Re-evaluation of lipogenesis from dietary glucose carbon in liver and carcass of mice

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Abstract We have estimated rates of fatty acid synthesis from glucose carbon and from all 2-carbon units in liver and carcass of mice using [U-^{14}C]glucose and $^{3}H_2O$ under four different nutritional states. The liver synthesized only a small fraction (2–9%) of the fatty acids that were formed from glucose carbon in mice that were fasted 24 hr, fasted–refed, or fed ad libitum. However, in fed–refed mice, the liver's role increased and now accounted for 40% of the fatty acids that were formed from glucose carbon. Under the latter conditions (fed–refed), the liver synthesized 50% of the fatty acids that were formed from all 2-carbon units. At least five-sixths of all the fatty acids synthesized de novo in the fed–refed mouse were derived from carbon fed in the glucose test meal. These studies, in contrast to most earlier studies, provide direct evidence in mice of the major contribution that dietary carbohydrate makes, especially in the liver, to the synthesis of fatty acids. In addition, we have shown that lipogenic inhibition (fasting) and activation (feeding) are most marked in liver and greater for glucose- than for non-glucose-carbon. Possible implications for dietary control of carbohydrate-induced hyperlipemia and obesity are discussed.

Supplementary key words fatty acids · semi-compartmental analysis · lipogenic activation · $^{3}H_2O$ · [U-^{14}C]glucose · nutritional control · fasting–refeeding

Despite extensive research on the conversion of dietary sugar into fat, the quantitative role of the liver in this process has not been established. Most evidence indicates that, in rodents, the liver's role is minor (1–15). We know of two exceptions (16, and some data in ref. 11), but in neither case was the incorporation of glucose carbon into liver FA vs. total carcass FA measured. The incorporation of glucose carbon into liver FA in vivo has been reported to be so slow that one group of investigators has stated ". . . experiments with [^{14}C]glucose do not provide a useful measure of hepatic FA synthesis, as blood glucose is of minor significance as a carbon source in mice . . . or rats. . . ." (17). In fact, because of the uncertainties of complex dilution phenomena in the intact animal, there has been a tendency to utilize $^{3}H_2O$ (or D_2O), rather than ^{14}C-labeled glucose, as the preferred tracer, and to focus upon total lipogenic rates.

Isotopic water has been used to evaluate the role of the liver with respect to total body FA synthesis (17–22). Most of these studies indicate, or allow one to calculate, that the liver's role is minor (18–22). However, we find one exception, namely, the recent extensive study of Hems et al. (17), who concluded that ". . . fatty acid synthesis in normal mice, expressed per whole animal, is about twice as rapid in liver as in free adipose tissue (i.e., in the discrete adipose organ)." Although [^{14}C]glucose was also used in these studies, no attempt was made to calculate rates of FA synthesis from glucose-C. Nevertheless, the authors deduced that only about 10% of the FA synthesized in the liver was derived from glucose-C in lean mice (17).

Of course, such a conclusion can only be drawn validly if rates of hepatic FA synthesis from glucose-C and from total carbon are simultaneously compared. Not only were the necessary rates from glucose-C not measured by Hems et al. (17), but the total extrahepatic tissues were not analyzed for labeled FA, either from [^{14}C]glucose or from $^{3}H_2O$. As noted earlier (14, 15), extrapolations based upon selected samples of adipose tissue can be highly misleading (2–4, 13, 14), especially when one is attempting to evaluate the quantitative role of the liver with respect to the whole body's transformation of carbohydrate into FA. These objections to the studies of Hems et al. (17), have led us to re-examine the questions that were raised by these authors and that have long troubled us in our own laboratory. For instance, it is not at all obvious to us that the liver of an animal ingesting a high carbohydrate diet should be synthesizing most of the body's FA from non-glucose carbon, especially when dietary glucose, by itself, can

Abbreviations: FA, fatty acids; TLFA, total lipid fatty acids; PE, petroleum ether; I. D., injected dose.

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activate lipogenesis dramatically and almost immediately in previously starved mice (23).

It is conceivable that the relative contribution of glucose-C and non-glucose carbon to total FA synthesis in the liver might depend upon the presence or absence of dietary amino acids. In the studies of Hems et al. (17), the animals were eating a carbohydrate-based breeding diet that presumably was rich in protein. Therefore, one could hypothesize that glucose derived from dietary starch had activated the liver to synthesize FA primarily from dietary amino acids. If that were the case, one would want to know whether the liver would still synthesize most of its FA from non-glucose carbon if the animals were eating neat glucose as the only carbon source.

We have tried to answer some of these questions and to re-evaluate the relative role of the liver with respect to the total rate of FA synthesis in hepatic and extrahepatic tissues of mice that were fasted, fasted–refed, fed ad libitum, or fed–refed (force-fed). At the same time, we have attempted to re-evaluate the quantitative importance of glucose-C to the total rate of FA synthesis in these animals. By establishing basal lipogenic rates in mice that were fed ad libitum or previously starved, we have also been able to ascertain, using doubly labeled tracer techniques, whether rapid lipogenic activation, which occurs following a small, glucose test meal (1, 12, 23, 24), reflects an increased rate of FA synthesis from all 2-carbon units, as well as from dietary glucose-C.

Our studies, reported here, provide direct evidence in mice that, after ingestion of glucose, most of the body's FA are synthesized from the dietary glucose-C. Although we have been unable to confirm the findings of Hems et al. (17) with respect to the liver's playing the primary role in FA synthesis from all 2-C units in animals that are eating ad libitum, we have observed that the role of the liver with respect to FA synthesis from dietary glucose-C changes drastically depending upon the exact dietary state of the animal. Rates of FA synthesis from dietary glucose-C by the liver are shown here to be so rapid that the liver can play a major quantitative role in the synthesis of fat from dietary carbohydrate in fully fed mice.

METHODS

Animals

Male Swiss-Webster/ICR mice (Hilltop Lab. Animals, Inc., Chatsworth, CA), 8-wk old, were housed in colony cages and maintained on experimental diets ad libitum for 3 weeks before each experiment. The range of body weights for all groups of animals was 35–42 g. No anesthesia was used in any of the studies.

Diets

In most experiments the mice were fed a fat-free, 58% glucose diet (12). In some initial studies patterned after Jansen, Zanetti, and Hutchison (6) half of the mice ate a 56% glucose–1% corn oil diet. Both diets contained 22% casein, 6% Hawk-Oser salt mixture, 2% liver Vio-Bin, 0.2% vitamin mix, and the remaining portion was non-nutritive cellulose. Since no significant differences were found between the data with respect to these two diets, results have been combined.

Radioactive tracer doses

^3H_2O (1–3 mCi, 0.1–0.2 ml per animal) was injected intraperitoneally. [U-^{14}C]Glucose (5 μCi) was administered in one of three ways: *1*) as described by Jansen et al. (5, 6), 250 mg of glucose was force-fed as a 50% w/v aqueous solution with an animal feeding needle; *2*) as described by Baker and Huebotter (23), tracer was homogeneously mixed with a solid test meal (120 mg glucose/20 g body wt., either as a 58% glucose fat-free diet or solid, neat glucose); or *3*) the tracer was injected intravenously in 50 μl of saline (without preservative; see ref. 25). Both isotopes were purchased from Dhom Products, Ltd., North Hollywood, CA. Purity of [U-^{14}C]glucose was ascertained by thin-layer chromatography (26). Test meals, when fed, were given between 8 AM and 12 noon.

