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Glucagon and Plasma Lipoprotein Lipase[1] (38254)

RAYMOND CAREN AND LUCILLE CORBO
(Introduced S. Rosenfeld)

Cedars-Sinai Medical Research Institute and the Division of Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, and the Department of Medicine, University of California at Los Angeles, Los Angeles, California 90024

Previous work from this laboratory suggested an endocrine control of lipid metabolism by the pancreatic alpha cells through secretion of glucagon. Destruction of rabbit pancreatic alpha cells with cobalt chloride resulted in elevation of plasma cholesterol and lipemia both of which returned to normal as the alpha cells regenerated (1). Further study showed that glucagon administration to normal dogs caused depression of plasma cholesterol and total esterified fatty acids, while glucose, insulin, and epinephrine had no effect (2). Amatuzia *et al.* demonstrated that glucagon lowered plasma cholesterol, triglycerides, and phospholipids in hyperlipemic people (3). Further studies showed that glucagon lowered plasma lipids *in vivo* and *in vitro* by transfer of plasma lipid to blood platelets with accompanying inhibition of epinephrine-induced platelet aggregation (4, 5).

The clearing of lipemic plasma following glucagon administration has been a consistent observation in this laboratory and suggested that glucagon increased plasma lipoprotein lipase activity. This study was done to determine the effect of glucagon on plasma lipoprotein lipase activity and showed that glucagon increased plasma lipoprotein lipase activity *in vivo* and *in vitro* when incubated with whole blood.

Materials and Methods. Fifteen normal people (9 men, 6 women) were studied. Informed consent was obtained from each individual in accordance with the USPHS guidelines regulating human experimentation. Dietary history revealed a daily fat consumption of 40%–50% and carbohydrate of approximately 40% of total caloric intake. No subject had taken any medication for at least 2 weeks prior to study, and none of the women had taken anovulatory medication for at least 6 mo. Fasting plasma glucose levels were normal in all subjects. Plasma triglycerides were normal in all but one who had slight elevation (Table I). Plasma lipoprotein electrophoretic pattern was normal in this individual.

Following an overnight fast, venous blood was taken using plastic syringes and sodium citrate (19%, 0.18 ml/10 ml blood). One milligram glucagon[2] dissolved in 1 ml normal saline was given subcutaneously and blood taken 1 hr later. The blood was centrifuged 15 min at 850*g* in a swinging bucket head at 22° and plasma kept at 4° until assayed for plasma lipoprotein lipase activity within 2 hr using a modification of a method for determination of post-heparin plasma lipolytic activity (6). Plasma (2.2 ml) was incubated at 37° in a Dubnoff apparatus with 5.5 ml 20% bovine albumin (Fraction V in 0.1 *M* ammonium sulfate and adjusted to pH 8.5) and 3.3 ml of a 1:10 normal saline dilution of Ediol.[3] Final pH was 8.4. Two 1.0 ml aliquots were removed at 5, 15, 30 and 45 min and added drop-

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[2] Glucagon for injection, Eli Lilly and Co.

[3] 50% coconut oil emulsion, CalBiochem., La Jolla, CA.

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TABLE I. Sex, Age, and Fasting and 1 hr Post-Glucagon Plasma Triglycerides (TG) of 15 Normal People.

Subject	Sex	Age	mg/dl plasma	
			0 Hr TG	1 Hr TG[a]
KL	F	19	73	37
NR	F	19	26	20
RO	M	23	35	14
ER	M	27	54	40
TC	M	21	62	34
JY	M	18	39	33
ES	F	42	76	70
SA	M	52	48	45
HR	M	18	51	20
JH	M	18	49	36
PO	M	52	118	22
MB	F	31	70	35
LC	F	45	99	76
RC	M	56	177	118
LN	F	36	24	17

[a] $P < 0.001$, paired data analysis.

wise to 5 ml extraction mixture (40 parts isopropanol: 10 parts heptane: 1 part *N* sulfuric acid). After standing 5 min, the tubes were shaken 2 min in a Kahn shaker. Three ml heptane and 2.0 ml distilled water were then added, and the tubes shaken 2 min. A 3.0 ml aliquot of the heptane phase was removed from each tube and free fatty acids (FFA) titrated (7). FFA concentration minus the 5 min value (zero-time blank) was plotted against time to give reaction velocity (μmoles FFA/min/ml plasma). Plasma triglycerides were determined on 0 and 1 hr post-glucagon plasma (8).

