[U-^{14}C]Glucose metabolism in vivo in rats rendered obese by a high fat diet

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Abstract Estimates of the glucose pool, the glucose space, the turnover rate, and the recycling of glucose were made after the injection of [U-^{14}C]glucose into (*a*) obese rats fed a high fat diet and (*b*) rats fed a carbohydrate diet. The specific activity–time curve consisted of two components. Physiological parameters were calculated by using a two-compartment model. The glucose pool and glucose space were the same in both groups of rats. The turnover rate was 1.96 mg · min^{-1} for the carbohydrate-fed rats and 1.55 mg · min^{-1} for the fat-fed rats. There was about 12% recycling in both groups. In the carbohydrate-fed group, another approach based on simultaneous use of [6-^{14}C]glucose and [6-^{3}H]glucose yielded nearly the same values for these parameters. Respiratory excretion of CO_2 and the incorporation of labeled glucose into lipids of some tissues were also measured. The rate of excretion of labeled CO_2 and the conversion of labeled glucose into fatty acids in fat-fed rats were lower than in the carbohydrate-fed rats by 50% and 80%, respectively. More glucose was diverted into glyceride glycerol in the fat-fed group. It is suggested on the basis of the results that glyceride glycerol can serve as a gluconeogenic substrate in these rats where the turnover rate of glucose is much higher than the daily intake of carbohydrates.

Supplementary key words glucose space · glucose pool · two-compartment model · turnover rate · glucose oxidation · glucose irreversible disposal · recycling · gluconeogenesis · perirenal and epididymal adipose tissue · fatty acid · glyceride glycerol · Cori cycle

It is well known that feeding rats a high fat, low carbohydrate diet produces a syndrome characterized by excess adiposity. The adipose tissue of such animals has been demonstrated to exhibit impaired glucose and pyruvate utilization (1–3). Lipogenesis is depressed whereas glyceroogenesis, "glyceroneogenesis," and lactate production are increased. A high fat diet alters two key enzymes of "glyceroneogenesis" in adipose tissue: phosphoenolpyruvate carboxykinase, which is increased (1), and pyruvate dehydrogenase, which is decreased (2), thus exerting a sparing action on carbohydrate utilization.

A high fat diet is also known to lower markedly the activity of hepatic enzymes involved in lipogenesis (malic enzyme, glucose dehydrogenase) (4, 5) and to increase gluconeogenic enzymes (5). Curiously, in the diaphragm, a high fat diet has little effect, if any, on the uptake of glucose, though oxidation of this substrate is strongly depressed (2). These effects that differ from one tissue to another in vitro have raised questions concerning in vivo glucose utilization in rats fed a high fat, low carbohydrate diet. This study was undertaken to provide quantitative information on glucose utilization and to investigate the following questions: (*a*) Is the rate of glucose turnover modified by such a diet? (*b*) Is there a sparing effect on oxidation of glucose to CO_2? (*c*) Does the in vivo effect of a fat diet on the incorporation of labeled glucose in tissues confirm the in vitro findings?

MATERIALS AND METHODS

Animals and diets

Rats of the Commentry France Wistar strain were purchased at weaning and fed ad lib. one of the following diets for 4–6 wk. (*1*) *High carbohydrate diet* (%, w/w): casein, 12.1; DL-methionine, 0.4; lard, 1.1; wheat flour, 77.1; salt mixture, 4.0; vitamin mixture, 2.2; bran, 3.0; water, 0.2 (proteins, 18%; carbohydrates, 59%; fat, 3%). (*2*) *High fat diet* (%, w/w): casein, 31.8; lard, 41.5; wheat flour, 7.4; salt mixture, 6.0; vitamin mixture, 3.3; bran, 4.5; water, 5.5 (proteins, 28%; carbohydrates, 7%; fat, 41%). The diets were formulated to provide the same amount of protein, vitamins, and salt per calorie, and the rats had access to food until they were administered the labeled glucose.

1. Turnover of glucose

(*a*) *Intraperitoneal injection of* [*U-*^{14}*C*]*glucose.* The rats were injected intraperitoneally at 9 a.m. with a tracer dose, 6 μCi, of [U-^{14}C]glucose (120–150 mCi/mmole) in 0.5 ml of saline with 0.5 mg of unlabeled glucose. In order not to disturb the glucose pool size, only 20 μl of

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blood was taken. For each time interval, the samples from four rats were pooled and collected in microtubes (Beckman) where they were deproteinized with 10% (w/v) trichloroacetic acid. After centrifugation, samples of the supernate were applied to a thin-layer plate impregnated with a silica gel for separation of glucose and lactate from other labeled compounds. The plates were developed in water–96% ethanol–25% ammonium hydroxide 12:100:16 (6). Spots of glucose and lactate, located with aniline and bromocresol green, respectively, were scraped off and eluted with water. These solutions were added to Bray's solution (7) and radioactivity was measured. The glucose concentration was measured before and/or after chromatography. Previous data have shown that there were no systematic differences in glucose recovery.

Mathematical analysis. Analysis of the data was performed on an IBM 360/91 computer (Columiba University Computer Center), using a least squares estimation of nonlinear parameters (8). A function fitting the observed data was obtained for each group of four pooled rats, and the following physiological parameters were calculated: glucose pool size, glucose space, rate constant (fraction of pool/min) of reversible and irreversible glucose, glucose turnover rate (mg of glucose/min), and glucose recycling. Determinations were carried out on four groups of four rats for each diet, and thus a mean and standard deviation were calculated for each parameter.

