Rapid activation and inactivation of fatty acid synthesis from glucose in vivo

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Abstract The flux of glucose carbon to total body fatty acids was measured in unanesthetized mice either after fasting or 50–80 min after they nibbled a small test meal containing 120 mg of glucose (fasted–refed). Flux was calculated from plasma [14C]glucose specific activity curves and from total body 14C-labeled fatty acid 30 min after intravenous injection of tracer [14C]glucose. Mobilization of liver glycogen, changes in the body glucose pool size, and total flux of carbon through the glucose pool during periods of fasting and refeeding were defined. Liver glycogen was almost completely depleted 8 hr after food removal. Body glucose pool size fell during fasting and increased after refeeding the test meal. Irreversible disposal rate of glucose C varied directly with body glucose pool size; but flux of glucose C into fatty acids increased exponentially as body glucose concentration increased. Within an hour after nibbling a small test meal, the flux of glucose C into total body fatty acids increased 700% in mice previously starved for 24 hr. However, flux of glucose C into fatty acids in postabsorptive mice (food removed for 2 hr; livers rich in glycogen) was only about 2% of the value calculated from published studies in which the incorporation of an intubated [14C]glucose load into total body fatty acid was measured in mice. A possible explanation for this phenomenon is presented.

Supplementary key words irreversible disposal · fasting · refeeding · control · lipogenesis · liver glycogen · [14C]glucose · isotope kinetics · mice

A VARIETY of experimental approaches have been used to study the conversion of sugar into fatty acids in vivo in mice and rats (1–10). These and other studies have established that the lipogenic machinery of the body varies with dietary state (11–19). However, the magnitude and time course of the changes in lipogenic rates which take place when rodents start to eat carbohydrate have not been established under any conditions. For example, Jansen and his colleagues (6, 7) have carried out a very thorough study in mice of the effect of fasting and refeeding on the conversion of 14C-labeled glucose loads to fatty acids. Their results were expressed as a percentage of the tracer incorporated into fatty acids; no estimates of the flux (e.g., μg of glucose C/min incorporated into fatty acids) were given. Similar studies of incorporation of tracer [14C]glucose into fatty acids in fasting rodents or in fasted–refed animals have been reported (2, 8–10, 12). If one converts the percentage incorporation found in the two experimental approaches (glucose load vs. nonload) into flux (μg of glucose C converted to fatty acids/min), it would appear that some immediate, dramatic changes in the flux of glucose C into fatty acids must occur each time an animal eats.[1] However, this phenomenon has never been reported in rats and mice. Indeed Yeh and Leveille (19) have recently stated that an immediate increase in flux of glucose C into fatty acids occurs when chicks are given a meal, but that such a rapid response does not occur in rodents. That this phenomenon may have been overlooked is clear not only from the statement of Yeh and Leveille (cited above), but also from the report of Hollifield and Parson, who showed that there was no appreciable change in lipogenic rates in vitro before and immediately after untrained, fasted rats ate a meal, and that an adaptation period of days was required for lipogenic mechanisms to respond rapidly to dietary carbohydrate (13; see also 21).

The present study is the first of a series of reports on the

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[1] For example, Baker and Huebotter (20) reported that negligible [14C]glucose was recovered as fatty acids 15 min after an intravenous injection of a tracer dose into postabsorptive mice. We may calculate that a maximum of 3 μg of glucose C/min could have been converted to fatty acids in those experiments (ad lib. fed mice, but stomachs empty). On the other hand, we may also calculate from the data of Jansen et al. (6) that the formation of fatty acids from glucose was at least 8200% greater than 3 μg of glucose C/min within just 10 min after their mice were given a glucose test meal.

rapid activation and inactivation of lipogenesis by dietary glucose in mice. In it we shall attempt to estimate the flux of glucose C into fatty acids during periods in which the body pool was approximately in a steady state: shortly after a night of ad lib. eating, after a brief fast, after an extended fast, and shortly after a brief nibbling period following an extended fast. It would appear from our in vivo studies, and from those of other investigators, that the major enzymatic mechanisms for synthesizing fatty acids from glucose become activated at the time that glucose is being actively absorbed from the intestine, and that they may become relatively inactive very quickly after the dietary carbohydrate has been absorbed.

