Lipogenesis from [2-^{14}C]Glucose in Squirrel Monkeys (37089)

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(Introduced by C. H. Beatty)

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Numerous investigations on lipogenesis *in vivo* have used the physiological metabolite glucose that can be incorporated into lipids by diverse pathways. The most desirable location for radioactive carbon within glucose would be where it is not easily lost during catabolism, yet will be incorporated into lipid products that, when degraded, reveal the metabolic pathways through which the carbon chain has passed. [2-^{14}C]Glucose rather admirably fulfills these requirements. It has been used for *in vitro* studies on carbohydrate metabolism (1–10), less often for *in vitro* studies on lipogenesis (11–13) but not, to my knowledge, for *in vivo* studies on mammalian lipogenesis.

The purpose of this work was to assess the usefulness of [2-^{14}C]glucose as a precursor metabolite for lipid synthesis *in vivo*, particularly to determine the different lipid moieties into which radiosubstrate could be incorporated, and to degrade each to obtain information on the pathways traversed by the glucose carbon.

Materials and Methods. Five male squirrel monkeys *(Saimiri sciurea)*, 0.5–0.7 kg, maintained on Purina monkey chow were fasted 48 hr and refed a fat-free diet 48 hr. This establishes optimal conditions for lipogenesis (14). After sedation with 0.6 mg Sernylan (phenylcyclidine hydrochloride, Parke, Davis and Co., Detroit, Mich.) per kg body weight, catheters were introduced into the femoral vein on one side and into the contralateral femoral artery. At time zero, 100 μCi in 1.7 μmole of [2-^{14}C]glucose (New England Nuclear, Boston, Mass.) were injected into the femoral vein. Blood samples were withdrawn at intervals from the femoral artery catheter. At 20 min, the monkeys were anesthetized with 9 mg Sernylan/kg body weight and selected tissues were removed and frozen in liquid nitrogen. Liver was sampled from several lobes; omental, epididymal, perirenal, and subscapular brown fat were freed of adherent connective and muscle tissue before freezing.

Lipids were extracted (15), purified (16), and separated on thin-layer chromatography (17). After elution from silica gel (chloroform for nonpolar lipids such as triglycerides and chloroform with methanol and water for phospholipids), aliquots were radioassayed and the rest of the lipid was hydrolyzed in ethanolic-KOH. A petroleum ether extract contained the nonsaponifiable lipids; after acidification, fatty acids were extracted with petroleum ether. The latter were methylated and separated on gas–liquid chromatography (HI-EFF, 2 BP [ethylene glycol succinate] from Applied Science, Inc., College Park, Pa.) and the peaks were trapped and radioassayed. Those with higher radioactivity were extracted for decarboxylation (13, 18). Glycerol from the aqueous portion of triglyceride hydrolysis was purified and degraded with periodate; carbon 2 as formic acid was reduced to formaldehyde and it and the formaldehyde carbons 1 + 3 were converted to their dimedone derivative for radioassay (13). This procedure does not differentiate between radioactivity in C-1 and C-3. [^{14}C] Acetate arising from [2-^{14}C]glucose was isolated and converted to phenylacetophenone that was degraded (13, 19) to determine radioisotope distribution between the methyl and carboxyl carbon.

Results. Within five minutes after injec-

TABLE I. Incorporation of [2-^{14}C] Glucose into Liver and Adipose Lipids.

	Lipid dpm/mg wet weight	
	Mean ± SEM	Range
Liver	9.5 ± 4.1	2.3– 25.6
Adipose tissue		
Omental	60.4 ± 23.8	10 –142
Perirenal	88.8 ± 50.4	15 –284
Epididymal	23.8 ± 6.7	8 – 35
Brown	152.6 ± 56.2	18 –272

Statistical analyses (p values by paired analysis; $n = 5$): Liver < brown (0.04); omental (0.05); epididymal (0.06); perirenal (0.03). Brown > epididymal (0.02); omental (0.08). Omental > epididymal (0.06).

tion, radioactivity in the blood decreased rapidly to about 10% of that injected; over the next 15 min, it decreased only slightly to 5–8% of the total. Measurable radioactivity appeared in serum lipids within one minute and increased during the experiment but remained too low for extensive analysis.

