Specific role of glucose in rapid lipogenic activation in vivo

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Abstract Lipogenesis from glucose C was previously found to be rapidly activated as soon as mice nibbled a fat-free, glucose-rich diet. We have studied here whether such rapid activation is a specific effect of dietary glucose. The flux of endogenous glucose C to total lipid fatty acids (TLFA) in mice fasted for 1 day was compared with the minimal average flux of exogenous dietary glucose to TLFA during a 40-min period after the ingestion of various glucose-rich test meals by previously fasted mice. The fasted mice were injected intravenously with [U-^{14}C]glucose, and the flux of glucose C to TLFA and to all "end products" was estimated from serial plasma glucose specific activity measurements and ^{14}C incorporation into TLFA 30 min after ^{14}C injection. Only 0.6 to 0.8 μg of glucose C/min/20 g body wt was converted to TLFA, whereas 208 ± 16 μg of glucose C/min/20 g body wt was converted to all "end products" in the fasted animals. Previously fasted mice were fed [^{14}C]glucose in small test meals as a neat solid, as a 30% aqueous solution, or as a fat-free, 58% glucose diet. During the next 40 min, the average flux of glucose C into TLFA increased at least 50- to 60-fold, regardless of the form in which glucose was fed; however, when glucose was fed as part of a complete fat-free diet, glucose was utilized at a much lower plasma glucose level than in mice fed either pure solid glucose or an aqueous glucose solution. Rapid activation of lipogenesis from glucose requires only glucose as a dietary constituent.

Supplementary key words irreversible disposal · fasting · refeeding · control · lipogenesis · [^{14}C]glucose · isotope kinetics · mice · glucose utilization · hyperglycemia

MANY DIETARY CONSTITUENTS are known to influence lipogenesis. For example, dietary sugars have been shown to increase (1, 2), and dietary fats to inhibit (3, 4), the lipogenic capacities of liver slices. There is also considerable evidence that the presence of dietary amino acids can enhance the secretion of insulin (5) and of insulinogenic gastrointestinal hormones (6). This increased secretion of insulin induced by dietary amino acids might be expected to stimulate both lipogenic activity (7, 8) and the rate of glucose utilization (9, 10).

Several earlier studies of the stimulatory effect of dietary carbohydrates upon lipogenesis in rodent tissues have indicated that many hours or days are required before the lipogenic adaptations are manifested (1, 2, 4, 7, 8, 11–13). However, we reported recently that fatty acid synthesis from glucose was rapidly and dramatically increased after fasted mice nibbled a small meal which included amino acids (casein), vitamins, and a salt mixture in addition to glucose (14, 15). In contrast, many of the earlier studies of the acute effects of carbohydrate refeeding upon lipogenesis involved the intubation of aqueous glucose solutions without other dietary constituents (2, 16). Thus, it seemed possible that tissues of previously fasted mice might show a rapid increase in their capacity to synthesize fatty acids from glucose C after ingestion of dietary glucose, but only when the dietary glucose was accompanied by other constituents such as amino acids.

In the present study we shall describe some large differences in the capacities of fasted mice to handle identical glucose loads depending upon the form in which the glucose test meal is fed. Moreover, we shall present evidence which confirms our earlier finding (14, 15) that lipogenesis from glucose C is rapidly activated as soon as a fasted mouse nibbles a small glucose load. The activation appears to be independent of other dietary constituents.

Abbreviations: TLFA, total lipid fatty acids; sp act, specific activity; TLC, thin-layer chromatography.

MATERIALS AND METHODS

Animals

Male mice (strain 129/J, Jackson Laboratory, Bar Harbor, Maine) weighing 16–21 g were housed individually in metabolism cages (Acme Metal Products, Chicago, Ill.) for 3–4 days, during which time they ate a fat-free, 58% glucose diet with water ad lib. The diet contained 22% casein, 6% Hawk-Oser salt mixture, 11.8% nonnutritive cellulose, 2% liver VioBin, and 0.2% vitamin mixture (14). The mice were deprived of food 18–28 hr before the experiment. After fasting, the mice weighed 13–20 g.

[U-^{14}C]Glucose

1 or 2 μCi of uniformly labeled tracer (New England Nuclear, 99% pure by TLC, 15.5 mCi/mmole) was administered in 50 μl of water either intravenously or by addition of 1 μCi to the test meals.