In a preliminary study, the time course of appearance of [^{14}C]glucose into the blood was measured. The [U-^{14}C]glucose was administered intraperitoneally. We chose this route of injection because it is much less stressful for the animals than intravenous injection.

Seven blood samples of 20 μl each were obtained within the first 5–30 min after the intraperitoneal injection, and they were placed in counting vials. 0.5 ml of Soluene (Packard) was added. After 1 hr at 50°C, radioactivity was counted in toluene–ethanol 4:1 (v/v) scintillation fluid containing 2.5 ml of diphenyloxazole (0.4%) and 1,4-bis(5-phenyloxazol-2-yl)-benzene (0.015%). It was assumed that in this early phase very little labeled carbon was present in compounds other than glucose.

(*b*) *Intravenous injection of [6-^{3}H]glucose combined with [6-^{14}C]glucose.* This study was performed only on control rats given a high carbohydrate diet. Animals were lightly anesthetized with ethyl ether. When they had been asleep for about 5 min they were injected via the jugular vein, after a small incision, with 10–15 μCi of [6-^{14}C]glucose (40–50 mCi/mmole) and 30–40 μCi of [6-^{3}H]glucose (2 Ci/mmole). At specified intervals (see Fig. 6), blood was collected from three or four rats, pooled, and treated as above. Radioactivity and glucose concentration were measured after thin-layer chromatography.

Mathematical analysis. The log of specific activity (dpm/μg of glucose) was plotted against the time, and a regression analysis was performed. The proportion of glucose recycled via the Cori cycle was calculated as described by Dunn, Chenoweth, and Shaeffer (9).

2. Respiratory studies

The injection schedule was as outlined above. Immediately after the injection, each rat was placed in a metabolic chamber and air was drawn in at the rate of 800 ml/min through a trap containing 50 ml of 1 N NaOH. At specified times the expired gases were diverted via a Y connection into fresh NaOH. The sodium hydroxide solutions were analyzed for radioactivity and carbon dioxide. Samples of the NaOH–carbonate solution (0.2 and 0.4 ml) were placed in 25-ml flasks sealed with serum stoppers fitted with disposable polyethylene center wells (Kontes Glass) containing 1 M Hyamine (Packard). 2 ml of 6 N H_2SO_4 was injected into the flasks. After shaking for 1 hr at room temperature, the center wells were transferred into counting vials with 10 ml of the toluene–ethanol scintillation fluid. Carbon dioxide was determined on 10-ml aliquots of the NaOH solution by titration with HCl using phenolphthalein and helianthin as indicators.

Mathematical analysis. The log of the specific activity (dpm/mmole of CO_2) was plotted against time. A linear regression line was fitted to the straight-line section over the period from 1 to 5 hr.

3. Tissue analysis

30 rats fed a high carbohydrate diet and 30 rats fed a high fat diet for 6 wk were injected intraperitoneally with 15 μCi of [U-^{14}C]glucose (120–150 mCi/mmole) dissolved in 0.5 ml of saline with 0.5 mg of glucose. Six rats from each group were decapitated at 45, 105, 165, 225, and 300 min, and heart, liver, and perirenal and epididymal adipose tissues were removed and weighed. The heart and a sample of liver (1 g) were homogenized in $CHCl_3$–CH_3OH 1:1 (v/v) by using a VirTis homogenizer. The insoluble material was collected by centrifugation and reextracted with $CHCl_3$–CH_3OH. Blood was not homogenized but was otherwise treated in the same way as the liver.

Lipids from adipose tissue samples (1 g) were extracted by shaking the tissues overnight with $CHCl_3$–CH_3OH 2:1 (v/v) and then for 2 hr with fresh solvent. All the combined chloroform–methanol extracts were adjusted to a $CHCl_3$–CH_3OH ratio of 2:1 (v/v) and washed by the method of Folch, Lees, and Sloane Stanley (10) to remove nonlipid radioactive material. An aliquot was dried under nitrogen, and the residue was weighed and used for measurement of ^{14}C incorporation into total lipids. The remainder of the lipid was saponified with ethanolic KOH and then acidified for the determination of ^{14}C-labeled fatty acid and ^{14}C-labeled glyceride glycerol. Fatty acids were counted in the toluene–ethanol scintillation fluid and the glycerol in Bray's solution (7).

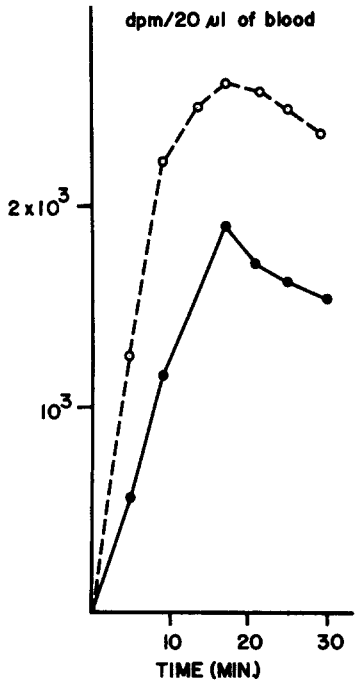

Fig. 1. Time course of appearance of [^{14}C]glucose in blood. *Closed circles,* rats fed a high carbohydrate diet; *open circles,* rats fed a high fat diet. Each point is the mean of three rats.

