Effects of adrenalectomy and dexamethasone on hepatic lipid metabolism[1]

Thomas G. Cole,[2] Henry G. Wilcox,[3] and Murray Heimberg[3,4]

Department of Pharmacology,
University of Missouri School of Medicine, Columbia, MO 65212

Abstract The influence of adrenalectomy and dexamethasone on hepatic free fatty acid metabolism was studied in isolated perfused livers from male rats. Adrenalectomy 1 week prior to perfusion did not affect uptake of oleate, output of triglyceride, or rate of ketogenesis compared to sham-operated match-fed controls. Livers from dexamethasone-treated rats (0–2 mg/kg per day for 7 days) removed less oleate from the perfusate, esterified more to total and very low density lipoprotein (VLDL) triglyceride, and oxidized less to ketone bodies, compared to match fed controls; additional studies with [1-^{14}C]oleate confirmed these findings. The output of glucose by livers from dexamethasone-treated rats was also stimulated. The output of VLDL triglyceride was correlated with output of total perfusate triglyceride (r = 0.77, $P < 0.001$). Prior to perfusion, dexamethasone livers accumulated more triglyceride than did control livers. Adrenalectomy did not affect the concentration of plasma free fatty acid or blood ketones and glucose; however, the plasma concentration of triglyceride was elevated. Dexamethasone increased the concentration of plasma free fatty acid, total triglyceride, and VLDL protein, triglyceride, phospholipid, and free cholesterol. No changes were observed in the concentration or composition of plasma low density lipoprotein (LDL) lipids. The concentration of plasma high density lipoprotein (HDL) protein and lipid, and plasma apoA-I, tended to increase; the ratio of total HDL cholesterol to LDL cholesterol was elevated with dexamethasone treatment. These observations suggest that augmented synthesis and secretion of VLDL triglyceride contribute to glucocorticoid-induced hypertriglyceridemia.—**Cole, T. G., H. G. Wilcox, and M. Heimberg**. Effects of adrenalectomy and dexamethasone on hepatic lipid metabolism. *J. Lipid Res.* 1982. **23:** 81–91.

Supplementary key words VLDL • LDL • HDL • lipoprotein lipase

Adrenal glucocorticoids can induce hypertriglyceridemia in experimental animals and man (3–6). Since, in the postabsorptive state, the liver is the primary source of plasma triglycerides secreted in the form of the very low density lipoprotein (VLDL), effects of glucocoticoids on hepatic production and secretion of the VLDL may contribute to the induced hypertriglyceridemia. In addition, hypertriglyceridemia may result from reduced peripheral clearance, and/or reduced activity of lipoprotein lipase (7). The results of previous investigations from this laboratory suggested that the synthesis and secretion of triglyceride by the perfused rat liver were stimulated following administration of cortisone to the animal (8, 9). Increased hepatic secretion of triglyceride has also been reported in the rat and mouse (10, 11) and increased numbers of VLDL-like particles are associated with the hepatic Golgi apparatus following glucocorticoid administration (10, 12, 13). Bagdade et al. (11) investigated the secretion of triglyceride by the liver in vivo and the activity of adipose tissue lipoprotein lipase (LPL) in rats treated with dexamethasone (DEX) under conditions of constant weight gain; they concluded that, although both processes contributed to the hypertriglyceridemia, the reduced utilization of triglyceride due to depressed LPL activity was the more important factor.

To clarify further the effects of adrenal glucocorticoids on hepatic metabolism of free fatty acid (FFA) and on the possible hepatic role in hypertriglyceridemia, the present study was undertaken. For these experiments, the metabolism of FFA by isolated perfused livers from adrenalectomized rats and from rats treated with various amounts of DEX was examined. In addition, the effects of DEX on the lipids and apolipoproteins of the plasma lipoproteins were determined. Preliminary reports of this work have been presented (1, 2).

METHODS

Male Charles River CD Sprague-Dawley rats (180–315 g) in weight-matched groups were used in all ex-

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; FFA, free fatty acid; DEX, dexamethasone; ADX, adrenalectomized.

