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Effect of Insulin on Plasma Triglyceride Concentration in Hepatectomized Geese

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The effect of insulin on the removal of plasma triglycerides was tested in hepatectomized geese. In this preparation plasma triglyceride concentration decreases exponentially following hepatectomy. Infusion of insulin (0.001 U/kg/min. for 60 minutes) had no significant effect on the rate of disappearance of plasma triglycerides in the hepatectomized animals. The conclusion is, therefore, reached that insulin does not affect the removal of plasma triglycerides by the extrahepatic tissues in the goose.

Insulin has a well established lipogenetic and antilipolytic effect in several mammalian species (12). However, studies by GOODRIDGE (7) and GRANDE (8) suggested that this hormone lacks antilipolytic effect in birds. Some clinical observations and experimental data in mammals suggest that insulin plays a role both in hepatic and peripheral metabolism of triglycerides (TGL) (1, 2, 13).

Since insulin is believed to enhance the removal of plasma TGL by activating adipose tissue lipoprotein lipase (1, 2, 13) it is of interest to determine whether this hormone stimulates the removal of plasma TGL in hepatectomized birds. It has been previously reported (14) that plasma TGL concentration falls exponentially in fasting hepatectomized geese.

It is the purpose of this paper to describe the effect of insulin infusion on plasma TGL concentration in fasting hepatectomized geese. In this preparation, a difference in the rate of all of plasma TGL between insulin treated and non-treated birds is expected to reflect the effect of this hormone on the removal of TGL by the peripheral tissues.

Materials and Methods

Adult domestic geese (males and non-laying females) were used. They were housed in a room lighted for 10 hours every day and fed *ad libitum* a commercial diet (Pellets Sanders for poultry). All the experiments were done in birds fasting for 16-18 hours. Anesthesia was induced with sodium pentobarbital (Nembutal, Abbott Laboratories) at the dose of

33 mg/kg (two-thirds i.v. and one-third i.m.). After anesthesia PE-90 gauge catheters were inserted into the wing veins, one for infusion and the other for the withdrawal of blood samples. Infusions were made with a continuous infusion pump (model Braun-Melsungen, Type 871022). Infusion rate was 12 ml/h. Appropiate dilutions of bovine crystalline insulin (lot g-10, 3, 2, 40 U/ml, Novo Laboratories) in phosphate buffer (0.004 M, pH 7.4) were prepared daily just before the beginning of the infusion.

The dose of insulin was 0.001 U/kg/min for 60 min. Control animals received phosphate buffer. Functional hepatectomy was performed by ligature of the liver vessels as described by RANNEY *et al.* (15) except that the ligatures were tied inside the abdomen of the anesthetized animal, as previously reported by OYA *et al.* (14).

Blood samples were taken 30 minutes and just before the completion of the ligature of the liver vessels and during and after infusion at various time intervals as indicated. Blood was collected in centrifuge tubes containing EDTA (1 %) and kept in bath of ice water. After removing an aliquot for sugar determination, the samples were centrifugated and the plasma was removed. Blood sugar (BS) was estimated in duplicate by Glucose Oxidase method (4). Plasma free fatty acids (FFA) were measured by the method of TROUT *et al.* (17). Plasma TGL was estimated by a transesterification method using a commercial kit (Dade Division, Miami, Florida) (16). Aliquots of the remaining serum were kept frozen at —20° C for determination of immunoreactive insulin activity (IRI). Serum insulin was assayed in triplicate by the ethanol precipitation method of HEDING (11). Hematocrit values were estimated using a standardized method (Micro Hematocrit capillary tube CHB-Berdram, Denmark). Data were analyzed by standard methods of statistical analysis. Unless otherwise stated, the levels of significance of the differences reported were calculated by the Student's test for paired variates.

Results

Figure 1 describes the changes in BS, FFA, IRI and TGL during typical experiments of insulin infusion in 7 hepatectomized (right pannel) and 5 control ani-

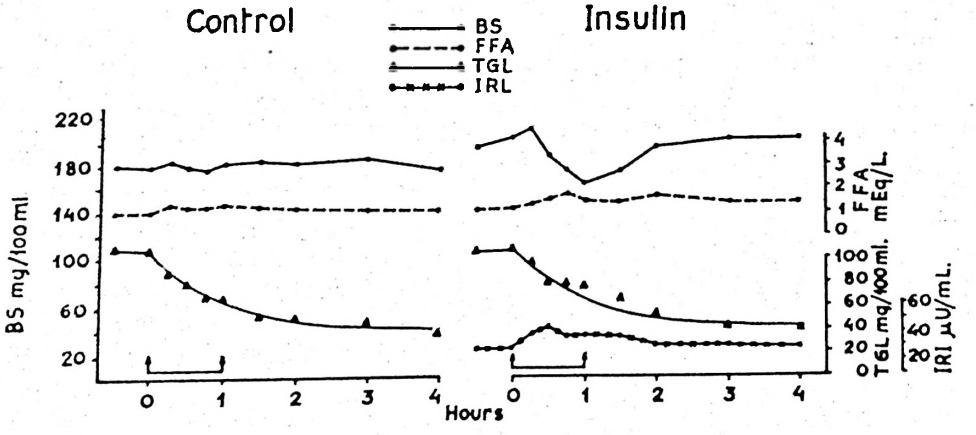

Fig. 1. *Plasma free fatty acids (FFA mEq/l), blood sugar (BS mg/100 ml), triglycerides (TGL mg/100 ml) and immunoreactive insulin (IRI µU/ml) in hepatectomized geese.* Left pannel means for 5 geese infused for 1 hour with phosphate buffer. Right pannel means for 7 geese infused for 1 hour with insulin (0.001 U/kg/min.).

mals (left pannel). No significant changes in neither BS of FFA were observed in the control experiment. Insulin infusion produced no change of plasma FFA in hepatectomized geese. BS decreased from preinfusion values of 195 mg/100 ml (SE±5.6) to 165 (SE±13). The BS decreased at the end of insulin infusion (33 mg/100 ml SE±9.7) was significant ($p < 0.05$).

