, the data were combined.

total lipid fatty acids during that 35-min period (cpm/min/20 g body wt). The calculation was simplified by the empirical finding that both a_{gluc} and $\Delta q/\Delta t$ were approximately constant during this period. The calculation is that used by other workers for a 2-compartment model (glucose → fatty acids) in which the contents of the first compartment are in a steady state and at a constant specific activity and the second compartment is considered as an "end-product." In that case, $dq_2/dt = R_{21}a_1$ (where the subscripts refer to the first and second compartments, respectively). By substituting $\overline{\Delta q/\Delta t}$ for the average of dq_2/dt and $\bar{a}_{gluc}$ for the average specific activity of compartment 1 during the 35-min period of study, we obtain

$$R_{21} \sim \frac{\overline{\Delta q/\Delta t}}{a_{gluc}} \qquad \text{Eq. 1}$$

during the same period.

Among the assumptions that we have made in this and in earlier studies (7), is that of rapid mixing of plasma with extraplasma glucose. Evidence to support this assumption has been presented in several of our earlier reports (for refs., see 5–7).

RESULTS

Plasma glucose concentrations

As shown in **Fig. 1,** the ingestion of a small glucose test-meal (150 mg/20 g) caused a marked hyperglycemia in starved gorging mice. After 40 min, the hyperglycemia was about 60% greater in mice fed pure glucose (either as a solid meal or intubated as a 30% aqueous solution) than in mice fed a fat-free 58% glucose diet. The hyperglycemic responses were similar to those reported previously in nibbling animals fed either pure glu-

TABLE 2. Influence of test meal composition on the absorption and utilization of dietary glucose in gorging mice, Series I

	Glucose (mg)						
			In Total Body[b]				
Test Meal[a]	Ingested	Absorbed from Gastrointestinal Tract in 40 min	Before Eating (a) (t = 0)	After Eating (b) (t = 40 min)	(b − a) Increment	Excreted in Urine in 40 min[c]	Utilized in 40 min[d]
Starved 25 hr	0	0	4.2				13
Solid glucose (neat)	80 ± 5.5	72 ± 2.5	4.2	30	26	2.1 ± 0.29	44
30% Aqueous solution by gastric intubation	90 ± 0	58 ± 6.6	4.2	30	26	1.9 ± 0.58	30
58% Glucose solid complete diet	72 ± 9.0	37 ± 8.5	4.2	23	19	1.0 ± 0.60	17

Four mice per group, average body wt 12.0 g; each value is the mean ± SE (n = 4 unless otherwise indicated).

[a] Units: 150 mg [U-^{14}C]glucose/20 g body wt intubated or offered as solid meals. Values in cols. 2–8 are based on actual mouse wts.

[b] Total body glucose before eating (7.0 mg/20 g body wt) was calculated from a separate isotope dilution study using another group of 25-hr fasted mice (6). Values were then adjusted for mean body wt of the mice used in the present study. Total body glucose after eating was estimated as follows:

$$\frac{[\text{plasma glucose (40 min after eating)}]}{[\text{plasma glucose (before eating)}]} \times \text{column a}$$

[c] n = 3.

[d] Glucose utilization estimated as follows: (glucose absorbed) − [(glucose increment in total body) + (glucose excreted in urine, in 40 min)]. Value for the fasted mice is based on calculation of irreversible disposal rate from intravenously injected [U-^{14}C]glucose in an earlier study (6).

cose or the 58% glucose test meal (7). However, the results differed from another study using gorging mice fed the 58% glucose diet only (6). In the latter study, the maximum hyperglycemia did not persist as long as in the experiments shown here. The cause of this variation is not known, but it seems unrelated to the manner of eating, for this variability has been noted subsequently in nibbling mice as well as in trained gorgers.[2]

