Biosynthesis of Fatty Acids in Obese Mice *in Vivo*. I. Studies with Glucose-1-^{3}H(1-^{14}C), Glucose-6-^{3}H(6-^{14}C), DL-Lactate-2-^{3}H(2-^{14}C), and Glycerol-2-^{3}H(1,3-^{14}C)*

W. W. Shreeve, E. Lamdin, N. Oji, and R. Slavinski

ABSTRACT: Rates and mechanisms of biosynthesis of fatty acids in the liver and in other tissues of the remaining carcass of obese hyperglycemic mice and their lean siblings have been investigated by isolation and counting of radioactivity in total fatty acids of mice sacrificed 90 min after intraperitoneal injection of trace amounts of carbohydrates labeled with tritium and carbon-14. The pairs of labeled carbohydrates injected were glucose-1-^{3}H and glucose-1-^{14}C, glucose-6-^{3}H and glucose-6-^{14}C, DL-lactate-2-^{3}H and DL-lactate-2-^{14}C, or glycerol-2-^{3}H and glycerol-1,3-^{14}C. All of the labeled carbohydrates (whether ^{3}H or ^{14}C) showed similar and highly significant increases of about five- to eightfold greater incorporation of radioisotope into liver fatty acids of obese mice than into those of lean mice. Incorporation into carcass fatty acids of obese mice was only about 1.5- to 2-fold greater than in lean mice. In the liver, tritium from lactate and glycerol was converted to fatty acids much more extensively than that from the first or the sixth carbons of glucose in regard to the percentage of administered amount and in relation to the amount of ^{14}C converted to fatty acids from the same carbohydrates. In the liver, glucose-1-^{3}H showed no more specificity of transfer to fatty acids relative to glucose-1-^{14}C than did glucose-6-^{3}H relative to glucose-6-^{14}C. For carcass fatty acids the ^{3}H:^{14}C ratio was higher for 1-labeled glucose than for 6-labeled glucose or pair-labeled lactate or glycerol. Conversions to ^{3}HOH from various ^{3}H-labeled carbohydrates were either the same or greater in obese mice than in lean mice. On the other hand, conversions of ^{14}C-labeled carbohydrates to $^{14}CO_2$ were either the same or lower in obese mice than in lean mice.

Earlier studies of transfer of ^{3}H and ^{14}C from position 1 of glucose to fatty acids of liver and carcass in lean and obese mice *in vivo* (Shigeta and Shreeve, 1964) suggested that hydrogen from this location was not a prominent source of reductive hydrogen *via* formation of NADPH.[1] Low labeling of fatty acids from glucose-1-^{3}H might be explained by a proton–hydrogen exchange between water and that reductive hydrogen which contributes to the odd-carbon position of fatty acids (Foster and Bloom, 1963) and/or by isotope discrimination against the transfer to NADP^{+} from glucose-1-^{3}H compared to glucose-3-^{3}H (Katz *et al.*, 1965). However, an additional reason for the finding of ^{3}H:^{14}C ratios of 1.0 or less from glucose-1-^{3}H(1-^{14}C) in both liver and carcass fatty acids *in vivo* (in contrast to ratios of 6.0 or 7.0 found with rat liver slices; Foster and Bloom, 1961) could be the availability of other major substrate sources for providing reducing equivalents for fatty acid synthesis.

Positive evidence for the existence of sources of reducing hydrogen for the constitution of fatty acids other than the NADPH generated in the pentose cycle pathway has been indicated by the findings of conversion to fatty acids of DL-lactate-2-^{3}H (Foster and Bloom, 1961; Lowenstein, 1961) and glycerol-2-^{3}H (Foster and Bloom, 1961) in rat liver slices, L-lactate-2-^{3}H (D'Adamo *et al.*, 1961) in perfused rat liver, DL-lactate-2-^{3}H (Shreeve, 1965) in human subjects *in vivo*, and succinate-2,2′-^{3}H (Whereat, 1965) in aortic mitochondria. Combined radioisotopic and balance studies (Flatt and Ball, 1964; Katz *et al.*, 1966) have further indicated that the reducing equivalents generated in the pentose cycle pathway could provide only about one-half to three-fourths of those needed for synthesis of fatty acids in adipose tissue under conditions of stimulation by insulin or refeeding. It has been suggested (Lowenstein, 1961; Young *et al.*, 1964; Leveille and Hanson, 1966) that a portion of metabolic NADPH may be derived by transhydrogenation through a coupling of NAD^{+}-linked malic dehydrogenase and NADP^{+}-linked "malic enzyme." The coupling of these two malic dehydrogenases from the supernatant fraction of liver homogenates has been demonstrated (Young *et al.*, 1964). Also, measurement of changes of enzyme activities in both liver (Young *et al.*, 1964) and adipose tissue (Young *et al.*, 1964; Leveille and Hanson, 1966) upon fasting and refeeding suggests involvement of this transhydrogenation mechanism in

* From the Division of Biochemistry, Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973, and Departments of Physiology and Medicine, University of Cincinnati, Cincinnati, Ohio 45219. *Received October 3, 1966.*

[1] Abbreviations used: NADP^{+} and NADPH, oxidized and reduced nicotinamide–adenine dinucleotide phosphates; CoA, coenzyme A; NAD^{+}, oxidized nicotinamide–adenine dinucleotide.