MATERIALS AND METHODS

Animals

Male mice (strain 129/J, Jackson Laboratory, Bar Harbor, Maine) weighing 18–26 g were housed 10 to 12 per cage and fed Purina Laboratory Chow and water ad lib. In studies of lipogenesis from glucose, the mice were transferred to individual metabolism cages the day before the experiment and fed as above.

Test meal

A commercial, synthetic, glucose-rich (58%) diet[2] was given as the test meal (see "Experimental approach and theoretical aspects," below).

Tracer injections and blood sampling

[14C]Glucose (New England Nuclear, 1 µCi, uniformly labeled, 5.4 mCi/mmole in 50 µl of water) was injected via a 100-µl syringe into a lateral caudal vein. Radiochemical purity (99%) was established by thin-layer chromatography (TLC) and by data supplied by the manufacturer. During the injection, the mice were unanesthetized but restrained in a close-fitting, ventilated, plastic tube. Serial samples (25 µl each) of ophthalmic sinus venous capillary blood (22, 23) were drawn for 30 min.

Glucose determinations

Total body glucose was estimated in two ways, directly and indirectly. In the direct method, mice were stunned, frozen in liquid nitrogen, and homogenized in 70% ethanol (24). 70% ethanolic extracts of carcass homogenates were deproteinized (25) and assayed enzymatically (24). In the indirect method, TLC was

used to separate glucose from plasma (24) obtained from the ophthalmic venous plexus 60 sec after intravenous tracer injections. Total body glucose was calculated as shown in Table 1 from the specific activity of the purified plasma glucose.

Plasma glucose concentrations were estimated in filtrates of deproteinized plasma or after TLC (24). Plasma glucose specific activity was measured after separation by TLC (modified as in Ref. 26).

Validity of methods for measuring total body glucose

The total body glucose could be predicted with great accuracy and reliability in the mouse by the simple indirect method in which the radioisotopic dilution of tracer glucose was determined 60 sec after intravenous injection. The indirect method is based upon earlier reports that the total body glucose pool in the mouse behaves approximately as a single, well-mixed pool (20), and that negligible metabolism of glucose occurs in 60 sec, when mixing of the tracer within the total pool appears to be virtually complete (20, 24). Directly measured whole-body glucose (24) in one population of mice was found to be exactly the same, within the error of the method, as that estimated by the simple isotope dilution technique (Table 1). Therefore, these two methods were used interchangeably. A number of experiments were carried out in which it was established that appreciable hepatic glycogenolysis did not occur when unanesthetized mice were stunned and dropped into liquid nitrogen. Thus, the body glucose pool size had not been artifactually increased by stunning the animals. Liver temperatures (implanted thermocouple) fell to 0°C within 17 sec after the mice were dropped into liquid nitrogen. In experiments using 6-hr-fasted mice, the total amount of body glucose, measured by direct, whole-body analysis, was the same in pentobarbital-anesthetized mice[3] (10.7 ± 0.45 mg/20 g body wt, mean ± SE, n = 16) and in unanesthetized, stunned mice (10.4 ± 0.36 mg/20 g body wt, n = 27). Further studies also showed that liver glycogen concentrations of fed mice were the same when the liver was dissected from animals rapidly and frozen

TABLE 1. Comparison of direct and simple indirect (isotope dilution) methods for measuring total body glucose pool size in mice

Group	Method	No. of Mice	Total Body Glucose
			mg glucose/20 g body wt
A	Direct assay	5	10.8 ± 1.0[a]
B	Indirect assay[b]	7	11.0 ± 0.3

[a] Mean ± SD.
[b] Indirect assay: body glucose = $100/(\%$ injected [14C]glucose/ mg glucose) at $t = 60$ sec.

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[2] Special diet no. 67127 (Nutritional Biochemicals Corp., Cleveland, Ohio): 58% glucose, 22% casein, 6% Hawk-Oser salt mixture, 11.8% nonnutritive cellulose, 2% liver VioBin, and 0.2% vitamin mixture.

as when the animals were first stunned and frozen and the livers subsequently dissected from the frozen carcasses.

Liver glycogen

Tissue samples were obtained from freshly killed mice and were immediately immersed in liquid nitrogen, or they were obtained from frozen mice which had been stunned and immersed immediately in liquid nitrogen. In the latter case, livers were dissected from the frozen carcasses. Glycogen was precipitated from alkaline digests of the tissues and assayed enzymatically (27).