Two aspects of the plasma glucose curves shown in Fig. 1 are especially relevant to the lipogenic studies reported below. First, the plasma glucose concentration during the first 15-min period following feeding is, on the average, only half that during the next 25 min. Thus, one would expect the rates of both glucose utilization and lipogenesis to be greater during a 40-min (Series I) than during a 15-min study (Series II and III). Second, the average specific activity of body glucose will be lower during the first 15 min after a labeled glucose test meal than during the next 25-min period. This follows from the facts that at zero time the plasma glucose specific activity is zero and that when glucose concentration is maximal, or shortly thereafter, the body glucose pool specific activity should approximate that of the labeled test meal (13, 14). This has been confirmed in mice in a separate study.[3]

Glucose absorption

The lower plasma glucose levels found in mice fed a 58% glucose diet compared to those fed the same amount of pure glucose can be attributed to the slower rate of glucose absorption in animals that ate the complete, fat-free diet. This is shown by the results in the 3rd column of **Table 2.** Only half as much glucose was absorbed by animals that ate the 58% glucose test meal as by those that ate about the same quantity of pure, solid glucose.

Utilization of exogenous glucose

By measuring (*1*) the unabsorbed glucose and, by difference, the glucose absorbed; (*2*) the plasma glucose before and 40 min after ingestion of a test meal, from which one may estimate the glucose that has accumulated in the total body glucose pool (Table 2, see also Ref. 7); and (*3*) the amount of glucose excreted in the urine in 40 min, one may estimate the amount of ingested glucose that was converted to other products; i.e., the exogenous glucose utilized in 40 min. These values are given in Table 2.

[2] Baker, N. Unpublished observations.
[3] Baker, N., and D. B. Learn. Unpublished observations.

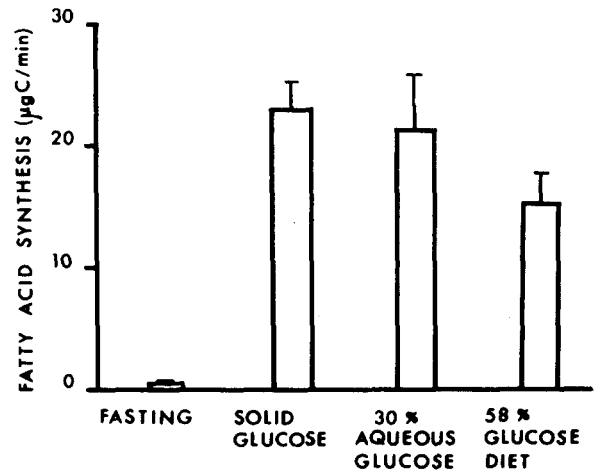

Fig. 2. Flux of glucose carbon into total lipid fatty acids in gorging mice fasted 25 hr or fasted 25 hr and re-fed a test meal (150 mg of glucose per 20 g body wt) containing the tracer. Rates are μg of glucose-C converted to fatty acid per minute per 20 g body wt in experiments of 40-min duration. Each value is the mean (± SE, vertical bars) of four mice.

Average rates of exogenous glucose utilization were greatest in mice fed pure, solid glucose (44 mg/40 min = 1.1 mg/min) and lowest in mice fed the 58% glucose test meal (17 mg/40 min = 0.43 mg/min). Glucose was utilized more rapidly in re-fed gorgers than in starved animals. The extent of this increase in glucose utilization rate following the ingestion of pure glucose, solid or aqueous, was similar to that previously reported for nibbling mice (7).

Activation of lipogenesis in gorging mice

The average minimal flux of glucose-C to total lipid fatty acids in the whole body during the 40 min following re-feeding of three different test meals was measured in the gorging mice. These values, expressed as μg of glucose-C converted to fatty acids/min/20 g body wt are shown in **Fig. 2,** and are compared with values previously reported for starved gorgers (6). The results were similar in mice re-fed each diet (15, 21, and 23 μg of glucose carbon/min/20 g) and were greatest in mice re-fed pure glucose. Fatty acid synthesis from glucose-C in the latter case was 40 times greater than the values previously reported for starved gorgers (6).