The material that was insoluble in chloroform–methanol was dried and digested in 30% (w/v) KOH. Total glycogen was precipitated with 1.2 vol of 95% ethanol and Na_2SO_4 (11). The precipitate was collected by centrifugation and dissolved in 0.9% NaCl. Glycogen was reprecipitated from the NaCl solution and washed successively with 95% ethanol and acetone. The washed precipitate was hydrolyzed in 2 ml of 2 N perchloric acid at 100°C for 1 hr. After neutralization with KOH, a sample of the hydrolyzate was added to 10 ml of Bray's solution (7). Radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer, and counts were corrected for quenching by using an external standard. When double labeling was used, the proportion of the measured disintegrations in the ^{3}H channel due to ^{14}C was determined.

Student's *t* test was used for statistical comparisons between two groups of data. Calculations were carried out on an Olivetti electronic calculator, Programma 101, with the help of the programs perfected by Lowy and Manchon (12).

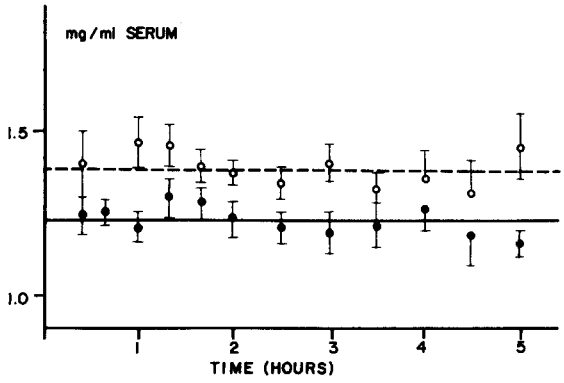

Fig. 2. Concentration of serum glucose after intraperitoneal injection of a tracer dose of [^{14}C]glucose. Each point is the mean of four pools of four rats each. *Closed circles,* rats fed a high carbohydrate diet; *open circles,* rats fed a high fat diet.

RESULTS

Mixing time

Fig. 1 shows the time course of diffusion of radioactive glucose from the intraperitoneal cavity into the blood. The peak of blood radioactivity was at 17 min in both groups of rats. With a single intravenous injection, the mixing time is shorter but not negligible (13, 14). Nevertheless, in order to make the mathematical models as simple as possible, no delay time in mixing was assumed. These results (Fig. 1) led us to the conclusion that in turnover rate studies blood sampling should not be started earlier than 25 min after the injection of isotope.

Stability of serum glucose concentration

Fig. 2 shows that there was no significant change in the serum glucose concentration during the time period studied. Therefore, it seems justified that further calculations should be based on a steady state system. Compared with controls, fat-fed rats had an increased serum glucose concentration ($P < 0.05$). This is in agreement with the results of Zaragoza and Felber (15).

Pool size and turnover rate

Experiments with [U-^{14}C]glucose. **Figs. 3** and **4** depict the curves of the decline in specific activity of blood glucose in rats fed the high carbohydrate diet and in rats fed the high fat diet, respectively. In these figures we report the experimental data, each individual computer-derived curve, and the computer-derived grouped curve, the equation for which is given in the legend.

In both groups of rats a rapid decrease of blood glucose specific activity was followed by a progressively slower rate of decline. The equation fitting the data is of the form: $\text{sp act}\,(t) = X_1e^{-\lambda_1 t} + X_2e^{-\lambda_2 t}$. The fall of the glucose specific activity was a little less steep in the blood of rats fed the high fat diet than in the blood of rats fed the high carbohydrate diet, as illustrated by the lower value ($P < 0.05$) of the first exponential slope in the fat-fed group. Neither the zero-time intercepts nor the second exponential slope was significantly affected as a result of feeding the rats the high fat diet.

Such a two-exponential function is compatible with a two-compartment model that makes possible a quantitative description of the behavior of glucose (**Fig. 5**). As-

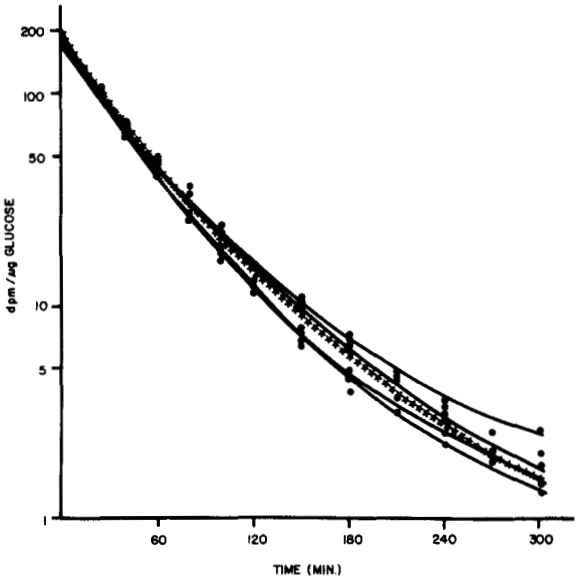

Fig. 3. Time course of blood glucose specific activity (dpm/μg glucose) in rats fed the high carbohydrate diet. ●, experimental data; ——, individual computer-derived curves (for each group of four rats); ×, computer-derived grouped curve (for four groups), whose equation is:

$$\text{sp act} = 171.2 \pm 12.7e^{-(0.0275 \pm 0.00248)t} + 27.5 \pm 12.9e^{-(0.00974 \pm 0.00258)t}$$