The effect of glucagon (insulin-free[4]) on plasma lipoprotein lipase activity *in vitro* was studied in 10 subjects (4 men, 6 women). The effect of insulin (glucagon-free[4]) was studied in 8 of the subjects. Three equal aliquots of fasting whole blood from each individual were incubated respectively with glucagon (10 μg/ml plasma), insulin (10 μg/ml plasma), and an equal volume of .005*N* HCL in which the hormones were dissolved for 1 hr at 22°. Another three aliquots of the same blood from each individual were simultaneously incubated with glucagon, insulin, and diluent at 37°. After incubation, the mixtures were centrifuged and plasma lipoprotein lipase activity assayed as previously described. The activity of the diluent controls was subtracted from the activity found in the glucagon and insulin vials to give net change in activity. Triglyceride levels were determined on plasma from diluent control blood and blood incubated with glucagon at 22°.

The effect of pH on plasma lipoprotein lipolytic activity of fasting and 1 hr post-glucagon plasma was determined. The pH of the 20% albumin was adjusted with ammonium hydroxide, and pH of the final assay mixture before incubation was measured in a Beckman Model G pH meter.

Standard deviation of the assay method for pre- and post-glucagon plasma lipolytic activity was ±0.001 μmoles FFA/min/ml plasma. Standard deviation of the *in vitro* glucagon study was ±0.032 μmoles FFA/ml plasma while the standard deviation for the *in vitro* insulin study was ±0.018 μmoles FFA/ml plasma.

Results. Figure 1 shows the effect of 1

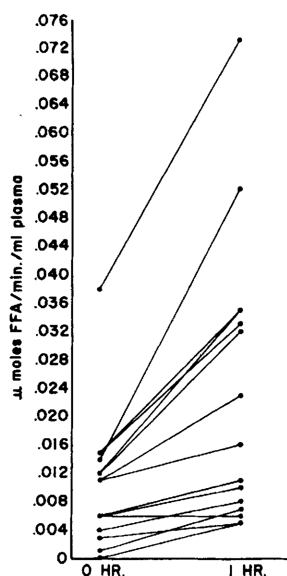

FIG. 1. Effect of 1.0 mg cs glucagon on lipoprotein lipase-like activity of 1 hr post-glucagon plasma of 15 people (9 men, 6 women).

[4] Supplied by Eli Lilly and Co.

mg subcutaneous glucagon on plasma lipoprotein lipase-like activity of 15 normal people (9 men, 6 women). The activity is termed lipase-like since coconut oil emulsion rather than isolated chylomicrons was used as substrate. Enzyme activity was present in fasting plasma of all people except one. The relationship between lipolysis and time of incubation in 0 hr and 1 hr post-glucagon plasma was linear in all subjects. Mean ± SE of the reaction velocity of pre-glucagon plasma for the 15 people was 0.010 ± 0.002 (range 0 – 0.038). Mean ± SE of 1 hr post-glucagon reaction velocity was 0.024 ± 0.005 (range 0.005–0.073). Plasma lipoprotein lipase-like activity was increased by glucagon in 14 of 15 subjects. The increase at 1 hr was statistically significant ($P < 0.001$, paired t test). Plasma triglycerides were significantly depressed by glucagon at 1 hr (Table I, $P < 0.001$, paired t test). There was no significant correlation between change in plasma triglyceride levels and plasma lipoprotein lipase-like activity by glucagon in 1 hr post-glucagon plasma ($r = -0.124$).

Figure 2 shows typical assay curves of fasting and 1 hr post-glucagon plasma of two individuals. The assay period for 1 hr post-glucagon plasma was extended to 90 min in six subjects. The relationship between lipolysis and time of incubation was linear to 90 min in all.

Figure 3 shows the effect of pH on plasma lipoprotein lipase-like activity (reaction velocity) of fasting and 1 hr post-glucagon plasma. Peak enzyme activity was obtained at pH 8.4 in both fasting and 1 hr post-glucagon plasma.

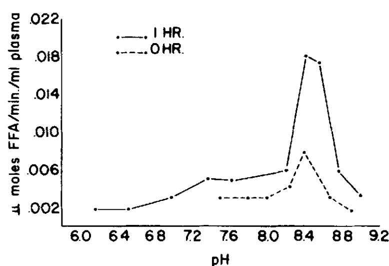

FIG. 3. Effect of pH lipoprotein lipase-like activity of fasting and 1 hr post-glucagon plasma of a normal man.