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[2] This research is taken in part from a dissertation presented to the faculty of the Graduate School of the University of Missouri in partial fulfillment of the requirements for the Ph.D. degree. Present address: Lipid Research Center, Washington University School of Medicine, St. Louis, MO 63110.

[3] Present address: Department of Pharmacology, University of Tennessee Center for the Health Sciences, Memphis, TN 38163.

[4] To whom requests for reprints should be sent.

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periments. The rats were housed individually in temperature and humidity-controlled rooms, in wire-bottom cages, with a light cycle from 0500 to 1700 hr, for at least 4 days prior to experimentation.

Rats under diethyl ether anesthesia were adrenalectomized bilaterally through small paravertebral incisions. The ADX rats were allowed free access to ground Purina Rodent Laboratory Chow #5001 and received 0.9% NaCl to drink instead of tap water, to maintain electrolyte balance. Control rats were subjected to sham adrenalectomy. Since adrenalectomy reduced food intake, the control rats were match-fed by group to the ADX rats and were allowed free access to tap water. After 7 days, livers from both groups of rats were perfused in vitro as described below.

To determine the effect of dose of DEX on hepatic metabolism, various groups of rats were injected subcutaneously daily for 7 days with 0–2.0 mg DEX/kg body weight, diluted with 0.9% NaCl, such that about 0.5 ml was injected. Control rats were injected with equivalent volumes of 0.9% NaCl. In this group of experiments, DEX rats generally consumed less food than normal rats when DEX animals had free access to Purina Chow and water. Control rats for each dose of DEX were therefore match-fed by group to the DEX rats. After 7 days of treatment, livers were removed for perfusion in vitro.

Livers were surgically isolated from the rats under light anesthesia with diethyl ether between 0900 and 1100 hr, approximately 22 hr after the last injection of drug or 7 days after adrenalectomy. The perfusion apparatus has been described previously in detail (8). A 5-ml blood sample was withdrawn from the abdominal aorta into a heparinized syringe just prior to cannulation of the portal vein. The perfusate composition, the oleate–albumin complex, and perfusion techniques were identical to those described by Keyes and Heimberg (14). Prior to introduction of the liver into the perfusion apparatus, a 2-ml sample of perfusate was taken for analysis of glucose. Immediately before the start of the infusion and at successive hourly intervals, 5-ml samples of perfusate were taken for analysis of FFA, triglyceride, glucose, and ketone bodies. After 3 hr, the livers were perfused with 20 ml of ice-cold 0.9% NaCl, cleansed of nonhepatic tissue, blotted, weighed, and then homogenized in ice-cold 0.9% NaCl at a concentration of 1 g liver/6 ml saline. Samples for extraction of lipids were taken immediately.

From the dose–response experiments, it was observed that 0.5 mg DEX/kg caused the greatest stimulation of secretion of triglyceride by the liver. In a third group of experiments the metabolism of [1-^{14}C]oleate was evaluated in two groups of rats. One group of rats was injected with 0.5 mg DEX/kg daily while a control group was injected daily with 0.9% NaCl. Both groups in this experiment consumed similar quantities of food. The livers from these rats were removed after 7 days of treatment and were perfused as described above, except that the initial volume of perfusate was 90 ml; after a 20-min equilibration period, a complex containing 3 g of bovine serum albumin, 1419 μmol oleic acid, and 20 μCi [1-^{14}C]oleic acid per dl was infused at a rate of 11.7 ml/hr (166 μmol oleic acid/hr). $^{14}CO_2$ generated during the experiment was collected in three traps in series, each containing 125 ml of 10% KOH (w/v). The KOH in each trap was changed hourly to insure complete collection of the radioactive CO_2. One hour after the infusion of FFA was started, 38 ml of perfusate was removed for analysis. The experiment was continued for another 3 hr at which time the remainder of the perfusate was removed. The liver was then treated as discussed above.