Test meals

120 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 (anhydrous granular dextrose, cat. no. 1916, J. T. Baker Chemical Co., Phillipsburg, N.J.); *3*) fat-free, 58% glucose diet (14).

[U-^{14}C]Glucose was incorporated into all test meals. Shortly before scheduled feedings, 50 μl of the labeled glucose dissolved in water was added to dry, weighed test meals in food cups, and the water was allowed to partially evaporate. Gentle mechanical mixing of the air-dried, but still moist, test meals yielded a rather homogeneous mixture and helped ensure proportional ingestion of both labeled and unlabeled glucose. The 30% glucose was given by gastric intubation via an animal feeding needle (Perfecktum, 16 gauge, Popper and Sons, New York).

Analyses

Analytical procedures for plasma glucose specific activity and concentration, as well as for glucose and ^{14}C-labeled fatty acid in tissues, have been described previously (14, 17, 18). ^{14}C-labeled TLFA in the whole mouse were calculated from separate analyses of the gastrointestinal tract and the rest of the carcass.

Production of $^{14}CO_2$ was estimated indirectly based upon the assumption that 100% of the ingested [^{14}C]-glucose minus the total ^{14}C remaining in alcoholic alkaline digests of mouse carcasses approximated the $^{14}CO_2$ which had been expired. Direct evidence to support this assumption has been presented in an earlier study (19). Highly alkaline samples were diluted eightfold with water before dissolving with scintillator. Radioactivity was measured in a liquid scintillation counter.

Calculations of glucose C flux to TLFA

Our study of the activation of fatty acid synthesis from glucose C is based on two types of tracer experiments, one for fasted mice (endogenous glucose) and the other for refed mice (exogenous glucose). In the case of fasted mice the flux of endogenous glucose C to TLFA in the whole animal was estimated by our modification of (14, 19) the noncompartmental analysis of Shipley et al. (20). The data required for this estimate are plasma glucose specific activity–time curves and ^{14}C-labeled TLFA at one point in time after i.v. [^{14}C]glucose injection. The equation used in this calculation, as well as the definitions of symbols, is given in Table 1. Although our calculation requires a number of assumptions (e.g., an oversimplified two-compartment model, negligible turnover of newly formed lipid fatty acids, rapid turnover of intermediates, etc.), the method we have used has been validated by a theoretically more accurate, multicompartmental analysis (14, 19). The limitations of our semicompartmental analysis have also been presented in these earlier studies.

To determine the extent to which fatty acid synthesis was activated in refed mice, we compared the milligrams of endogenous glucose C per minute converted to TLFA in fasted mice (above) with the average minimum flux of *exogenous* glucose C to TLFA during a 40-min period after ingestion of a test meal labeled with [U-^{14}C]glucose. The latter flux in refed mice was calculated as follows:

$$\text{Avg mg glucose C/min converted to TLFA} = q_{fa}(t)/a_0(t - t_0) \quad \text{(Eq. 1)}$$

where $q_{fa}(t)$ = total radioactivity (cpm) in TLFA at

TABLE 1. Irreversible disposal of glucose C and flux of glucose C into total body lipid fatty acids in 25-hr-fasted mice: definitions of symbols and summary of data

Definition	Symbol	Mean Value ± SD (n)
Irreversible disposal of glucose C	R_1	208 ± 16 μg glucose C/min/20 g (4)
Incorporation of [^{14}C]glucose into fatty acid (observed at time t)	$q_{fa}(30')$	0.24 ± 0.064% of injected ^{14}C (4)
	$q_{fa}(60')$	0.25 ± 0.010% of injected ^{14}C (4)
Incorporation of [^{14}C]glucose into all "end products" (calculated at time t)	$q_n(30')$	62% of injected ^{14}C
	$q_n(60')$	87% of injected ^{14}C
Flux of glucose C into total lipid fatty acids	R_2 (calculated at t = 30′)[a]	0.80 ± 0.096 μg glucose C/min/20 g (4)
	R_2 (calculated at t = 60′)	0.60 ± 0.072 μg glucose C/min/20 g (4)

[a] $R_2 = R_1[q_{fa}(t)/q_n(t)]$; t = min after i.v. [U-^{14}C]glucose injection.