Tissues

Blood samples were obtained from an opthalmic venous capillary sinus (27, 28). The capillary tubes were centrifuged for 2 min to obtain plasma. Animals were killed by cervical fracture; livers were quickly removed, weighed, and immersed in 30% (50% ethanolic) KOH solution that had previously been chilled to −16°C. Carcasses were immediately frozen in liquid nitrogen and stored at −16°C for subsequent saponification. No detectable loss of labeled FA occurs under these conditions.[2]

Analyses

Carcasses and livers were saponified in 30% (50% ethanolic) KOH, 100 and 10 ml, respectively, for 2–3 hr under reflux at 80°C. Longer periods of standing in alkali, followed by prolonged refluxing in ethanolic KOH, as described by Jansen, Zanetti, and Hutchison (7), did not increase the yield of FA. Nonsaponifiable lipids were removed from the digests by two extrac-

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tions with petroleum ether (PE). After acidification with HCl, FA were then extracted with PE. The PE–FA extracts were twice backwashed with water to minimize ^3H_2O contamination, and, after evaporation of the PE, the FA were assayed for ^{14}C and ^3H.

Plasma glucose specific activity was determined as previously described (25, 26). Plasma glucose concentration was determined by enzymatic assay of deproteinized samples of blood plasma (28). The total blood removed (prior to the terminal sample) for these analyses represented about 5% of the blood volume.

Body (plasma) ^3H_2O specific activity was defined by radioassay of diluted terminal plasma and expressed as activity per unit mass of water, measured by weighing plasma before and after drying in air. Negligible counts remained in plasma solids after evaporation.

^{14}C- and ^3H-Labeled samples were dissolved in scintillator solution [Instagel:toluene 1:1 (v/v)] and assayed with a Beckman Model LS 3133P liquid scintillation spectrometer. Instagel was purchased from Packard Instruments Corp., Downers Grove, IL. Appropriate ^{14}C and ^3H standards, including aliquots of all doses, were counted alone, and selected samples were spiked with [^3H]- and [^{14}C]palmitic acid as internal standards to obtain quench curves. Appropriate corrections were applied.

OUTLINE OF EXPERIMENTAL DESIGN

The approach used was a composite of several tracer techniques that we and others have developed over a number of years to study rates of FA synthesis from glucose-C in vivo (15, 23, 29, 30) and from all 2-C sources using ^3H_2O as tracer (20). Modifications were necessary, as described below, to treat those cases in which lipogenic rates were increasing due to the feeding of a glucose test meal.

Nutritional states

The following six nutritional conditions were studied: *1*) 24-hr fasted ($n = 6$ for ^{14}C studies; $n = 26$ for ^3H studies); *2*) 24-hr fasted, refed a test meal of 58% glucose diet ($n = 3$); *3*) 24-hr fasted, refed a test meal of solid, neat glucose ($n = 3$); *4*) 24-hr fasted, refed a test meal of 50% glucose as an aqueous solution ($n = 37$); *5*) fed ad libitum ($n = 6$ for ^{14}C studies; $n = 31$ for ^3H studies); *6*) fed ad libitum and also intubated with a 50% aqueous glucose solution (fed–refed; $n = 41$).

Rates of FA synthesis from all 2-C units using ^3H_2O as tracer

Tracer ^3H_2O was injected intraperitoneally at zero time (t_0). (Refed mice were also given a [U-^{14}C]glucose test meal 15 min later, as described below.) The following measurements were made: *1*) liver TLFA-^3H at $t = 0$, 15, 25, 40, and 75 min; *2*) carcass TLFA-^3H at the same five times; *3*) plasma ^3H_2O specific activity at $t = 75$ min.; this specific activity was assumed to be equal to that of the total body water. Separate experiments showed that the equilibrium between plasma H_2O and body H_2O was sufficiently rapid for this assumption to be valid in our mice. Specifically, the plasma ^3H_2O specific activity approached a plateau value corresponding to a body water space of 70 ± 1.4% of body wt. ($n = 10$); the plateau was reached at approximately $t = 10$ min. A similar time course was reported in rats by Stansbie et al. (31).

The rates of FA synthesis from all 2-C units in liver and carcass (nmol FA/min) were calculated as follows based upon the assumptions of Windmueller and Spaeth (20):

$$\text{Rate} = \frac{^3H_{cpm} \text{ into TLFA between } t_1 \text{ and } t_2}{^3H_{cpm}/\text{g-atom H in body water}} \times \frac{10^9}{13.3} \times \frac{1}{t_2 - t_1}$$

The factor, $10^9/13.3$, corrects for isotopic discrimination and converts g-atoms of H into nmol of 16-C FA formed. The calculation assumes that one-half of all the H atoms in a newly synthesized FA are derived from H_2O (20). In our preliminary experiments, two calculations were carried out to obtain minimal and maximal estimates of lipogenic rates in the case of fasted–refed mice that had been injected with ^3H_2O at t_0, fed glucose at $t = 15$ min, and killed at $t = 75$ min. The minimal rate was obtained by assuming linearity from t_0 to t_{75}; therefore, a 75-min interval was used in the above calculation of the FA synthetic rates. A maximal estimate was obtained by assuming that the incorporation of ^3H_2O into FA was negligible in the fasted mice until glucose was fed and that thereafter FA synthesis was rapid and linear for the next 60 min. On the basis of our subsequent, more detailed experiments, a better approximation appears to be intermediate between these two values. Accordingly, the mean of these limits, based upon a 67.5-min interval, was used to interpret the preliminary experiments.

Rates of FA synthesis from glucose carbon

Two types of experiments were carried out. In the first (see *A*, below), we used mice that were either fasted 24 hr or fed ad libitum. No test meals were given. In the second (see *B*, below), labeled test meals were given.

A. Experiments using mice either fasted 24 hr or fed ad libitum but not given test meals.

Tracer [U-^{14}C]glucose was injected intravenously at zero time. The following measurements were then made: *1*) plasma glucose concentration; *2*) plasma glucose specific activity at t = 1, 5, 15, 30, and 60 min (after tracer glucose injection); *3*) liver TLFA-^{14}C at t = 60 min; *4*) carcass TLFA-^{14}C at t = 60 min.

The rates of FA synthesis from glucose-C (nmol FA/min) in the liver (R_1) and in the carcass (R_2) were then calculated by a semi-compartmental analysis (30). The latter was based upon the relationship

$$R_1 = \text{I.D.R.} \times \frac{m}{q_n} \times k_1$$

or

$$R_2 = \text{I.D.R.} \times \frac{n}{q_n} \times k_1$$

where I.D.R. is the glucose carbon irreversible disposal rate (30, 32), m is the percentage of the injected dose in liver TLFA at t = 60 min, n is the percentage of the injected dose in carcass TLFA at t = 60 min, q_n is the percentage of the injected dose in all end-products at t = 60 min, and k_1 = 1/0.192 (the factor that converts μg glucose-C to nmol FA synthesized from glucose-C; 1 nmol of a FA with 16 carbons, if synthesized entirely from glucose-C, would require 0.192 μg glucose-C).

I.D.R. was calculated from the plasma glucose specific activity–time curve. It equals the reciprocal of the area under that curve (30, 32). If specific activity is expressed as the fraction of the injected dose per μg glucose carbon, then the units of I.D.R. = μg glucose carbon/min. The quantities m and n were measured directly. The value for q_n, which includes the radioactivity in all end-products, in the liver and in the carcass, as well as in expired CO_2, was calculated as described previously (30). This value (q_n) was estimated on the basis of a 2-compartment model using the slopes (g_1 and g_2) of the plasma glucose specific activity–time curve. The latter curve was fit to a bi-exponential function of the following form:

$$\text{Sp. act. } (t) = a_1 e^{-g_1 t} + a_2 e^{-g_2 t}$$

where the units of g_1 and g_2 are reciprocal time (min^{-1}). The relationship between $q_n(t)$, g_1, and g_2, described previously (30), is as follows:

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

B. Experiments using mice fasted 24 hr and then either fed a solid test meal or intubated with 50% glucose (fasted–refed) or fed ad libitum and then intubated with glucose (fed–refed).

