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On the turnover of long-chain fatty acids in plasma

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SUMMARY

The turnover of fatty acids released by lipolysis in the body was estimated by measuring the lipolytic activity of plasma and the rise in fatty acid concentration after an injection of heparin. The mixture of fatty acids liberated endogenously appeared to be cleared at the same rate as has been previously reported for albumin-bound, C^{14}-labeled fatty acids.

Long-chain fatty acids[1] in plasma appear to transport a large quantity of material and energy (1). Their flux, as estimated from the turnover of tracer molecules (Table 1) and from the concentration of fatty acids in plasma (about 800 μeq per liter), amounts to about 250 μeq per liter per minute, or in caloric terms to about 0.6 calorie per liter per minute. Whether the flux actually delivers all of this energy directly to working cells, or whether, as appears more likely, it is in part a mixing process involving lipids of plasma and tissue, remains uncertain. In either case the apparent turnover is impressively rapid.

These estimates, however, are open to the objection that they involve only the clearance of fatty acids carried in artificially prepared fatty acid-protein complexes (2). It has seemed reasonable to believe that tracer acids injected in this form will blend indistinguishably with the native fatty acids carried on blood proteins, but the possibility remains that a complex prepared in a test tube might not be quite native, and might be cleared at an abnormal rate. An additional objection to the tracer technique is that it measures the clearance of only a single acid. Although the acids that have been measured are the major components of the fatty acid mixture (3), it is admittedly an approximation to combine the turnover of a subfraction with the concentration of a whole mixture for an estimate of total flux.

The present study has provided an estimate of the turnover of the fatty acid mixture released by lipolysis in the body. As is well known, an injection of heparin in lipemic subjects causes a transient rise in fatty acid concentration (4). If it is assumed that the rise is wholly due to lipolysis and that the clearance of fatty acids is independent of concentration at levels above 700 μeq per liter (5), their turnover can be calculated from the rate of lipolysis and the changes in concentration:

$$K = \frac{P - C'}{\Delta C} \times 10^2$$

In this expression K (per cent per minute) is the turnover rate; P (μeq per liter per minute) is the rate of lipolysis as determined by incubating a sample of postheparin blood at 37°; C' (μeq per liter per minute) is the rate of change of fatty acid concentration as determined in serial samples of blood taken with an inhibitor (6), diethyl-*p*-nitrophenyl-phosphate,[2] to prevent *in vitro* lipolysis (7); and ΔC (μeq per liter) is the increment in concentration above the preheparin base line. Each of the values (P, C', ΔC) refers to a given instant of time; blood taken for determination of P has a stable lipolytic activity as shown by a linear

[1] The term "long-chain fatty acids," or (when the chain length is understood) simply "fatty acids," is used in the present paper as a synonym for "nonesterified" or "unesterified" fatty acids. The term "free" fatty acids has been avoided since the fatty acids in question are not free until separated from protein by laboratory methods. The short-chain fatty acids in plasma might be considered as free, but usually they are excluded from the category of "free fatty acids."

The simple term "fatty acids" should be unobjectionable since molecules linked to glycerol or cholesterol by a covalent bond have lost their acid function. Esterified compounds are fatty esters, not acids; if a monocarboxylic structure is acidic, then obviously it is not esterified.

[2] Also known as Compound E 600, or Paraoxan. Obtained from Dr. Schrader, Farbenfabriken Bayer, Wuppertal-Elberfeld, West Germany.

TABLE 1. TURNOVER OF FATTY ACIDS AS ESTIMATED FROM THE CLEARANCE OF C^{14}-LABELED, ALBUMIN-BOUND FATTY ACID

Species	Acid	Half Time	Turnover	Ref.
		min	*%/min*	
Dog	Palmitic	2.0	35	(9)
Dog	Palmitic	2.1	33	(10)
Human	Palmitic	1.7	41	(11)
Human	Oleic	3.1	23	(11)
Human	Palmitic	2.5	28	(5)
Human	Oleic	2.3	30	(5)
Human	Linoleic	2.4	29	(5)

increase in concentration of fatty acids during a 30-minute period of incubation (7). This rate of production of fatty acids was assumed to be equal to that occurring in the circulating plasma at the moment of taking the blood sample. Serial blood samples taken with inhibitor for determination of fatty acid concentration of circulating plasma were plotted against time, and the values of C' (slope) and ΔC (increment of height), corresponding to the times of sampling for lipolytic activity, were estimated graphically.

Twelve medical students served as subjects. About 8:00 A.M. they ingested 240 ml of heavy cream. For the next 5 hours they took only water *ad libitum*. Starting about 1:00 P.M., samples of blood were taken at 30, 25, and 15 minutes and immediately before an injection of heparin (10 mg intravenously). After the injection, samples were taken at 5, 15, and 30 minutes. In six subjects, studied under identical conditions, but on other days, samples of blood were taken without inhibitor at 3 and 30 minutes after an injection of heparin for determination of lipolytic activity. The fatty acid concentrations in all samples were determined by a single extraction method previously described (8).

TABLE 2. TURNOVER OF FATTY ACIDS ESTIMATED FROM LIPOLYTIC ACTIVITY AND THE RISE OF FATTY ACID CONCENTRATION AFTER INJECTION OF HEPARIN

Time	P	C′	ΔC	K	
min	*μeq/liter min*	*μeq/liter min*	*μeq/liter*	*per cent/min*	
3	72.8 ± 18.3	28.2 ± 6.0	141 ± 30.1	31.7 ± 15.3	28.4 ± 9.5
30	53.3 ± 12.1	−3.9 ± 3.2	228 ± 88.6	25.1 ± 11.2	

The results, and the standard errors of the mean values, are shown in Table 2. Two estimates of turnover rate were obtained: the first was calculated from the rapid rise of fatty acid concentration during the initial 5 minutes after injection of heparin, while the second was obtained from the data of the last 15 minutes, during which time the concentration had passed its peak and was falling slowly. The standard error of turnover was calculated from the variances of the three mean values (P, C', ΔC) according to the formula:

$$\sigma_K^2 = \left(\frac{\partial K}{\partial P}\right)^2 \sigma_P^2 + \left(\frac{\partial K}{\partial C'}\right)^2 \sigma_{C'}^2 + \left(\frac{\partial K}{\partial \Delta C}\right)^2 \sigma_{\Delta C}^2$$

The two values of turnover given in Table 2 are not significantly different. Combining them yields the final estimate, 28.4 ± 9.5% per minute. This value agrees remarkably well with the turnovers that have been calculated from the clearance of labeled palmitic, oleic, and linoleic acids (Table 1).

The present results thus tend to validate some of the assumptions involved in the use of tracer acids. It must be emphasized, however, that the equality of turnover rates means only that the fatty acid-protein complexes manufactured in the test tube are essentially the same as those formed in the blood stream. It remains for future work to determine whether or not this kind of complex accounts for all of the long-chain fatty acid in circulation.

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