Total body fatty acid

Frozen carcasses of mice were partitioned with a guillotine and digested in 40 ml of hot (90–98°C) 30% KOH. Cool digests were diluted 1:1 with alcohol and extracted with 3 vol of petroleum ether (bp 30–60°C) to remove nonsaponifiable lipids. Washed alcoholic digests were then acidified with HCl and extracted with 2 vol of petroleum ether. Aliquots of the extract were dried and assayed for ^{14}C in a scintillation counter.

Experimental approach and theoretical aspects

In the present study, conditions were sought in which the body glucose pool would remain relatively constant during the course of an isotopic experiment at various periods of fasting and after a brief period of refeeding. (The exact periods of fasting were not determined; food was routinely removed at 8 AM. Although we shall refer to mice as having been "fasted" for, for example, 2 hrs, this represents only the minimum fasting period, since we did not determine when the animals actually ingested food prior to 8 AM.) Liver glycogen concentrations were also determined in order to provide an independent reference which might define the dietary conditions of the mice during the early postabsorptive (2-hr-fasted), late postabsorptive (4–6-hr-fasted), and briefly starved (24-hr-fasted) states.

In order to study lipogenesis from glucose in untrained, fasted–refed mice, it was necessary to offer food to 24 mice in order to obtain 6 mice which would actually nibble a 210-mg test meal when it was offered, in a period of 12 ± 6 min. One of the six mice ate only 160 mg of the test meal. Most of the food was ingested in the first 5 min in each case.

The method used to calculate the approximate flux of glucose C to fatty acids was that of Shipley et al. (8):

$$R_2 = R_1 \times \frac{q_{f.a.}(t)}{q_n(t)} \qquad \text{(Eq. 1)}$$

where: R_1 = irreversible glucose C disposal rate (mg C/min/20 g mouse).

R_2 = flux of glucose C into fatty acids (mg C/min/20 g mouse).

$q_{f.a.}(t)$ = % of injected [^{14}C]glucose incorporated into total body fatty acids at a time, t, when most of the injected ^{14}C had been converted to "end products."

$q_n(t)$ = % of injected [^{14}C]glucose incorporated into all "end products" at time t.

We have modified the approach of Shipley et al. (8) in one important way. Whereas they isolated and assayed all of the ^{14}C-labeled "end products," $q_n(t)$, we have calculated this value, as well as R_1, from the plasma glucose specific activity–time curves using a two-compartmental model:

$$\rightarrow \text{glucose} \rightleftarrows \text{intermediate pools} \rightarrow \text{"end products" } (q_n).$$

Although calculation of R_1 appears to be practically independent of the assumed model in the case of glucose (8, 28), calculation of $q_n(t)$ is model-dependent in that all "end products" are assumed to be formed from a common pool of intermediates whose average specific activity can be calculated from measurements of plasma glucose specific activity. This assumption is obviously an oversimplification; nevertheless, our method of analysis yields rates of fatty acid synthesis from glucose C which agree within 10%[4] of those calculated by the noncompartmental approach suggested by Shipley et al. (8). In the case of the above model, the percentage of injected [^{14}C]glucose converted to total "end products" at any time, $q_n(t)$, is given by the following expression:

$$q_n(t) = 100\left(1 + \frac{g_2 e^{-g_1 t} - g_1 e^{-g_2 t}}{g_1 - g_2}\right)$$

where g_1 and g_2 are the slopes of the two exponential components of the plasma glucose specific activity–time curve, which is defined by the following equation:

$$\text{Sp. Act. } (t) = A_1 e^{-g_1 t} + A_2 e^{-g_2 t}$$

Thus, R_1 and $q_n(t)$ are calculated from the plasma glucose specific activity data, while $q_{f.a.}(t)$ is directly measured at 30 min. Our modification, which, of course, is not restricted to glucose metabolism, allows one to estimate the flux of a distant precursor into one of many "end products" without having to actually isolate and assay all of the so-called end products. This approach is not strictly correct because it treats such compounds as glycogen and fatty acids as end products when, in fact, they are not (29). However, in the case of fatty acids, with which we are concerned here, the turnover rate is so slow

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[3] 2.5 mg/20 g body wt administered subcutaneously approximately 10 min prior to removal of liver specimens.