1. Preliminary experiments (effect of dietary protein and other dietary inclusions on hepatic and extrahepatic lipogenesis). In these studies, the solid test meal consisted of either neat glucose or 58% glucose fed as part of the regular fat-free, 58% glucose diet (120 mg glucose/20 g body wt in either case). The test meal was labeled with [U-^{14}C]glucose. The mice were allowed to eat for about 10 min; they ate the entire test meal in each case. Zero time was taken as the time mice started to eat. (Mice were injected 15 min earlier with ^3H_2O to estimate total rates of FA synthesis, as described above.) The following measurements were made: *1*) specific activity of the ingested glucose; *2*) liver TLFA-^{14}C at t = 60 min; *3*) carcass TLFA-^{14}C at t = 60 min.

The rate of FA synthesis from dietary glucose carbon (R_3) in either liver or carcass was estimated as described earlier (23) using the following equation:

$$R_3 = \frac{\text{TLFA-}^{14}\text{C} \times 1/0.192 \times 1/60 \text{ min}}{\text{sp. act of fed glucose dose (\% dose per } \mu\text{g C)}}$$

where TLFA-^{14}C = % dose at 60 min in liver or carcass. Again, the factor 0.192 converts μg glucose-C to nmol FA synthesized from glucose-C.

2. Detailed study as a function of time. The test meal consisted of glucose (250 mg glucose = 100 mg glucose-C per mouse, fed as a 50% aqueous solution). It was labeled with [U-^{14}C]glucose and fed by gastric intubation 15 min after injection of ^3H_2O. The following measurements were made: *1*) plasma glucose concentration 30 min after feeding; *2*) glucose specific activity in the test meal; *3*) liver TLFA-^{14}C at 0, 10, 25, and 60 min after feeding the glucose test meal; *4*) carcass TLFA-^{14}C at the same four times.

The rate of FA synthesis from dietary glucose-C in liver or in carcass (nmol FA/min) was calculated as in the preliminary experiments, except that a better estimate of the rate of ^{14}C incorporation into the liver and carcass FA was used in the calculations. Thus,

$$R_4 = \frac{k_2}{\text{sp act fed glucose carbon}} \times \frac{1}{0.192}$$

where k_2 is the average slope of glucose-^{14}C incorporation into TLFA-^{14}C after lipogenic activation (% fed ^{14}C/min) for liver or for carcass. The slope was calculated from a least-squares fit to a straight line of data points for the interval, t = 25 to 60 min after feeding glucose. The specific activity of the fed glucose

TABLE 1. Effect of protein and other dietary inclusions on the contribution of fed glucose carbon to fatty acids newly synthesized in liver and carcass of fasted–refed mice

Tissue	Interval Used for Calculations	Test Meal (a) Neat Glucose (n = 3)	Test Meal (b) 58% Glucose Diet (n = 3)	(a + b) Grand Mean (n = 6)	Liver as % of Total Rate
	min	*nmol FA/min*			
Rate from all 2-C units in:					
Carcass	75	260 ± 59	230 ± 27	270 ± 74	
	60	330 ± 74	280 ± 33		
Liver	75	47 ± 14	50 ± 8.3	55 ± 18	17
	60	59 ± 17	63 ± 10		
Rate from glucose-C in:					
Carcass	60	310 ± 140	230 ± 19	270 ± 96	
Liver	60	11 ± 7.6	9.7 ± 1.4	10 ± 5.4	4.0

120 mg glucose/20 g body wt; test meal varied according to actual weight of each animal. Results expressed as mean ± SEM. Grand mean represents average rate calculated on the basis of an average 67.5-min interval (see Methods).

carbon is expressed as percent of the ingested dose per μg glucose-C.

The assumptions and limitations of our approach have been detailed in our earlier work (23, 24). That the rate of incorporation of ^{14}C into TLFA is an approximately linear function (after an initial lag period) has been verified (24); moreover, the assumption that the load of labeled glucose in the stomach behaves as a source of constant specific activity glucose, as in a constant infusion experiment, during the first hour after feeding has also been validated (24). This approach gives a minimal estimate of the average rate of FA synthesis from exogenous glucose-C. The estimate is minimal 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 and therefore of little practical significance in our calculations (23). It is an average rate, because it relies upon the fitting of a straight line to just two points. This simplified approach has been shown to approximate closely the rate of total FA synthesis from body glucose in fasted–refed mice (24) and is capable of demonstrating rapid and dramatic changes in lipogenic rates (23, 24). However, this method cannot be used by itself to calculate net rates of FA synthesis from glucose-C (33) and should be interpreted with caution. Nevertheless, additional data using ^3H_2O, shown below, tend to further substantiate the value of this simple approach.

All of the tracer techniques used here assume that there has been negligible redistribution of the newly synthesized FA from liver to carcass or vice versa during the short-term period under study. Evidence to support this assumption has been published (5, 11); however, we cannot rule out the possibility that such transport has occurred.

RESULTS

Effect of protein (and other dietary inclusions) on the contribution of fed glucose carbon to FA newly synthesized in liver and carcass of fasted–refed mice

Approximate average rates of FA synthesis from glucose-C (exogenous) and from non-glucose-C in liver and extrahepatic tissues (whole body minus liver) in 24-hr fasted mice refed a glucose test meal (120 mg glucose/20 g body wt) are shown in **Table 1.** One group of mice was fed a test meal of neat glucose; the other group was fed a 58% glucose, fat free diet (12). Rates of de novo FA synthesis were calculated over two time intervals after ^3H_2O injection (see Outline of Experimental Design). As shown in Table 1, there was no significant difference between groups fed neat glucose and those fed the 58% glucose test meal that included protein. In both groups, the livers of the mice synthesized only a minor fraction of the total fatty acids. This is most clearly seen in the last column of Table 1 where both groups have been combined and a grand mean of all values presented for rates of synthesis from all carbon sources (based on labeled water data) and from glucose-C in liver and in carcass. Only 4% of the FA synthesized from dietary glucose-C and only 17% of the FA synthesized from all sources of carbon were formed in the liver.

Of the total of 325 (270 + 55) nmol FA that were formed de novo from all 2-C units each min, 280 (270 + 10), or 86% was derived from exogenous

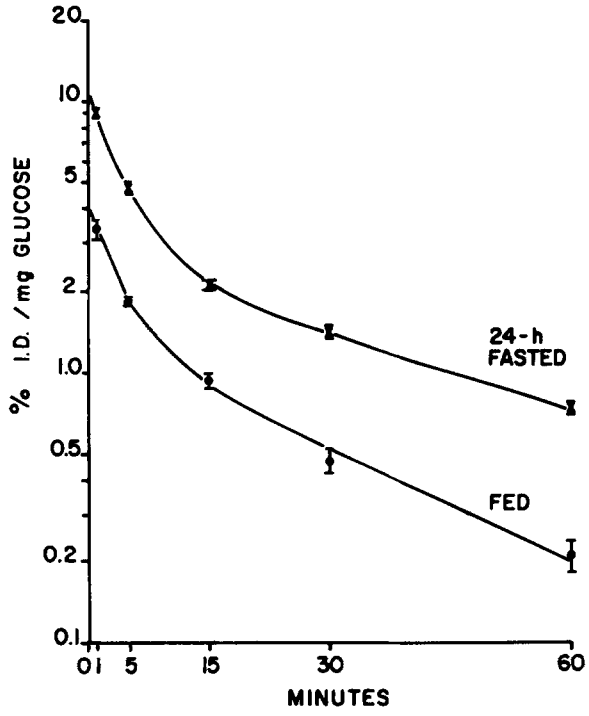

Fig. 1. Plasma glucose specific activity (% of injected dose/mg glucose) after intravenous injection of tracer [U-^{14}C]glucose into mice fasted 24 hr or fed ad libitum. Each value is the mean ± SEM of six mice previously fed a fat-free, 58% glucose diet for 3 wk. Each curve drawn is a least-squares computer fit of a biexponential function.

glucose-C. Thus, these estimates indicate that 86% of the total FA that were being synthesized derived their carbon from the dietary glucose that was fed in the test meal. As noted above, this was true whether the mice were fed neat glucose or glucose plus a potential source of amino acids. No evidence was found to support the view that the liver synthesized most of the body's FA in either case.