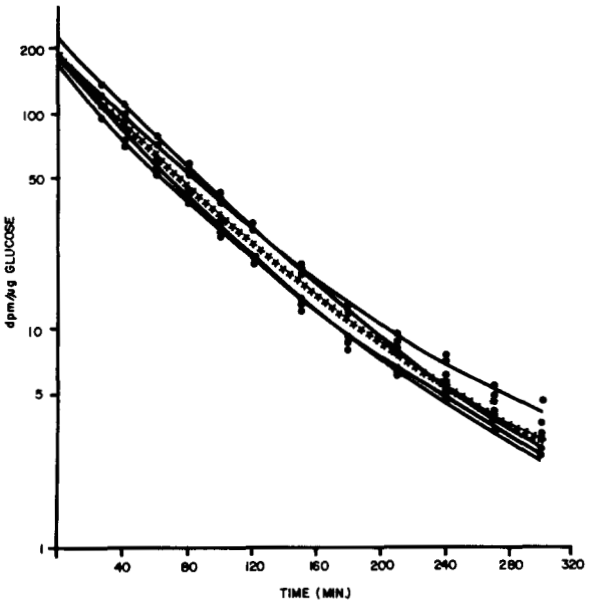

Fig. 4. Time course of blood glucose specific activity (dpm/μg glucose) in rats fed the high fat diet. ●, experimental data; ——, individual computer-derived curves (for each group of four rats); ×, computer-derived grouped curve (for four groups), whose equation is:

$$\text{sp act} = 157.6 \pm 19.81e^{-(0.0188 \pm 0.00304)t} + 20.2 \pm 12.03e^{-(0.00688 \pm 0.00329)t}$$

suming that tracer is injected into compartment I, this model predicts that a prompt transfer of ^{14}C from glucose to nonglucose (compartment II) is followed by recycling of ^{14}C back into glucose. k_{01} represents the rate constant of irreversible loss of ^{14}C. k_{21} represents the rate constant of reversible glucose, and k_{12} the rate constant of recycling from a nonglucose compartment. Nonlabeled glucose replenishes the system via ρ^{10}. The values of these parameters are given by the experimental two-exponential function according to the following relations (16):

$$X_1 = \frac{(\lambda_1 - k_{12})a_{s(0)}}{\lambda_1 - \lambda_2} \qquad X_2 = \frac{(k_{12} - \lambda_2)a_{s(0)}}{\lambda_1 - \lambda_2}$$

$$\lambda_1 + \lambda_2 = k_{01} + k_{12} + k_{21} \qquad \lambda_1\lambda_2 = k_{01}k_{12}$$

in which λ_2 and X_2 are the slope and the zero-time intercept of the second exponential, respectively; λ_1 and X_1 are the slope and the zero-time intercept of the first exponential, respectively; and $a_{s(0)}$, specific activity at zero time, is provided by $X_1 + X_2$. The pool was estimated by the ratio between the total radioactivity injected and $a_{s(0)}$. The distribution space is defined as the volume of body water in which the pool is distributed, with the assumption that the concentration of glucose in this space is similar to that of serum.

Physiological parameters are presented in **Table 1.** The size of the glucose pool is comparable in both groups of rats. As body fat increases in fat-fed rats, the distribution space becomes smaller, though the difference between the groups is not significant. Although serum glucose is higher in fat-fed rats, total turnover rate of glucose is decreased by 20%. This repressive effect of the fat diet on glucose utilization concerns only irreversible disposal. No significant difference is seen between fat-fed and carbohydrate-fed rats either in turnover rate of reversible disposal of glucose or in percentage of recycling. About 12% of the glucose that leaves the glucose pool is recycled back into the pool in both groups.

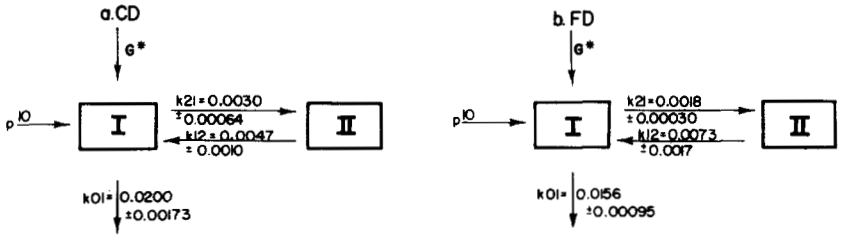

Fig. 5. Two-compartment model used to represent the kinetics of labeled glucose. *a*, values of rate constants in rats fed a high carbohydrate diet (CD); *b*, values of rate constants in rats fed a high fat diet (FD).

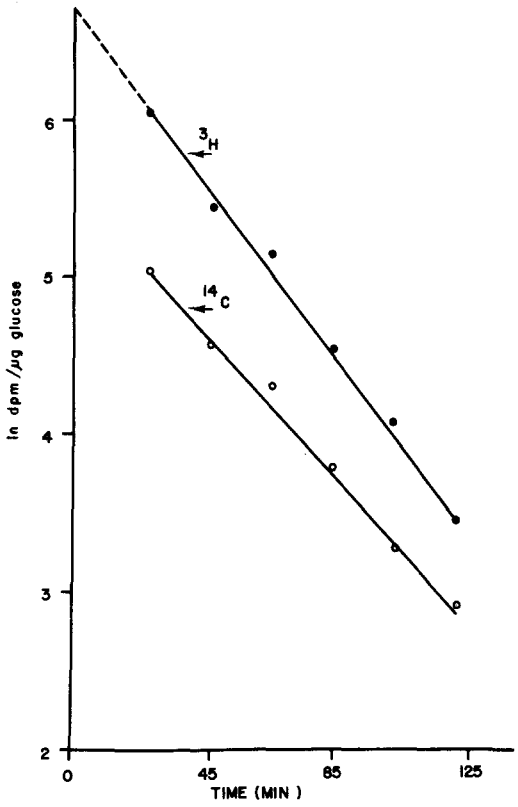

Fig. 6. Time course of glucose specific activity (dpm/μg glucose) of a representative experiment in which rats received an intravenous injection of [6-^{3}H]glucose and [6-^{14}C]glucose. Each point is the mean of two determinations on pooled blood from four carbohydrate-fed rats.

