Effects of Cortisol and ACTH Administration on Some Aspects Of Lipid Metabolism in the Hamster.* (32309)

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The effects of cortisol and ACTH administration on lipid metabolism *in vivo* have received less attention than the effects of these hormones on gluconeogenesis and carbohydrate metabolism. The clear lipolytic response of isolated adipose tissue to cortisol and ACTH(1,2,3) is clouded by contradictory reports of *in vivo* effects(4,5,6). The present study describes effects on liver and plasma total lipid and sterols produced in hamsters by a 3-day cortisol regimen or an acute ACTH administration. The resulting effects on the concentration of total lipid and sterols and the *in vivo* incorporation of label from acetate-C^{14} into total lipid are reported.

Procedures. Healthy male golden Syrian hamsters weighing 110 g or more were used.

Cortisol (Sigma), dissolved in a small volume of acetone and mixed with corn oil, was injected subcutaneously 4 times a day for 3 days. The total daily dose was 0.5 mg/kg. Controls received only the corn oil vehicle.

ACTH (H.P. ACTHAR GEL, Armour) was emulsified with corn oil or dissolved in 0.9% saline. Ten minutes before the acetate-C^{14} was administered the hormone was given both intraperitoneally (1.7 I.U. in saline) and subcutaneously (1.7 I.U. of the emulsion) in an effort to attain prompt and sustained ACTH levels during the experiment. Controls received a deactivated emulsion (heated to 90°C for 15 minutes) and saline. The brief, single dose period was chosen to minimize the possibility of responses due to endogenous corticoid release and to avoid the complex physiological and metabolic changes of adrenalectomy.

Sodium acetate-2-C^{14} (New England Nuclear) dissolved in saline was injected intraperitoneally after the last hormone administration in a dose of 30 mg (240 μC)/kg. One hour later the hamsters were sacrificed under pentobarbital anesthesia. Cardiac blood and liver samples were collected within 7 minutes following anesthesia.

Analytical methods. Total lipid extracts of plasma and liver samples were prepared according to Folch *et al*(7). Free and esterified sterols were separated from the total lipid extract by silicic acid column chromatography as described by Havel *et al*(8). The free sterol used for radio-assay was separated from other lipids present in the eluate fraction by digitonide formation according to Sperry and Webb(9) after addition of 1.5 mg carrier cholesterol. The eluate fraction containing esterified sterol was saponified and the liberated sterol extracted by the method of Abell *et al* (10). Sterol concentrations were measured colorimetrically by the method of Rosenthal *et al*(11) against recrystallized standards (12) which were chromatographed concurrently with the tissue extracts.

Radio-assays were carried out in the solution of Bray(13) in a refrigerated scintillation spectrometer (Packard). A statistical counting accuracy(14) of 97% or greater was attained on all samples.

Liver glycogen was isolated essentially by the method of Good *et al*(15) and measured with the anthrone reagent described by Roe (16).

Results and discussion. The experimental results are presented in Tables I and II. Data are not given for the specific activities of the sterol fractions; the spread among individual values precluded statistically meaningful results.

No change in hepatic glycogen stores was detected following the single administration of ACTH. The activity of the ACTH emulsion was established in a separate experiment in which hamsters treated with the emulsion for 3 days showed significant ($p<0.01$) increases in liver glycogen.

The lower specific activity of plasma total lipid produced by both cortisol and ACTH

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TABLE I. Liver Glycogen Concentration, Net Body Weight (BW) Change and Liver/BW Ratio in Hamsters Treated with Cortisol for 3 Days.

	Control	Cortisol	n	p
Liver glycogen (mg/g)	61.9 (± 4.5)*	85.7 (± 5.9)	34	.01
Liver/BW × 100	4.27 (± .15)	5.03 (± .22)	24	.01
Net BW change (g)	+3	−2	40	—

* ( )—Standard error of the mean difference.
n—Number of observations equally divided between experimental and control groups.
p—Probability based on Student's *t* distribution for random groups.

TABLE II. Effects of Cortisol and ACTH on Liver and Plasma Total Lipid and Sterols One Hour After the Injection of Acetate-2-C^{14} into Hamsters.