Since these results were in such striking contrast to the findings of Hems et al. (17), with respect to the role of the liver in relation to total FA synthesis, a more thorough study was carried out in which rates of FA synthesis from total carbon sources and from dietary glucose-C were calculated based upon more extensive data (serial measurements of tracer incorporation into FA). These data were related to rates of FA synthesis in mice that were either starved for 24 hr or fed ad libitum (no test meals) but injected intravenously with tracer [U-^{14}C]glucose and intraperitoneally with $^{3}H_2O$.

Rates of body glucose carbon replacement (irreversible disposal) and conversion to FA and other "end-products" in fasted mice and in mice fed ad libitum

In order to calculate rates of FA synthesis from glucose-C in animals that were not given labeled glucose test meals, tracer glucose was injected intravenously and rates of FA synthesis in liver and carcass were calculated by semi-compartmental analysis (30). This approach requires measurement of the plasma glucose specific activity–time curve (**Fig. 1**) and incorporation of [^{14}C]glucose into FA at one point in time (60 min, in this case). As shown in Fig. 1, there was little variation within each group of mice, as reflected by the small standard error in either fasted or fed animals. In the fed mice the specific activity curves were lower initially and fell as fast as or faster than in the fasted mice. This reflects both a greater body pool size in fed mice, as reported earlier in another mouse strain (29), and a greater rate of glucose irreversible disposal in the fed mice (29). The curves are defined by the following equations:

$$\text{Fasted:} \quad \text{sp. act} = 8.2\,e^{-0.26t} + 2.7\,e^{-0.022t}$$

$$\text{Fed:} \quad \text{sp. act} = 2.6\,e^{-0.28t} + 1.4\,e^{-0.033t}$$

(Units of specific activity are shown in Fig. 1, ordinate.)

Body glucose was calculated based upon the specific activity at $t = 1$ min, which closely approximates the extrapolated specific activity value at t_0; the rationale and validation of this technique have been presented earlier (30). The body glucose contents of fasted and fed mice were 6.2 ± 0.3 and 16 ± 1.3 mg/20 g body wt, respectively (mean ± SE; $n = 6$/group). The irreversible glucose disposal rates, calculation of which is independent of the estimated body glucose pool size, are shown in **Table 2.** The values, expressed

TABLE 2. Rates of body glucose-carbon replacement (irreversible disposal) and conversion to fatty acids and other end-products in fasted and ad libitum-fed mice

Parameter	Dietary State	
	24-hr Fasted	Fed Ad Libitum
Rate of irreversible disposal of glucose-C (μg glucose-C/min)	260 ± 2.0	770 ± 8.4
Incorporation at 60 min of injected glucose-^{14}C (% injected dose) into:		
Carcass FA	0.78 ± 0.050	2.30 ± 0.13
Liver FA	0.018 ± 0.0011	0.203 ± 0.083
Total end-products	71 ± 1.1	84 ± 1.2
Rate of glucose-C conversion (μg glucose-C/min) to:		
Carcass FA (R_2)	2.9 ± 0.02	21 ± 0.2
Liver FA (R_1)	0.065 ± 0.00057	1.8 ± 0.01
Rate of fatty acid synthesis from glucose-C (nmol FA/min) in:		
Carcass (R_4)	15 ± 0.13	111 ± 1.0
Liver (R_3)	0.34 ± 0.0029	9.6 ± 0.088

Mean ± SEM ($n = 6$/group). Mice were given intravenous injections of 5 μCi of [U-^{14}C]glucose (30) and the incorporation into end-products calculated from data in Fig. 1. The rates of lipogenesis were determined as described in Methods.

as μg glucose carbon/min, averaged 770 in fed mice and 260 in fasted animals; thus, glucose was being removed three times faster in the fed than in the fasted mice.

The incorporation of ^{14}C into FA in liver and carcass of both groups of mice 60 min after injection also is shown in Table 2. The incorporation into carcass FA was three times faster in fed than in fasted animals; however, the incorporation of glucose-C into liver FA was an order of magnitude faster in the fed than in the fasted mice. The liver was clearly of much greater quantitative significance in the fed than in the fasted animals; however, even in the fed state, the liver accounted for only 8% of the total ^{14}C-labeled FA.

Values for the incorporation of [^{14}C]glucose into total "end-products" at 60 min were calculated from the curves shown in Fig. 1 and the coefficients and exponents of the equations (above) that describe these curves. Using these values (71 and 84% in fasted and fed mice, respectively), we could calculate the rates of FA synthesis from glucose-C (Table 2). These rates will be discussed below in relation to rates of FA synthesis in mice given a glucose test meal. However, it is noteworthy that the difference between fed and fasted rates of FA synthesis from glucose-C are much more striking than one would estimate simply by inspecting the ^{14}C incorporation data into FA. Rates of FA synthesis were almost an order of magnitude faster in the carcasses of fed than of fasted mice, and were 31 times faster in livers of fed than of fasted animals.

The data in Fig. 1 also provide additional information about the body glucose pool, its rates of replacement and recycling, and the like; however, such studies in another strain of mice have been presented before, and the present data are in close agreement with the other published studies (29, 30). Here, we wish to focus primarily upon the conversion of glucose- and non-glucose-C to FA.

Lipogenic activation from glucose carbon in fasted–refed mice

The rates of FA synthesis from glucose-C in fasted mice (Table 2) may be compared with the corresponding values in fasted–refed mice (Table 1). These data establish that, in this strain of mice, as in the Bar Harbor 129J strain studied earlier (12), there is an extremely rapid and large activation of FA synthesis from glucose-C immediately after the fasted mice nibble a small glucose test meal. In the carcass the increase was from 15 to 270 nmol FA/min, and in the liver the increase was from 0.34 to 10 nmol FA/min synthesized from glucose-C (fasted vs. fasted and then refed either neat glucose or the 58% glucose test meal). A more accurate estimation of the extent and rapidity of lipogenic activation in liver and carcass is provided by the data and analyses that follow; moreover, by including data obtained after ^3H_2O injection in our analyses, we have been able to compare lipogenic activation from non-glucose-C with that from glucose-C.

Incorporation of ^3H_2O into FA of liver and extrahepatic tissues of fasted, fasted–refed, fed, and fed–refed mice

In order to evaluate the relative contribution of glucose-C into FA under various dietary states and to calculate total rates of FA synthesis, the incorporation of ^3H_2O into hepatic and extrahepatic tissue FA was measured. These measurements were carried out at various times after injection of the tracer in order to take into account changes in the rates of FA synthesis that would be expected to occur after ingestion of a glucose test meal.

First, it may be useful to review the experimental format briefly. Four groups of mice were studied in the next series of experiments. These groups and their approximate plasma glucose concentrations ($\bar{x} \pm SE$) are listed below (n refers to the small number of animals used for plasma glucose analyses).

I. Fasted 24 hr, 1.1 ± 0.080 mg glucose/ml plasma (n = 2).
II. Fasted 24 hr, then refed 50% glucose in H_2O (250 mg glucose/mouse), 5.5 ± 0.82 mg glucose/ml plasma (n = 3) at 30 min after glucose intubation.
III. Fed ad libitum, 2.2 ± 0.10 mg glucose/ml plasma (n = 3).
IV. Fed ad libitum, then refed 50% glucose in H_2O (250 mg glucose/mouse), 3.2 ± 0.29 mg glucose/ml plasma (n = 3) at 30 min after glucose intubation.

All four groups were injected intraperitoneally with ^3H_2O at zero time; groups II and IV also received [U-^{14}C]glucose, added to the oral glucose load, 15 min later. Each study was terminated 75 min after injection of ^3H_2O (60 min after the labeled glucose test meal in groups II and IV).