time t; a_0 = specific activity of the ingested glucose (cpm/mg glucose C); and $(t - t_0)$ = time interval (min) between the time, t_0, that the mice began to eat and the time, t, that they were killed. This equation is derived from the following considerations:

a_0 = $q_{gluc}(t_0)$/mg glucose C in the test meal (Eq. 2)

where $q_{gluc}(t_0)$ = cpm of [^{14}C]glucose in the test meal. $q_{fa}(t)/q_{gluc}(t_0)$ = fraction of the exogenous glucose C which is recovered in TLFA at time t; and

$[q_{fa}(t)/q_{gluc}(t_0)]$ · mg glucose C in test meal = mg ingested glucose C converted to TLFA in time interval $t - t_0$ (Eq. 3)

Substitution of Eq. 2 in Eq. 3 and division by the time interval $t - t_0$ give Eq. 1, which is a minimal estimate because it does not take into account: (*a*) dilution of exogenous glucose C by either endogenous glucose C or intermediate C prior to incorporation of the exogenous glucose C into TLFA or (*b*) the fractional turnover of TLFA, which is slow (19, 20) and therefore of little practical significance in our flux estimates (14, 19).

RESULTS

Irreversible disposal rate of glucose C and flux to TLFA in 25-hr-fasted mice

In order to estimate the flux of glucose C to fatty acids in fasted mice by a semicompartmental approach (14, 19), only two kinds of data are required: the plasma glucose specific activity–time curve after a single injection of [U-^{14}C]glucose and the percentage of the injected ^{14}C incorporated into TLFA at one point in time. These data are shown in Fig. 1 and Table 1, respectively. From the data in Fig. 1, one obtains the equation:

$$\text{Plasma glucose sp act} = A_1e^{-a_1t} + A_2e^{-a_2t} = 16.6e^{-0.81t} + 4.8e^{-0.035t}$$

From this equation, we have estimated (20–22) irreversible disposal rate of glucose C (i.e., the conversion in μg of glucose C/min to "end products" which do not recycle appreciable ^{14}C to glucose during the experiment). This flux averaged 208 ± 16 μg of glucose C/min/20 g body wt. The plasma glucose specific activity data were also used to estimate the percentage of injected ^{14}C in all "end products" (q_n), as described previously (14); according to this calculation, 62% and 87% of the injected ^{14}C were converted to all "end products" at 30 and 60 min, respectively, after tracer glucose injection.

Only one-fourth of 1% of the injected ^{14}C was found in TLFA at 30 and 60 min in the fasted mice (Table 1). From these values, using the equation of Shipley et al. (20), $R_2 = R_1\,[q_{fa}(t)/q_n(t)]$ (see Table 1 for definition of symbols), we may calculate that only 0.6–0.8 μg of glucose C/min/20 g was converted to TLFA in fasted mice (Table 1). These values are about half those previously reported for fasted mice that had been maintained on Purina laboratory chow (14) instead of the fat-free, 58% glucose diet. In the latter study (14), we found that 1% [(0.40/39) × 100], and in the present study 0.4% [(0.24/62) × 100], of the radioactivity in all "end products" was incorporated into TLFA at 30 min after [^{14}C]-glucose injection.

Plasma glucose concentrations after feeding glucose test meals

The plasma glucose concentration of 25-hr-fasted mice averaged 1.15 mg/ml (Fig. 2). When 120 mg of glucose/20 g body wt was eaten as a solid or intubated as an aqueous solution, the plasma glucose concentration increased about sevenfold and reached a maximum, at 25 min, of about 7–8 mg/ml. This hyperglycemic response of mice fed pure glucose was correlated with marked glucosuria (Table 2). There was no significant difference between the plasma glucose levels of mice fed solid glucose and of mice intubated with aqueous glucose. However, a much lower glycemic response was observed when mice ate the same quantity of glucose in the form of their regular fat-free, 58% glucose diet. In the latter group the maximum plasma glucose concentration averaged 4.3 mg/ml (Fig. 2), and glucosuria was considerably lower than in mice fed solid or aqueous glucose alone. Thus, some component in the diet either inhibited the absorption of glucose or augmented, perhaps indirectly, the rate at which glucose was removed from the circulation. The data in Table 2 indicate that both explanations may be true. Only two-thirds of the glucose was absorbed in 40 min when mice were fed 90 mg of

