# The Effect of Hormones on Hepatic Cholesterol Ester Synthesis *in Vitro* (33997)

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Androgens, estrogens, and thyroxine modify the hepatic synthesis of cholesterol as well as the chemical nature of the lipoprotein complex which contains the major part of the plasma cholesterol and cholesterol esters (1–6). Cholesterol esters have a much longer half-life than free cholesterol. As such, the esterified cholesterol may represent an important transport or sequestered form. Plasma as well as liver contain enzyme systems that perform esterification of free cholesterol with fatty acids (1, 2, 7–10). Conversion of cholesterol to cholesterol esters may be influenced by hormonal agents that act either by direct activation of the esterase or by changing the biochemical factors which indirectly control the enzymatic esterification process. In the present report we describe the *in vitro* effect of testosterone, 17$\beta$-estradiol, glucagon, and L-thyroxine on the formation of cholesterol palmitate, oleate, and linoleate by the cholesterol ester synthetase of rat liver microsomes.

*Materials and Methods.* Male Sprague-Dawley rats, age 12 weeks, were killed by decapitation. A microsomal enzyme fraction was prepared from the livers as described by Goodman *et al.* (10). One $\mu$mole of cholesterol-7$\alpha$-$^3$H (5.8 $\mu$Ci) and 0.3 $\mu$moles each of potassium oleate, palmitate, and linoleate were placed in a 10-ml Erlenmeyer flask. After the addition of 1 $\mu$mole of coenzyme A, 9 mg defatted of human serum albumin, 18 $\mu$moles of ATP, 1.4 mg of microsomal protein, and 0.1 *M* potassium phosphate buffer, pH 7.4, to each flask, the incubations were performed in a Dubnoff metabolic incubator at 37° for 2 hr with air as gas phase. The final total incubation volume was 3 ml in each flask with or without the appropriate quantities of hormones added as indicated in Table I. Glucagon and sodium L-thyroxine were dissolved in buffer before addition. Testosterone and 17$\beta$-estradiol were added to the flask in acetone solution. After evaporation of the acetone, 9 mg of defatted human serum albumin in 2 ml of buffer was added and the steroids were dissolved by shaking for 18 hr at 4°. The incubations were terminated by the addition of 1 ml of methanol and stored at —20°. Cholesteryl-4-$^{14}$C 4-$^{14}$C oleate, palmitate, and linoleate [200 $\mu$g (12 000 dpm) of each] were added as carrier. Solvent extraction and separation of the cholesterol ester fraction from unesterified cholesterol by column chromatography on alumina (Woelm, grade II; eluting solvent: benzene–petroleum ether 3:7) were done as described by Goodman *et al.* (10). The individual cholesterol esters were separated and purified to radiochemical homogeneity (constant $^3$H/$^{14}$C ratio) by thin-layer chromatography on silica gel G impregnated with $AgNO_3$ (25 g of silica gel plus 55 ml of 0.7% $AgNO_3$ in water) with benzene–hexane 1:1 (v/v) and hexane–chloroform 10:1 as the ascending solvents. The plates were scanned for radioactivity with a Packard model 7201 radiochromatogram scanner. According to the location of the $^{14}$C-tracer, the silica gel with the radioactivity was scraped into counting vials and suspended in 15 ml of scintillation gel (5 g of Cab-O-Sil thixotropic gel powder, 400 mg of PPO, 10 mg of POPOP, 100 ml of toluene). Each sample was counted in a model 3375 Packard Tri-Carb liquid scintillation spectrometer. Based on the recovery of $^{14}$C-tracers, the losses of $^3$H-labeled metabolites incurred during the extraction and chromatographic procedures could be calculated. The data listed in Table I are correspondingly corrected.

*Results.* The effect of different hormone concentrations on the *in vitro* synthesis of cholesterol esters by a rat liver microsomal preparation is shown in Table I. Without

TABLE I. The Effect of Hormones on Cholesterol Ester Synthesis by Rat Liver Microsomes.

| Concentration of drug ($M$) | | ($m\mu$moles) Cholesterol palmitate formed | Cholesterol oleate formed | Cholesterol linoleate formed |
|---|---|---|---|---|
| Control (no addition) | | 11.6 ± 0.4[a] | 12.5 ± 0.9 | 8.3 ± 0.3 |
| Testosterone | $11.6 \times 10^{-5}$ | 22.2 ± 0.9 | 25.7 ± 1.0 | 4.6 ± 1.5 |
| | $23.2 \times 10^{-5}$ | 18.7 ± 0.8 | 27.8 ± 0.8 | 16.7 ± 1.1 |
| 17$\beta$-Estradiol | $1.2 \times 10^{-6}$ | 10.2 ± 0.1 | 40.7 ± 0.8 | 4.7 ± 0.2 |
| | $1.2 \times 10^{-5}$ | 10.6 ± 0.2 | 35.9 ± 0.5 | 16.4 ± 0.4 |
| | $1.2 \times 10^{-4}$ | 15.6 ± 0.3 | 20.1 ± 1.5 | 30.1 ± 0.7 |
| | $2.4 \times 10^{-4}$ | 3.4 ± 0.4 | 26.8 ± 0.3 | 8.1 ± 0.9 |
| L-Thyroxine | $2.1 \times 10^{-6}$ | 22.1 ± 0.7 | 22.8 ± 0.2 | 32.9 ± 0.9 |
| | $10.5 \times 10^{-6}$ | 26.9 ± 0.1 | 29.9 ± 0.3 | 44.8 ± 1.5 |
| | $2.1 \times 10^{-5}$ | 18.1 ± 0.5 | 24.4 ± 0.2 | 26.7 ± 0.3 |
| Glucagon | $0.5 \times 10^{-6}$ | 21.4 ± 0.3 | 29.1 ± 0.5 | 35.4 ± 0.9 |
| | $2.5 \times 10^{-6}$ | 12.7 ± 0.3 | 16.5 ± 0.5 | 15.0 ± 0.4 |
| | $5.0 \times 10^{-6}$ | — | 14.5 ± 0.4 | 13.0 ± 0.6 |