The results of our experiments are shown in **Figs. 2** and **3.** The incorporation of ^3H_2O into carcass FA was approximately linear in the fasted mice. The rate of incorporation increased significantly following intubation of glucose (Fig. 2*A*). A definite lag period prior to activation of lipogenesis was observed in the fasted–refed mice, as noted earlier in studies using labeled glucose-C to study lipogenic activation (24). The fed–refed mice showed an even greater initial rate of tracer incorporation into extrahepatic tissue

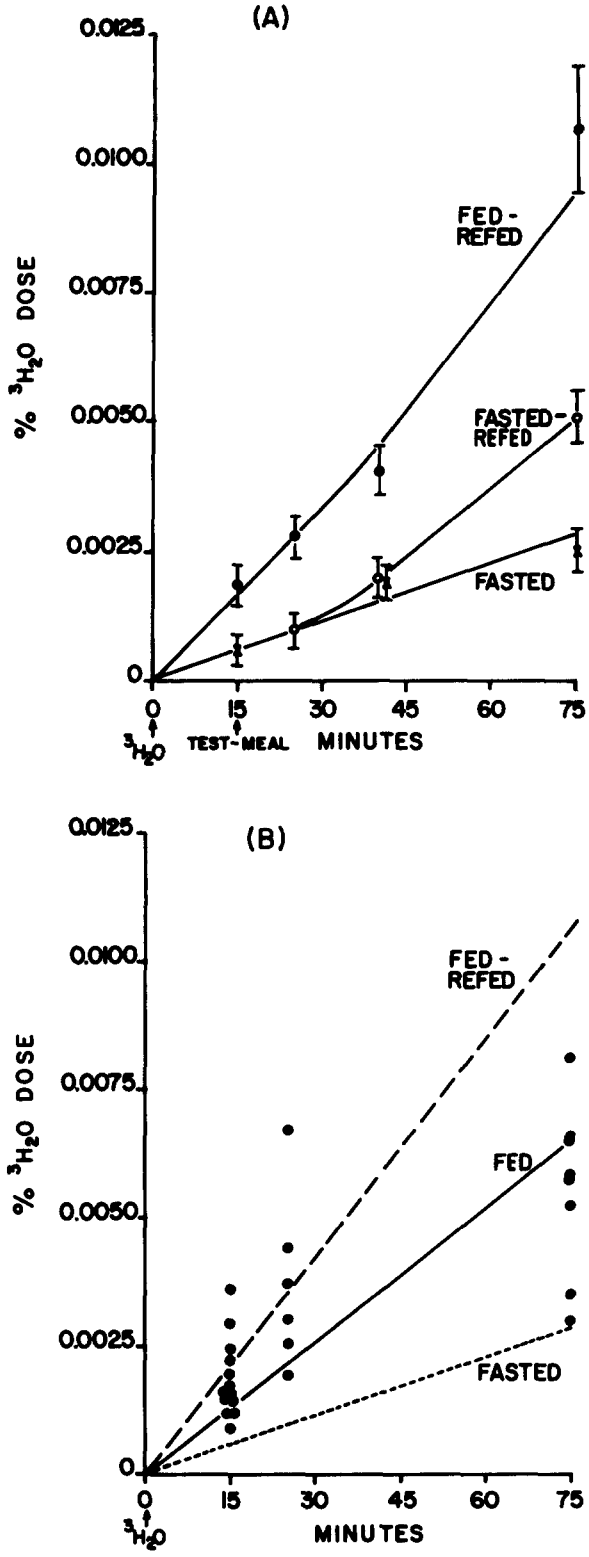

Fig. 2. Incorporation of ^3H (% dose) into extrahepatic (carcass) TLFA of mice fasted 24 hr, or fasted 24 hr and then refed, or fed ad libitum and refed (*A*), and of mice fed 58% glucose diets ad libitum but not refed (*B*). Mice were injected intraperitoneally with ^3H_2O (~2 mCi) at $t = 0$. Refed animals were force-fed 250 mg of glucose (50% solution) at $t = 15$ min. Mice had been maintained on 58% glucose diets (see text) for 3 weeks. Curves represent least-squares fit of data to a straight line from 25 to 75 min for refed animals and 0–75 min for fasted and fed (but not refed) animals. In *B*, each point represents data from one fed mouse, and the broken lines are the slopes already shown in Fig. 2*A* for the fasted and the fed–refed mice. In this latter case, the terminal slope has been transposed to zero time. These rates are only shown for reference. Mean ± SE; *n*, 6–13 mice/point (*A*).

FA and an additional enhancement of lipogenesis after intubation of the glucose test meal in comparison to the fasted and fasted–refed mice (Fig. 2*A*). The corresponding data were much more scattered in mice fed ad libitum as shown in Fig. 2*B*. The mean curves for the fasted and fed–refed (terminal slope after activation) are included for comparison. Most of the fed values fell between the two reference curves; moreover, the least-squares fit of the data to an assumed straight line function gave a slope between that of the fasted and the fed–refed groups (Fig. 2*B*). The data obtained for the mice fed ad libitum indicate that some of them were actively absorbing glucose whereas others were probably in the postabsorptive state; most of our mice seemed to be in the post-absorptive state during the day (34, 35).

The corresponding data for ^3H incorporation into liver FA in each state are shown in **Fig. 3,** *A* and *B*. As shown in Fig. 3*A*, the differences between fasted, fasted–refed, and fed–refed groups are much more pronounced in liver than in carcass. Moreover, marked changes in rates of incorporation into liver FA of both fasted and fed mice after intubation of the glucose test meal were observed, accompanied by a distinct lag period of about 10 min prior to activation of lipogenesis by the test meal in both groups of mice. As in the case of extrahepatic tissues, the incorporation of label into liver FA was highly variable in the mice fed ad libitum, as shown in Fig. 3*B*. Again, the linear least-squares fits for the fasted and for the fed–refed mice (terminal slope) are included for reference. Most of the data fell between these two limits, and the least-squares fit to a straight line gave a slope that was also intermediate in value. In each case (Figs. 2*A*, *B* and 3*A*, *B*) a zero-time blank has been subtracted.

Although we shall postpone detailed discussion of these data until rates of FA synthesis, which were calculated from these tracer incorporation data, are presented (below), it is of interest at this point to compare the slopes in Figs. 2*B* and 3*B*, where two phenomena are especially pronounced. First, the inhibitory effect of fasting (compared to fed–refed values) was much greater in liver than in carcass. Second, whereas the liver synthesized an almost negligible proportion of the total FA in fasted mice, the liver synthesized FA as fast as all the rest of the body combined in the fed–refed animals.

Incorporation of exogenous glucose carbon into hepatic and extrahepatic FA of mice previously fed or fasted 24 hr

Curves defining the rates of incorporation of [U-^{14}C]glucose (fed by gastric intubation) into extrahepatic and hepatic tissue FA of previously fed and fasted mice are shown in **Fig. 4,** *A* and *B*, respectively. As noted previously in fasted mice, there was a marked delay after feeding glucose before activation of lipogenesis was evident (24). That such a lag also occurs in mice fed ad libitum suggests that the fed mice were probably in an early postabsorptive state, consistent with the data obtained after injection of $^{3}H_2O$, above. The incorporation of glucose-C into FA was significantly faster in the fed than in the fasted mice; however, this difference was considerably more pronounced in liver than in carcass. In order to calculate rates of FA synthesis from exogenous glucose-C (fed in the test meal), the slope between 25 and 60 min after feeding the labeled test meal was used (24). These values are summarized and discussed below.