According to Shipley et al. (17), total irreversible disposal of glucose may be calculated from the numerical expression of the curve of specific activity of glucose, without requirement of a physiological model, by an adaptation of the Stewart-Hamilton dilution principle. It consists of dividing the dose of tracer by the mean specific activity (area under the curve divided by time). In our experiment, the values given by this method are 1.72 ± 0.103 mg · min^{-1} for carbohydrate-fed rats and 1.38 ± 0.121 mg · min^{-1} for fat-fed rats ($P < 0.10$). These values are strikingly similar to those obtained by a two-compartment model.

TABLE 1. Effect of diet on weight of rats, serum glucose, glucose pool, glucose space, and glucose turnover rate

	CD[a]	FD[b]
Weight of rats (g)	254 ± 2.29	265 ± 2.6[c]
Serum glucose (mg/ml)	1.24 ± 0.040	1.38 ± 0.022[d]
Glucose pool (mg)	86 ± 6.4	89 ± 5.9
Glucose space (% of body wt)	28 ± 2	24 ± 1.6
Turnover rate of glucose (mg · min^{-1})	1.96 ± 0.0739	1.55 ± 0.143[c]
Irreversible disposal (k_{01} × pool)	1.71 ± 0.115	1.38 ± 0.120[d]
Reversible disposal (k_{21} × pool)	0.25 ± 0.064	0.17 ± 0.035
Reversible disposal/total turnover rate	13%	11%

Each value is the mean ± SD of four experiments (16 rats pooled by 4).
[a] Rats fed a high carbohydrate diet.
[b] Rats fed a high fat diet.
[c] Differs from CD group, $P < 0.05$.
[d] Differs from CD group $P < 0.10$.

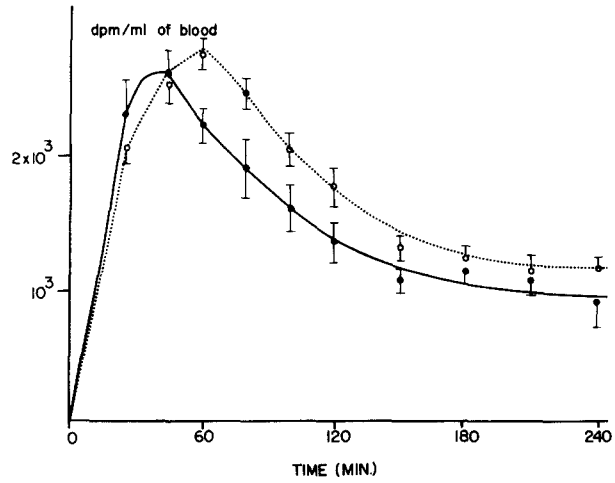

Fig. 7. Time course of incorporation of [U-^{14}C]glucose carbon atoms into blood lactate. *Closed circles,* rats fed a high carbohydrate diet; *open circles,* rats fed a high fat diet. Each point is the mean ± SEM of four groups of four rats each.

Experiments with [6-^{14}C]glucose combined with [6-^{3}H]glucose. In the above studies the tracer was injected intraperitoneally in order to have the animals as quiet as possible. Particularly in fat-fed rats, which have large stores of fat, restraining or anesthesia must be avoided (until the effects of these conditions on turnover rate of glucose in obese rats have been studied). However, with intraperitoneal injection, the question is raised about the loss of label because there is a lag in mixing. Moreover, our previous calculations were based on a two-pool model that is oversimplified and not entirely correct. In order to test the validity of this approach, we have carried out a study of the turnover of glucose using [6-^{3}H]glucose in combination with [6-^{14}C]glucose administered intravenously in lightly anesthetized rats. This comparison has been made only on control rats. According to Dunn et al.

TABLE 2. Some parameters of glucose metabolism measured by the simultaneous use of [6-^{3}H]glucose and [6-^{14}C]glucose in rats fed a high carbohydrate diet

Rate constant (min^{-1})	
[6-^{3}H]Glucose	0.0249 ± 0.000868
[6-^{14}C]Glucose	0.0205 ± 0.000896
Glucose pool size (mg)	81 ± 1.73
Glucose space (% of body wt)	27 ± 0.7
Turnover rate of glucose (mg · min^{-1})	2.013 ± 0.0633
Cori cycle (calculated according to Dunn et al. (9)	18%

Each value is the mean ± SD of three experiments (three or four rats per experiment). The rats weighed 223 ± 2.4 g; the serum glucose concentration was 1.36 ± 0.045 mg/ml.