The increased rates of glucose-C conversion to fatty acids that occurred in gorging mice after a meal resulted not only from increased rates of glucose utilization but also from a greatly increased fraction of the utilized glucose directed towards fatty acid synthesis. From the results summarized in Table 2 and Fig. 2, we may calculate that only 0.3–0.4% of the glucose utilized was converted to fatty acids in the fasting state. After ingestion of

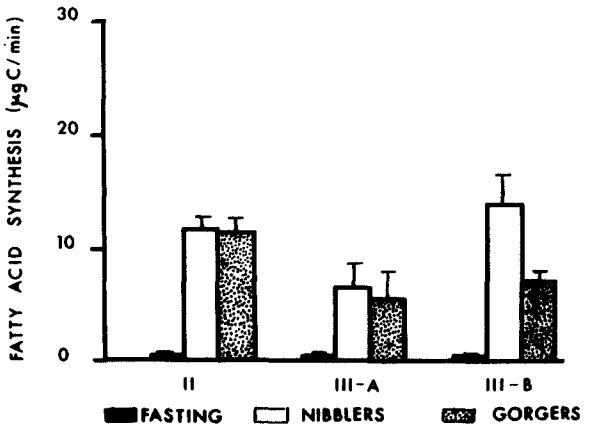

Fig. 3. Flux of glucose carbon into total lipid fatty acids in nibbling and gorging mice fasted 25 hr or fasted 25 hr and re-fed a 30% glucose solution (120 mg glucose/20 g body wt), containing tracer, by gastric intubation. Rates are μg of glucose-C converted to fatty acid per minute per 20 g body wt in experiments of 15-min duration. Each value is the mean (± SE, vertical bars) of 6–12 mice. See text for details of feeding regimens.

glucose, the percentage of utilized exogenous glucose converted to fatty acids increased to about 4% (3–5%) in the gorgers, regardless of the type of test meal. From previously published data (7), we have calculated that this order-of-magnitude shift in the pattern of glucose utilization that occurred almost as soon as the animals ingested a small glucose-containing test meal was, on the average, greater in nibblers than in gorgers for each kind of test meal.

Incorporation of dietary glucose-C into fatty acids in nibbling and gorging mice

Results of the short-term (15 min) studies (Series II and III) are shown in **Fig. 3.** The reader should bear in mind that the rates shown in the figure are average, minimum values and could have been expressed simply as percent of the test meal glucose-C incorporated into total body fatty acids in 15 min. Because of time delays for glucose absorption, insulin release, activation of lipogenic enzymes, and metabolic transformations, the incorporation is expected to be very slow initially. Experiments that confirm this point are described below (Series IV). Nevertheless, by studying relative "rates" of lipogenesis from dietary glucose-C in nibblers and gorgers at such early times, one minimizes the complication of redistribution within the body of newly synthesized fatty acids. Identical "rates" of glucose-C conversion to fatty acids were found in both nibbling and gorging mice in Series II. Series III showed that neither a longer training period (2 weeks) nor feeding twice daily to reduce emaciation and to decrease the periods of intermittent starvation increased lipogenesis from glucose-C in gorgers relative to nibblers. In fact, the gorgers that were allowed to eat 2 meals/day for 2 weeks were eating twice as much food/day as those that ate only 1 meal/day (Table 1, Expts. II, IIIA, and IIIB); yet, "2-meal gorgers" incorporated only half as much of the dietary glucose-C into fatty acids in 15 min as did the "1-meal gorgers."