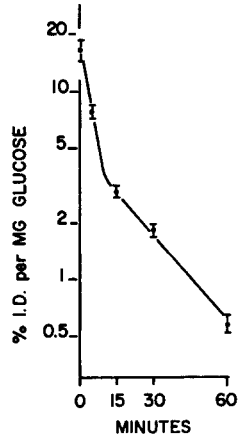

FIG. 1. Mean glucose specific activity (% of injected ^{14}C/mg glucose) curve after intravenous injection of tracer [U-^{14}C]glucose into 25-hr-fasted mice. Each value is the mean (± SE, vertical bars) of four mice, adjusted to 20 g body wt. I.D., injected dose.

glucose as a 58% glucose diet, whereas 90% of the glucose was absorbed when it was fed either neat or as a 30% aqueous solution. On the other hand, about 49 ± 5 mg of glucose was utilized in 40 min (610 μg of glucose C/min/20 g) regardless of the form in which glucose was fed (Table 2). This may represent a faster relative rate of utilization in the case of mice fed 58% glucose since plasma glucose concentrations in that group were much lower than those of mice in the other two groups. The average minimum rate of exogenous glucose utilization after ingestion of the glucose test meals was several times greater than the rate of irreversible glucose disposal in the fasted mice (610 vs. 209 μg of glucose C/min/20 g body wt).

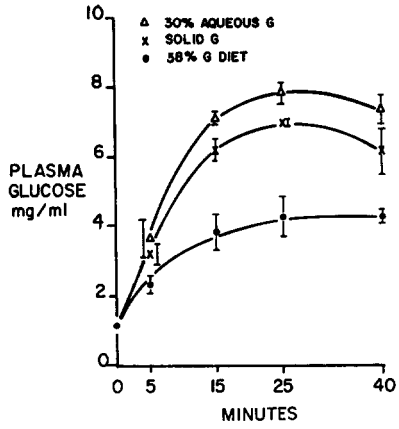

FIG. 2. Plasma glucose concentration curves in mice fasted 25 hr and refed 120 mg of glucose/20 g body wt as shown, at $t = 0$. Each value is the mean (± SE, vertical bars) of four mice.

Comparison of glucose C flux to TLFA in fasted and glucose-refed mice

In the experiment shown in Fig. 2, tracer [U-^{14}C]-glucose was added to each of the three different test meals. This allowed us to estimate in each case the *minimum* average flux of glucose C into fatty acids after ingestion of glucose. As shown in Table 3, about 4 ± 1% of the absorbed [^{14}C]glucose was found in ^{14}C-labeled TLFA in 40 min regardless of the dietary form in which glucose was fed. Very little of the labeled fatty acids (about 2–5% of the ^{14}C present in fatty acids of the rest of the carcass, including liver) was found in the tissues of the gastrointestinal tract (Table 3). The flux of glucose C into fatty acids before and after ingestion of the test meal was estimated from the data in Tables 1 and 3. As shown in Fig. 3, the minimum flux of glucose C into fatty acids increased by at least 50- to 60-fold during the 40-min period after ingestion of the test meal. There was no statistically significant difference in this lipogenic response among the three groups of mice fed solid glucose, aqueous glucose, or 58% glucose diet. The minimum flux of glucose C to CO_2 was also estimated, indirectly (19), after the feeding of glucose test meals. There was no effect of nonglucose constituents in the 58% glucose diet upon the oxidation of ingested glucose to CO_2. Thus, of 36 mg of glucose C ingested, an average of 365 ± 30 (n = 4) and 410 ± 40 μg (n = 4) of glucose C/min were oxidized to CO_2 by mice that ate the 58% glucose test meal and solid glucose, respectively. In the animals fed solid glucose neat or as part of a diet, the minimal flux of glucose C to CO_2 was 10 times greater than the flux to fatty acids. Moreover, after these test meals were eaten, the flux of glucose C to CO_2 was more than double the total flux of glucose C to all "end products," including CO_2, in the unfed, fasted mice (Table 1). The increase in the flux of glucose C to CO_2 apparently oc-

TABLE 2. Influence of test meal composition on the absorption and utilization of dietary glucose

	Glucose (mg)							
			In Total Body[b]					
			(a)	(b)				
Test Meal[a]	Ingested	Absorbed from Gastrointestinal Tract in 40 min	Before Eating ($t = 0$)	After Eating ($t = 40$ min)	(b − a) Increment	Excreted in Urine in 40 min	Utilized in 40 min[c]	Oxidized to CO_2 in 40 min
30% aqueous solution by gastric intubation	90	80 ± 3.1	5.0	31	26	4.7 ± 0.94	49	*d*
58% glucose, solid complete diet	90	58 ± 3.0	4.7	18	13	0.3 ± 0.16	45	27 ± 2.5
Solid glucose (neat)	90	77 ± 5.4	4.9	26	21	2.3 ± 0.41 (n = 3)	54	31 ± 2.8

Four mice per group, average body weight, 15.5 g; each value is the mean ± SE (n = 4 unless otherwise indicated).