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

that no appreciable error could result from treating body lipid fatty acids as "end products." For example, Jansen, Hutchison, and Zanetti (6) have reported that the fractional turnover rate constant of carcass lipid fatty acids is only 0.00016/min in mice after intubation of labeled glucose. Such slow turnover would not be detected in experiments of several hours' duration. We have confirmed this under our experimental conditions. No significant fall in ^{14}C-labeled fatty acid occurred between 60 and 180 min after injection of [U-^{14}C]glucose in 5-hr-fasted mice.[4] Nevertheless, one must recognize that we are approximating here the transfer rate of glucose C into the slowly turning over lipid fatty acids. Newly synthesized fatty acids which are oxidized to shorter chain-length metabolites before they are converted to lipid esters would not be included in our estimates of fatty acid synthesis.

Another limitation of our method is the assumption that the second slope of our plasma [^{14}C]glucose specific activity–time curves is the terminal slope and that no further recycling of ^{14}C from so-called end products to glucose occurs during our experiment. We have evaluated the magnitude of error which we may have introduced by our method of calculation by adding a hypothetical third component having a slope of one-half that of our "terminal" slope, from $t = 60$ min to ∞. A corresponding third compartment which could receive ^{14}C from compartment 2 and recycle it to compartment 1 was also added. The results were not significantly different from those obtained by our present analysis in any of the four conditions which we studied.

RESULTS

Liver glycogen

The concentration of liver glycogen in mice fasted for up to 8 hr is shown in Fig. 1. No significant net glyco-
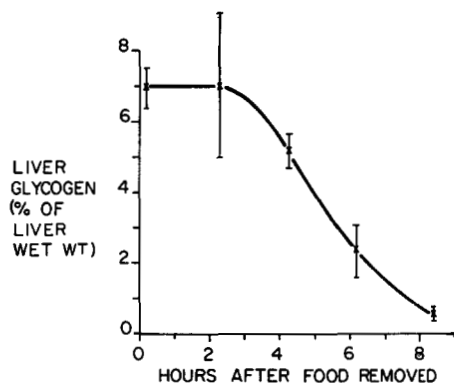

FIG. 1. Mean glycogen concentration in liver during an acute period of fasting in mice. Each value is the mean (± SD, vertical bar) of four mice.

genolysis occurred during the first 2 hr; however, between 2 and 8 hr the liver glycogen concentration fell rapidly. By 8 hr most of the liver glycogen had been mobilized.

Total body glucose

The total body glucose pool decreased by about 30% within 7 hr after removal of food. The exact time of the fall appeared to be variable, but occurred at about the time glycogen was being depleted from the liver. This is shown first of all by a composite graph of all body glucose measurements, carried out over a period of years, in groups of mice deprived of food for various time periods (Fig. 2). About 10 mg of glucose were found in the total body pool of an early postabsorptive 20-g mouse; this agrees closely with earlier direct and indirect measurements in mice (20). Considerable variability was seen from population to population of mice deprived of food for 4–6 hr. Such variability might be anticipated if there were a "sudden" fall of plasma glucose during or shortly after the time of hepatic glycogen mobilization. Although these data do not establish the detailed time course of such a decline in the body glucose pool, it is evident that the body glucose pool does fall during a 24-hr fast and, as shown below, is replenished after the mice nibble a glucose-rich meal for several minutes.

Three separate experiments designed to determine the time course of the early decline in body glucose pool size in individual fasting mice were carried out using the single injection, isotope dilution technique. The results, shown in Fig. 3, indicate that the body glucose pool size fell in two out of three experiments. The variability in the data of both Figs. 2 and 3 during the period 4–6 hr after fasting may reflect the fact that the exact time at which the mice last ate was not known in any experiment, even though food was invariably removed at 8 AM. Nevertheless, it appears that the body glucose may fall within 5 hr after removal of food, as shown by experiment III, Fig. 3. In the latter experiment, each mouse served as its own internal control in the determination of body glucose (e.g., 2 hr vs. 5 hr after removal of food). As shown in Fig. 3, there was a tendency for plasma glucose to fall during experiments in which the body glucose also fell; however, the decrease was statistically significant only in experiment III ($P < 0.01$ at 5, 7, or 9 hr relative to 2-hr values).[5]