Rates of FA synthesis from glucose- and from non-glucose-carbon in hepatic and extrahepatic tissues of fasted and fed mice

The data presented in Table 2 and Figs. 1–4 were used to calculate rates of FA synthesis, summarized in **Table 3.** Note that the rates calculated for synthesis from glucose-C refer to exogenous glucose in the case of the refed animals, whereas in the fasted and fed (ad libitum) groups, the rates refer to "endogenous" body glucose (traced by intravenous injection studies). Among the many comparisons that may be made using the data in Table 3, we shall first focus upon the relative role of the liver under each condition. In the fasted state, only 3.3% of the total FA synthesized was made in the liver. The liver's relative role increased to 50% of the total in the fed–refed state. If one looks specifically at the FA synthesized from glucose-C, the liver again accounted for only 2.2% of the total in the fasted state but increased to 39% in the fed–refed condition. Only in this latter condition did glucose-C play a major role with respect to FA synthesis. This is interesting because this dietary state is the one least likely to be chosen for a tracer study because of the potential difficulties involved in analyzing the data.

The liver appeared to synthesize all of the FA that were synthesized from non-glucose-C in both the fasted–refed and the fed–refed states. Conversely, all of the FA synthesized in the carcass was derived from the dietary glucose carbon in mice that were actually absorbing glucose from the test meal. This

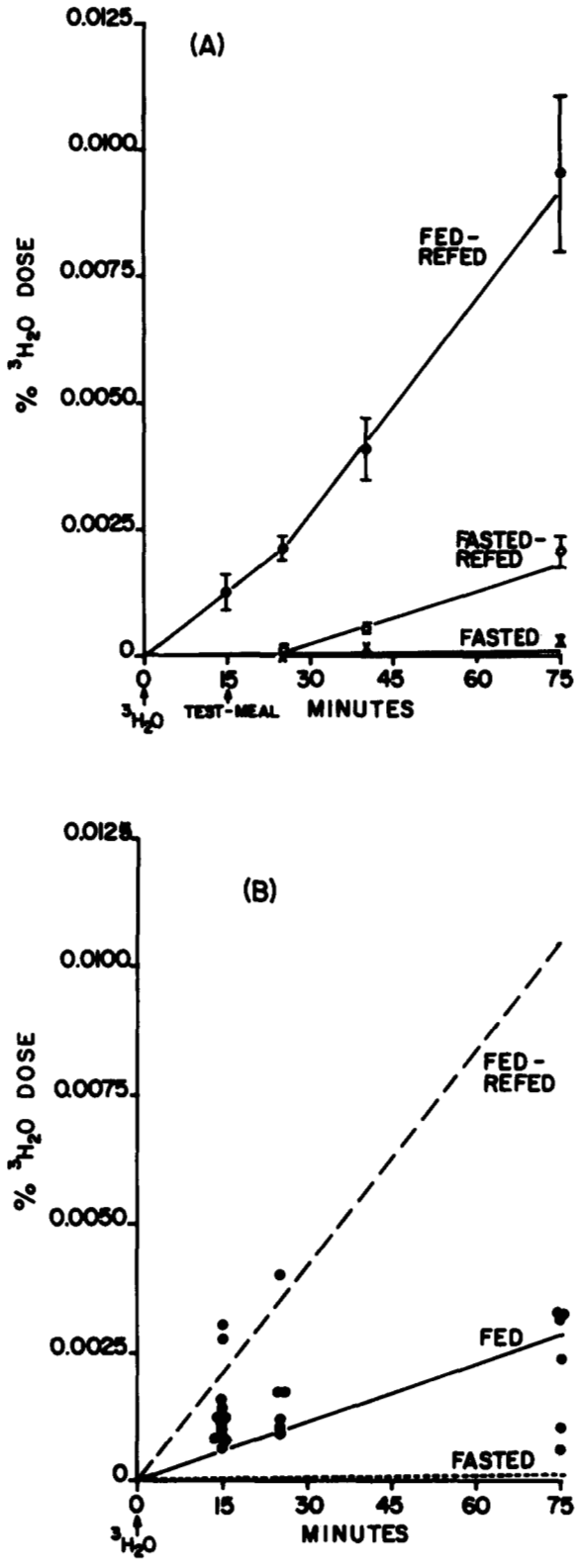

Fig. 3. Incorporation of ^{3}H (% dose) into liver TLFA of mice fasted 24 hr or fasted 24 hr and then refed, or fed ad libitum and refed (*A*), and of mice fed 58% glucose diets ad libitum but not refed (*B*). Conditions and data analysis are exactly the same as those of corresponding carcasses in Figs. 2*A* and 2*B*, including both the fasted and the fed–refed terminal slopes.

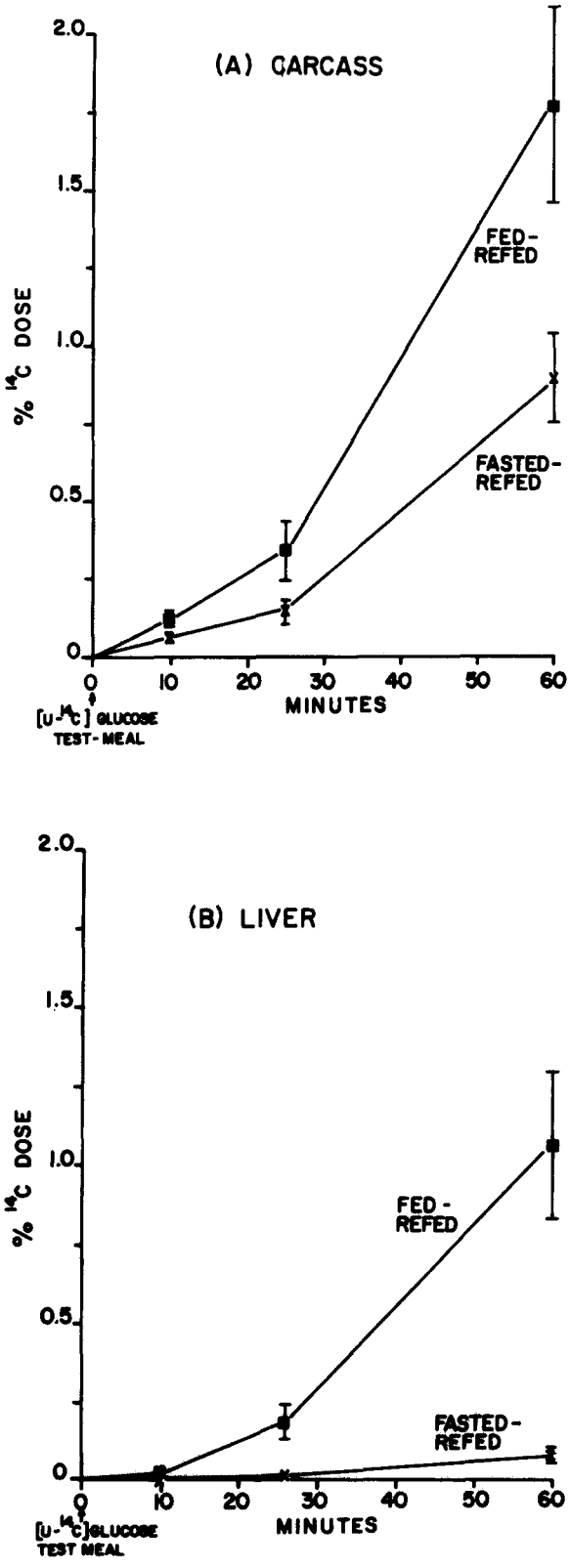

Fig. 4. Incorporation of ^{14}C into extrahepatic ("carcass"; panel *A*) and hepatic (panel *B*) TLFA of mice fasted 24 hr or fed ad libitum. Both groups of mice were force-fed 250 mg of [U-^{14}C]-glucose as a 50% solution at $t = 0$. Data are mean ± SE; $n = 7$ or 8. Mice had been maintained on diets as described in Figs. 2 and 3.

