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Research Article

THE CHARACTERISTICS OF THE PERIPHERAL TRANSPORT OF C^{14}-LABELED PALMITIC ACID *

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Previous studies of the uptake and release of free fatty acid (FFA) of various tissues (1, 2) have indicated that during the basal state there is usually a net release of FFA from the leg, but a more variable response in the forearm. This variability of forearm arteriovenous FFA differences has raised the issue of whether or not arteriovenous differences of total FFA alone provide an accurate index to the dynamics of FFA transport across peripheral areas. The studies reported here indicate that the characteristics of C^{14}-labeled palmitate transport may be disparate with arteriovenous differences of total FFA, and that evidence of tissue uptake of FFA may be apparent in the presence of negative arteriovenous differences of total FFA.

METHODS AND MATERIALS

Studies were carried out in male hospital patients who were above age 35 and who had been fasted for 10 to 15 hours. All but one subject were free of serious disease and none was diabetic.

Sixteen subjects were studied by infusing into an arm vein 0.004 to 0.010 mc of albumin-bound palmitic acid-1-C^{14} by means of a Bowman constant infusion pump.[1] Individual dosage was determined according to age. One seriously ill aphasic patient with a progressive degenerative central nervous system disorder received 0.030 mc over a period of 3 hours. The albumin-bound palmitic acid-1-C^{14} was prepared as previously described (3) and mixed with an appropriate amount of physiological saline solution so that the desired amount of label could be administered at the rate of 2.1 ml of solution per minute for a varying length of time (20 minutes to 3 hours). Simultaneous 6 ml arterial and venous blood samples were collected in syringes at suitable intervals from indwelling needles either from a brachial artery and a convenient antecubital vein or from a femoral artery and vein in the same limb. No samples were drawn from the arm being infused and no attempt was made to sample from veins which were thought to drain specifically deep forearm tissue or muscle. In 6 subjects, sampling was continued after the infusion was stopped. The use of a tourniquet to collect venous blood was generally avoided; however, when the use of a tourniquet became necessary no inconsistencies were noted in the results.

FFA concentration was determined by the method of Dole (4). Palmitic acid-1-C^{14} was extracted, separated from other lipids, and measured as previously reported in a windowless gas-flow counter (3). Later a Packard tri-carb liquid scintillation spectrometer was used. Samples were prepared for this detector essentially in the same manner with the following exceptions: the heptane extracts of fatty acids, together with 12.5 mg of palmitic acid carrier, were put into scintillation vials. The solvents were evaporated by blowing a stream of cool air from a hair dryer across the mouth of the vials. Ten ml of toluene-phosphor solution was then added and the samples counted to a maximum error of 3 per cent. Addition of an internal standard revealed no significant quenching.

RESULTS

During constant infusion the arterial level of label rose rapidly at first and reached a value in about 12 to 15 minutes[2] from which it increased

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[1] Palmitic acid-1-C^{14}, specific activity, 1 mc per mmole, obtained from Volk Radiochemical Co., Chicago, Ill.

[2] If a substance is injected into a fixed volume "v" at the rate of "a" units per unit time, and a constant fraction "K" of the amount present is removed per unit time "t," the amount of the substance "x" present at any time is equal to $(a/K)\ (1-e^{-Kt})$. Also, if "c" is the concentration of label, and "v" its volume of distribution, $c = (a/Kv)\ (1-e^{-Kt})$ (5).

When $t = \infty$, $e^{-Kt} = o$ and $x = a/K$.

When the concentration of label becomes constant, (at $t = \infty$) the rate of influx equals the rate of efflux. If one selects arbitrary values for t, a, and K, and plots $x = (a/K)\ (1-e^{-Kt})$, x will rise rapidly at first and then

TABLE I

Fractional disappearance rates and plasma flux of free fatty acids (FFA) calculated by the method of constant infusion and data used in these calculations

Subject	Arterial plasma FFA concentration	C^{14} activity per estimated plasma vol. of 3,000 ml	Rate of infusion of palmitic acid-1-C^{14}	Fractional disappearance	Plasma FFA flux
	μmoles/L	*cpm*	*cpm/min*	*rate/min*	*μmoles/min*
J.C.	610	386,000	87,300	0.23	420
J.Q.	660	454,000	121,200	0.28	550
P.B.	450	375,000	115,500	0.31	420
C.H.	335	457,000	106,700	0.24	250
F.D.	830	233,000	74,000	0.32	850
C.A.	540	129,000	56,800	0.43	700
W.C.	720	225,000	49,300	0.22	480
A.W.	230	99,000	45,300	0.45	310
R.C.	712	115,000	35,700	0.31	660
H.P.	854	82,700	27,700	0.33	850