Group A. Cortisol for 3 days before acetate-C^{14}

		Control	Cortisol	n	p
		cpm/mg			
Total lipid	liver	12933 (±1518)	15691 (±1617)	28	.4
" "	plasma	17438 (±2210)	8991 (±1390)	22	.01
		mg/g or /100 ml			
" "	liver	33.2 (± 1.6)	28.4 (± 1.0)	24	.025
" "	plasma	503 (±22)	532 (±49)	22	.5
Free sterol	liver	1.24 (± .04)	.98 (± .04)	20	.001
" "	plasma	25.4 (± 1.8)	23.6 (± 1.2)	20	—
Esterified sterol	liver	.15 (± .01)	.16 (± .02)	24	—
" "	plasma	46.4 (± 3.3)	61.0 (± 5.4)	20	.05

Group B. ACTH 10 min before acetate-C^{14}

		Control	ACTH	n	p
		cpm/mg			
Total lipid	liver	5003 (± 880)	1936 (±247)	14	.025
" "	plasma	12366 (±1860)	3677 (±730)	14	.005
		mg/g or /100 ml			
" "	liver	32.3 (± .8)	34.4 (± 1.6)	14	.4
" "	plasma	551 (±15)	604 (±21)	14	.1
Free sterol	liver	1.41 (± .05)	1.39 (± .05)	16	.5
" "	plasma	22.7 (± 1.1)	21.8 (± 1.5)	14	.5
Esterified sterol	liver	.33 (± .08)	.31 (± .13)	16	.5
" "	plasma	47.5 (± 3.1)	38.5 (± 2.4)	14	.05

Symbols explained in Table I.

treatments is consistent with the view that both hormones promote lipolysis in adipose tissue(1,2,3). The release of pre-existing, non-labelled lipids into the blood would dilute or lower the specific activity of plasma total lipid, as indeed was observed following the administration of both hormones.

The lower specific activity of liver total lipid in ACTH treated hamsters may reflect depressed liver lipid synthesis arising from the stimulation of peripheral lipolytic processes. An elevation of plasma non-esterified fatty acids in the ACTH treated hamsters, as reported for the rat(6), could have led to an excessive generation of fatty acyl-Co A in the liver. Acyl-Co A derivatives of fatty acids have been shown to competitively inhibit purified rat liver acetyl-Co A carboxylase, an enzyme involved at the initial step of fatty acid synthesis(17). The operation of this mechanism in the ACTH treated hamsters could have inhibited liver lipid synthesis. The lower specific activity of plasma total lipid in ACTH treated hamsters also could have contributed to the lower specific activity of liver total lipid to the extent that labelled plasma lipids were utilized in hepatic lipid synthesis.

The higher mean specific activity of liver total lipid in cortisol treated hamsters was

not statistically significant because of the spread of sample values, although both this and the total C^{14} incorporation into liver total lipid (specific activity × mg/g × liver/B.W.) otherwise appeared to increase. The fact that liver total lipid specific activity at least was not lowered by cortisol treatment suggests that the liver may have compensated for the probable initial inhibitory effect of cortisol-induced lipolysis on hepatic lipid synthesis. It has been reported elsewhere that cortisone administered for 7 days to adrenalectomized rats followed by an intravenous injection of palmitate-C^{14} did not change the incorporation of isotope into liver total lipid, but the incorporation into liver neutral lipids (triglycerides) was increased (18). The lower concentration of liver total lipid observed in cortisol treated hamsters is notable since cortisone administration to adrenalectomized rats was reported to produce fatty livers as well as to increase liver size (18). The appearance of fatty livers in cortisone treated animals may be dose-dependent; the dose used in the study cited was more than 60 times greater than that employed in the present study.

The decline in liver free sterol concentration in cortisol treated hamsters may be related to the greater liver mass. This would be expected if liver free sterol is largely a structural component and cortisol produces liver hypertrophy but not an increase in liver cell population. In rats, cortisone administration was reported to produce hepatomegaly but not to increase the number of liver cells (19).