The body glucose pool, which fell during a 24-hr fast,

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[5] The percentage decline of plasma glucose concentration during fasting was less than that of total body glucose in experiments II and III. We do not understand why this occurred. We would expect the plasma glucose concentration to reflect the changes in total body glucose if all of the body glucose consists of a single, well-mixed pool (8, 20). Further studies of this phenomenon, using trained, meal-fed animals, will be the subject of a later report.
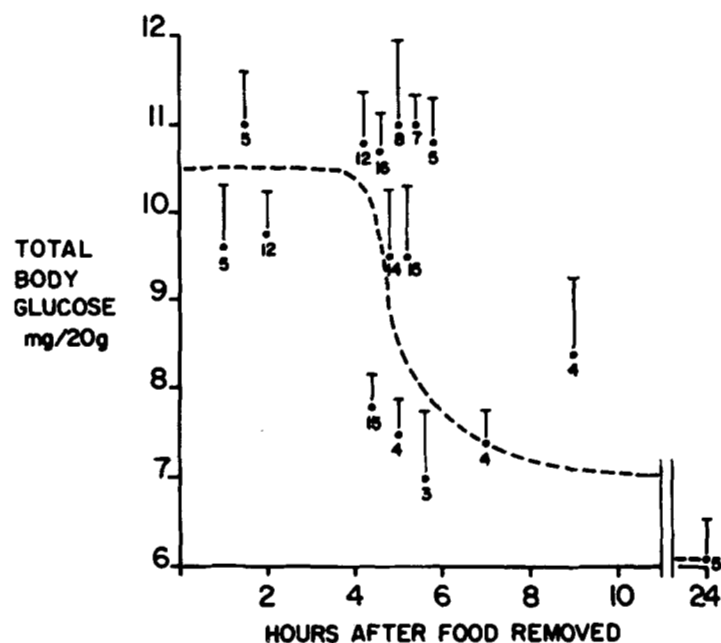
Fig. 2. Mean total body glucose levels in mice during an acute period of fasting or brief starvation; each value is the mean (+ SE, vertical bar) for the number of mice indicated. Data are normalized to 20 g body wt.

was rapidly replenished when the starved mice were refed (Fig. 4). Within 30 min after the mice began to nibble their 210-mg test meals, their plasma glucose concentrations more than tripled and remained relatively high for at least 50 min (Fig. 4). Between 50 and 80 min after the brief refeeding period, the plasma glucose concentration remained stable (2.6 ± 0.1 mg of glucose/ml). This permitted an isotopic experiment to be carried out in the steady state (approximately) during this period even though the mice had been refed a large load of glucose and were hyperglycemic. The slow decline of the plasma glucose curve in briefly starved–refed mice is a reflection of the decreased glucose tolerance in these animals, as shown by Jansen et al. (6).
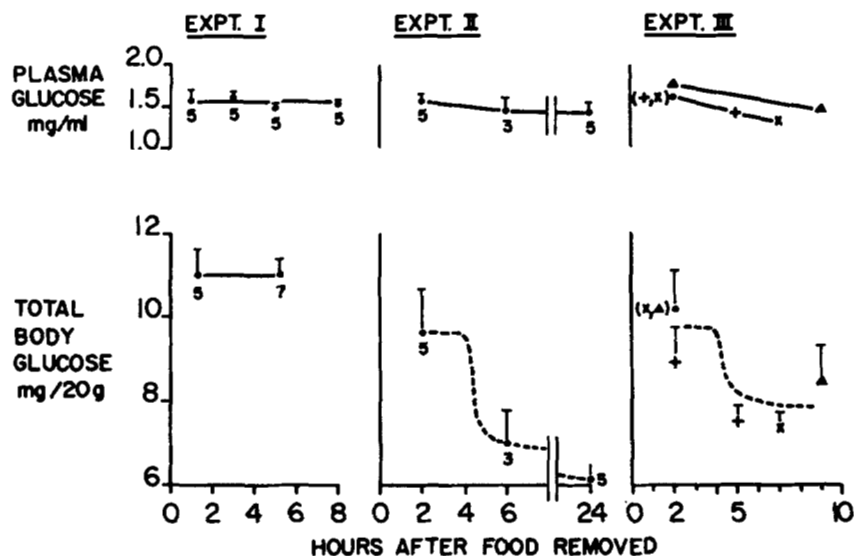

Fig. 3. Mean plasma glucose concentrations and total body glucose levels (per 20 g body wt) during periods of acute fasting and brief starvation in mice. Vertical bars, + SE; number of mice per group is indicated. In Expt. III, two measurements were carried out using each mouse, first in the early postabsorptive state and then after a brief fasting period of 5 (+), 7 (×), or 9 (▲) hr. Four mice were in each group; the mean values for each group are shown. Standard errors for mean plasma glucose concentrations are not shown (Expt. III) because they were less than the height of the symbols.