(9), the use of [6-^{3}H]glucose in turnover studies corrects for recycling and gives an accurate measurement of new glucose production. Combined with [6-^{14}C]glucose, it provides a simple means of estimating the Cori cycle. As shown in **Fig. 6,** the decay rates of both specific activities plotted logarithmically with respect to time are linear. In agreement with the results of Dunn et al. (9), the decay rate of ^{3}H-labeled glucose is faster than that of ^{14}C-labeled glucose. **Table 2** shows that the rate constant of [6-^{3}H]glucose is significantly higher ($P < 0.05$) than the rate constant of [6-^{14}C]glucose. Glucose pool size, glucose space, and turnover rate of glucose, as measured with [6-^{3}H]glucose, are very similar to the results provided by a mathematical analysis of a two-pool model (Table 1). The recycling due to the Cori cycle (Table 2) is of the same magnitude as the recycling yielded by the two-pool model. These observations led us to conclude that recycling into the circulation of ^{14}C from labeled glycogen is very small.

Lactate labeling from glucose

The time course of incorporation of [^{14}C]glucose carbon atoms into blood lactate in fat-fed and carbohydrate-fed rats is illustrated in **Fig. 7.** The amount of ^{14}C incorporated into lactate is expressed as dpm/ml of blood, because the blood lactate concentration was not measured during the time period studied. Maximum labeling of blood lactate occurred more quickly in the carbohydrate-fed rats than in the fat-fed rats. This is in agreement with a higher turnover rate in the carbohydrate-fed rats. In the absence of data on the specific activity of lactate, it can be concluded only that there is a rapid turnover of lactate in both groups.

Oxidation of glucose

The rate of expiration and the total recovery of $^{14}CO_2$ in 4 hr after the intraperitoneal administration of [U-^{14}C]glucose is shown in **Fig. 8.** As for lactate, the maximum labeling of CO_2 occurred before 60 min in the carbohydrate-fed rats whereas in the fat-fed rats it occurred later. The regression of the log of the specific activity of CO_2 with time was linear for both groups of rats in the period from 1 to 5 hr after the injection. The high fat diet affected very significantly ($P < 0.01$) the constant and the coefficient of this single-exponential function. In the rats given the fat diet, the labeling of CO_2 as well as the rate constant of oxidation of [U-^{14}C]glucose to $^{14}CO_2$ was decreased by 50%. Similar results have been reported by Mayes and Felts (18). The high fat reduces the oxidation rate of glucose more than the turnover rate of glucose. Indeed, irreversible disposal in the fat-fed rats is decreased by 20% while, within 4 hr, 66% of labeled glucose is converted into CO_2 in the rats given the high carbohydrate diet vs. 36% in the rats given the high fat diet (Fig. 8).

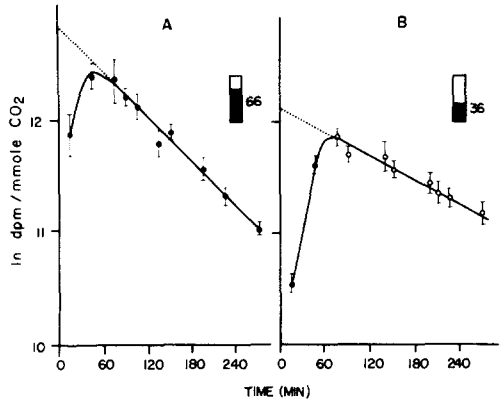

Fig. 8. Time course of specific activity of CO_2 from rats given [U-^{14}C]glucose. Means ± SEM from eight rats. The percentage recovery, over 4 hr, of radioactivity injected is shown in the rectangle. *A,* rats fed a high carbohydrate diet; *B,* rats fed a high fat diet. Observed values from 1 to 5 hr fit the following equations: *A,* ln sp act = $-(0.0070 \pm 0.00065)t + 12.875 \pm 0.101$; *B,* ln sp act = $-(0.0035 \pm 0.00041)t + 12.118 \pm 0.071$.

Incorporation of radioactivity from [U-^{14}C]glucose into different tissues

The time course of accumulation of ^{14}C into glycerol and fatty acid moieties of lipids is shown in **Fig. 9.** Radioactivity in plasma lipids was low (<0.002% of total injected radioactivity per ml of blood), indicating that recir-

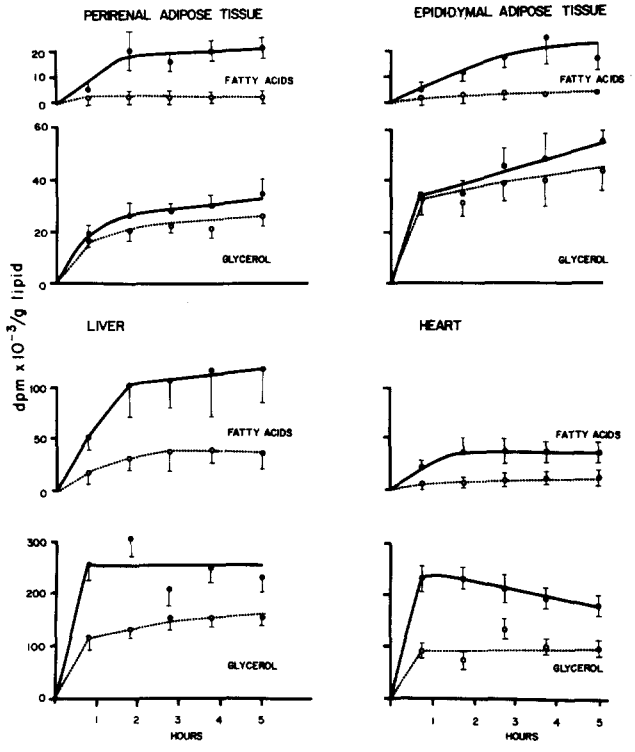

Fig. 9. Time course of incorporation of radioactivity (dpm × 10^{-3}/g lipid) into glycerol and fatty acid moieties of lipids in epididymal and perirenal adipose tissues, liver, and heart. *Closed circles,* rats fed a high carbohydrate diet; *open circles,* rats fed a high fat diet. Each point is the mean ± SEM of six rats.