Total rate of fatty acid synthesis from glucose-C during activation of lipogenesis

The total rate of glucose-C conversion to fatty acids in previously starved nibbling and gorging mice during the period of activation of lipogenesis, shortly after intubation of the labeled glucose test meal, was also found to be the same in nibblers and gorgers. The conditions were identical to the study of Series II, except that data were obtained throughout a 45-min period after gastric intubation of 120 mg of glucose in water, and plasma glucose concentrations and specific activities were measured as a function of time. Values for each parameter at each time were not significantly different from one another in nibblers and gorgers (**Table 3**). Therefore, all data were pooled. Plasma glucose concentration rose from 0.98 mg/ml to a maximum of 6.52 mg/ml at 25 min. The composite curve was very similar to that shown in Fig. 1

TABLE 3. Plasma [^{14}C]glucose specific activities, plasma glucose concentrations and total body ^{14}C-labeled fatty acid in previously fasted nibbling and gorging mice after gastric intubation of [U-^{14}C]glucose[a]

Time[b] (min)	Nibblers (n = 3)	Gorgers (n = 3)
Plasma glucose relative specific activity (percent of dietary glucose specific activity, mean ± SE)		
7.5	68 ± 6.8	72 ± 7.4
10.0	69 ± 7.9	76 ± 9.2
15.0	76 ± 6.4	70 ± 11.5
25.0	87 ± 1.8	82 ± 10.1
35.0	85 ± 9.0	95 ± 0.5
45.0	89 ± 3.2	89 ± 1.1
Plasma glucose concentration (mg/ml, mean ± SE)		
0.0	1.08 ± 0.070	0.88 ± 0.056
10.0	6.19 ± 0.42	4.67 ± 0.91
25.0	7.43 ± 0.49	5.60 ± 1.76
45.0	6.57 ± 0.81	4.93 ± 0.90
^{14}C-Incorporation into total body fatty acids (percent of intubated dose/20 g body wt, mean ± SE)		
10.0	0.059 ± 0.008 (3)	0.083 ± 0.020 (3)
15.0	0.40 ± 0.045 (9)	0.48 ± 0.070 (9)
25.0	0.51 ± 0.20 (3)	0.87 ± 0.47 (3)
45.0	1.7 ± 0.52 (9)	2.5 ± 0.63 (9)

[a] 120 mg/20 g.
[b] Time after gastric intubation.

("glucose"). At 45 min the mean plasma glucose concentration was 5.75 mg/ml and appeared to be nearly constant between 10 and 45 min (average, 5.9 mg/ml); thus, a near-steady state was attained during this 35-min interval.

Plasma glucose specific activities for both nibblers and gorgers were identical, within experimental error, at all times (Table 3). The composite curve, expressed as percent of the glucose specific activity in the test meal, is shown in **Fig. 4.** Plasma glucose specific activity rose rapidly after feeding and within 7-1/2 min was 70% that of the fed [^{14}C]glucose. During the 35-min interval between 10 and 45 min the specific activity remained almost constant (82 ± 6% of the dietary glucose specific activity, mean ± SE), as shown in Fig. 4.

The incorporation of [^{14}C]glucose into total body fatty acids is shown in Table 3 and Fig. 4. Incorporation was low for 10 min after feeding the labeled glucose; however, thereafter the rate of incorporation was nearly linear in both nibblers and gorgers. The percent incorporation into fatty acids varied greatly at 45 min in this particular experiment (gorgers, 2.5 ± 0.63% of the dose, n = 9; nibblers, 1.7 ± 0.52% of the dose, n = 9, mean ± SE). The difference of the means (0.80%) was not statistically significant. Values were nearly identical in the nine nibblers and nine gorgers at 15 min (0.48 ± 0.070 and 0.40 ± 0.045, gorgers and nibblers, respectively). In Series IV the slope for the composite data during the 35-min period chosen for analysis (t = 10–45 min) was 0.057%/min per 20 g body wt (Fig. 4).

From these data and using Eq. 1 (see Calculations above), we estimate that in this particular experiment the overall average rate of conversion of total body glucose-C to fatty acids was 32 μg glucose-C/min/20 g body wt in nibblers and gorgers during the 35-min period. This value is similar to values obtained in present and previous studies of exogenous glucose-C conversion to fatty acids during a 40-min period. Earlier estimates would be erroneously low in that an initial lag, though recognized, was not taken into account. It is now clear from the data in Fig. 4 that the overall rate of conversion of glucose-C to fatty acids is only 15–20% faster than the rate of exogenous glucose-C conversion to fatty acids between 10 and 45 min and that no significant, differential dilution of exogenous glucose-C occurs in the body glucose pool of gorging compared to nibbling mice.