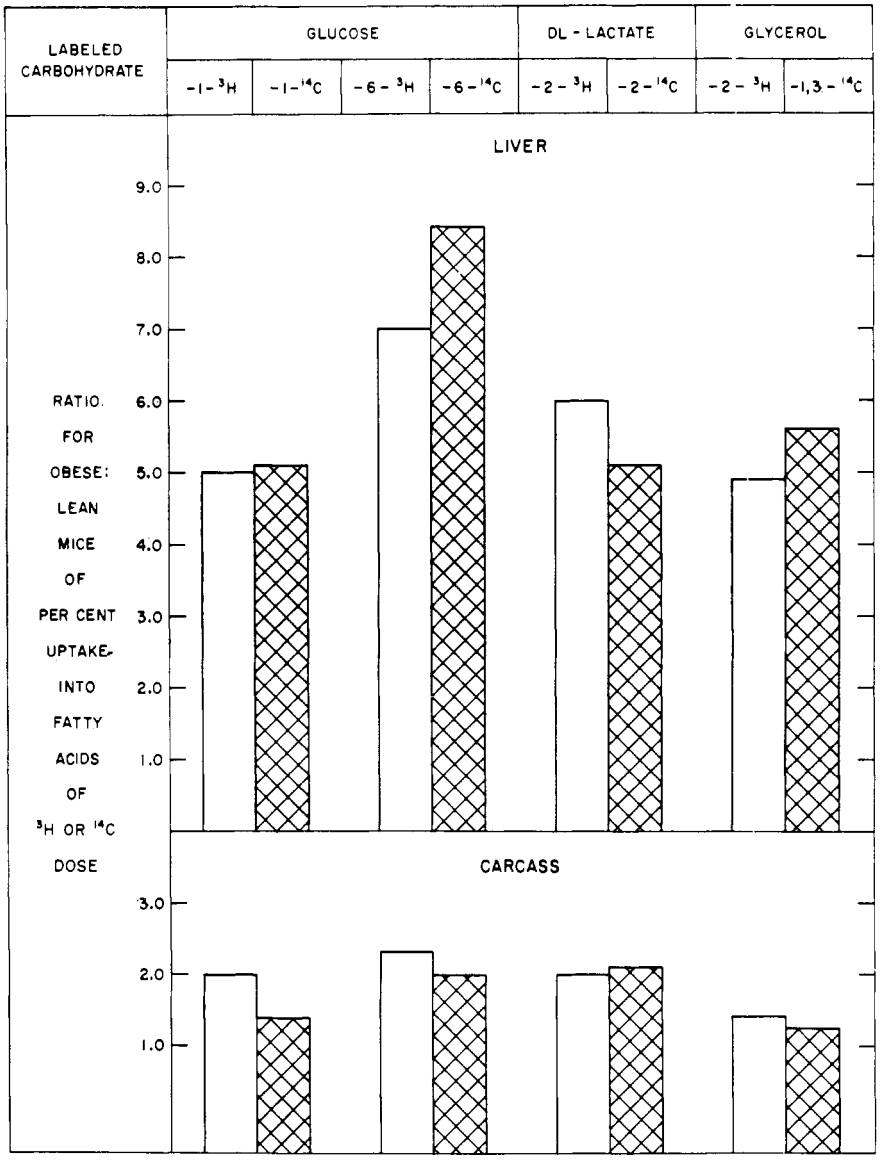

FIGURE 1: Incorporation of ^{14}C and ^{3}H into fatty acids of mice.

biosynthesis of fatty acids.

We have investigated isotopic yields of ^{3}H and ^{14}C in the liver and carcass fatty acids of lean and obese mice after intraperitoneal injection of various labeled carbohydrates in addition to glucose-1(^{3}H-1-^{14}C) (Shigeta and Shreeve, 1964) in order to assess incorporation of ^{3}H by pathways other than the pentose cycle.[2] Findings described in this paper indicate that *in vivo* incorporation into liver fatty acids of tritium from glucose-6-^{3}H is similar to that from glucose-1-^{3}H, while that from DL-lactate-2-^{3}H or glycerol-2-^{3}H is substantially greater than from either labeled glucose compound. On the other hand, for carcass fatty acids, glucose-1-^{3}H shows the highest labeling. Ratios of differences in extent of isotope incorporation between lean and obese mice were similar for all of these ^{3}H- or ^{14}C-labeled carbohydrates.

Materials and Methods

The methods used were essentially those previously described (Shigeta and Shreeve, 1964). The obese hyperglycemic mice of the Bar Harbor strain and their lean siblings used in the present studies ranged in age from 8 to 24 weeks. The weight of the obese mice varied from 34 to 64 g (mean of 52 g) and the weights of two (paired) lean mice varied from 30 to 55 g (mean of 44 g). Mice of both sexes were used, predominantly female. Neither age nor sex appeared to affect signifi-

[2] Preliminary reports have appeared in *Federation Proc. 23*, 165 (1964), *24*, 343 (1965), and *Ann. N. Y. Acad. Sci. 131*, 464 (1965).