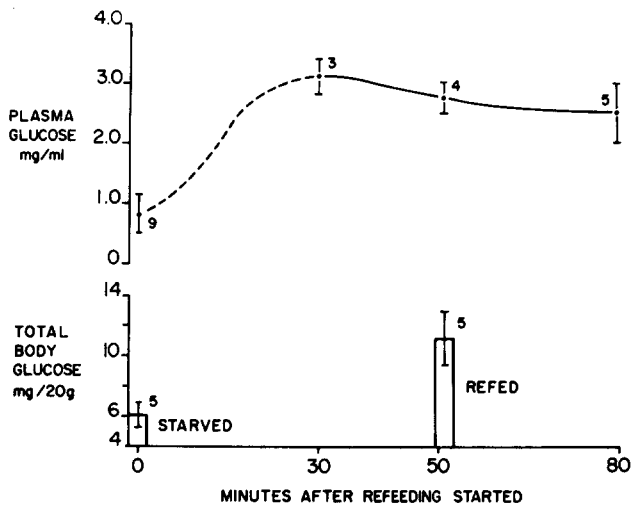*Baker and Huebotter* **Rapid Activation–Inactivation of Lipogenesis from Glucose** 333

Fig. 4. Mean plasma glucose concentrations and total body glucose levels (indirect method) in mice which had been briefly starved (24 hr) or starved 24 hr and refed a 210-mg meal at $t = 0$. Each value is the mean value ($\pm$ SD, vertical bars) of number of mice indicated. Abscissa, minutes after mice started to nibble their test meal.

Net rate of irreversible glucose carbon disposal during acute fasting

The net rate of conversion of glucose carbon to temporary "end products" such as fat depots, glycogen, and protein as well as to true end products such as CO_2 can be estimated from the plasma glucose specific activity–time curves after tracer [¹⁴C]glucose injection (8, 28, 30) The latter curves, obtained from four groups of mice fasted for varying periods of time, are shown in Fig. 5. In the early postabsorptive period (2-hr-fasted, steady-state), the net rates of irreversible glucose carbon disposal (and production) averaged 0.3 mg of glucose carbon/ min/20 g of body weight. The rate of irreversible glucose

disposal was found to be directly proportional to the total body glucose pool size under the conditions studied and was increased two- to threefold within 50 min after a briefly starved mouse started to nibble some food (Table 2).

Fatty acid synthesis from glucose during brief starvation and after the nibbling of a small meal

The incorporation of [¹⁴C]glucose into total body lipid ¹⁴C-labeled fatty acid and into all of the so-called end products of irreversible glucose disposal in mice after 2, 6, and 24 hr fasting, as well as in mice fasted 24 hrs and then allowed to nibble a small meal, is shown in Fig. 6. In all dietary states, even after a night of eating, the conversion of [¹⁴C]glucose into fatty acids is an extremely slow process relative to the conversion of glucose to other substances. For example, at 30 min after injection of [U-¹⁴C]glucose into 2-hr-fasted mice, 60% of the injected ¹⁴C was converted to "end products," but only 1% of the injected ¹⁴C was found in total body fatty acids. The incorporation of [¹⁴C]glucose into fatty acids was clearly reduced by starvation, and the incorporation was markedly augmented within 50 min after a period of brief refeeding (about 12 min nibbling of 160–210 mg of food).

A more meaningful estimate of the rates of fatty acid synthesis from glucose can be given from the relation suggested by Shipley et al. (8) (see Equation 1 in "Experimental approach and theoretical aspects," above).

