The Depletion and Restoration of Post-Heparin Lipolytic Activity in the Human Forearm[1] (34906)

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The enzyme lipoprotein lipase appears to be necessary for the transfer of plasma triglyceride fatty acids into tissues. It is released into the plasma in response to circulating heparin and when various tissues including the human forearm (1, 2), and hearts from rats (3) and humans (4) are perfused with heparin, post-heparin lipolytic activity (PHLA) can be detected in the effluent circulation within the first minute. However Enser *et al.* (5) have found that when the rat heart is continuously perfused with heparin, the release of enzyme diminishes strikingly within minutes yet only a proportion of the heart's total enzyme activity is released.

The rate at which enzyme activity is depleted and restored has been measured in rat adipose tissue where it has been found to have a half-life of 1–2 hr (6, 7). A number of procedures and hormones influence this turnover and insulin is particularly effective in raising the activity of the enzyme or preventing its decline in tissues such as adipose tissue (8, 9).

The present study was undertaken to determine how rapidly PHLA is exhausted during a constant infusion of heparin into the human forearm, the period required for recovery to occur, and the influence of preceding infusions of insulin.

Methods. Clinical Procedures. Seven healthy young men were studied. They had fasted overnight and had not smoked. A brachial artery was cannulated and a polythene catheter was inserted proximally against the direction of blood flow. Catheters were also inserted into forearm veins in the direction of flow. In subjects 1, 2, 4, and 6 a vein in the antecubital fossa was selected and from its position and the dark color of the blood, it was thought to drain predominantly muscle. A superficial vein draining fat and skin was cannulated in subjects 3, 5, and 7. Further evidence about the sites of venous drainage was derived from the arterio–venous differences in free fatty acid (FFA) concentrations, which were markedly negative only in subjects 3, 5, and 7.

Both catheters were kept patent by infusions of solutions delivered by a constant infusion pump at a rate of 0.2 ml/min. In some subjects the initial solution was 0.15 *M* saline and in others, porcine "glucagon-free" insulin (Ely Lilly, Indianapolis) was delivered at a rate of 4 mU/min. After 1 hr, heparin was added to the initial solution and delivered at a rate of 100 μg/min for a further 20–30 min (the dose was 50 μg/min in subject 7). The solutions were then alternated as shown in the Figures so that heparin was delivered with either saline or insulin after a preceding period of the other solution (except in subject 1 in whom two consecutive infusions of saline plus heparin were compared and in subject 7 in whom heparin was infused continuously for 80 min). Blood flows were measured in 4 subjects by infusing Evans blue dye at a constant rate and measuring the dilution in the vein (10), the main purpose being to estimate the variation in flow during the entire procedure. This was found to be no greater than 11%. Since only one vein was cannulated, equilibration of the dye within the venous system of the forearm could not be established. Nevertheless the range of estimated plasma flows was 18–30 with a mean of 25 ml/min. This would result in concentrations of heparin in the brachial

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arterial plasma of approximately 4 μg/ml.

Blood was sampled from the brachial artery and forearm vein simultaneously at various times as indicated in the Figures. Approximately 10 ml blood was obtained in 10 sec. The hand was excluded during sampling by inflating a narrow arterial cuff at the wrist.

Laboratory Procedures. Blood was also placed into iced heparinized tubes for measurement of FFA concentrations (11) and PHLA content. The latter was measured in duplicate: 0.2 ml plasma was added to a mixture of 0.1 ml of 1 *M* Tris buffer at pH 8.2; 0.6 ml of 16% (w/w) bovine serum albumin in 0.1 *M* $(NH_4)_2$ SO_4 at pH 8.2; and 0.1 ml of a preincubated mixture (1:1) of 16 mg of triglyceride as Ediol and fresh human serum. The FFA released during a 1-hr incubation at 37° was measured and the PHLA was expressed as μ*M* FFA/min/ml plasma.

Results. When heparin was infused for the first time into the brachial artery at the rate of 100 μg/min, PHLA appeared rapidly in the veins of the forearm and more slowly in the brachial artery (Figs. 1 and 2). Peak PHLA was usually reached in the veins after about 10–15-min infusion. In most subjects venous PHLA then declined with a decreasing veno–arterial difference indicating that the local release of PHLA into the forearm veins diminished rapidly. Local release of PHLA was very small in subjects 2 and 5 after 30 and 20 min, respectively, and in subject 7 who received heaprin continuously for 80 min. Preceding infusions of insulin did not alter the pattern of appearance and disappearance of locally released PHLA. The amount of PHLA released locally and the duration of this response were similar in veins draining predominantly fat and in those draining muscle and presumably fat as well. Systemic PHLA did not reach the same level as local PHLA and in several subjects appeared to be reaching a peak by 20–30 min.