TABLE I: Comparative Transfers of Tritium or Carbon-14 from Various Carbohydrates (and Water) into Liver and

Carbohydrate	Glucose							
Position and Kind of Label	-1-^{3}H		-1-^{14}C		-6-^{3}H		-6-^{14}C	
Type of Mouse	L	O	L	O	L	O	L	O
% dose in liver fatty acids (no. of expt)	0.035 ± 0.003[c] (8)	0.175[a] ± 0.011 (6)	0.038 ± 0.007 (5)	0.193[b] ± 0.060 (5)	0.025 ± 0.004 (6)	0.176[a] ± 0.020 (5)	0.027 ± 0.003 (8)	0.228[a] ± 0.074 (5)
% dose in carcass fatty acids (no. of expt)	0.62 ± 0.10 (8)	1.25[a] ± 0.16 (5)	1.20 ± 0.30 (5)	1.67 ± 0.23 (4)	0.24 ± 0.04 (4)	0.56 ± 0.13 (5)	0.88 ± 0.07 (8)	1.72[a] ± 0.22 (5)
% dose in body water (no. of expt)	62.7 ± 3.4 (7)	78.8[b] ± 6.4 (5)			44.6 ± 2.0 (5)	42.2 ± 2.1 (5)		
% dose in respiratory CO_2 (no. of expt)			45.7 ± 1.5 (3)	43.0 ± 7.0 (2)			39.8 ± 1.8 (8)	30.8[b] ± 2.1 (4)

[a] $P < 0.01$ for obese *vs.* lean mice. [b] $P < 0.05$ for obese *vs.* lean mice. [c] SE = $\sqrt{\frac{\Sigma(x - x)^2}{n(n-1)}}$.

cantly the results with any of the labeled compounds. However, too few mice were available for adequate comparison of sex differences.

Glucose-1-^{14}C (sp act. 2–3 mc/mmole), glucose-1-^{3}H (500 mc/mmole), glucose-6-^{14}C (2 mc/mmole), DL-sodium lactate-2-^{3}H (670 mc/mmole), glycerol-1,3-^{14}C (12 mc/mmole), and glycerol-2-^{3}H (200 mc/mmole) were obtained from New England Nuclear Corp. Glucose-6-^{3}H (470 mc/mmole) and DL-zinc lactate-2-^{14}C (2–5 mc/mmole) were obtained from Volk Radiochemical Co. ^{14}C (2–5 μc) and/or 8–40 μc of ^{3}H were administered to one obese mouse or pair of lean mice.

Mice were fasted for 5 hr (approximately 9 AM–2 PM) prior to injection intraperitoneally with a trace amount (no carrier addition) of one of the carbohydrates. Substrates were labeled with ^{3}H or ^{14}C or both and were contained in 1.0 ml of 0.9% NaCl/obese mouse or 0.5 ml/lean mouse. One obese mouse or two lean mice were placed in a metabolic cage for collection of $^{14}CO_2$ in the expired air. Tissues from these paired lean mice were also combined for analysis. Liver weights of the obese mice ranged from 2.1 to 5.5 g (mean of 3.6 g) and the two livers of the paired lean mice together weighed from 1.3 to 2.5 g (mean of 1.9 g). Liver:body weight ratio was, therefore, about 50% higher in obese than in lean mice, as noted previously (Shigeta and Shreeve, 1964).

After 90 min the mice were sacrificed by decapitation with immediate collection of neck blood for analysis of ^{3}HOH and, in some instances, of glucose. Livers were removed, weighed, and placed in alkaline-alcohol digesting mixture. The remaining carcasses were chopped into small pieces and also placed in 30% aqueous KOH–ethanol (1:1, v/v). Saponification, extraction with petroleum ether (bp 30–60°), acidification, and further extraction of fatty acids were performed as previously described (Shigeta and Shreeve, 1964). In later experiments the final petroleum ether extract was clarified of an occasional fine precipitate by shaking with dilute HCl solution, centrifuging, and separating off the top ether layer by pipet.

Radioactivity of all samples was measured in duplicate by liquid scintillation counting with separation of ^{14}C and ^{3}H counts by a discriminator-ratio method (Okita *et al.*, 1957). Aliquots of the administered solutions were simultaneously counted, and corrections for quenching in samples made by the use of internal standards.

The techniques used for analysis of ^{14}C in respiratory CO_2 and of glucose and ^{3}HOH in the blood have been previously reported (Shigeta and Shreeve, 1964). Further measurement of dilution of injected ^{3}HOH in five lean and five obese mice suggested that in relatively large obese mice the body water space was less than the value of approximately 40% earlier found (Shigeta and Shreeve, 1964). Therefore, in the present series the extent of conversion of ^{3}H from carbohydrates to ^{3}HOH was calculated on the basis that the volume of distribution of labeled water was 40% of body weight for obese mice of 50 g or less, 35% for 50–55 g, 32% for 55–60 g, and 30% for 60 g or more. A value of 55% of body weight for lean mice was used.