only very slowly (Figures 1 and 2). In all subjects the venous level remained less than the arterial level by an amount which varied considerably from subject to subject, but the amount of the arteriovenous difference did not exceed 50 per cent of the arterial level. Table II lists the mean values of the labeled palmitate and titrated total plasma FFA for arterial and venous blood after equilibrium had been reached. With constant infusion of label there was always a positive arteriovenous difference of palmitic acid-1-C^{14} regardless of whether the total FFA arteriovenous difference was positive (Figure 1A) or negative (1B). In those subjects in whom there was a positive arteriovenous difference of total FFA, the relative magnitude of the arteriovenous difference of label exceeded the total FFA arteriovenous difference; the amount of label extracted always pointed to a greater removal of fatty acid than was indicated by the plasma FFA arteriovenous difference. Examination of Figure 1 shows that palmitic acid-1-C^{14} levels, both arterial and venous, seem to rise very slowly during prolonged administration. Since some of the data suggest that arteriovenous difference might become narrower with time, one subject (L.C.) was infused with tracer for 3 hours to see whether or not the positive arteriovenous difference could be abolished. However, the arteriovenous difference was maintained for the entire time with minor fluctuations. We have no explanation for this gradual very slow rise, which was not a constant observation. The possible roles of recirculating label, of nonremovable label and of changing physiological state might be considered.

When the constant infusion was stopped, the arterial and venous concentrations of label fell rapidly and the positive arteriovenous difference immediately disappeared or, in most instances, became slightly negative (Figure 2). These events are not felt to be the result of recirculation of label; the mean circulation time through the arm as measured by Freis, Schnaper and Lilienfield (7, 8) is greater than 30 seconds. In a system in which arterial level is falling rapidly a delay of this magnitude would make it appear as if a positive arteriovenous difference were abolished.

slowly approach limit a/K asymptotically. The behavior of constantly infused label appears to be analogous. From a practical standpoint, the concentration changes very little or rises slowly enough so that the level of label can be considered to be a/K after about 30 minutes. At an average FFA fractional disappearance rate of 0.3 per minute the level of label can be expected to reach 99 per cent of the limiting value a/K in 15 minutes.

It might be tempting to calculate flux of FFA from constant infusion data and to compare these calculations with values obtained by single injection methods. However to do this one would have to know the volume of distribution of FFA, or v. This quantity is not necessarily identical with the intravascular volume. Furthermore, the average concentration of palmitic acid-1-C^{14}, c, cannot be obtained readily because the concentration of this label in the blood is anything but uniform; arterial level is not representative of the blood as a whole and neither is the mixed venous blood which contains the newly infused label. The best one can achieve is an approximation. These approximations measured from our data do fall within the range of the values obtained by single injection (6) but we do not feel that these are reliable calculations (Table I). Using the same equation, an estimate of the volume of distribution of FFA indicates that this volume is about the same size as the blood volume or slightly larger.

Furthermore, the slow component of the forearm circulation which produces a delay of about 2 minutes might play an important role in apparent recirculation of label. These observations will be discussed elsewhere in greater detail (9).

DISCUSSION

The objective of previous studies of arteriovenous differences of total titratable free fatty acids in the peripheral areas during the basal state has been to delineate the direction and, to some extent, the magnitude of FFA transport in those areas. The arteriovenous difference across the extremities during conditions of relatively unchanging blood flow has been felt to represent an index of tissue utilization of the substance under study (1, 2). Andres, Cader and Zierler used arteriovenous differences of glucose across the forearm to calculate that component of oxygen consumption of the forearm accounted for by glucose utilization (10). The low value of 7 per cent seemed to indicate that oxidation of other substrates was primarily responsible for the oxygen consumption, and it was their suggestion that "the small fraction of fatty acids which is unesterified" may be such an energy source. The majority of Gordon's observations of the arteriovenous differences of total FFA were consistent with this hypothesis (1) but not in all instances. Even though blood was sampled from "deep" veins several negative arteriovenous differences were observed and the presence of these negative arteriovenous differences across the fore-