TABLE 3. Rates of fatty acid synthesis from glucose-C and from non-glucose-C (relative role of the liver) in fasted and fed mice

Dietary State	Rate (nmol FA/min) from: Total Carbon	Glucose-Carbon	Non-glucose-Carbon
Fasted			
Carcass	83	15	68
Liver	2.8	0.34	2.5
(Liver as % of total)	(3.3)	(2.2)	(3.5)
Fasted–Refed			
Carcass	190	190	0
Liver	71	18	53
(Liver as % of total)	(27)	(8.7)	(100)
Fed			
Carcass	190	110	80
Liver	83	10	73
(Liver as % of total)	(30)	(8.3)	(48)
Fed–Refed			
Carcass	330	350	0
Liver	330	220	110
(Liver as % of total)	(50)	(39)	(100)

indicates that the feeding of glucose suppressed lipogenesis from non-glucose-C in the carcass, but not in the liver. Even though the total rate of FA synthesis was more than twice as high in the fed as in the fasted mice (neither group given a test meal), no significant difference was observed in the rates of FA synthesis from non-glucose-C in extrahepatic tissues of these animals. In the fed–refed mice, at least two-thirds of the total FA synthesized in the liver and 83% of that made in the whole body was derived from exogenous glucose-C. These relationships are brought out more clearly in Figs. 5*A* and 5*B*, as discussed below.

Effect of fasting on FA synthesis in liver and carcass

In **Fig. 5*A***, which is derived from Table 3, four relationships are emphasized. First, fasting for 24 hr reduced the rate of FA synthesis from all 2-C units in the whole mouse from 660 to 86 nmol FA/min, a decrease of 88%. Second, the corresponding inhibition in liver was 99%, from 330 to 2.8 nmol FA/min. Third, the rate of FA synthesis from glucose-C in the whole mouse after a 24-hr fast fell from 570 to only 14 nmol FA/min, a 98% inhibition. Fourth, the rate of FA synthesis from glucose-C in the liver fell from 220 to 0.3 nmol FA/min following this 24-hr period of starvation. The minimal estimate (12) of glucose-C conversion to FA in the livers of the fully fed mice was 70,000% faster than that of the fasted mice in vivo.

Effect of a single, small glucose test meal on lipogenesis (FA synthesis) in previously fasted mice

Again, four relationships derived from Table 3 have been selected for emphasis and are summarized in

Fig. 5*B*. First, the total rate of FA synthesis from all 2-C units in the whole mouse was only increased 3-fold by ingestion of the test meal. This contrasts markedly with our earlier reports that a single test meal causes an order-of-magnitude increase in the rate of FA synthesis from glucose-C (12, 23, 24). The latter activation was confirmed in the present study, both in the whole mouse and in the liver. On the average, lipogenesis from glucose-C was activated 14-fold in the whole body and 60-fold in the liver (Fig. 5*B*). The total rate of FA synthesis from all 2-C units in the liver was increased 25-fold by a single test meal, from 2.8 to 71 nmol FA/min.

DISCUSSION

A major discrepancy has existed in the literature with respect to the role of the liver in FA synthesis from glucose carbon and from other carbon sources. As we noted in our introduction, Hems et al. (17) have calculated that at least 60% of all FA synthesized de novo from all carbon sources is made by the liver. However, in the 30 years that preceded this report (17), all relevant experiments utilizing ^{14}C-labeled glucose have shown that the liver synthesizes only a minor fraction of the body's FA, even under conditions of induced (nondiabetic) hyperglycemia in both rats and mice (1–10; 12–15). In the present study, we have attempted to resolve these discrepant observations by using quantitative, in vivo tracer techniques and by varying and controlling both the previous and the immediate nutritional status of our animals.

Our first preliminary experiment, using fasted–refed mice, confirmed an earlier observation from our laboratory (23); namely, that lipogenic activation by dietary glucose in previously fasted mice, as measured by the incorporation of labeled glucose carbon into FA, is independent of dietary amino acids (at least when the latter are fed as casein). Our present data now extend this observation to include FA synthesis from all sources of carbon, as judged by the incorporation of ^3H_2O into both carcass and liver FA in fasted–refed mice. On the other hand, our experiments with fasted–refed mice, during periods of rapid lipogenic activation by dietary glucose, differed markedly from the findings of Hems et al. (17) that the livers of fed mice synthesized most of the body's FA. As in our earlier studies with glucose carbon as tracer, the liver's role was not dominant, even when ^3H_2O was used to estimate lipogenic rates. In more detailed experiments that included mice which had been fasted, fasted–refed, and fed ad libitum, we still found

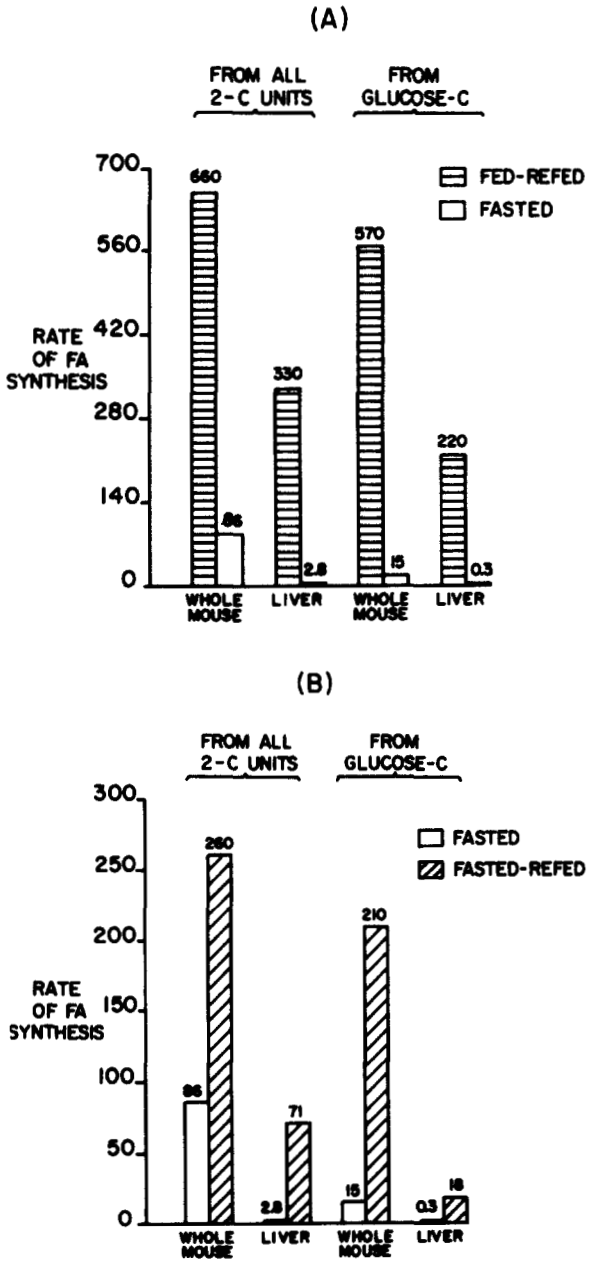

Fig. 5. Effect of fasting (*A*) and of refeeding glucose to previously fasted mice (*B*) on fatty acid synthesis from all 2-C units and from glucose carbon in the whole mouse and in the liver. Rates of FA synthesis are expressed as nmol FA/min and are derived from the data shown in the previous figures and Tables 2 and 3.

that the liver contributed less than half of all FA synthesized, regardless of the carbon source. Of these three groups, the liver's lipogenic role was greatest in the animals that had been fed ad libitum; however, even in the latter mice, the liver made only 30% of the total FA that were synthesized in the whole animal (i.e., from all 2-C sources) and contributed only 8% to the total FA synthesized from glucose carbon. These fed animals had been eating a fat-free, 58%

glucose diet—one that might be expected to maximize the liver's role as a lipogenic organ. Thus, our findings were, to a large extent, in agreement with most studies that have been reported in the literature. For example, we have converted data recently reported by Yen et al. (22) into the units used here (nmol FA/min formed from all 2-C units, based upon their ^3H_2O studies). In each of six different groups of mice that were fed ad libitum, the liver accounted for only 25–33% of the total body's FA synthesis (22); moreover, absolute rates of FA synthesis in both carcass and liver were almost identical to those reported here for mice fed ad libitum or fasted–refed, especially if a small correction is made to allow for tracer ^3H_2O equilibrium in our studies (intraperitoneal injection) vs. theirs (intravenous injection).