[a] Units: 120 mg of [U-^{14}C]glucose/20 g body wt.

[b] Total body glucose before eating (6.0 mg/20 g body wt) was calculated (14) from a separate isotope dilution study using another group of 25-hr-fasted mice (see Fig. 1). Values were then adjusted for mean body weights of the mice used in the present study. Total body glucose after eating was estimated as follows: (plasma glucose 40 min after eating/plasma glucose before eating) × column a.

[c] Glucose utilization was estimated as follows: glucose absorbed − (glucose increment in total body + glucose excreted in urine in 40 min).

[d] Indirect estimates of $^{14}CO_2$ in this group were highly variable and therefore of questionable validity (range, 5.9–22).

TABLE 3. Incorporation of [^{14}C]glucose into total and gastrointestinal tract lipid fatty acids in fasted mice refed glucose test meals

Test Meal[a]	No. of Mice	Incorporation of Ingested [^{14}C]Glucose into: Total Lipid Fatty Acids[b]	Incorporation of Ingested [^{14}C]Glucose into: Gastrointestinal Tract Lipid Fatty Acids	Incorporation of Absorbed [^{14}C]Glucose into: Total Lipid Fatty Acids[b]	Incorporation of Absorbed [^{14}C]Glucose into: Gastrointestinal Tract Lipid Fatty Acids
		%			
30% aqueous solution	4	2.72 ±0.388	0.0425 ±0.0058	3.35 ±0.366	0.0529 ±0.0058
58% glucose diet	3	2.96 ±0.303	0.153 ±0.0721	4.67 ±0.186	0.263 ±0.143
Solid glucose (neat)	4	3.37 ±0.587	0.164 ±0.0367	3.90 ±0.587	0.188 ±0.0375

Values are means ± SE.
[a] Units: 120 mg of [U-^{14}C]glucose/20 g body wt.
[b] Includes gastrointestinal tract ^{14}C-labeled fatty acid.

curred very rapidly after the mice began absorbing glucose.

DISCUSSION

There is considerable evidence in the literature that the feeding of carbohydrate increases lipogenesis in various tissues by both rapidly acting and slowly acting mechanisms (1, 2, 11–16, 23–25). However, as yet there have been no quantitative in vivo studies published on the flux of dietary glucose C to fatty acids in any tissue under any physiological condition. Most investigators have avoided such calculations because of complications which arise from the interpretation of radioisotopic data during non-steady state conditions (i.e., the rapidly rising and falling blood glucose levels following ingestion of a carbohydrate-rich meal). The approach which we have taken in this study allows us to make some minimal estimates of the flux of glucose C to total body lipid fatty acids in fasted mice and in fasted mice immediately after the ingestion of various glucose-rich meals. More accurate estimates could, and certainly should, be made using additional data and a more complex mathematical analysis; however, we have chosen to use the simplest experimental design and calculations in order to establish whether or not the predicted (14) rapid and large changes in fatty acid synthesis from glucose C during absorption of dietary glucose actually occurred and, if so, to determine whether the changes in flux were dependent upon dietary constituents other than glucose.

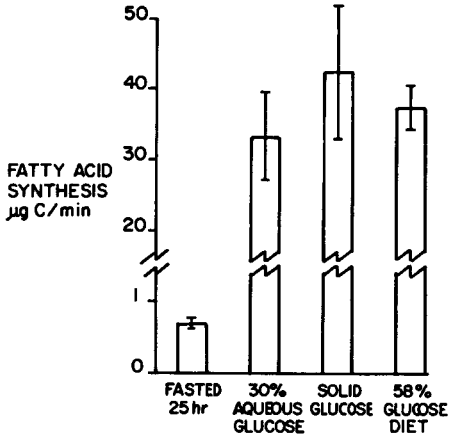

FIG. 3. Flux of glucose carbon into total lipid fatty acids in 25-hr-fasted mice or in mice fasted 25 hr and then refed a test meal containing the tracer. The rates in the refed groups are calculated on the basis of 120 mg of glucose given per 20 g body wt and represent the *average* values during experiments of 40-min duration. Each value is the mean (± SE, vertical bars) of four mice except for the right-hand bar (n = 3).