The values for irreversible glucose C disposal rate, R_1, under each of the four dietary conditions described above have already been presented in Table 2. From these rates and the data for $q_{f.a.}(30')$ and $q_n(30')$ shown in Fig.
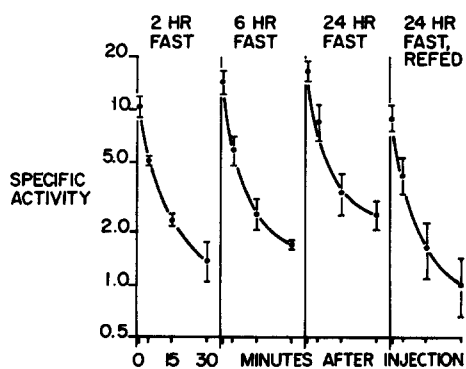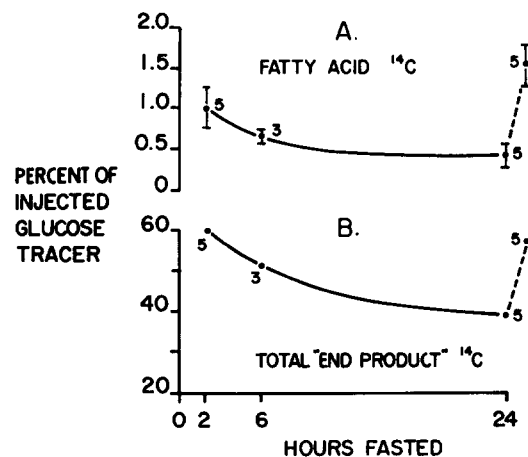

Fig. 5. Mean plasma glucose specific activity–time curves for mice during an acute period of fasting, brief starvation, or refeeding after a brief starvation. Each value is the mean ($\pm$ SD, vertical bars) of five mice, except in the 6-hr-fasted group (n = 3). Time points for each group are at 1, 5, 15, and 30 min. Specific activity is expressed as (% of the injected [¹⁴C]glucose/mg glucose)(body wt/20).

Fig. 6. Conversion of tracer glucose to ¹⁴C-labeled products in mice during an acute period of fasting, brief starvation, or refeeding after brief starvation (dashed line). In A, each value is the mean percentage of the injected [¹⁴C]glucose incorporated into total body fatty acids in 30 min, $\pm$ SD (vertical bars); number of mice as indicated. In B, the percentage of injected [¹⁴C]glucose incorporated in 30 min into total "end product ¹⁴C" is also shown. The latter values were calculated from the data in Fig. 5.

TABLE 2. Glucose irreversible disposal rate and conversion to fatty acids in fasted and fasted–refed mice

Condition	Plasma Glucose Specific Activity[a] $(A_1e^{-g_1t} + A_2e^{-g_2t})$				Irreversible Disposal of Glucose C (R_1)[b]	Incorporation[a] of [14C]Glucose into:		Flux of Glucose C to Total Body Fatty Acids (R_2)[b]
	A_1	g_1	A_2	g_2		Fatty Acid 14C $q_{f.a.}(30')$	"End Products" $q_n(30')$	
Early postabsorptive	6.44	0.26	3.96	0.035	290	1.0	60	4.9
Late postabsorptive	10.4	0.29	3.86	0.027	220	0.66	51	2.9
Briefly starved	12.0	0.20	4.55	0.020	140	0.40	39	1.4
Briefly starved–refed	6.18	0.24	2.73	0.033	370	1.55	57	10.0

Standard deviations or errors are given in Figures.

[a] Units: A_1, A_2, (% of injected 14C per mg glucose)(g body wt/20); g_1, g_2, min^{-1}; $q_{f.a.}$ (30'), % injected 14C in total body fatty acids at t = 30 min (observed); $q_n(30')$, % injected 14C in total "end products" at t = 30 min (calculated).

[b] Units: μg C/min/20 g body wt.

6, the approximate rates of glucose conversion to fatty acids were calculated using Equation 1. These rates are summarized in Table 2. Only about 5 μg of glucose carbon/min/20 of g body weight is converted to fatty acids in mice which have nibbled ad lib. all night (Table 2). After a fast of 6 hr, sufficient to mobilize most of the liver glycogen, this rate of fatty acid synthesis fell by 40%. After a more prolonged period of starvation (24 hr), the rate fell to 30% of that in the early postabsorptive state. Within 50 min after nibbling a small meal, the flux of glucose carbon being converted to fatty acids was increased 700%. As can be seen from Fig. 7, the rates of fatty acid synthesis increase exponentially as (A) the body glucose pool size per 20 g of body weight and (B) the rates of irreversible disposal of glucose carbon increase.