There was virtually no PHLA in the forearm veins and brachial artery after 1 hr without heparin. With the second infusion of heparin the venous level of PHLA rose less than with the first infusion especially in subjects 2 and 5 and the veno–arterial differences were smaller. This indicates a diminished release of PHLA from forearm tissues and that much of the activity in the veins was derived from the arterial side. The systemic release of PHLA as judged by the rise in arterial PHLA was only moderately diminished in some subjects and remained constant in subject 7 in whom heparin was infused continuously for 80 min with and without added insulin. Perfusion of the forearm with insulin for 1 hr before the second heparin infusion did not appear to change the pattern of response. The concentration of insulin in venous blood was from 47–81 μU/ml in 4 subjects in whom the measurements were made.

Discussion. These studies showed that release of PHLA from human adipose tissue and muscle declined rapidly during constant perfusion with heparin. This has also been shown with the isolated rat heart but since the total released activity accounted for only about half of the heart's total enzyme content, Robinson and Jennings (3) suggested that the remainder might have been present in a less accessible site or in a different form. The rate at which enzyme activity was depleted in the present studies was probably related to the local concentration of heparin since release of PHLA was still occurring from other tissues when that in the infused forearm had virtually ceased. It is less likely that tissues elsewhere than in the forearm are not as readily depleted of enzyme activity or that the concentration of heparin in the brachial artery (approx 4 μg/ml) was inhibitory.

The forearm tissues had regained their capacity to release PHLA when heparin was infused for a second time 1 hr after the first infusion had ended. The total response was less with the second infusion and varied considerably among the 6 subjects. Since the second response, as judged by the earliest levels of locally released PHLA during each infusion, was from 12 to 147% (av 65) of the initial value, it is likely that the pool of enzyme which was available for release, had been replenished at a rate which is comparable to that observed from *in vitro* studies of the turnover of the enzyme in adipose tissue