The incorporation of radiosubstrate into lipids of liver and adipose tissue is shown in Table I. The range was extreme among the five animals but was more consistent within each animal, *i.e.*, the ratio of incorporation by the organs was similar for each monkey. Because of the wide range, the standard error of the mean shown in Table I, as well as Tables II and III, was too great to yield meaningful statistical analyses in most instances. Therefore, comparisons of the statistical significances of the data reported in Tables I through III were based on paired analysis of incorporation or distribution within the organs of each monkey; this minimized the great variations among the monkeys. Incorporation of [2-^{14}C]glucose into lipids was 2.5–16 times greater in adipose tissue than in the liver. Incorporation into liver was statistically less (by paired analysis) than all adipose tissues, epididymal was less than brown, omental, and perirenal adipose tissue, and omental was less than brown adipose tissue. The amount of white adipose tissue was relatively sparse in these squirrel monkeys that had been starved and refed in this regimen. Total lipid radioactivity in liver and adipose tissue was estimated at no more than 1% of that injected. Other organs examined—*e.g.*, aorta, brain, adrenals, testes, *etc.*—usually contained radioactivity too low to allow analyses; lipids from the carcass musculature, a major site of lipogenesis from [^{14}C]glucose (20), were not examined in the current work.

The biological variation among these animals was less evident when tissues were examined for specific metabolic incorporation into lipids. The distributions of [2^{14}C]glucose into the phospholipids and triglycerides from TLC are shown in Table II; these two accounted for 75 to 85% of the ^{14}C incorporation into lipids. According to expectations, triglycerides in adipose tissue had the greatest amount of radioactivity. The incorporation of radiosubstrate into liver triglycerides

TABLE II. Distribution of ^{14}C from [2-^{14}C] Glucose into Phospholipids and Triglycerides of Liver and Adipose Tissues.

	Specific activity[a]	
	Triglyceride	Phospholipid
Liver	3.1 ± 1.0 (0.9–6.9)	3.7 ± 1.6 (0.7–10.0)
Adipose tissue		
Omental	44.8 ± 17.1 (7.5–97)	2.9 ± 1.2 (0.9–7.4)
Perirenal	71.7 ± 39.3 (11.4–221.8)	4.5 ± 2.3 (1.7–13.6)
Epididymal	15.3 ± 3.5 (5.6–24.6)	2.8 ± 1.1 (0.7–5.5)
Brown	115.5 ± 45.0 (15–253)	15.1 ± 10.0 (1.1–54.7)

[a] Specific activity = arithmetic mean of disintegrations per minute in lipid/mg wet weight ± standard error of the mean; the range of values is given in parentheses. Statistical analyses (p values by paired analysis; $n = 5$): Triglycerides: Liver < omental (0.02); epididymal (0.02); perirenal (0.02); brown (0.06). Brown > omental (0.09). Epididymal < perirenal (0.10); brown (0.03); omental (0.05). Phospholipids: Liver < brown (0.06). Brown > omental (0.08); epididymal (0.10); perirenal (0.08). Perirenal > epididymal (0.09).

TABLE III. Distribution of ^{14}C from [2-^{14}C] Glucose into Glycerol Moiety of Triglycerides and Phospholipids.

	Triglycerides		Phospholipids
	%[a]	Specific activity[b]	%[a]
Liver	24	0.55 ± 0.06 (0.33–0.65)	35
Adipose tissue			
Omental	26	8.6 ± 2.3 (2.1–14.1)	69
Perirenal	30	23.9 ± 12.2 (1.7–62.8)	76
Epididymal	42	7.0 ± 2.3 (2.1–15.0)	86
Brown	44	56.6 ± 24.2 (54.2–134.2)	71

[a] % = glycerol moiety dpm ÷ glycerol moiety dpm + fatty acid dpm. The remaining percentage not listed was in the fatty acids.

[b] Specific activity = lipid dpm/mg wet wt tissue × % in triglyceride or phospholipid × % in glycerol moiety. Values are the mean ± SEM with the range given in parentheses. No specific activities are given for phospholipids since only 2 to 3 samples had enough radioactivity for analysis.

Triglyceride statistical analyses (p values with paired analysis; $n = 5$): Liver < brown (0.05); omental (0.002); epididymal (0.02); perirenal (0.08). Brown > omental (0.09); epididymal (0.08).

was significantly less than into any of the adipose tissues. Among the adipose tissues, epididymal had significantly less incorporation than into brown or omental whereas the difference into perirenal was less significant; brown was greater than omental. Among phospholipids, liver differed only from brown adipose tissue and among the adipose tissues, brown was greater than the other adipose tissues only at $p = 0.08$–0.10 and perirenal was greater than epididymal at $p = 0.09$.

Radiosubstrate is incorporated into lipids by divergent pathways. The glycerol backbone of neutral- and phospho-glycerides arises a few enzymatic steps after the cleavage of hexose to triose, whereas fatty acids are synthesized after further catabolism of the trioses to acetyl units. The hydrolysis of the lipids into a glycerol moiety and fatty acids gives some indication of the activities of the two pathways. Among the triglycerides (Table III), the major incorporation of ^{14}C was 70–75% into the fatty acids of liver, and omental and perirenal adipose tissue; epididymal and brown fat had a majority of only 55–57% in the fatty acids. On the basis of specific activities, liver incorporated less radioactivity into glycerol than that incorporated into adipose tissues; brown was greater than omental and epididymal adipose tissues. Phospholipid glycerophosphate had much less radioactivity than in all the adipose tissues; not enough phospholipid samples were available for analysis of specific activity.

