Metabolic Effects of Human Chorionic Gonadotropin (HCG) in Rats (35043)

ROBERT FLEIGELMAN AND GEORGE H. FRIED
(Introduced by W. Antopol)

Biology Department, Brooklyn College of City University of New York, Brooklyn, New York 11210

Human chorionic gonadotropin (HCG), employed clinically because of its influence on gonadal function, has also been used successfully in the treatment of obesity. Simeons (1) asserts that an enhancement of the mobilization of fat occurs during HCG treatment, although no rationale for this effect has been determined. The present study is an attempt to establish a biochemical basis for the action of HCG by assessing the effects on three enzymes which are involved in linking glycolysis to the esterification and synthesis of fatty acids in possible target tissues: soluble alpha-glycerophosphate dehydrogenase (AGPD), glucose-6-phosphate dehydrogenase (G6PD), and lactic dehydrogenase (LDH). The significant role of these enzymes in directing lipid synthesis has been discussed (12).

Materials and Methods. Hooded rats obtained from the Brooklyn College stock colony were maintained on Rockland Rat Chow *ad libitum*, with free access to water. Injections of 50 IU of HCG (Lot No. 6837, Bioline Labs, Brooklyn) were given ip each day for 7 days. Controls received 0.2 ml of saline. Alternative routes of administration have been employed in other studies of the metabolic effects of HCG (13, 14).

Male rats were weighed and then sacrificed by decapitation, following by exsanguination. The liver, both epididymal fat pads, and the gastrocnemius muscle from both legs were removed, blotted dry, and then frozen by placing the tissue on aluminum foil boats immersed in an acetone–Dry Ice saturated solution. In selected instances, fresh tissues were assayed and compared with frozen samples.

Tissues were homogenized with nine parts distilled water in a Tr-R homogenizer using Teflon pestles and glass tubes. The crude 10% homogenates were centrifuged at 12,000*g* for 30 min in a Sorvall high-speed refrigerated centrifuge. The clear supernates were then brought to final concentrations of 1% for muscle, 5% for fat, and 0.5% for liver.

The assays for AGPD were carried out according to the tetrazolium method of Fried *et al.* (2). Assays for G6PD and LDH were carried out according to a modification of the tetrazolium technique, involving substitution of Tris buffer (pH 7.4) for G6PD (3) and appropriate substitution of substrates. In all assays homogenate blanks were run to determine endogenous activity. Thirty per cent trichloroacetic acid (0.2 ml) was used to stop the reactions.

The formazan was extracted with 5 ml of ethyl acetate, and the optical densities were determined in a Coleman Junior II spectrophotometer (Model 6/20) at 490 nm. Each successive reading was first blanked with pure ethyl acetate. Calculation of enzyme activity was based upon a calibration curve obtained with chemically reduced iodoformazan. Nitrogen determinations were carried out on aliquots of homogenate according to a modification of the micro-Kjeldahl method utilizing direct nesslerization. Enzyme activity was expressed as micrograms of formazan per μg of homogenate nitrogen (3).

Results. The effect of HCG is most manifest in adipose tissue as demonstrated by significant decreases in activities of all three enzymes (Table I). In liver, there was a decrease of 93% in G6PD activity, while LDH and AGPD were unaffected. Muscle,

TABLE I. Effects of HCG on Enzyme Activity.

	No. of rats (wt)	Adipose	Liver	Muscle
LDH				
Control	4 (441 g)	23.22 ± 0.56[a]	21.10 ± 3.13	23.46 ± 2.39
HCG	6 (482 g)	15.09 ± 0.57[b]	22.43 ± 1.65	24.96 ± 1.93
G6PD				
Control	4 (441 g)	7.83 ± 0.52	11.26 ± 1.28	No activity
HCG	6 (482 g)	4.04 ± 0.25[b]	1.05 ± 0.02[b]	No activity
AGPD				
Control	4 (441 g)	21.08 ± 1.59	8.93 ± 0.55	7.92 ± 0.32
HCG	6 (482 g)	3.23 ± 0.48[b]	7.70 ± 0.16	2.86 ± 0.16[b]

[a] All enzyme activities expressed as micrograms formazan/μg nitrogen (± SE).

[b] Probability <0.001 for the null hypothesis using Student's t test to compare control and HCG groups.

which shows no activity of G6PD under control or experimental conditions exhibited a 64% decrease in AGPD activity. LDH showed no change. In adipose tissue, specific decreases in activity of 35, 48, and 85% were found for LDH, G6PD, and AGPD, respectively. Thus, HCG treatment demonstrates more marked effects on AGPD and G6PD than on LDH. Fresh tissues showed activities essentially similar to the frozen samples.