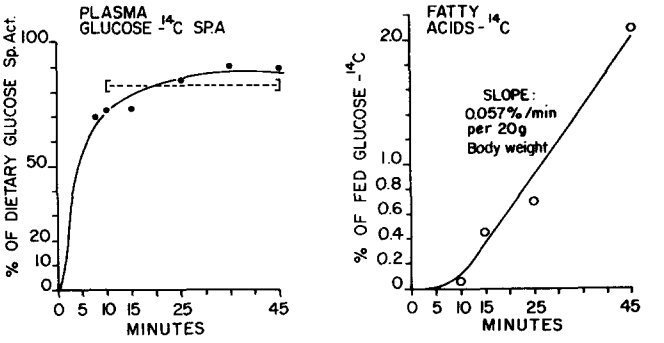

Fig. 4. Composite plasma glucose specific activities and ^{14}C-labeled fatty acids during the first 45-min period following intubation of aqueous [U-^{14}C]glucose in previously starved nibbling and gorging mice. Values for nibblers and gorgers were not significantly different from each other and were combined to obtain a best estimate of plasma glucose specific activity (82 ± 6%, mean ± SE, shown as a dashed line, 18 mice, 9 nibblers and 9 gorgers, t = 10–45 min). Percent incorporation of the fed [^{14}C]glucose into total body fatty acids for the combined nibblers and gorgers is based on data from 24 nibblers and 24 gorgers. Values have been corrected to 20 g body wt (see text).

DISCUSSION

When rats are converted from nocturnal nibblers to daytime gorgers, they have been reported to show dramatic increases in lipogenic capacity (1–4). This phenomenon is of great practical interest, for it has been inferred that a meal-eating pattern may be a major factor in the deposition of excess fat (15–18). However, a critical review of the literature has led us to conclude that there is insufficient evidence available, especially in the intact animal, to evaluate the contribution of hyperlipogenesis to the development of obesity. As an initial step towards reevaluating this problem, we have chosen the mouse as an experimental model and semiquantitative tracer techniques to compare lipogenic rates in nibbling and gorging animals.

We have used two techniques in these experiments. One method estimates the minimum rate of exogenous glucose-C conversion to fatty acids (R_{20}); the second estimates the total rate of exogenous plus endogenous body glucose-C conversion to fatty acids (R_{21}) during the same period. The first method is by far the simpler since it does not require serial measurements of plasma (body) glucose specific activity. Moreover, it gives the same relative information as the more complex technique, and even the absolute values are comparable to those obtained by the more complicated approach. As shown here, R_{20}/R_{21} is about 0.85 in both nibblers and gorgers. That is, the rate of exogenous glucose-C conversion to fatty acids is actually about 85% of the total rate (R_{21}). This is not surprising since most of the glucose that turns over in rodents during periods of intestinal glucose absorption is known to be derived from exogenous glucose (13, 14).

We have concentrated on the early changes that occur following the feeding of a glucose test meal to previously starved mice. It is clear from our studies that a very marked and comparable activation of lipogenic

enzymes occurs in both nibbling and gorging mice within minutes after feeding. An apparent 10-min time delay occurs in these previously starved mice; however, this is due, in part, to greater dilution of the labeled exogenous glucose by the relatively unlabeled body glucose pool at early times. It also must reflect a delay in the time required for absorption of glucose, release and transport of insulin and other messengers, and activation (release of inhibition) of lipogenic enzymes.