Experimental Results

Blood Glucose Concentration. The concentration of blood glucose at the time of sacrifice was measured only occasionally. Among 12 pair of lean mice the range was 44–82 mg/100 ml with a mean of 65. Among 18 obese mice the range was 72–286 mg/100 ml with a mean of 178. Since no values above 300 mg/100 ml were found in obese mice of this series, such mice were not separated into "mild" and "severe" hyperglycemic as formerly (Shigeta and Shreeve, 1964).

Conversion of Tritium-Labeled Carbohydrates to Body Water. Results of measurement of volatile tritium in the blood water after injection of glucose-1-^{3}H, glucose-6-^{3}H, DL-lactate-2-^{3}H, and glycerol-2-^{3}H together with values for the body water space found

Carcass Fatty Acids, Body Water, and Respiratory CO_2 of Lean (L) and Obese (O) Mice.

DL-Lactate				Glycerol				Water	
-2-^{3}H		-2-^{14}C		-2-^{3}H		-1,3-^{14}C		^{3}HOH	
L	O	L	O	L	O	L	O	L	O
0.303 ± 0.058 (8)	1.825[a] ± 0.236 (6)	0.086 ± 0.021 (7)	0.440[a] ± 0.070 (6)	0.193 ± 0.036 (6)	0.939[a] ± 0.133 (6)	0.021 ± 0.004 (6)	0.118[b] ± 0.032 (6)	0.001 ± 0.0002 (3)	0.0157 ± 0.0068 (3)
0.15 ± 0.02 (6)	0.30[a] ± 0.01 (6)	0.99 ± 0.24 (5)	2.06[b] ± 0.30 (6)	0.17 ± 0.01 (6)	0.24[b] ± 0.02 (6)	0.57 ± 0.06 (6)	0.71 ± 0.08 (6)	0.0093 ± 0.0016 (3)	0.0303 ± 0.0067 (3)
73.1 ± 1.9 (7)	100.0[a] ± 3.3 (5)			65.5 ± 1.2 (6)	66.8 ± 2.8 (6)				
		74.2 ± 2.5 (6)	64.2 ± 5.4 (6)			48.5 ± 3.3 (4)	35.7[b] ± 3.8 (3)		

with tritiated water (*cf.* Methods) were used to calculate the per cent dose in body water given in Table I. Obese mice appeared to show significantly higher incorporation into body water from glucose-1-^{3}H and DL-lactate-2-^{3}H with no difference for glucose-6-^{3}H or glycerol-2-^{3}H compared with lean mice. In a previous study from this laboratory no significant differences between lean and "mild-hyperglycemic" obese mice were found for glucose-1-^{3}H (Shigeta and Shreeve, 1964).

Conversion of ^{14}C-Labeled Carbohydrates to Expired Carbon Dioxide. The total excretion of respiratory $^{14}CO_2$ by the mice, as per cent of the amount of injected radioactivity, up to 90 min after administration of glucose-1-^{14}C, glucose-6-^{14}C, DL-lactate-2-^{14}C, or glycerol-1,3-^{14}C is given in Table I. With glucose-1-^{14}C no differences were found between lean and obese, which confirms the previous observation (Shigeta and Shreeve, 1964). With glucose-6-^{14}C the obese mice appeared to excrete significantly less $^{14}CO_2$ than did the lean mice. The metabolism of glycerol-1,3-^{14}C to $^{14}CO_2$ appeared to be significantly lower in obese mice, while that of DL-lactate-2-^{14}C was also lower but not significantly so.

Conversion of Labeled Carbohydrates to Total Fatty Acids of the Liver. PER CENT DOSE. Table I contains the data on per cent of injected ^{14}C or ^{3}H ("per cent dose") converted to fatty acids from glucose-1-^{3}H(1-^{14}C), glucose-6-^{3}H(6-^{14}C), DL-lactate-2-^{3}H(2-^{14}C), and glycerol-2-^{3}H(1,3-^{14}C). There was fivefold greater incorporation of glucose-1-^{3}H or glucose-1-^{14}C into total liver fatty acids of obese mice than for lean mice (Figure 1). The present differences between lean and obese mice of seven- and eightfold for glucose-6-^{3}H and glucose-6-^{14}C, respectively, appear to be higher than for the 1-labeled glucose species, but may not be significantly so, since earlier studies showed differences greater than fivefold between lean and obese mice for 1-labeled glucose (Shigeta and Shreeve, 1964). Both tritium and carbon-14 from the other two carbohydrates (lactate and glycerol) also show similar differences between lean and obese in extent of conversion to liver fatty acids (Table I and Figure 1).

The transfer of glucose-1-^{3}H to liver fatty acids is only slightly higher than that of glucose-6-^{3}H for lean mice and no higher for obese mice. From Table I it is clear that much higher percentages of tritium injected in the form of DL-lactate-2-^{3}H or glycerol-2-^{3}H are transferred to liver fatty acids than from either of the labeled glucose species. Since D-lactic acid is probably not oxidized through the mediation of a pyridine nucleotide (Tubbs, 1965), the percentage of the natural (L) isomer incorporated is presumably twice that given in Table I as "per cent of dose in liver fatty acids." Thus, almost 4% of L-lactate-2-^{3}H may be converted to fatty acids in the obese liver. Glycerol-2-^{3}H appears to have about one-fourth the extent of transfer of L-lactate-2-^{3}H in regard to per cent of dose.