Discussion. HCG is a glycoprotein found in the urine of pregnant women. Its most significant clinical role has been in influencing such gonadal functions as the differentiation of Leydig cells, induction and maintenance of testicular androgens, and treatment of Froehlich's syndrome (1, 4). While the effects suggest a significant mediating role for the gonads in HCG action, direct effects on metabolic parameters of HCG have been reported in castrated monkeys (14). Histochemical comparisons of enzyme activity in normal and castrated rats tested with HCG suggested a direct action of this hormone on liver metabolism (15). A mediating role for the adrenals has not been reported.

For the past 15 years, Simeons has used HCG in the treatment of obesity (5–7). Maintenance on a strict 500 cal/day diet and daily injections of HCG (125 IU) produced weight loss from "abnormal" fat deposits, while alleviating the sensation of hunger. HCG was believed to act by mobilizing fat from excessive fat stores in a selective manner. The present studies suggest an enzymatic basis for this action.

In adipose tissue, triglyceride synthesis is probably dependent on AGPD conversion of dihydroxyacetone phosphate (DHAP) to AGP (active glycerol), due to the absence of a glycerol-kinase (8). The 85% decrease in activity of adipose tissue AGPD, an enzyme which links glycolysis with esterification of fat, is consistent with a marked diminution of fatty acid esterification during HCG treatment. Consequent increases in the pool of FFA in this tissue would account for mobilization and transport to such sites as the liver. The dramatic decrease of G6PD in liver may signify a negative feedback mechanism operating in response to the increased FFA since this metabolite is known to diminish lipogenesis (9). Reduction of G6PD activity in adipose tissue indicates that at least in terms of pentose-shunt generation of NADPH for fatty acid synthesis there is reduced lipogenesis. Thus, an enzymatic predisposition for lowered FFA production and diminished esterification is associated with HCG administration, which may account for its action in reducing the adipose mass. Why abnormal fat deposits are more vulnerable to this treatment than normal fat is still puzzling. MacDonald and Barry's finding that in dieting humans there is a selective withdrawal of fatty acids from adipose depots (10), suggests the possibility of differential susceptibilities to lipid-mobilizing agents by adipose

masses whose fatty acid composition are different.

The diminution of G6PD in liver is in sharp contrast to previous findings of a marked increase in oxidative enzymes in this tissue after direct incubation with HCG in the medium (11). Although *in vivo* and *in vitro* dosages cannot be readily equated, the tendency for oxidative enzyme activity to rise while pentose-shunt activity declines is suggestive of possible control mechanisms regulating metabolite flow in either a catabolic or anabolic direction.

1. Simeons, A. T. W., "Pounds and Inches. A New Approach to Obesity," 110 pp. Private Publication, Salvator Mundi International Hospital, Rome (1968).

2. Fried, G. H., Greenberg, N., and Antopol, W., Proc. Soc. Exp. Biol. Med. **107**, 523 (1961).

3. Fried, G. H., and Antopol, W., Amer. J. Physiol. **211**, 1321 (1966).

4. Turner, C. D., "General Endocrinology," 579 pp. Saunders, Philadelphia (1966).

5. Simeons, A. T. W., Lancet **2**, 946 (1954).

6. Simeons, A. T. W., Amer. J. Clin. Nutr. **12**, 197 (1963).

7. Simeons, A. T. W., Amer. J. Clin. Nutr. **15**, 188 (1964).

8. Lochaya, S., Hamilton, J. C., and Mayer, J., Nature (London) **197**, 182 (1963).

9. Langdon, R. G. *in* "Lipid Metabolism" (K. Bloch, ed.), pp. 238–290. Wiley, New York (1960).

10. MacDonald, J., and Barry, P. J. C., Amer. J. Clin. Nutr. **15**, 158 (1964).

11. Kuzin, A. M., and Lebedeva, N. E., Probl. Endokrinol Gormonoter **9**, 34 (1963).

12. Fried, G. H., Schreibman, M. P., and Kallman, K. D., Comp. Biochem. Physiol. **28**, 771 (1969).

13. Nagai, K., Matsumoto, K., Kotoh, K., Hanai, J., and Okano, K., Med. J. Osaka Univ. **16**, 321 (1966).

14. Janni, A., Laura, A., and Arcoraci, A., Boll. Soc. Ital. Biol. Sper. **40**, 443 (1964).

15. DeGregorio, G., Jr., Gallippi, G., and Quattrocchi, G., Boll. Soc. Ital. Biol. Sper. **40**, 459 (1964).

Received March 9, 1970. P.S.E.B.M., 1970, Vol. 135.