Nevertheless, when we took precautions to insure that fed mice were actually absorbing glucose by the time of the study, a condition emphasized by Favarger and Gerlach (1), Jansen et al. (5–7), and others (12, 29, 36), a dramatic change occurred in our animals. The liver was found to synthesize half of the body's total FA; two-thirds of these FA newly synthesized by the liver could be shown to be derived from dietary glucose carbon; and all of the FA synthesized in the carcass appeared to be derived from the exogenous glucose carbon with negligible dilution. In the whole animal, at least five-sixths of the newly synthesized FA was derived from dietary glucose carbon under these conditions (fed–refed). We have obtained additional support for our finding that the liver can synthesize a very large fraction of the total body's FA from dietary glucose carbon in a completely independent study of lipogenesis in cancerous and control mice. We used conditions similar to those reported here (fed–refed mice) and found that the liver contributed about 30% of the total body FA newly synthesized from glucose carbon.[3] Clearly, the values that we are finding in our laboratory (30–39%) for the liver's minimum, relative contribution to total FA synthesis from glucose carbon in fully fed, nibbling mice are higher than any other values reported in the literature.

Despite our unequivocal demonstration of the liver's important role in the synthesis of FA from glucose carbon, and despite the fact that this evidence was only obtained under the fed–refed condition used by Jansen et al. (5, 6), our findings are in conflict with those reported by the latter investigators. They observed (5, 6) that only about 10% of the total FA synthesized in 1 hr from dietary glucose carbon was formed in the livers of fed–refed mice. They also found a similarly minor role of the liver in rats under comparable conditions; i.e., 5–7% of the total synthesis from dietary glucose carbon occurred in the liver. Our data also differ from those of Jansen et al. in that a distinct lag in the incorporation of [U-^{14}C]glucose into carcass FA was observed in our fed–refed mice, whereas they found no lag at all. We have no explanation for these differences between our results and those of Jansen et al.; however, as noted earlier, we have never been able to find the remarkably rapid rates of incorporation of glucose carbon into carcass FA reported by Jansen et al. even though we have tried to use conditions identical to theirs with respect to mouse strain, diet composition, test meal, and method of saponification.[3] However, our values for the incorporation of exogenous glucose carbon into hepatic FA in fed–refed mice are comparable to those reported by Jansen et al. (5, 6). Thus, it appears as though under conditions of extremely rapid lipogenesis (no lag, 13% incorporation of a large glucose load into carcass FA in 60 min), the liver again plays a minor role with respect to total body FA synthesis from glucose carbon. This possibility is supported by the studies of Lequin and Steyn-Parvé (2) with insulin-treated rats that were known, and also shown by the authors, to be hyperlipogenic compared to normal controls. Only about 2% of the total FA synthesized from glucose carbon fed as a test meal was recovered in the liver of animals that had been fasted for only 3 hr prior to glucose intubation in these insulin-treated animals (2). Our earlier quantitative studies of 25-hr fasted, meal-fed mice also indicated that the liver played a minor role in the conversion of glucose carbon to FA, even after the mice gorged a glucose-rich meal for an hour of nearly continuous eating (12). In those studies only about 7% of the FA newly synthesized from glucose carbon was found in the liver 60 min after the mice had finished ingesting the 1.2 g meal. In summary, then, the liver may play a quantitatively major role in the synthesis of FA, both from dietary glucose carbon and non-glucose carbon during alimentary hyperglycemia in previously non-fasted, nibbling mice, but this has not been observed regularly by other workers under a wide range of conditions, each of which was associated with rapid lipogenesis in mice and rats. Thus, a key variable that determines the relative role of hepatic lipogenesis remains to be elucidated.

On the basis of our findings in fed–refed mice, it seems necessary to reexamine a common fallacy that has arisen as a result of two decades of failure to demonstrate rapid lipogenesis from glucose carbon in the liver, and, in fact, very low rates of incorporation of glucose into any major end-products other than

[3] Kannan, R., D. B. Learn, and N. Baker. Unpublished results.

glycogen and CO_2. These observations have led some workers to play down the importance of carbohydrate as a carbon source for FA synthesis in the liver (see quote from ref. 17 in introduction, above). We have calculated from our present data, assuming a minimal value for the glucose irreversible disposal rate in fed–refed mice, that only about 10% of the glucose metabolized by both carcass and liver is converted to fatty acids, even under the most favorable conditions. (This agrees with the early work of Masoro, Chaikoff, and Dauben (37), but not with that of Stetten and Boxer (18) who estimated, on the basis of D_2O studies and several assumptions, that 30% of the ingested glucose had been converted to fatty acids in previously fasted rats.) Moreover, the liver accounts for only a minor fraction of the total body's utilization of glucose in mice (34). Nevertheless, the liver may play a major role in the body with respect to both total glycogen synthesis (2, 7, 18), and, as we've shown here, total FA synthesis from dietary glucose.

From our present data we are unable to distinguish how much of the exogenous glucose carbon, either in the hepatic or extrahepatic tissues, was first converted to lactate and other compounds (perhaps in muscle), then recycled to liver and to adipose tissue, and finally converted to FA by this indirect route. Numerous studies have shown that a major part of lactate is derived from glucose (e.g., 38–41); therefore, under the conditions of our experiments (24), one expects lactate to become highly labeled. Moreover, lactate has been shown to be a highly effective precursor of FA in adipose tissue in vitro (42).

Our data confirm and extend the earlier work of Jansen et al. (5–7) with respect to the effects of fasting on FA synthesis in mice in vivo. Fasting inhibits FA synthesis drastically in both hepatic and extrahepatic tissue, but the effect is much greater in liver than in the carcass (adipose tissue). We found a greater sensitivity of liver to fasting whether we measured FA synthesis from all 2-C units or from glucose carbon; yet, lipogenesis from glucose carbon was inhibited both in liver and carcass to a greater extent than it was from non-glucose carbon. The converse was also true; namely, lipogenic activation in previously fasted mice was more pronounced in liver than in carcass, and a greater percentage increase was noted for glucose carbon than for all 2-C units. The molecular basis for these differences is being studied, but the data are consistent with the earliest studies of glucose and acetate conversion to FA in liver slices (43, 44) and in adipose tissue (45) in fasted and refed animals.

There is an important potential implication of the present study in relation to carbohydrate-induced hyperlipemia and obesity. Earlier studies had indicated that after a period of fasting, the ingestion of carbohydrate would lead to a condition of hyperlipogenesis both in liver and in adipose tissue. Hyperlipogenesis in liver could lead to increased secretion of lipoproteins into the blood and possibly increased deposition of lipids, both in arterioles and in fat depots. However, our data strongly suggest the opposite relationship. That is, we would expect a much smaller fraction of the calories ingested as carbohydrates in the previously fasted animal to be converted to FA than in the case of nonfasted mice. If this is, in fact, the case, and, if it holds true in human subjects, one might consider avoiding the ingestion of carbohydrates except after periods of intermittent fasting—periods long enough to reduce the liver's and adipose tissue's capacity to synthesis FA from glucose carbon. Further studies are required to establish whether there are any practical implications of our findings with respect to human nutrition, dietary management, and prevention of disease states.

We wish to thank Dr. Ramaswamy Kannan and Mr. James M. Mead, Jr., for their valuable contributions toward the design and execution of the initial pilot studies on which this paper was based. This work was supported in part by VA Medical Research, VA Geriatric Research, Education and Clinical Center, and NIH-USPHS Grants CA 15813 and Am 16673. Computing assistance was obtained from the Health Sciences Computing Facility, UCLA, supported by NIH Special Research Grant Resources RR-3.

Manuscript received 8 September 1977; accepted 15 February 1978.

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