DISCUSSION

In the present study, we have compared rates of fatty acid synthesis from glucose C in untrained mice which had eaten all night as well as after various periods of fasting and refeeding. The extremely slow rate of fatty acid synthesis from glucose (5 μg of glucose C/min) observed in mice, only 2 hr after removal of food and at a time when the animals' livers were still rich in glycogen (7% of the wet liver wt), must be contrasted with reports that 2.3% of a 250-mg glucose load can be recovered as fatty acids in mice 10 min after gastric intubation (6). This represents a minimal average conversion rate of 250 μg of glucose C/min, a rate 50-fold greater than that which we have observed in the early postabsorptive state (2-hr-fasted). Even larger percentage conversions of orally fed glucose meals have been reported by Masoro, Chaikoff, and Dauben (2). Although a 700% increase in the rate of fatty acid synthesis from glucose was observed in our studies within 50–80 min after a brief period of nibbling a 58% glucose meal, the rate still seems much lower than that which occurred when a [14C]glucose load was fed to mice (2, 6). We have confirmed the latter observations[4] and are led to the conclusion that lipogenesis from glucose C may be almost an order of magnitude greater during absorption of a glucose meal than in the postabsorptive period. Thus, we infer that lipogenesis from glucose C in mice may be accelerated and decreased during and after ingestion of food much more rapidly than one would surmise from earlier studies (15, 31–33).

Although rapid changes in lipogenic rates in vivo during ingestion of a glucose-rich meal have not been studied in great detail, they were recognized in 1958 in two independent studies (11, 12). Both groups of investigators used animals trained to eat meals. Subsequently, many workers studied the rapid induction of lipogenic mechanisms in trained meal-fed animals and attention has come to be focused upon differences between trained meal eaters and nibblers and upon long-term adaptations of the lipogenic enzymes during training periods (13, 17, 18). As a result, the changes which occur in normal, nibbling animals as they ingest their food and shortly thereafter have not received adequate attention, and some authors have even suggested that rapid changes in lipogenic rates during feeding and nonfeeding periods exist in chicks but not in untrained rodents (19). How-
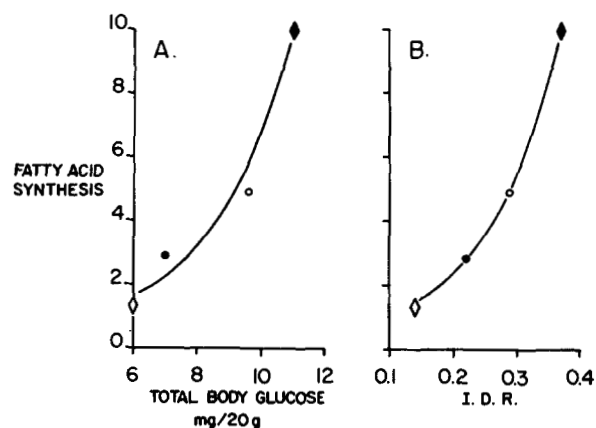

FIG. 7. Mean flux of glucose carbon into total body fatty acids (μg glucose C per min per 20 g body wt) as functions of (A) total body glucose concentration (mg/20 g) or (B) irreversible disposal rates (I.D.R.) of glucose carbon in mice which had been acutely fasted, briefly starved, or starved and refed. Symbols: $\Diamond$, briefly starved; $\bullet$, late postabsorptive; $\bigcirc$, early postabsorptive; $\bullet$, briefly starved–refed. Units of I.D.R., mg C/min/20 g body wt.

ever, the present study demonstrates that the untrained mouse, even when previously starved, is able to respond to a small glucose-rich meal by dramatically increasing the rate at which it converts glucose to fatty acids. The increase is all the more significant since starvation is known to inhibit glucose utilization (7, 31, 32).

Other authors using ^2H_2O (1) or ^3H_2O (34) have reported dramatic, insulin-dependent increases in lipogenic rates in rats after ingestion of a meal; however, the relationship between the time of ingestion of a meal and the time at which lipogenesis was increased was not examined in detail. Acute studies of conversion of ^3H_2O to fatty acids before, during, and after ingestion of a meal should prove interesting. Of course, ^3H_2O is not a suitable tracer for studying the flux of glucose C into fatty acids, but the combination of [^{14}C]glucose with ^3H_2O should prove extremely interesting provided the complexities of analyzing the ^{14}C data during non-steady states can be overcome. An additional factor which may prove to be of considerable importance is that of diurnal variation. Although lipogenesis is clearly most rapid at night (35), it has not been established whether this variation is a fundamental enzymic rhythm unrelated to the pattern of food ingestion or whether, as we are suggesting here, this rhythm reflects primarily the fact that lipogenesis from glucose may occur at a rapid rate only when animals are actually absorbing dietary glucose. Studies designed to answer some of these questions using nibbling mice will be the subject of a subsequent report.

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