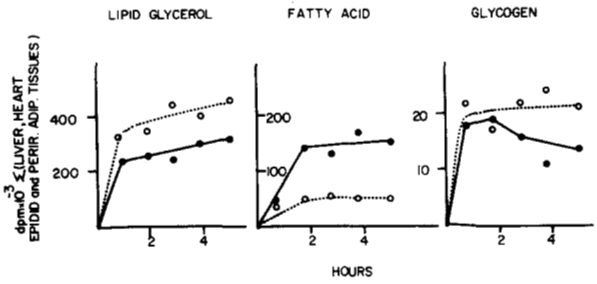

Fig. 10. Time course of incorporation of ^{14}C from ^{14}C-labeled glucose (dpm $\times 10^{-3}$) into lipid glycerol, fatty acid, and glycogen in liver + heart + perirenal and epididymal adipose tissues. *Closed circles,* rats fed a high carbohydrate diet; *open circles,* rats fed a high fat diet. Each point is the mean of six rats.

culation of lipids synthesized in tissues is small. The curves of incorporation of radioactivity into organs resemble each other except for the glyceride glycerol of heart. All curves rise sharply during the first 1–2 hr and then flatten out. The flattening can be explained by the "sink effect" of the pool, where entering ^{14}C is so thoroughly diluted that its loss with effluent is not detectable (17). Labeling of lipid glycerol in adipose epididymal tissue is significantly greater than that observed in perirenal adipose tissue ($P < 0.05$). This result agrees with the in vitro studies of Durham, Miller, and Holmes (19), who have reported an esterification rate significantly lower in perirenal tissue than in epididymal tissue. Labeling of the glycerol moiety of lipids is greater than labeling of the fatty acid. This result is different from the distribution found in the incubated fat pad (2). When results are expressed per gram of lipid, the radioactivity of lipid glycerol is found to be lowered by the fat diet in heart and liver but not in adipose tissues. On the other hand, fatty acid labeling is markedly reduced in all organs of rats given the fat diet. Lipogenesis in adipose tissue is depressed to a much greater extent by dietary fat than is lipogenesis in liver and heart. This decrease of in vivo lipogenesis in obese fat-fed rats parallels the decrease of in vitro lipogenesis from glucose or pyruvate (1, 2) and that of lipogenic enzyme activities (4, 5).

TABLE 3. Weight and lipid content of tissues from rats fed a high carbohydrate or a high fat diet for 6 wk[a]

	Heart	Liver	Adipose Tissue	
			Perirenal	Epididymal
	g of tissue			
CD[b]	0.685 ±0.011	8.856 ±0.162	3.02 ±0.163	3.28 ±0.140
FD[c]	0.881 ±0.0125[d]	10.651 ±0.218[d]	5.56 ±0.253[d]	5.34 ±0.208[d]
	g lipid/100 g tissue			
CD	2.06 ±0.086	3.50 ±0.068	88.74 ±0.964	88.17 ±0.682
FD	2.51 ±0.104[d]	6.47 ±0.027	90.80 ±0.978	88.7 ±0.825

[a] 30 animals in each group; results are means ± SD.
[b] Carbohydrate-fed rats; mean weight, 270.6 ± 3.1 g.
[c] Fat-fed rats; mean weight, 330.6 ± 4.0 g.
[d] Significantly different from the corresponding CD value ($P < 0.01$, Student's t test).

Table 3 points out that perirenal and epididymal adipose tissues are increased in weight to the same extent by the fat diet. Heart and liver are very significantly heavier in rats given the fat diet than in rats given the carbohydrate diet, partly due to an increase of lipids in these organs.

As shown in **Fig. 10,** the level of incorporation of radioactivity into lipid glycerol in the whole rat is much greater in the rats fed the high fat diet than in carbohydrate-fed rats. Whatever the basis of reference, fatty acid labeling is strongly depressed by the fat diet. In the organs studied, the incorporation of radioactive glucose into glycogen was minor and the turnover was slow (Fig. 10).

DISCUSSION

In using [U-^{14}C]glucose, this study points out that the logarithm of specific activity of [^{14}C]glucose in blood is a complex function of time. This fact is in agreement with the work of Shipley et al. (17). Nevertheless, this curve is linear in the first 2 hr. Several authors have thus reported linear regression of this parameter with time in the rat in short-term experiments (9, 20). But, if this linear portion is the basis for extrapolation to zero time in order to calculate the pool and space of glucose, an overestimation arises. In this study, the value of 28 ml/100 g body weight in control rats, obtained from a two-exponential function, is very similar to that previously reported in the literature (17, 21).