Cortisol administration apparently increased the concentration of plasma esterified sterol, while ACTH administration apparently reduced it. Cortisone treatment has been reported to raise plasma cholesterol in the rabbit without changing the total body cholesterol (20). If a similar response occurred in the cortisol treated hamsters, the apparent increased concentration of plasma esterified sterol might well arise from a re-distribution of esterified sterol from extra-vascular locations to the vascular compartment. The apparent decline in the concentration of plasma esterified sterol after acute ACTH treatment suggests a direct ACTH effect, one not mediated by a secondary stimulation of adrenal corticoid secretion. The basis for this apparent ACTH effect is unknown.

Summary. Acetate-C^{14} was given intraperitoneally to hamsters which had received cortisol for 3 days or ACTH only 10 minutes previously. 1. Cortisol administration reduced the liver total lipid concentration in contrast to reports that cortisone administration to rats produces fatty livers. The contrasting response in hamsters and rats receiving similar hormones may either be attributable to species differences or depend on the amount of hormone given. 2. Cortisol administration did not lower the specific activity or incorporation of label into liver total lipid. Hepatic lipid synthesis may have compensated during the 3-day dose period for a probable, initial inhibitory effect. 3. Plasma esterified sterol concentration was elevated by cortisol treatment. The increased concentration may result from a redistribution of sterol to the vascular compartment, as found in the cortisone-treated rabbit. Plasma esterified sterol concentration was lowered by the brief ACTH treatment. This response, opposite to that produced by the prolonged cortisol treatment, indicates a direct effect, but one the basis of which is presently unknown. 4. Both ACTH and cortisol administration reduced the specific activity of plasma total lipid, an observation consistent with the lipolytic action of these hormones. 5. ACTH administration decreased the specific activity of liver total lipid. This effect could arise from the mobilization of free fatty acid and a consequent excessive generation of fatty acyl-Co A in the liver, inbihiting lipid synthesis.

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Inhibition of Lipolysis by Hypoxia in Puppies.* (32310)

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In the neonatal mammal, heat and energy requirements are large, met primarily by aerobic metabolism(1,8). Lipid is an important source of energy for these aerobic pathways in fasting mammals. Therefore, it might be anticipated that diminished mobilization and utilization of free fatty acid (FFA) might handicap the very young mammal.

Many cardiopulmonary disorders of the human neonate may result in acute, severe hypoxia. Heat(2) and energy(14) production are curtailed during acute hypoxia. Although defective oxidation of FFA could, in part, explain these difficulties, the role of FFA mobilization has not been investigated. It is the purpose of this report to describe experiments in puppies which demonstrate that inhibited FFA mobilization is associated with acute hypoxia.

Methods and materials. Ten puppies, 21-35 days of age and weighing 900-1200 g, were the subjects of this study. (Dogs of this age group were chosen because a) the neonate's ability to respond to hypothermia with an increase in oxygen consumption and heat production develops rapidly in the first week of life(5), b) inhibition of these metabolic responses to cold stress by acute hypoxia is not apparent until the responses mature(7), and c) increase in heat production and oxygen consumption occurring with cold stress has been inhibited up to 40 days of age in mammals similar to the dog(2).) The puppies were 3-5 hours post-prandial at the beginning of the study. Each animal was studied in the constant thermal environment that resulted in the lowest oxygen consumption for that specific animal (ambient temperature range 27-29°C). Although very young puppies are less likely to shiver, a neutral thermal environment was sought in order to further minimize this possibility. Innovar®-Vet† was administered in amounts sufficient to produce a degree of sedation that would allow only occasional limb movement, although permitting arousal on painful stimulation. This degree of sedation resulted in an arterial PO_2 of at least 75 mm Hg during the control and recovery periods when room air was breathed. A mixture of 8% oxygen and 92% nitrogen was administered for 45 minutes to produce hypoxia. A plastic hood was sealed over the animal's head and the respired gas mixture was pulled through at

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† Each ml of Innovar®-Vet contains .4 mg fertanyl citrate and 20 mg droperidol.