After the beginning of insulin infusion, IRI levels stabilized throughout the whole period up to 120 minutes (fig. 1). The TGL displayed a sustained decrement both during the control and insulin infusion experiment. However, when plotted in a semilogaritmic graph these decrements were expressed by a stright line. The average slope of the TGL decay lines (15-120 min.) in the control experiments was —0.0023 (SE±0.0004), and with insulin —0.0024 (SE±0.0007). The difference between these slopes was not statistically significant (t test for non paired variates). No differences in hematocrit values between the start and the end of the experiments were recorded.

Discussion

These experiments demonstrate that insulin did not modify the rate of decrease of TGL after hepatectomy. As shown by BAGDADE *et al.* (1) in man the peripheral removal of TGL by insulin seems to be due to activation of the intravascular lipoprotein lipase by the hormone, perhaps mediated by the adenylcyclase system (18). However, the present results do not give support to the view that insulin enhances removal of plasma TGL in the extrahepatic tissues in birds.

These results confirm previous observations (8) regarding the lack of antilipolytic effect of insulin in birds as indicated by the virtually identical plasma FFA levels in both experimental situations. The hypoglycemic effect of heterologous insulin in birds, reported by HAZELWOOD (10) is confirmed by these results. Determination of IRI levels during the infusion of insulin showed a correlation between the hypoglycemic effect and the plasma insulin levels.

Recent reports suggest that in birds the regulation of adipose tissue metabolism is mediated through the adenyl cyclase system, but there are differences between mammals and birds in this respect. In the domestic fowl, FRÖHLICH (6) showed that insulin failed to decrease cyclic AMP levels in plasma, muscle and liver in contrast with the results obtained in other species (5). Also previous studies by GRANDE *et al.* (9) showed that theophylline, a drug known to increase cyclic AMP intracellular levels through phosphodiesterase inhibition, did not potentiate the lipolytic effect of glucagon in birds. A similar lack of lipolytic enhancement was obtained with methylpyrazol (3).

Resumen

Se estudia en gansos hepatectomizados el efecto de la insulina sobre la extracción de triglicéridos plasmáticos. Con esta técnica la concentración de triglicéridos plasmáticos decrece exponencialmente después de la hepatectomía.

La infusión de insulina (0,001 U/kg/min., durante 60 minutos) no tiene efecto significativo sobre la velocidad de desaparición de los triglicéridos plasmáticos en el animal hepatectomizado. Se concluye que la insulina no afecta la extracción de triglicéridos plasmáticos por los tejidos extrahepáticos en el ganso.

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References

1. BAGDADE, J. D., PORTE, D. and BIERMAN, E. L.: *Diabetes*, **17**, 127-132, 1968.

2. BORENSZTAJN, J., SAMOLS, D. R. and RUBINSTEIN, A. H.: *Amer. J. Pysiol.*, 223, 1271-1275, 1972.
3. CARMENA, R. and GRANDE, F.: *Biochem. Pharmacol.*, 19, 1838-1841, 1970.
4. CAWLEY, L. P., SPEAR, F. F. and KENDALL, R.: *Am. J. Clin. Path.*, 32, 195-200, 1959.
5. EXTON, J. H. and PARK, C. R.: *J. Biol. Chem.*, 244, 1424-1433, 1969.
6. FRÖHLICH, A. A. and MARQUARDT, R. R.: *Biochem. Biophys. Acta*, 286, 396-405, 1972.
7. GOODRIDGE, A. G. and BALL, E. G.: *Comp. Biochim. Physiol.*, 16, 367-379 1965.
8. GRANDE, F.: *Proc. Soc. Exp. Biol. Med.*, 130, 711-713, 1969.
9. GRANDE, F., PRIGGE, W. F. and OYA, M. DE: *Proc. Soc. Exp. Biol. Med.*, 141, 774-781, 1971.
10. HAZELWOOD, R. L., KIMMEL, J. R. and POLLOCK, H. G.: *Endocrinology*, 83, 1331-1336, 1968.
11. HEDING, L. G.: *Horm. Metab. Res.*, 1, 145-146, 1969.
12. JEANRENAUD, B.: *Metabolism*, 10, 535-581, 1961.
13. NIKKILA, E. A.: *Adv. Lipid. Res.*, 7, 63, 1969.
14. OYA, M. DE, PRIGGE, W. F. and GRANDE, F.: *Proc. Soc. Exp. Biol. Med.*, 136, 107-110, 1970.
15. RANNEY, R. E., CHAIKOFF, I. L. and DOBSON, E. L.: *Amer. J. Physiol.*, 165, 588-595, 1951.
16. ROGER, M. E. and KO, H.: *Anal. Biochem.*, 29, 405, 1969.
17. TROUT, D. L., ESTES, E. H. and FRIEDBERG, S. J.: *J. Lipid. Res.*, 1, 199-102, 1960.
18. WING, D. R. and ROBINSON, D. S.: *Biochem. J.*, 109, 841-849, 1968.