Post-Heparin Lipolytic and Monoglyceridase Activities in Fasted Man[1] (35665)

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(Introduced by Charles S. Davidson)

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Animal studies reveal diminished adipose tissue "clearing factor" lipase after starvation (1, 2); however, no measurements of heparin-releasable lipases have been made in fasted humans. Since plasma post-heparin lipolytic activity is used clinically to assess defects in the removal of triglycerides (3), it seemed essential to recognize the physiologic factors that affect such activity. Accordingly, we measured plasma post-heparin activity against a soy bean oil emulsion and micellar mono-olein in individuals fasted for 10 days, and then after refeeding. Prolonged fasting in man causes a significant decrease in post-heparin lipolytic and monoglyceridase activity. Plasma triglyceride fell in the face of these declines in post-heparin lipolytic activity.

Methods and Materials. Six euthyroid, obese volunteers were fasted on a metabolic ward after at least 3 days of an equilibration diet consisting of 40% mixed carbohydrate, 20% protein, and 40% fat (60% unsaturated, 40% saturated; approximately 700 mg cholesterol). Patients were studied before fast, and on days 3, 7, and 10 of total caloric restriction during which daily intake consisted of 50 mEq NaCl, 87 mEq KCl, and 2000 ml of water. Patients were restudied between 8 and 9 a.m. after 1 day of refeeding with 1500 calories. Nonobese euthyroid controls ($N = 15$) recruited among hospital personnel and convalescing patients were studied after an overnight fast. All subjects were free of renal, hepatic, or lipid disorders, and none was pregnant or taking oral contraceptives. The nature of the studies was fully explained and signed consent obtained.

On the morning of study, subjects were given 0.1 mg/kg of sodium heparin (Lipo-Hepin, Riker) intravenously and four venous plasma samples were obtained at 2-min intervals between 6 and 12 min after injection. Samples were centrifuged at 4°, the post-heparin plasma pooled and stored at −20°. Post-heparin lipolytic activity (PHLA) was determined by a modified Frederickson method (3) using a soy bean oil emulsion (Intralipid, Vitrum, Stockholm) as described previously (4). Post-heparin monoglyceridase activity (PHMA) was determined using a mono-olein micelle solution. Glyceryl monooleate, 90% (Calbiochem[2]) was sonicated (Sonifier Cell Disrupter, model W140D, Ultrasonics, Inc.) in a solution of 4% taurodeoxycholic acid (sodium salt, Sigma) in KRP buffer at ph 7.4 to give a final concentration of 3%. Post-heparin plasma (0.4 ml) was incubated with 0.6 ml of the substrate at 37° for 90 min. Free fatty acids were measured by the method of Dole (5). Pre-heparin plasma triglycerides were measured as described previously (4).

Partial thromboplastin times (6) were performed on pre- and 10-min post-heparin (0.1 mg/kg) samples after 9 days of starvation to rule out heparin antagonism in the evaluation of post-heparin lipases. Statistical analyses were made on the Mathatron 4280 computer.

Results. In 15 nonobese volunteers, the mean ± SEM PHLA was 6.4 ± 0.4 μM FFA/ml/hr and PHMA was 8.4 ± 0.4 μM

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[2] Thin-layer chromatography (methanol-washed silica gel H) of the monooleate substrate yielded pure mono-olein.

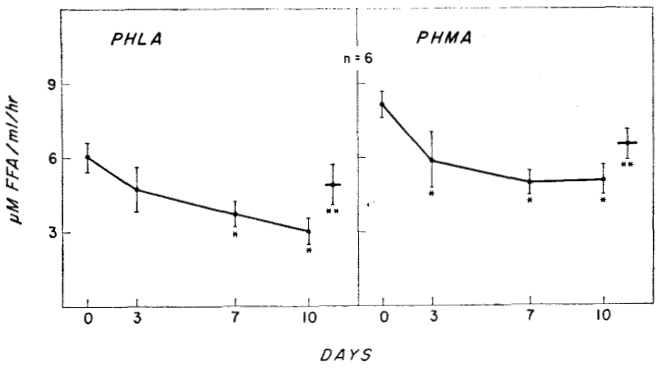

FIG. 1. Post-heparin lipolytic activity (PHLA) and post-heparin monoglyceridase activity (PHMA) in six obese subjects. Both enzymes fall significantly (*) during a 10-day fast and are restored significantly (**) after 1 day of refeeding.