The amount of tritium transferred from tritiated water to liver fatty acids (Table I) is not in any case as much as 10% of the lowest incorporation from any organic labeled compound, and is usually much less. Therefore, even though proton exchange with reductive hydrogen may be extensive (Foster and Bloom, 1963), it seems highly unlikely that any significant amount of tritium in liver fatty acids after administration of labeled organic compounds derives from labeled water formed at the site.

^{3}H:^{14}C RATIO. When ratios of ^{3}H:^{14}C conversion are compared, as in Figure 2,[3] it is seen that the 1-labeled glucose series has no higher a ratio than does the 6-labeled glucose series. Thus, no preferential

[3] For the representations of Figures 2 and 3 only those experiments were included in which animals received the simultaneous injection of carbohydrates labeled with both ^{3}H and ^{14}C. Therefore, the number of experiments in several cases is less than the total used to compile the data of Figure 1.

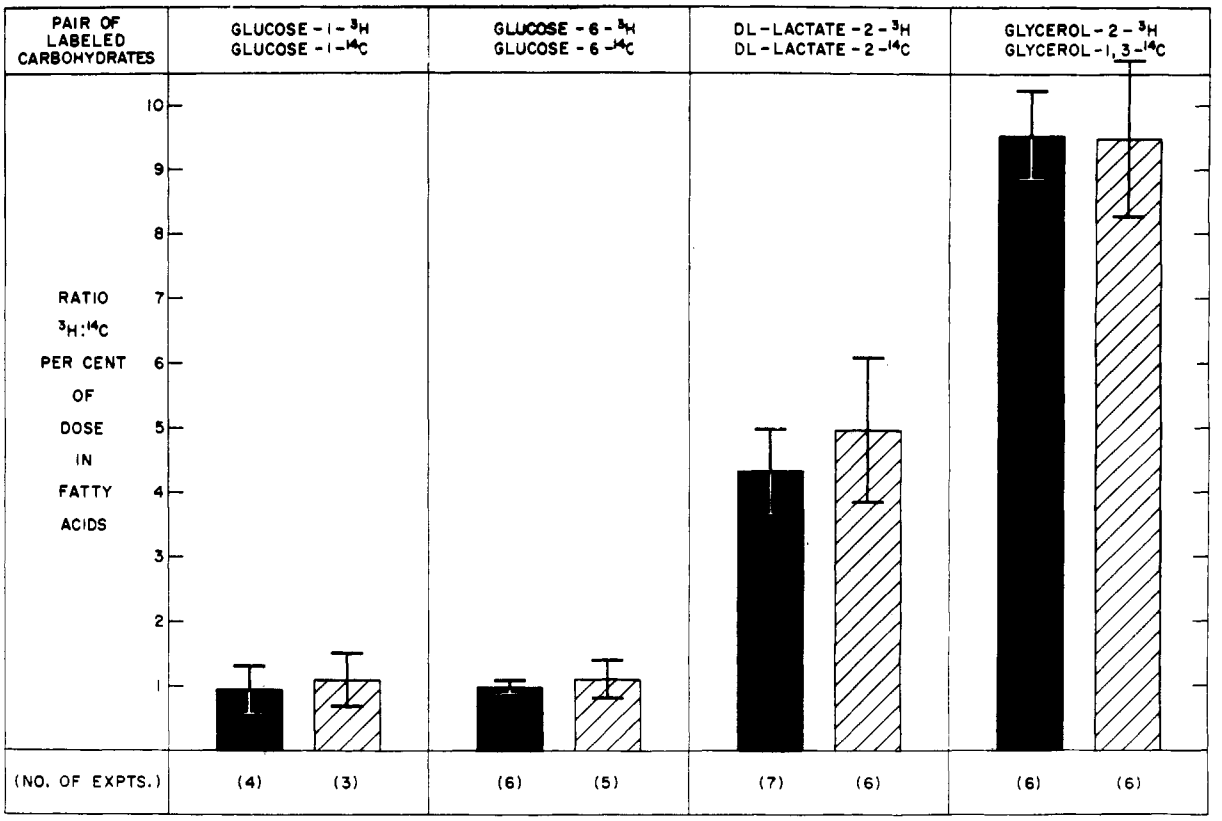

FIGURE 2: Incorporation of tritium relative to ^{14}C from doubly labeled carbohydrates into fatty acids of lean (■) and obese (▨) mice.

transfer *via* NADPH of glucose-1-carbon-bound hydrogen to fatty acids of liver *in vivo* is suggested by these data. This is in marked contrast to the similar ratio studies with rat liver slices (Foster and Bloom, 1961), which indicated that 98.5% of the tritium incorporated into fatty acids from glucose-1-^{3}H was transferred through NADPH by the action of glucose 6-phosphate dehydrogenase.