Degradation of the glycerol portion of triglycerides revealed randomization of ^{14}C from the carbon 2 position. Liver and brown, omental, and perirenal fat had 83–86% of the glycerol label in carbon 2 with 17–14% in carbons 1 + 3. Epididymal fat had 78% in carbon 2 with 22% in carbons 1 + 3, significantly different at $p \lesssim 0.04$ from the other tissues.

Over 90% of the radioactivity in fatty acid was in 14:0, 14:1, 16:0, 16:1, 18:0, and 18:1; in Table IV the percentage distribution is based on these six fatty acids only. Triglyceride fatty acids provide the main basis for comparison since they were in the greatest concentration in adipose tissue. Statistically significant differences were found only when liver fatty acids were compared with adipose tissue; the differences between the saturated fatty acids (myristic, palmitic, and stearic) were more notable than in the unsaturated. Omental and perirenal fat were quite similar; brown adipose tissue was intermediate between liver and white adipose tissue in incorporation. Liver phospholipid fatty acids varied from those in the liver triglycerides, again most significantly in the saturated fatty acids.

Decarboxylation ratios for all fatty acids were greater than theoretical. The disparity of actual and theoretical was linked to the approximately 15% randomization of ^{14}C in-

TABLE IV. Incorporation of [2-^{14}C] Glucose into Fatty Acids.

	Percentage[a]				
	Triglyceride				Phopholipid
Fatty acid	Omental	Perirenal	Brown	Liver	Liver
14.0	4.0 ± 0.6	5.2 ± 0.8	5.6 ± 1.3	10.2 ± 1.4	2.8 ± 0.4
14:1	0.5 ± 0.3	0.3 ± 0.3	0.2 ± 0.1	0.8 ± 0.3	2.1 ± 0.8
16:0	44.6 ± 4.5	49.4 ± 2.4	54.4 ± 4.5	62.9 ± 5.2	54.1 ± 0.5
16:1	4.0 ± 0.7	4.4 ± 0.7	6.7 ± 2.5	9.1 ± 2.1	8.5 ± 0.6
18:0	15.5 ± 2.2	16.4 ± 1.4	12.5 ± 2.0	2.3 ± 0.5	17.0 ± 3.6
18:1	31.5 ± 4.9	24.3 ± 3.5	20.7 ± 5.9	14.6 ± 2.2	15.6 ± 3.1
n^b =	4	3	3	4	2

[a] Percentage is based only on the distribution of ^{14}C among the fatty acids listed.

[b] Values are the mean ± SEM with n the number of animals analyzed. Statistical differences (p values) for Liver vs Adipose tissues:

14:0 liver > omental (0.01); perirenal (0.02); brown (0.06).

16:0 liver > omental (0.04); perirenal (0.06).

18:0 liver < omental, perirenal, and brown all <0.01.

16:1 liver > omental (0.06); perirenal (0.09).

18:1 liver < omental (0.02); perirenal (0.07).

to the methyl carbon. Correction of decarboxylation ratios for that randomization indicated *de novo* synthesis of myristic, palmitic, and palmitoleic acids. Stearic and oleic acid decarboxylation ratios showed that no more than half of the radioactivity could be incorporated by *de novo* synthesis; this likely represents a composite ratio with some of the fatty acids synthesized totally *de novo* and another fraction only by elongation.

Discussion. The suitability of using [2-^{14}C] glucose to study lipogenesis *in vivo* has been assessed in monkeys. There are several advantages to this substrate. The ^{14}C location in the carbon 2 position is not immediately respired from the glucose during catabolism, though complete catabolism of the glucose chain easily yields $^{14}CO_2$ in a manner similar to other ^{14}C glucoses (3). The use of glucose with only a single labeled carbon reduces complications inherent in following specific carbons during metabolism of uniformly labaled glucose. The ^{14}C is carried through metabolic pathways to locations within lipids that are amenable to chemical degradation techniques. These latter are particularly useful for assessing the degree of randomization within glyceride glycerol and for determining fatty acid synthetic mechanisms that utilize ^{14}C acetate catabolized from [2-^{14}C]glucose. Termination 20 min after radiosubstrate injection allowed time for the appearance of ^{14}C in lipids, yet minimized extensive randomization of label.