FFA/ml/hr. In six obese subjects overnight-fasted PHLA was 6.1 ± 0.5 μM FFA/ml/hr and PHMA was 8.1 ± 0.6 μM FFA/ml/hr. PHMA fell significantly ($p < .05$) by Day 3 of starvation; PHLA fell significantly by Day 7 (Fig. 1). Mean PHLA fell to 1/2 its baseline activity on Day 8 of starvation. After 1 day of refeeding 1500 calories of a mixed diet, both enzymes rose significantly (p. vs Day 10 = $< .05$).

Mean triglycerides (±SEM) before and after fasting and 1 day after refeeding, are shown in Table I. The partial thromboplastin time on Day 10 of the fast in a representative subject 10 min after heparin injection (0.1 mg/kg) was not significantly different from that in overnight-fasted controls in our laboratory.

Discussion. Animal studies have substantiated the importance of the nutritional status on tissue lipoprotein lipase. Fat pads and extracts of adipose tissue taken from starved animals release and contain less enzyme than similar preparations from fed animals (1, 2). In contrast, lipoprotein lipase of cardiac and diaphragm muscle increases during starvation (7). Incubating the adipose tissue from starved animals with glucose and insulin restores lipoprotein lipase activity (8).

TABLE I. Serum Triglycerides (mg/100 ml) in Six Obese Subjects During Fasting and Refeeding.

	Day			
Prefast	3	7	10	Refeed
138 ± 30[a]	118 ± 4	107 ± 10	98 ± 2	79 ± 11

[a] Mean ± SEM.

Plasma post-heparin lipolytic activity (PHLA) is felt to reflect adipose tissue stores of lipoprotein lipase (9). Our studies reveal that in man PHLA declines significantly after a week of starvation. These data confirm the study of Persson *et al.* (10) that reveals a decline in adipose tissue lipoprotein lipase activity in fasted man and suggest that in the instance of starvation, a parallelism exists between PHLA and the enzyme content of adipose tissue.

Despite the fall in PHLA during starvation, plasma triglycerides did not rise. Thus, in starvation as well as in hyperthyroidism (11) a diminished response in PHLA is not accompanied by an accumulation of endogenous triglycerides. These situations contrast with those of pregnancy (12, 13), hypothyroidism (14), and non-nephrotic uremia (15) where diminished PHLA is accompanied by increased levels of triglyceride. The failure of triglycerides to increase during starvation may simply reflect diminished hepatic production, but may also suggest that enzymes other than adipose tissue lipoprotein lipase play a role in triglyceride clearing.

Heparin is known to release lipolytic enzymes other than "clearing factor" lipase. Among these are enzymes which act against phospholipid (16) and monoglyceride substrates (17). Our studies indicate that PHMA differs in thermal stability and is

inhibited by different substances than PHLA (18). During starvation post-heparin monoglyceridase activity (PHMA) declines in parallel with that of PHLA.

PHLA and PHMA levels return toward prefast values after one day of refeeding a mixed 1500 calorie diet. This finding suggests a rapid synthesis of tissue lipoprotein lipase and again highlights the role of insulin and substrate in stimulating the manufacture of enzymes (8). Since the diets used in refeeding were of mixed composition, the relative importance of carbohydrate, protein or fat in inducing enzyme production cannot be assessed.