The glucose-6-^{3}H:-6-^{14}C ratio in liver fatty acids of about 1.0 in the present series is much higher than that of about 0.1 found with rat liver slices (Foster and Bloom, 1961) or of 0.3 for rat mammary gland slices (Abraham *et al.*, 1963). Since acetyl-CoA derived from carbons 5 and 6 (or 1 and 2) of glucose could not theoretically give a higher ^{3}H:^{14}C ratio in long-chain fatty acids than about 0.4 (Abraham *et al.*, 1963), a major share of the ^{3}H incorporated into liver fatty acids from glucose-6-^{3}H (and from glucose-1-^{3}H) *in vivo* evidently derives through metabolic reductive hydrogen, but probably not that formed in the pentose cycle pathway.

Pair-labeled lactate and glycerol show much higher transfer to liver fatty acids of ^{3}H relative to ^{14}C than do either of the pair-labeled glucose series. Whereas Figure 2 suggests that glycerol-2-^{3}H is more "efficiently" utilized than DL-lactate-2-^{3}H with respect to the ^{14}C analogs, two factors in particular modify this interpretation. Although, as mentioned above, D-lactate-2-^{3}H is probably not converted to fatty acids, some D-lactate-2-^{14}C is probably incorporated through the action of D-α-hydroxy acid dehydrogenase in the liver and elsewhere (Tubbs, 1965). This factor would cause the observed ^{3}H:^{14}C ratio to be too low. Secondly, the label in position 2 of lactate is not comparable to the labeled positions of 1 and 3 in glycerol for the formation of acetyl-CoA and thence fatty acids. Position 1 of glycerol (position 3 of glucose) would not be incorporated into fatty acids, except through complex, and probably minor, reactions of transketolation and transaldolation. Thus, the ^{3}H:^{14}C ratio for glycerol is too high relative to that for lactate because of the latter factor.

Conversion of Labeled Carbohydrates to Total Fatty Acids of the Carcass. PER CENT DOSE. The data of Table I, and the bar graphs in Figure 1, show that total fatty acids of the carcass of obese mice incorporate generally about twice as much tritium or carbon-14 from all of the labeled carbohydrates as do those of lean mice. The difference between lean and obese mice may be less for glycerol-2-^{3}H and glycerol-1,3-^{14}C than for the other compounds. Earlier results with glucose-1-^{3}H and glucose-1-^{14}C showed slightly greater differences than do the present findings (Shigeta and Shreeve, 1964). The differences in the present series were only sporadically significant and less so than the differences between lean and obese for the