In using [6-^{3}H]glucose, a tracer that corrects for recycling, the extrapolation to zero time of the logarithm of specific activity can provide an accurate measure of glucose pool size and glucose space. Of interest is the fact that these two methods give identical values, although the route of administration was intraperitoneal in the first experiment and intravenous in the second experiment. Estimates of the glucose space are likely to be very sensitive to any lag in mixing. The intraperitoneal route of injection used in this study therefore appears to be valid, a conclusion in agreement with that of Vernon and Walker (20).

Turnover rate and recycling of glucose in this study were also calculated by two methods in control rats: mathematical analysis of a two-pool model or studies in which [6-^{3}H]glucose and [6-^{14}C]glucose were administered simultaneously, a method that dispenses with a compartmental model. The two methods yielded identical values for the turnover rate of glucose. It is interesting to compare values thus obtained with those of Katz and Dunn (13), who used [2-^{3}H]glucose, an irreversible trac-

er. The rate constants in their experiments in postabsorptive rats were between 2.3 and 3.1 min^{-1} vs. 2.1 and 2.6 min^{-1} in our study. (The mean body weight was comparable in both studies.) For recycling, the model used led us to the conclusion that 13% of the labeled glucose that leaves the pool is recycled back into the pool. In our experiments, we have seen that hepatic glycogen is poorly labeled; therefore, the recycling is probably due to the Cori cycle. Indeed, the Cori cycle estimated by the method of Dunn et al. (9) is comparable. This agrees well with the estimates of von Holt et al. (22), who investigated glucose resynthesis by measuring the randomization of label from C_6 to C_1 and reported 12% resynthesis. Still higher values for recycling have been reported; for example, Katz and Dunn (13) as well as Baker et al. (14) calculated that one-third of the ^{14}C derived from glucose is recycled.

In the fat-fed rats, metabolic parameters of glucose utilization are not all affected to the same extent. The turnover rate of glucose is decreased by 20% ($P < 0.05$) and is equal to 1.55 $mg \cdot min^{-1}$. The utilization of glucose in these rats is much greater than was estimated from some in vitro studies (for example, uptake of glucose per gram of tissue is reduced by 80% in adipose tissue).

Glucose that leaves the pool is channeled to metabolic pathways other than oxidation and fatty acid synthesis. Indeed, compared with rats given a high carbohydrate diet (if irreversible disposal is decreased by 20% in fat-fed rats), oxidation to CO_2 and incorporation into fatty acid are decreased by 50% and 80%, respectively, in fat-fed rats; this suggests some other major route must be increased in these animals. Therefore, a fat diet exerts a sparing effect on the truly irreversible disposal of glucose. The repression of pyruvate dehydrogenase and pyruvate kinase shown in the tissues of these rats could be one of the mechanisms involved (2, 23).

Another result of this study was the surprising fact that the Cori cycle was equally active in both groups of rats. Indeed, assuming that in fat-fed rats the glucose turnover rate (1.55 $mg \cdot min^{-1}$) remains constant throughout the day, such rats will utilize about 2.2 g of glucose/day. Rats fed a high fat, low carbohydrate diet consume daily 10 g of food, which provides 0.7 g of carbohydrate. This implies significant gluconeogenesis, a result in agreement with enhanced glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activity previously reported in these animals (1, 5).

As recycling of glucose via the Cori cycle is not increased, fat-fed rats must be synthesizing glucose from other sources, such as amino acids and glycerol. Because hepatic transaminases are not increased in the rats fed the high fat diet,[2] further investigation is required in order to determine whether or not amino acids are used for gluconeogenesis in this case. Glycerol could be a gluconeogenic substrate in the fat-fed rats. Indeed, tissue analysis has shown that in these rats an important amount of the glucose is channeled into lipid glycerol as fat depots become larger. In adipose tissue, glucose is needed to ensure an adequate supply of α-glycerophosphate because this tissue lacks glycerol kinase (24) or has a very low glycerol kinase activity (25). This permits the uptake and storage of dietary fatty acids that are liberated by hydrolysis of chylomicrons by lipoprotein lipase. When further hydrolysis of adipose tissue lipids occurs, glycerol returns to the liver, where it may serve as a gluconeogenic substrate. Because the pool where it is diluted is capacious, recycling of glycerol back into glucose was not detectable under our conditions, and this reversible pathway appeared irreversible.

It is interesting to compare glucose metabolism under our nutritional conditions (high fat diet) with glucose metabolism in physiological situations associated with a high utilization of lipids. Baker et al. (26) reported that oxidation of glucose was lower in alloxan-diabetic rats than in normal rats. According to Dunn et al. (9), there are no marked differences in either percentage recycling or the turnover rates between fasted and postabsorptive rats. For Baker et al. (14), irreversible disposal, per 100 g of body weight, is about 0.6 mg/min for fasted rats and 1 mg/min for postabsorptive rats, and recycling seems to be more important in the latter animals. In suckling rats, which consume a high fat, low carbohydrate diet, the glucose turnover rate is lower than that of 30-day-old rats (20). Most of these studies indicate that a high utilization of lipids is generally associated with a decreased turnover rate of glucose.

In summary, our experiments indicate that fat feeding results in (*1*) a weakly enhanced plasma glucose concentration but no significant changes of glucose pool size or glucose space, (*2*) a diminished turnover rate of glucose that is nevertheless much higher than the dietary intake of carbohydrate, (*3*) a normal contribution of the Cori cycle to glucose production, (*4*) a sparing effect on glucose oxidation, (*5*) a repression of lipogenesis, and (*6*) an enhanced incorporation of glucose into glyceride glycerol, which may be a storage form of gluconeogenic precursors.

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