Examining lipogenesis by this pulse-labeling method yields information on one specific moment in an ongoing process. Most of the [2-^{14}C]glucose is rapidly removed from the blood. Liver had much less radioactive lipid than white adipose tissues, a reflection that although both tissues have enzymic capabilities for lipid synthesis, white adipose tissues can also serve as depots for lipids synthesized elsewhere. Brown adipose tissue, though similar to white adipose tissues in its preponderance of radioactivity in the triglycerides, is known to have higher metabolic capacities than white adipose tissues. The higher incorporation in brown may reflect either greater synthetic ability or smaller pool size of lipid precursors. However, the white adipose tissues taken from various sites in these squirrel monkeys also differed in their radioactive lipid content. The differences were more pronounced in the amounts of radioactivity present in the phospholipids and triglycerides and in the distribution of the ^{14}C among the glycerol and fatty acid portions in the triglycerides. It has been pointed out (21–23) that white adipose tissues from different sites do differ in fatty acid concentrations and esterification rates when incubated *in vitro*. The

concentrations of various lipid metabolites and precursors may vary within each tissue and this, added to any innate metabolic differences, can affect the actual amount of radiosubstrate incorporated into lipids; further each adipose tissue *in situ* is exposed to a variable concentration of metabolites as determined by changing blood flow (24). Thus, it is not unexpected that adipose tissues vary among themselves both in synthetic and storage capabilities. If these procedures mainly measure *in situ* synthesis, then the higher lipogenic activity of adipose tissue, even though sparse because of the dietary regimen, could still contribute as much towards total body lipids as that of liver. The significant differences in fatty acid patterns between liver and all adipose tissues would indicate that the *in situ* lipid production in the adipose tissues likely accounts more for these differences than the preferential removal of fatty acids synthesized elsewhere and removed from the blood for storage in the adipose tissues.

In this *in vivo* work, the ^{14}C from [2-^{14}C] glucose appeared mainly in the fatty acid moiety of adipose tissue triglycerides though not in the phospholipids. Others have found more radioactivity from [^{14}C]glucose in the glycerol moiety of triglycerides synthesized *in vitro* by adipose tissue (11, 23) and liver (25). The randomization of label in glyceride-glycerol *in vivo* was similar to that reported for the epididymal fat pads *in vitro* (6, 11). However, the *in vivo* results presented here also indicate that omental and perirenal had significantly less randomization of the radiocarbon than the epididymal white adipose tissue; this suggests a variability in pentose-cycle activity among the adipose tissues. Mechanisms that explain label randomization *via* the pentose cycle (2, 8, 9, 11) propose that carbons 1 and 3 of fructose 1, 6-diphosphate arising from pentose cycle randomization of [2-^{14}C]glucose should contain the randomized ^{14}C with C-2>>C-1>C-3. Splitting fructose 1,6-diphosphate, followed by triose isomerization, would yield glyceraldehyde 3-phosphate with label in C-2>>C-1<C-3. Conversion of the triose to acetyl CoA would cause the loss of triose carbon 1 and the appearance of carbon 3 ^{14}C in the methyl carbon of acetate with the majority of activity from the carbon 2 ^{14}C of triose in the carboxyl carbon of acetate. The similarity of the 15% randomization with acetate and adipose tissue glycerol indicates that most of the ^{14}C initially randomized from [2-^{14}C]glucose appeared in C-1 of hexose phosphate and then C-3 of the triose phosphates; the lesser amount of ^{14}C in the triose C-1 would be lost in the conversion of pyruvate to acetyl CoA. This indicates that the pentose pathway enzymes and substrates are not identically operative in liver and adipose tissue and not even among adipose tissue from different anatomical sites.

Summary. [2-^{14}C]Glucose was injected into squirrel monkeys *(Saimiri sciurea),* tissues were removed and frozen at 20 min, and analyzed for the incorporation of ^{14}C into lipids. The specific activity of radiosubstrate incorporation was 2.5–16 times greater into adipose tissues than into liver. There were significant differences among adipose tissues in the distribution of ^{14}C in triglycerides and phospholipids and also among that incorporated into the glycerol versus fatty acid moieties of triglycerides. Of the ^{14}C present in all triglycerides and in liver phospholipids, 56–76% was in the fatty acid moiety, whereas only 14–31% of the ^{14}C was present in the fatty acids of the adipose tissue phospholipids. There were significant differences between liver and adipose tissues as well as among some of the adipose tissues. Fatty acids 14:0, 16:0, and 16:1 were synthesized *de novo* whereas no more than half of 18:0 and 18:1 would have arisen by *de novo* synthesis. [2-^{14}C]Glucose can be a useful metabolite for *in vivo* lipogenic studies when there is concomitant understanding of the specific location of the ^{14}C within the lipids.

I thank Lynne Bonnett and JoAnn Wolff for their technical assistance. This work was supported by U.S. Public Health Service Grants No. AM-12601 (C.F.H.), HE-09744 (Dr. O. W. Portman), and RR-00163 (Oregon Regional Primate Research Center). Publication No. 632 from the Oregon Regional Primate Research Center.

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Received Sept. 26, 1972. P.S.E.B.M., 1973, Vol. 142.