Table II shows the *in vitro* effect of insulin-free glucagon (10 subjects) and glucagon-free insulin (8 subjects) on plasma lipoprotein lipase-like activity of whole blood at 22° and 37°. Incubation of glucagon with fasting blood at 22° caused significant increase of plasma enzyme activity $P < 0.001$, paired t test), while 37° incubation partially or completely inhibited enzyme activity in aliquots of the same blood from all individuals except one. Insulin did not significantly increase plasma lipoprotein lipase-like activity at either 22° or 37°. Figure 4 shows significant correla-

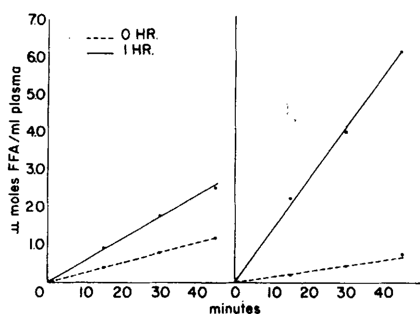

FIG. 2. Typical assay curves of plasma lipoprotein lipase-like activity of fasting and 1 hr post-glucagon plasma (2 subjects).

TABLE II. Effect of Insulin-Free Glucagon and Glucagon-Free Insulin on Plasma Lipoprotein Lipase-Like Activity of Whole Blood *in Vitro* at 22° and 37°.

	μmoles FFA/ml plasma			
	Glucagon[a]		Insulin[a]	
Subject	22°[b]	37°	22°	37°
RC	0.223	0	−0.009	0.068
CT	0.184	0.093	0.067	0.068
RR	0.112	0.008	−0.101	−0.132
LC	0.077	0.069	−0.009	—
SA	0.068	−0.005	0.023	0
JZ	0.203	0.037	0.048	0.044
CM	0.189	−0.048	0.050	0.050
FU	0.113	0.021	0.002	−0.003
VL	0.216	—	—	—
RO	0.101	—	—	—

[a] 10 μg/ml plasma.
[b] $P < 0.001$, paired data analysis.

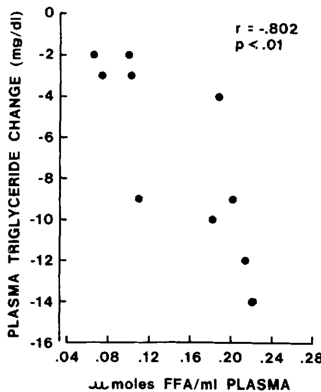

FIG. 4. Correlation between depression of plasma triglycerides and increase of plasma lipoprotein lipase-like activity by glucagon when incubated with whole blood.

tion ($r = 0.802$, $P < 0.01$) obtained between depression of plasma triglycerides and increase of plasma lipoprotein lipase-like activity when glucagon was incubated with whole blood at 22°.

Discussion. This study showed that glucagon increased plasma lipoprotein lipase-like activity when administered to human subjects. The hormone also increased the plasma enzyme activity *in vitro* when added to whole blood. A linear relationship between enzyme activity and incubation time was found indicating zero order kinetics. It appears that the enzyme is a plasma lipoprotein lipase since glucagon administration resulted in clearing of post-glucagon plasma and depressed all plasma lipid fractions with greatest depression of plasma triglycerides (4, 5). The effect of glucagon on plasma lipoproteins is further supported by the decrease of beta and pre-beta lipoprotein bands of post-glucagon plasma as shown by plasma lipoprotein electrophoresis (9).

The lack of correlation between plasma triglyceride depression and increase of plasma enzyme activity following glucagon administration would appear to indicate that the depression of plasma triglycerides was not related to an increase of plasma enzyme activity. However, glucagon administration causes other responses such as growth hormone secretion (10), insulin secretion (11), and glycogenolysis with hyperglycemia (12), all of which affect the level of plasma FFA. These effects of glucagon administration may influence uptake and release of plasma lipid by the liver and adipose tissue which may alter plasma triglyceride concentrations thus masking a correlation between plasma triglyceride depression and increase of plasma lipolytic activity. The above responses of glucagon administration were eliminated by *in vitro* study. Incubation of glucagon with whole blood resulted in an increase of plasma lipoprotein lipase-like activity and a depression of plasma triglycerides that were significantly correlated (Fig. 4). The correlation indicates that a glucagon-induced plasma lipoprotein lipase-like activity may be involved in the regulation of plasma triglycerides.