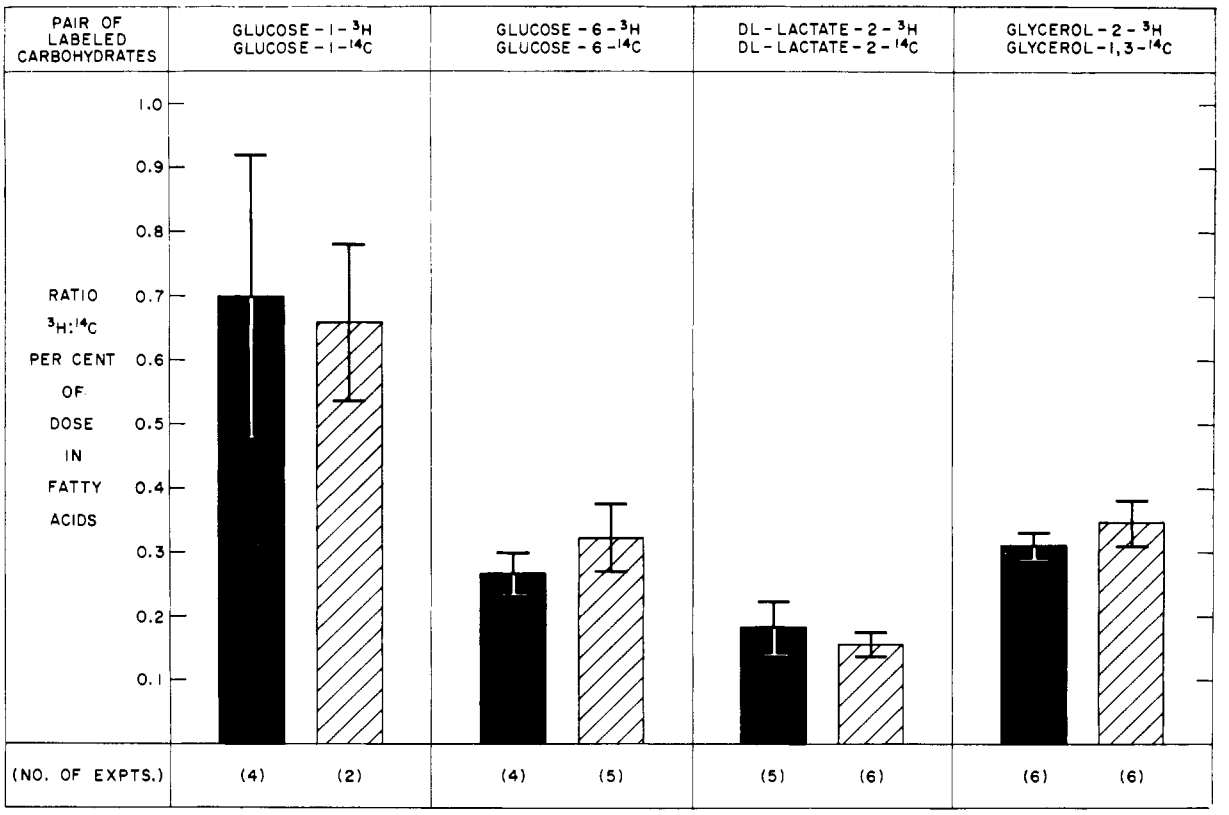

FIGURE 3: Incorporation of tritium relative to ^{14}C from doubly labeled carbohydrates into carcass fatty acids of lean (■) and obese (▨) mice.

fatty acids of liver (Table I).

^{3}H:^{14}C RATIO. The pattern among the various labeled carbohydrates of the ratio of incorporation of ^{3}H to ^{14}C into carcass fatty acids (Figure 3) is very much different from that for the liver fatty acids (Figure 2). In the mixture of tissues comprising the carcass (including adipose tissue as a site of fatty acid synthesis; Feller, 1954) there are for both lean and obese mice clearly higher ^{3}H:^{14}C ratios for 1-labeled glucose than for 6-labeled glucose. Since the ^{3}H:^{14}C ratio is only about 0.3 for 6-labeled glucose in carcass fatty acids, it is possible that most, if not all, of the ^{3}H was incorporated *via* attachment to carbon (Abraham *et al.*, 1963). The ^{3}H:^{14}C ratios for lactate and glycerol as precursors of carcass fatty acids are only one-twentieth to one-thirtieth of the corresponding values for liver fatty acids, which suggests major differences between liver and other tissues in the utilization of hydrogen from these two substrates for reductive synthetic processes.

Discussion

The present results substantially add to the previous evidence (Foster and Bloom, 1961; Lowenstein, 1961; D'Adamo *et al.*, 1961; Shreeve, 1965) that hydrogen transferred during oxidation of lactate and glycerol is available for reduction of fatty acid intermediates, particularly in the liver rather than in other tissues. Furthermore, comparisons between lactate-2-^{3}H and glycerol-2-^{3}H, on the one hand, and glucose-1-^{3}H, on the other, suggest that in the liver *in vivo* the three-carbon carbohydrates are much better sources of metabolic reductive hydrogen than is glucose. This appears to hold even though comparisons between glucose-1-^{3}H and glucose-3-^{3}H (Katz *et al.*, 1965) indicate that due to isotope discrimination, tritium may be transferred at only about one-half of the rate of transfer of natural hydrogen from the 1 position of glucose to fatty acids.

The reason for the much lower transfer *in vivo* of glucose-1-^{3}H to fatty acids relative to glucose-1-^{14}C and relative to labeling from tritiated lactate and glycerol than was observed with liver slices (Foster and Bloom, 1961) is not at all clear. However, one possibility is that when injected *in vivo* glucose is initially metabolized to a much greater extent by peripheral tissues rather than by liver, while circulating lactate and glycerol are metabolized more prominently by the liver. Intermediate carbohydrates (*e.g.*, lactic acid) formed by peripheral metabolism of labeled glucose could recirculate to the liver, where subsequent conversion to fatty acids would lower the ^{3}H:^{14}C ratio from glucose-1-^{3}H(1-^{14}C). Other possibilities are greater isotope exchange with water (Foster and Bloom, 1963) or isotope discrimination against glucose-

1-^{3}H (Katz *et al.*, 1965) *in vivo* than *in vitro*. Still another and interesting possibility is that the mechanisms for intermediate hydrogen transfer from lactate and glycerol are more active *in vivo* relative to the mechanisms for transfer from glucose (*via* the pentose cycle pathway) than they are *in vitro*.

That the latter possibility may be true is suggested by the ^{3}H:^{14}C ratio in fatty acids formed from glucose-6-^{3}H(6-^{14}C) in the liver. In liver slices this ratio was only about 0.1 (Foster and Bloom, 1961) while in the current study *in vivo* the ratio was about 1.0. Thus, most of the tritium transferred to fatty acids from glucose-6-^{3}H *in vivo* must be derived through reductive processes and very much more actively relative to the rate of conversion of glucose carbon to fatty acids than *in vitro*. Glucose-6-^{3}H is converted by glycolysis to pyruvate-3-^{3}H. Pyruvate may then be carboxylated to oxaloacetate in the mitochondria where it may be subsequently reduced to malate (Haynes, 1965; Shrago and Lardy, 1966). Malate (or fumarate) may then cross the mitochondrial membrane to become available to deliver hydrogen for metabolic reductive purposes (Haynes, 1965; Shrago and Lardy, 1966).