Several causes for diminished PHLA and PHMA with prolonged starvation warrant consideration. A reciprocal relationship between hormone-sensitive and heparin-responsive lipases in adipose tissue has been demonstrated (19). The augmented activity of the hormone-sensitive system during starvation (20) might exert an inhibitory action on those systems sensitive to heparin. Since heparin affected the partial thromboplastin time during fasting, no evidence for the presence of an antagonist to heparin exists (21), except if such an antagonist be selective against the lipase system. Since the post-heparin lipase activities were measured against artificial substrates, the possibility that activity against natural substrates is not affected warrants testing.

These studies emphasize that in man any assessment of post-heparin lipolytic activity must consider the nutritional status of the patient. Conflicting observations, such as the role of PHLA in the pathogenesis of alcoholic lipemia, may reflect the significance of these observations (22, 23). Likewise, the studies point out that diminished responses in lipolytic activities after the administration of heparin are not universally associated with hypertriglyceridemia. Certainly, further study is necessary to define the relation of post-heparin lipolytic activity and the clearing of plasma triglycerides.

Summary. Prolonged fasting in man causes a significant decrease in post-heparin lipolytic and monoglyceridase activities. Activity is restored after refeeding. Plasma triglycerides declined in the face of diminished clearing factor lipase.

1. Cherkes, A., and Gordon, R. S., Jr., J. Lipid Res. **1**, 97 (1959).

2. Robinson, D. S., J. Lipid Res. **1**, 133 (1960).

3. Frederickson, D. S., Ono, K., and Davis, L. L., J. Lipid Res. **4**, 24 (1963).

4. Schreibman, P. H., Wilson, D. E., and Arky, R. A., N. Engl. J. Med. **281**, 981 (1969).

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

6. Spector, I., and Corn, M., J. Amer. Med. Ass. **201**, 75 (1967).

7. Hollenberg, C. H., J. Clin. Invest. **39**, 1282 (1960).

8 Salaman, M. R., and Robinson, D. S., Biochem. J. **99**, 640 (1966).

9. Nestel, P. J., and Havel, R. J., Proc. Soc. Exp. Biol. Med. **109**, 985 (1962).

10. Persson, B., Hood, B., and Angervall, G., Acta Med. Scand. **188**, 225 (1970).

11. Arons, D. L., Downs, P., Schreibman, P. H., Arky, R. A., and Braverman, L. E., Program of the Endocrine Society, 52nd Meeting, No. 199 (1970).

12. Fabian, E., Stork, A., Kucerova, L., and Sponarova, J., Amer. J. Obstet. Gynecol. **100**, 904 (1968).

13. Suanborg, V., and Vikrot, Q., Acta Med. Scand. **178**, 615 (1965).

14. Porte, D., Jr., O'Hara, D., and Williams, R. H., Metabolism **15**, 107 (1966).

15. Bagdade, J. D., Porte, D., Jr., and Bierman, E. L., N. Engl. J. Med. **279**, 181 (1968).

16. Vogel, W. C., and Bierman, E. L., J. Lipid Res. **8**, 46 (1967).

17. Greten, H., Levy, R. I, and Frederickson, D. S., J. Lipid Res. **10**, 326 (1969).

18. Schreibman, P. H., Arons, D. L., and Arky, R. A., Clin. Res. **18**, 464 (1970).

19. Patten, R. L., J. Biol. Chem. **245**, 5577 (1970).

20. Vaughan, M., J. Biol. Chem. **237**, 3354 (1962).

21. Glueck, C. J., Kaplan, A. P., Levy, R. I., Greten, H., Gralnick, H., and Frederickson, D. S., Ann. Internal Med. **71**, 1051 (1969).

22. Losowsky, M. S., Jones, D. P., Davidson, C. S., and Lieber, C. S., Amer. J. Med. **35**, 794 (1963).

23. Wilson, D. E., Schreibman, P. H., Brewster, A. C., and Arky, R. A., J. Lab. Clin. Med. **75**, 264 (1970).

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