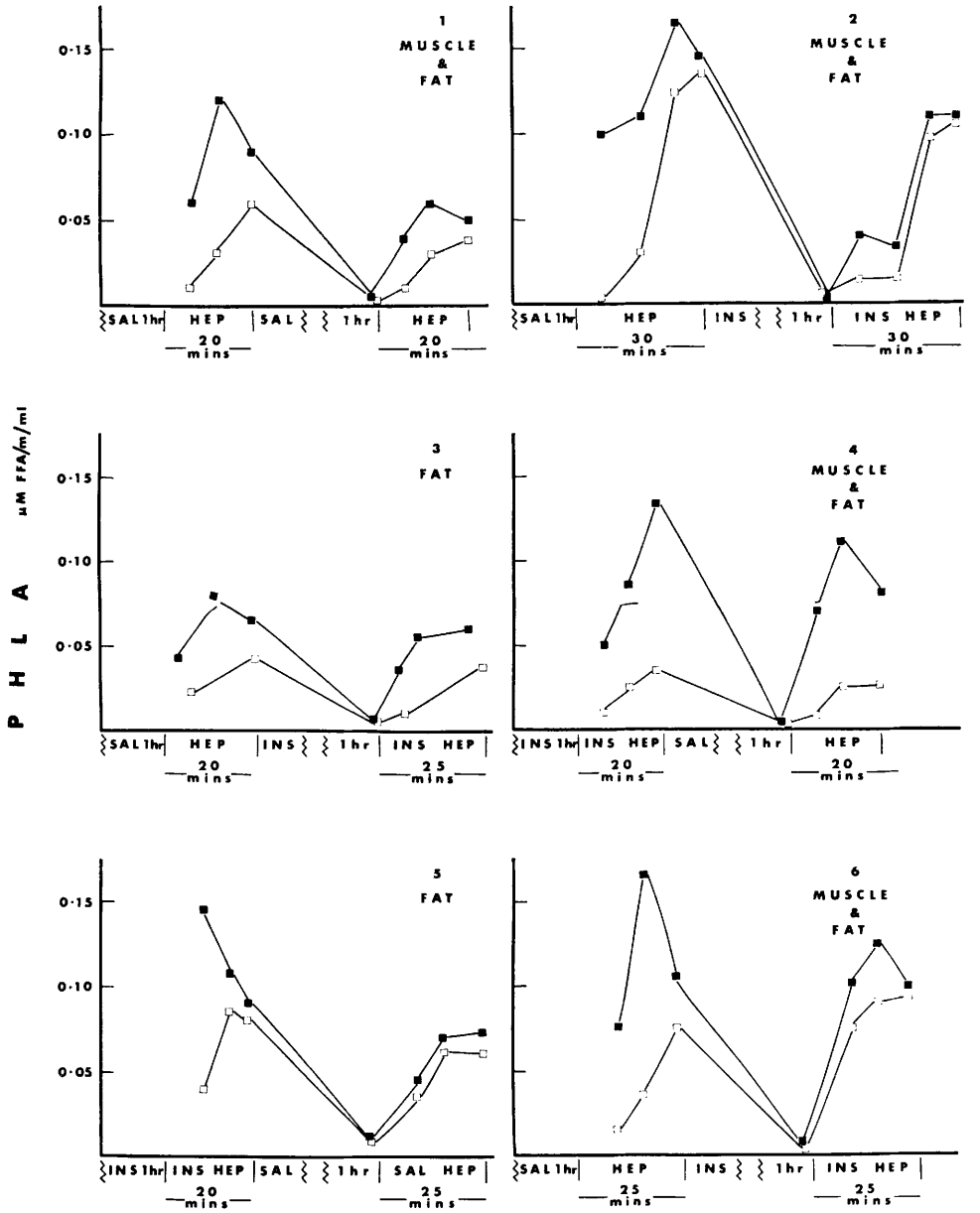

FIG. 1. Post-heparin lipolytic activity (PHLA) in the brachial artery (□); and forearm vein (■) during the perfusion of the forearm with solutions containing 0.15 *M* NaCl, insulin, and heparin, given separately or together.

(6, 7). This is based on the assumption that this pool had been restored by *de novo* synthesis or that the remaining enzyme had been converted to a form or transferred to a site that led to its release by further heparin. The possibility has not been excluded that some of the enzyme released from the forearm on the second occasion had initially been released elsewhere in the body and subsequently taken up by the tissues of the forearm, but this is less likely. Although the first infusion of heparin was stopped 1 hr before the second infusion, circulating heparin would have persisted for some time (12) and this might

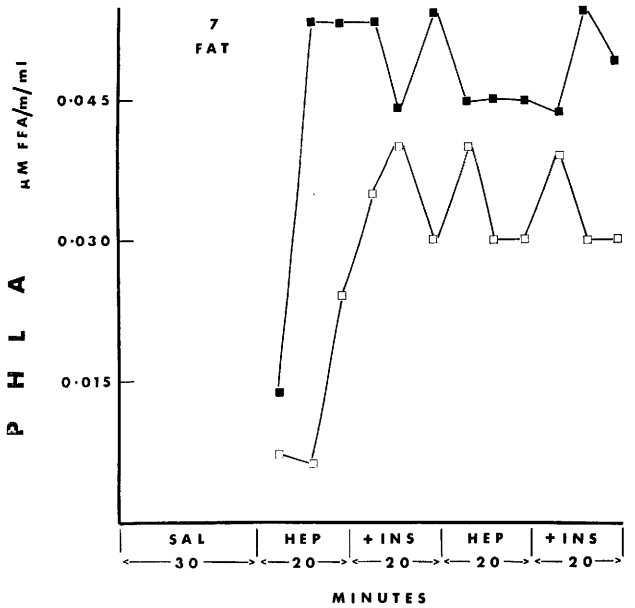

FIG. 2. PHLA in brachial artery (□); and a superficial forearm vein (■) during an 80-min perfusion with a saline solution containing heparin with or without insulin.

have partly accounted for the markedly diminished response to the second infusion in some subjects. Recovery of locally released PHLA did not occur while heparin was infused continuously (subject 7).

The failure of physiological concentrations of insulin to influence the release of PHLA might have been due to the short duration of insulin administration since at least 3 hr are required to demonstrate an effect when adipose tissue is incubated *in vitro* (6, 8, 9). Longer studies were not possible because the arterial catheters became painful after about 3 hr.

Summary. Post-heparin lipolytic activity (PHLA) was measured in venous blood in the human forearm during the constant infusion of heparin into the brachial artery. PHLA appeared rapidly and then diminished despite the continued administration of heparin. The replenishment of PHLA appears to be rapid in that a second infusion of heparin, 1 hr after the first infusion had ended, led to a further, though reduced release of PHLA. Prior 1-hr infusions of insulin in physiological concentrations did not appear to augment the release of PHLA.

1. Fraser, J. R. E., Lovell, R. R. H., and Nestel, P. J., Clin. Sci. **20**, 351 (1961).

2. LeQuire, V. S., Hamilton, R. L., Adams, R., and Merrill, J. M., Proc. Soc. Exp. Biol. Med. **114**, 104 (1963).

3. Robinson, D. S., and Jennings, M. A., J. Lipid Res. **6**, 222 (1965).

4. Muir, J. R., Clin. Sci. **34**, 261 (1968).

5. Enser, M. B., Kunz, F., Borensztajn, J., Opie, L. H., and Robinson, D. S., Biochem. J. **104**, 306 (1967).

6. Wing, D. R., and Robinson, D. S., Biochem. J. **106**, 667 (1968).

7. Nestel, P. J., and Austin, W., Life Sci. **8**, 157 (1969).

8. Wing, D. R., Salaman, M. R., and Robinson, D. S., Biochem. J. **99**, 648 (1966).

9. Austin, W., and Nestel, P. J., Biochim. Biophys. Acta **164**, 59 (1968).

10. Andres, R., Cader, G., and Zierler, K. L., J. Clin. Invest. **35**, 671 (1956).

11. Dole, V. P., J. Clin. Invest. **35**, 150 (1956).

12. Eiber, H. B., and Danishefsky, I., Nature (London) **180**, 1359 (1957).

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