The question arose whether the increase of post-glucagon plasma lipoprotein lipase-like activity was due to stimulation of insulin secretion by glucagon since it has been suggested insulin maintains or increases lipoprotein lipase activity of adipose tissue (13–16). However, all studies indicating that insulin maintains or increases adipose tissue lipoprotein lipase involve either insulin administration to decompensated diabetic people or animals or incubation of adipose tissue of fasted or diabetic animals with insulin and glucose. There are no reports showing that insulin administration increases plasma lipoprotein lipase activity in normal people or intact animals. The present study showed that insulin, unlike glucagon, did not significantly increase plasma lipoprotein lipase-like activity when incubated with whole blood (Table II) suggesting that insulin secretion stimulated by glucagon was not responsible for the increase of post-glucagon plasma lipoprotein lipase-like activity.

Previous work showed that the depression of plasma lipids by glucagon when incubated with whole blood at 22° was partially or completely inhibited when aliquots of the same blood were incubated with glucagon at 37° (4). The present study showed that incubation at 37° partially or completely inhibited the increase of plasma lipoprotein lipase-like activity observed at

22°. The inhibitory effect of 37° incubation on plasma lipid depression and increase of plasma lipoprotein lipase-like activity suggests that the two effects of the hormone may be related. No explanation can be given at this time for the inhibitory effect of 37° on plasma lipid depression and plasma lipoprotein lipase-like activity.

Increase of plasma lipoprotein lipase-like activity by glucagon when incubated with whole blood suggests that the hormone acted on one or all three types of blood cells or activated existing plasma enzyme. The latter possibility does not appear likely since studies now in progress indicate that glucagon does not increase plasma lipoprotein lipase-like activity when added to "cell-free" plasma (9). If blood cells are involved in the mechanism by which glucagon increases plasma lipoprotein lipase-like activity *in vitro*, the hormone may act by either release of preformed enzyme from one or all three types of blood cells or by enzyme induction or activation in the nucleated blood cells. This suggests the interesting possibility that blood cells may be a source of plasma lipoprotein lipase and may play a role in regulation of plasma triglycerides.

Summary. Subcutaneous administration of 1.0 mg glucagon to 15 fasted normal people caused significant increase of plasma lipoprotein lipase-like activity in 1 hr. The activity is termed lipase-like since coconut oil emulsion rather than isolated chylomicrons was used as substrate. A linear relationship between lipolysis and time of incubation obtained indicating zero order kinetics. pH optimum of enzyme activity was 8.4. There was no correlation between depression of plasma triglycerides and increase of plasma lipoprotein lipase-like activity following glucagon administration. This was thought to be due to other effects of glucagon administration which alter plasma lipid levels. Incubation of whole blood with glucagon caused an increase of plasma lipoprotein lipase-like activity and a depression of plasma triglycerides that were significantly correlated. The *in vitro* action of glucagon when incubated with whole blood suggests that blood cell(s) may be a source of glucagon-induced plasma lipoprotein lipase and that they may play a role in the regulation of plasma triglycerides. Increase of plasma lipoprotein lipase-like activity by glucagon when added to whole blood suggests that the hormone acted by releasing preformed enzyme from blood cell(s) or by enzyme induction in the nucleated blood cells.

1. Caren, R., and Corbo, L., J. Clin. Endocrinol. Metabol. **16**, 507 (1956).

2. Caren, R., and Corbo, L., Metabol. **9**, 938 (1960).

3. Amatuzia, D. S., Grande, F., and Wada, S., Metabol. **11**, 1240 (1962).

4. Caren, R., and Corbo, L., Metabol. **19**, 598 (1970).

5. Caren, R., and Corbo, L., Metabol. **20**, 1057 (1971).

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

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

8. Fletcher, M J., Clin. Chim. Acta **22**, 393 (1968).

9. Caren, R., and Corbo, L., unpublished observations.

10. Mitchell, M. L., Byrne, M. J., and Silver, J., Lancet **1**, 289 (1969).

11. Samols, E., *in* "The Nature and Treatment of Diabetes" (B. S. Leibel and G. A. Wrenshall, eds.), Excerpta Medica Foundation, p. 227. Amsterdam (1965).

12. Lipsett, M. B., Engel, H. R., and Bergenstal, D. M., J. Lab. Clin. Med. **56**, 342 (1960).

13. Robinson, D. S., J. Lipid Res. **1**, 332 (1960).

14. Hollenberg, C. H., Can. J. Biochem. Physiol. **40**, 703 (1962).

15. Kessler, J. I., J. Clin. Invest. **42**, 362 (1963).

16. Kessler, J. I., J. Lab. Clin. Med. **60**, 752 (1962).

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