That the metabolism of malate, lactate, and glycerol is interrelated through pyridine nucleotides which serve as common coenzymes for all these substrates is indicated by the way in which the redox equilibria for these carbohydrates and their immediate oxidation products vary in parallel in response to cellular metabolic changes (Hohorst *et al.*, 1965). Interchange of hydrogen from lactate or glycerol to malate is indicated by the fact that lactate-2-^{2}H (Hoberman, 1958) and glycerol-2-^{2}H (Hoberman and D'Adamo, 1960) can contribute deuterium to position 6 of glucose, presumably by coupling of lactic acid or glycerol phosphate dehydrogenases with malic acid dehydrogenase (Lowenstein, 1961; Hoberman, 1958). Lowenstein (1961) proposed that oxidation of lactate was coupled to reduction of oxaloacetate to malate, from which the tritium was then transferred to $NADP^+$ *via* the "malic enzyme" reaction of oxidative decarboxylation to pyruvate and CO_2. This theory has received further support from the studies of Young *et al.* (1964) which demonstrated coupling of NAD^+-dependent malic dehydrogenase with malic enzyme in supernatant fractions of rat liver homogenate. Moreover, various investigators (Young *et al.*, 1964; Leveille and Hanson, 1966) have determined that malic enzyme varies in activity with nutritional and hormonal changes in direction and extent consistent with a role in fatty acid biosynthesis.

The lower ^{3}H:^{14}C ratios for incorporation of tritium into carcass fatty acids than into liver fatty acids for labeled lactate and glycerol and for 6-labeled glucose, and particularly in relation to the ratio for 1-labeled glucose, suggest that a system which serves in common to transfer tritium from those sources other than glucose-1-^{3}H has much less activity in tissues comprising the carcass than in liver. However, malic enzyme itself shows even higher activity in adipose tissue than in liver (Young *et al.*, 1964) and in the former tissue, as in the latter, varies in activity in proportion to changes in fatty acid biosynthetic activity (Young *et al.*, 1964; Leveille and Hanson, 1966). Hohorst *et al.* (1965) suggested that in skeletal muscle there may be a functional compartmentation of the NAD^+-dependent redox system to explain the fact that in skeletal muscle, unlike liver, certain changes in the redox ratio of one substrate pair were not accompanied by similar changes in another. This observation may be related to the paucity of tritium transfer from various carbohydrates to fatty acids of the carcass.

The present data lend further support to the previous indication (Shigeta and Shreeve, 1964) that hyperlipogenesis is a more pronounced abnormality in the livers of these obese mice than in the tissues of the carcass on the whole. The twofold higher incorporation of radioisotopes into carcass fatty acids of obese than of lean mice is similar to differences noted for total lipids of the fat pad after intraperitoneal injection of glucose-U-^{14}C (Stauffacher *et al.*, 1965). The particular indications of specific increase of glycerol kinase in adipose tissue of obese hyperglycemic mice (Treble and Mayer, 1963; Lochaya *et al.*, 1963) do not seem to be reflected in any unusually high incorporation of glycerol-2-^{3}H or glycerol-1,3-^{14}C into fatty acids of carcass in the present study.

The much higher extent of conversion of glucose-1-^{3}H to ^{3}HOH than of glucose-6-^{3}H presumably indicates the operation of the pentose cycle pathway. The difference between glucose-1-^{14}C and glucose-6-^{14}C in formation of $^{14}CO_2$ appears to be somewhat less. It is possible that some of the tritium of glucose-1-^{3}H may be exchanged to water by phosphomannose isomerase (Topper, 1957). Whatever accounts for preferential conversion of glucose-1-^{3}H to ^{3}HOH may be relatively more active in the obese mice, since the latter show significantly higher conversion of glucose-1-^{3}H but not of glucose-6-^{3}H to ^{3}HOH as compared with lean animals.

In contrast to the findings with glucose-1-^{14}C, in both the present as well as earlier (Shigeta and Shreeve) studies, the conversion of glucose-6-^{14}C to $^{14}CO_2$ is lower in obese than lean mice. This may be associated with the observation that the ratio between lean and obese mice for incorporation into liver fatty acids appears greater for 6-labeled glucose than for 1-labeled glucose. Although these comparisons between lean and obese mice of formation of $^{14}CO_2$, ^{3}HOH, and liver fatty acid-^{14}C suggest a more active pentose cycle pathway in obese mice, such is not indicated by any special increase for obese mice in conversion of glucose-1-^{3}H to fatty acids either of liver or carcass.

The lower conversions to $^{14}CO_2$ for obese *vs.* lean mice of glucose-6-^{14}C, glycerol-1,3-^{14}C, and (to a lesser extent) DL-lactate-2-^{14}C appear even more significant in comparison to the relative rates of formation of ^{3}HOH from the corresponding tritium-labeled compounds. This difference of formation of $^{14}CO_2$ and ^{3}HOH is similar to that observed with obese human subjects after intravenous injection of glucose pairs labeled at the 1 position (Shreeve, 1965).

Addendum

After preparation of this manuscript there appeared a report (Abelin *et al.*, 1966) of differences between liver and carcass of albino mice in the incorporation of ^{3}H and ^{14}C from position-labeled glucose and glycerol into fatty acids *in vivo*. These differences are qualitatively, but not quantitatively, similar to those found in our study with the obese strain of mice.

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