[a] Standard deviation of 3 determinations.

hormone addition, cholesterol palmitate and oleate were synthesized at a higher rate than cholesterol linoleate. This is in good agreement with the results obtained by Goodman *et al.* (10) demonstrating the similarity of our microsomal enzyme preparation. The following observations are pertinent to the problem investigated: (i) Testosterone had a stimulating effect on cholesterol palmitate and oleate formation. Cholesterol linoleate synthesis was also increased at the higher hormone concentration; however, a marked inhibitory effect was noted at the lower hormone concentration. (ii) 17$\beta$-Estradiol drastically increased formation of cholesterol oleate at all hormone concentrations. Its effect on cholesterol palmitate and linoleate formation varied depending on the hormone concentration. (iii) L-Thyroxine uniformly increased the formation of all cholesterol esters at all hormone concentrations. (iv) The synthesis of the three cholesterol esters was slightly elevated by glucagon.

*Discussion.* In order to study and interpret the effects of hormones on the hepatic cholesterol esterifying activity *in vivo*, it is necessary to take into account any hormonal effects on biochemical parameters, such as the liver pools of cofactors and precursors as well as the concentration of active enzyme. These effects would be difficult to define. We have, therefore, chosen an *in vitro* system which allowed us to assay the esterase activity directly, eliminating any biochemical variables as much as possible.

The present studies revealed a marked effect of various hormones on the esterification of cholesterol with fatty acids by liver microsomes. Up to now, most studies of the hormonal effects on cholesterol esterification were done under *in vivo* conditions using animals pretreated with hormone. In several *in vivo* studies, a decreased hepatic cholesterol and cholesterol ester synthesis from acetate-$^{14}$C was observed after the administration of estrogens (5, 11, 12). In addition, estrogenic hormone caused a marked decrease of plasma cholesterol esters with an accompanying increase of liver cholesterol esters, suggesting a transfer of cholesterol esters from the plasma to the liver. Boyd (13) investigated the relative change of the fatty acid component of the cholesterol esters of human plasma after the administration of ethinylestradiol. He found a decreased concentration of cholesterol linoleate with a significant increase of cholesterol oleate and palmitate. Similar results have been obtained with estrogen treated rats (1). It is most difficult at present to correlate these results

with our data from *in vitro* experiments. But it is of interest to note that the *in vitro* effects of 17$\beta$-estradiol at a concentration approaching physiological levels could explain the relative changes of the individual plasma cholesterol esters observed under *in vivo* conditions. In essence, from the available evidence it may be concluded that the estrogenic hormones produce their effects at three levels: (a) biosynthesis of cholesterol from acetate or mevalonate, (b) esterification of cholesterol, and (c) lipoprotein transport complex.

Nothing is known of the effects of glucagon on cholesterol biosynthesis. Glucagon, however, stimulates the release of free fatty acids from lipid depots and could in this way actually increase the concentration and availability of circulating free fatty acid precursors.

One of the major effects of L-thyroxine appears to be the stimulation of plasma cholesterol degradation and excretion. In the liver L-thyroxine activates the synthesis of cholesterol by increasing the rate of incorporation of acetate into cholesterol (14). A uniform change in the fatty acid moiety of plasma cholesterol esters has been observed after the administration of thyroid hormone to humans (1). A relatively consistent activation of the rate of cholesterol esterification by hepatic microsomes under the influence of thyroxine was noted in our studies. The increase in esterification activity may play a role in the mechanism by which this hormone reduces plasma cholesterol levels.

At any one time, composition, concentration, and synthesis of cholesterol and its fatty acid esters in the liver are the net resultant of several processes. Our results suggest that in addition to other known effects, hormones exert their action through direct control of the enzymic interconversion of free cholesterol, fatty acids, and cholesterol esters.

*Summary.* The effects of hormones on the *in vitro* biosynthesis of cholesterol palmitate, oleate, and linoleate by rat liver microsomes from cholesterol and fatty acids was studied. It appears that L-thyroxine and glucagon in general stimulate the synthesis of all three esters. Testosterone increases cholesterol palmitate and oleate formation. Its effect on cholesterol linoleate formation varies, being stimulatory at the highest concentration and inhibitory at the lowest concentration of hormone used. The action of 17$\beta$-estradiol on cholesterol esterification also depended on the concentration of the hormone used. However, cholesterol oleate synthesis was markedly stimulated at all concentrations of 17$\beta$-estradiol.

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