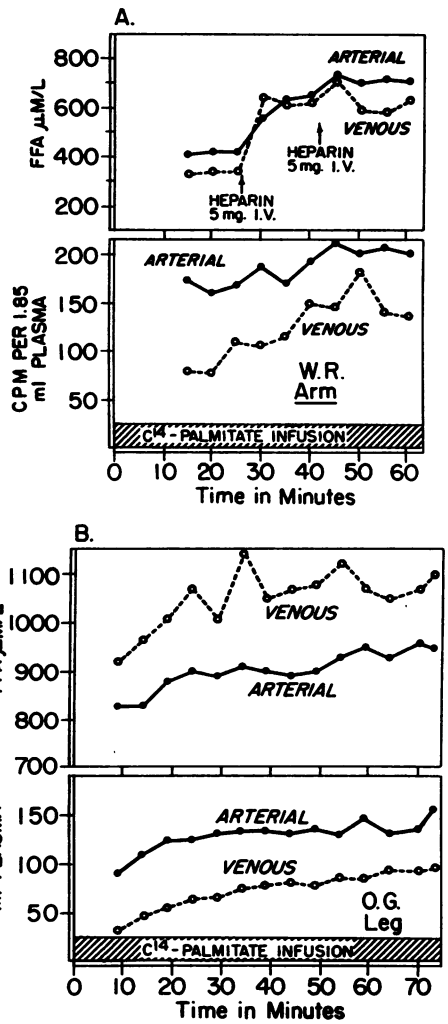

FIG. 1. SIMULTANEOUS ARTERIOVENOUS DIFFERENCES OF PALMITIC ACID-1-C^{14} AND TOTAL PLASMA FREE FATTY ACIDS (FFA) DURING CONSTANT INFUSION OF PALMITIC ACID-1-C^{14}. A. Positive arteriovenous difference of label and positive arteriovenous difference of total FFA. FFA level was raised with heparin to show that behavior of label is independent of FFA concentration. B. Positive arteriovenous difference of label and negative arteriovenous difference of total FFA.

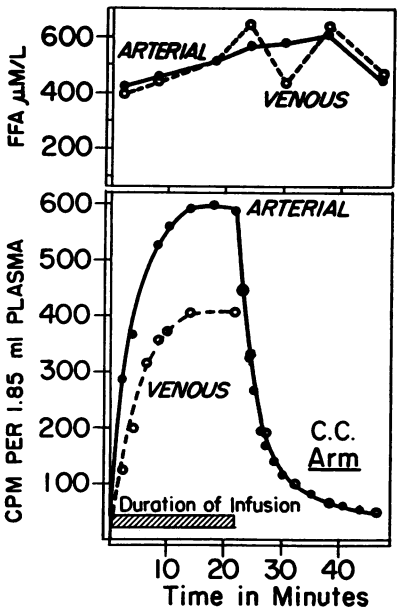

FIG. 2. SIMULTANEOUS ARTERIOVENOUS DIFFERENCES OF PALMITIC ACID-1-C^{14} AND TOTAL PLASMA FFA DURING CONSTANT INFUSION OF PALMITIC ACID-1-C^{14} AND FOLLOWING CESSATION OF INFUSION. Marked uptake of label is shown, while there is no significant difference in venous and arterial total plasma FFA concentration. When infusion is stopped, venous and arterial concentrations of label become almost identical.

TABLE II

Mean equilibrium levels of arterial and venous total plasma free fatty acids and palmitic acid-1-C^{14} during constant infusion of palmitic acid-1-C^{14} *

Subject	Arterial palmitic acid-1-C^{14} activity	Venous palmitic acid-1-C^{14} activity	Arterio-Venous difference of isotope	Arterial FFA conc.	Venous FFA conc.	Arterio-venous difference of FFA
	cpm/1.85 ml plasma		%	*μmoles/L*		%
R.N. leg	170	105	+39	415	475	−14
O.H. leg	130	80	+41	905	1,090	−20
C.D. leg	100	85	+18	540	700	−29
C.C. arm	590	405	+31	480	480	0
E.A. arm	700	430	+38	1,050	830	+21
L.C. arm	740	655	+12	275	305	−12
C.T. arm	890	730	+18	190	210	− 8
H.W. arm	825	735	+11	460	455	0
W.W. arm	240	175	+27	115	125	−11
J.Q. arm	270	155	+44	660	585	+12
J.C. arm	225	185	+19	610	595	+ 2
C.H. arm	290	255	+11	335	355	− 6
F.D. arm	150	95	+35	830	710	+14
C.R. arm	100	80	+16	885	840	+ 5
W.R. arm	225	145	+36	415	335	+20
P.B. arm	90	45	+50	450	440	+ 2

* Values represent means of three or more individual levels.

arm in the resting state properly raised the question of whether or not a negative arteriovenous difference of FFA meant that no FFA was being extracted; Gordon recognized that negative arteriovenous differences in blood sampled from deep forearm veins might indicate simultaneous release of FFA in excess of the fraction removed (1).