However, the major finding of the present study is our failure to observe any dramatic increases in lipogenesis from glucose-C in gorging mice. We have now carried out eight studies of nibbling mice and eight studies of gorging mice under a variety of conditions. The data are summarized in **Table 4** and Fig. 4. Although some of the studies, including several in the present report, have been done at separate times (5–7), composite results establish that both nibbling mice and gorging mice, when previously fasted, respond to a given test meal in a comparable fashion. Under our conditions, no greater rates of fatty acid synthesis from glucose-C were found in gorging mice compared to nibbling mice after they were fed test meals of glucose that contained 20 times the total body glucose. This was true whether the animals were fed a test meal with or without protein, whether the gorgers were trained to eat 1 or 2 meals per day, whether trained 4 or 14 days, whether the training diet was fat-free or regular lab chow, and whether the fasting period was 16 or 22 hr prior to feeding the test meal. Our conclusion also holds true when dilution of the exogenous glucose-C by the body glucose pool in two groups of mice is taken into account. Thus, the rate of fatty acid synthesis from total body glucose-C (as well as from exogenous glucose-C) is not significantly greater in gorging than in nibbling mice under our conditions. On the basis of an entirely independent study of nibbling and gorging (2 meals/day) mice, Favarger and Gerlach (19) also have concluded that there is no dramatic difference in the rates of labeled fatty acid formation from ^{14}C-labeled glucose between the two groups.

In view of the apparent striking difference in the lipogenic response of starved gorging mice and rats (4) to a carbohydrate-rich test meal, one immediately wonders whether the marked weight loss and decreased caloric intake noted in our gorging mice prevented the large hyperactivation of lipogenesis that occurs in gorging rats. We think that this is not the case for two reasons. First, the gorging rats in which a 200-fold increase in adipose tissue lipogenesis has been reported (4) were not pair-fed with the nibbling rats; the gorging rats lost weight and ingested fewer calories than the nibblers. Secondly, when we allowed our gorging mice to consume twice as many calories by training them to consume two large meals a day, weight loss was diminished, but activation of lipogenesis from glucose-C was not enhanced. Thus, lipogenesis is not orders of magnitude faster in gorging than in nibbling mice under the conditions of our experiments. It is enigmatic that two rodent species should respond so similarly with respect to a gorging eating pattern (diminished food intake and initial loss of body weight); yet, in only one species (the rat) does gorging seem to induce a 200-fold greater lipogenic rate from glucose-C than nibbling controls that have consumed far more calories daily than the gorgers. However, it is important to bear in mind that a number of differences exist between the mouse and the rat studies. Some of these variables include the amount of food ingested in the test meal, the relation of the fasting period to the different metabolic rates of each species, the emphasis upon liver and epididymal fat in the rat studies compared to the analysis of the whole mouse total lipid fatty acids, differences in the length

TABLE 4. Rate of glucose carbon conversion to fatty acid in 14 experiments with nibbling and gorging mice

Diet	Training	Test Meal	Length of Study (min)	μg glucose-C to fatty acid per min per 20 g body wt			
				Nibblers	(Ref)	Gorgers	(Ref)
58% Glucose	4 days	Fasted	30	0.56	(5)	0.63	(6)
58% Glucose	4 days	30% aq. glucose	40	33	(7)	21	(P.S.)[a]
58% Glucose	4 days	Solid glucose	40	42	(7)	23	(P.S.)
58% Glucose	4 days	58% glucose diet	40	37	(7)	15	(P.S.)
58% Glucose	4 days	30% aq. glucose	15	11	(P.S.)	11	(P.S.)
58% Glucose	14 days[b]	30% aq. glucose	15	14	(P.S.)	6.5	(P.S.)
Commercial Chow	14 days[c]	30% aq. glucose	15	6.6	(P.S.)	5.3	(P.S.)

[a] Present study (P.S.).
[b] Series IIIB; see text for details.
[c] Series IIIA.

of time after ingestion of the test meal, and the nature of the tracer techniques used. Further studies are required to elucidate the basis of this apparent species difference and to clarify the relationships between gorging patterns of food intake, hyperlipogenesis, and the deposition of fat in various species.

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