The interpretation of our data is based upon the comparison of two different experimental approaches. First, we estimated the flux of glucose C to TLFA in fasted mice, in the steady state, by conventional radioisotopic techniques (14). Then, we fed fasted mice various test meals containing ^{14}C-labeled glucose and estimated the average flux of *exogenous* glucose to TLFA in a 40-min period immediately after ingestion of the test meals. Comparison of the results of these two approaches shows that a rapid increase in the flux of glucose C to fatty acids in previously fasted mice occurred, regardless of the dietary form in which glucose was fed. The average minimum increase was 50-fold, and this change occurred during the 40-min period in which the test meal was being absorbed. We may surmise from earlier work that most of the newly formed fatty acids were synthesized in extrahepatic tissues in both fasted (15) and refed mice (15, 26, 27); however, only a small fraction of the newly synthesized fatty acids were found in the gastrointestinal tracts of the refed mice. Extension and refinement of our present approach should allow one to evaluate the extent to which various extrahepatic tissues participate in the

rapid lipogenic response that follows the ingestion of dietary glucose.

Although the main aim of our study was to evaluate the flux of glucose C to fatty acids before and after ingestion of various test meals, other quantitative aspects of glucose metabolism were observed which deserve discussion. First, it is important to realize that the amount of glucose contained in each test meal (120 mg/20 g body wt) was approximately equivalent to that which a mouse normally ingests at about 40-min intervals throughout the night.[1] Since a 20-g mouse has only about 8 mg of glucose in his total body (14), he ingests at least an order of magnitude more glucose than is present in his total body pool each time he nibbles such a diet. Yet, the normal mouse excretes only trace amounts of glucose during feeding periods.[1] Apparently, relatively massive quantities of glucose can be absorbed without inducing a pronounced and prolonged hyperglycemia.

On the other hand, it has been reported that, when comparable loads of aqueous glucose are administered by gastric intubation to 16-hr-fasted mice, plasma glucose may reach levels of 500 mg/100 ml (27). However, this intensive hyperglycemia is maintained long enough so that a second glucose load, if given 40 min after the first, would be expected to augment the hyperglycemic state and maintain it well above the maximum tubular reabsorption level of the kidney. Our present observations show that glucose, when ingested as part of a complete meal, is handled differently from glucose that is fed either neat or as an aqueous solution. The difference is particularly striking when repeated test meals are fed. For example, we have found[1] that plasma glucose concentrations reached levels of 1000 mg/100 ml after two intubations of aqueous glucose (120 mg/20 g body wt/intubation at 40-min intervals). In contrast, only a mild hyperglycemic response occurred when mice were fed two test meals containing the same amounts of glucose, but in the form of a 58% glucose diet (15). In the latter case, ingestion of one (or more) of the nonglucose dietary constituents permitted a relatively large load of glucose to be utilized or cleared from the circulation as fast as it was being absorbed from the gastrointestinal tract. However, in the present study the average flux of exogenous glucose C to fatty acids, to CO_2, and to all other metabolic products did not seem to be influenced by the form of the test meal. During the period of alimentary hyperglycemia induced by each of the three types of glucose test meals, there was at least a 100% increase in the flux of glucose (mg/min) from the extracellular fluid into the cells as compared with the flux of glucose just prior to the ingestion of the test meals. This increased flux of glucose C during absorption of the test meal occurred at a lower blood sugar level in the mice fed 58% glucose than in those fed solid or aqueous glucose. There are at least two possible interpretations of this phenomenon. Either glucose was being utilized at a nearly maximal rate in the mice fed 58% glucose or glucose was being utilized more efficiently in these animals due to the ingestion of one (or more) of the nonglucose constituents in the 58% glucose diet. Further studies of glucose conversion to various products after the feeding of single and multiple glucose test meals are required in order to gain a better understanding of dietary factors that influence glucose clearance from the circulation and glucose metabolism in the absorptive period. Additional experiments are also required in order to elucidate the mechanisms by which dietary and hormonal factors bring about the rapid changes in rates of glucose metabolism, especially in the flux of glucose C to fatty acids, that we have observed in these studies.

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

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