The present observations on the arteriovenous differences of infused C^{14}-labeled palmitate add a new dimension to this problem and are interpreted in the following way. In all of our studies, a positive arteriovenous difference was observed, even when the total chemically determined FFA arteriovenous difference was negative. The positive arteriovenous difference of palmitic acid-1-C^{14} is the result of the disappearance of tracer and of endogenous FFA from plasma and the entry of these substances into peripheral tissues, probably mostly muscle. If the studies of Bragdon and Gordon can be applied to man, very little, if any, tracer would be expected to enter fat depots (11). The fact that arteriovenous difference is maintained after prolonged infusion indicates that the arteriovenous difference represents continuous irreversible uptake of palmitic acid-1-C^{14}. In addition, FFA of negligible or zero specific activity from fat depots is added to the venous blood. The effect of this increment of low or zero specific activity may be sufficiently great to produce a negative arteriovenous difference of chemically determined endogenous FFA even though there is a positive arteriovenous difference of palmitic acid-1-C^{14}. At this point interpretation must remain qualitative, and these data cannot be used to measure the amount of FFA extracted. Concerning this qualitative interpretataion, several factors should be pointed out. In these experiments blood was drawn from randomly selected veins. This blood would have perfused regions varying in quantity of fat and muscle, and, therefore, the blood from these regions would also be expected to vary with respect to its composition of chemically determined total plasma FFA and palmitic acid-1-C^{14}. Another factor which could militate against making a quantitative interpretation should be mentioned. Since muscle contains many fat cells, it is conceivable that these cells could add FFA to the blood distal to the point of arterial sampling but proximal to muscle cells so that the blood perfusing these muscle cells might contain a higher concentration of FFA than does the arterial blood. If such an anatomical arrangement exists, the data presented could not be used to quantitate the amount of FFA extracted even if flow rate were known and even if venous blood were homogeneous in concentration. In spite of these variables the results consistently showed a positive arteriovenous difference of label, a difference which was always greater than any posi-

tive arteriovenous difference of chemically determined total plasma FFA. We have concluded, therefore, that in employing the technique of a constant infusion of C^{14}-labeled palmitate, the uptake of fatty acid by peripheral tissue may be adjudged to occur in the presence of evidence of net lipid mobilization from the same peripheral area.

The role which recirculation of label might play in the observed magnitude of the arteriovenous difference of palmitic acid-1-C^{14} is a moot point, since there is no direct evidence that FFA, an insoluble material requiring albumin for its transport, behaves like a freely diffusible ion.

The fate of the removed palmitic acid-1-C^{14} is not answered by these observations. Simultaneous measurement of the arteriovenous difference of labeled $C^{14}O_2$ might provide further information.

SUMMARY AND CONCLUSIONS

1. The arteriovenous difference of palmitic acid-1-C^{14} was measured in the human arm and leg during constant infusion of this isotope. Total plasma free fatty acid arteriovenous differences were measured simultaneously in the same blood.

2. The results indicate that in the resting, fasted subject, palmitic acid-1-C^{14} was extracted by the arm or leg even when there was a net release of total free fatty acids.

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REFERENCES

1. Gordon, R. S., Jr. Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. J. clin. Invest. 1957, **36**, 810.
2. Butterfield, W. J. H., and Schless, G. Observations on the peripheral metabolism of nonesterified fatty acids. Diabetes 1959, **8**, 450.
3. Friedberg, S. J., Harlan, W. R., Jr., Trout, D. L., and Estes, E. H., Jr. The effect of exercise on the concentration and turnover of plasma nonesterified fatty acids. J. clin. Invest. 1960, **39**, 215.
4. Dole, V. P. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. clin. Invest. 1956, **35**, 150.
5. Jokipii, S. G., and Turpeinen, O. Kinetics of elimination of glucose from the blood during and after a continuous intravenous injection. J. clin. Invest. 1954, **33**, 452.
6. Fredrickson, D. S., and Gordon, R. S., Jr. The metabolism of albumin-bound C^{14}-labeled unesterified fatty acids in normal human subjects. J. clin. Invest. 1958, **37**, 1504.
7. Freis, E. D., Schnaper, H. W., and Lilienfield, L. S. Rapid and slow components of the circulation in the human forearm. J. clin. Invest. 1957, **36**, 245.
8. Freis, E. D., and Schnaper, H. W. The effects of a variety of hemodynamic changes on the rapid and slow components of the circulation in the human forearm. J. clin. Invest. 1958, **37**, 838.
9. Estes, E. H., Jr. The influence of the peripheral vascular bed on the disappearance of injected materials. In preparation.
10. Andres, R., Cader, G., and Zierler, K. L. The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. clin. Invest. 1956, **35**, 671.
11. Bragdon, J. H., and Gordon, R. S., Jr. Tissue distribution of C^{14} after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat. J. clin. Invest. 1958, **37**, 574.