In certain experiments, livers from normal fed rats were perfused with a medium containing DEX. After the 20-min equilibration period, a pulse dose of DEX in 0.9% NaCl was added to the medium and infusion of an oleate–albumin complex containing DEX was started immediately, to maintain a desired concentration of DEX in the erythrocyte-free perfusate. Assuming no degradation of DEX had occurred during the perfusion, sufficient DEX was added to maintain steady state concentrations of 10^{-9}, 10^{-7}, 10^{-5}, 10^{-4}, and 10^{-3} M throughout the 3-hr perfusion period. Similar volumes of 0.9% NaCl or of the diluent for the DEX were added to the medium in control experiments.

To determine hepatic triglyceride concentrations prior to perfusion, livers from rats treated for 7 days with either 0.5 mg DEX/kg body weight or 0.9% NaCl were isolated as for perfusion but, instead, were homogenized immediately and extracted.

VLDL was isolated from the erythrocyte-free perfusate by ultracentrifugation, without density adjustment, in a 50.2 Spinco rotor at 39,000 rpm for 18 hr at 10°C. The top 3–5 ml were removed and recentrifuged as before through saline containing 0.001 M EDTA. The isolated VLDL was dialyzed against 100 volumes of redistilled water (0.001 M EDTA–0.01% NaN_3, pH 7.0) for 24 hr.

VLDL, LDL, and HDL were isolated from plasma by ultracentrifugation after sequential density adjustments with solid NaBr (15). VLDL was collected at a density of 1.006 g/ml after 18 hr centrifugation at 10°C and 39,000 rpm in a Beckman-Spinco ultracentrifuge using a Type 40 rotor. The isolated VLDL was recentrifuged as before to free the VLDL from contamination with higher density proteins (principally albumin). LDL was collected in the density range 1.006–1.063 g/ml following an 18-hr centrifugation and was not recentrifuged. The isolated HDL was recentrifuged under the same conditions. The lipoproteins were dialyzed at 4°C overnight as above.

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HDL was delipidated with chloroform–methanol 2:1 (v/v) (16), and isoelectric focusing of the apolipoproteins was carried out in 16.0 × 0.7 cm tubes using 50 μg HDL protein, essentially as described by Gidez, Swaney, and Murnane (17). Ampholines (LKB pH 4–6 and 3.5–10) were combined in the ratio of 3:2 to provide a wider pH range, but with an expanded pH 4–6 region. Gels prepared with both ammonium persulfate and riboflavin as the catalyst for polymerization were consistently more uniform. Electrophoresis was carried out for 16 hr at 400 volts and 5°C. The extruded gels were stained at 65°C for 45–60 min in a solution of 0.15% Coomassie Blue R250, 30% methanol, 12.5% trichloracetic acid, and 4% sulfosalicylic acid. Destaining was carried out by diffusion in 10% ethylacetate, 7% ethanol, and 5% acetic acid for 48–72 hr and gels were stored in 5% methanol–7% acetic acid (18). Gels were scanned at 550 nm using a Gilford 2520 gel scanner and peak areas were estimated by triangulation. The areas of the individual polymorphic forms of apoA-I, apoE region (includes apoA-IV) and apoC were combined and are reported as groups.

Lipids were extracted from liver homogenates, arterial plasma, lipoproteins, and erythrocyte-free perfusate by the method of Folch, Lees, and Sloane Stanley (19). Neutral glycerides were separated from phospholipids on 3-g silicic acid columns by elution with chloroform (20). In experiments utilizing [1-^{14}C]oleate, lipids were isolated by thin-layer chromatography (silica gel plates, 250 μm, Analtech, Inc., Newark, DE) using petroleum ether–diethyl ether–glacial acetic acid 84;15:1 (v/v/v) for development. Neutral glycerides, ketone bodies, and glucose were determined as described previously (14). FFA was measured using the extraction mixture and copper reagent of Stajner and Suva (21) and the sodium diethyldithiocarbamate reagent of Duncombe (22). ^{14}C in ketone bodies was measured according to the method of Bieberdorf, Chernick, and Scow (23). Measurement of $^{14}CO_2$ production was described previously (24). Protein content of the lipoproteins was carried out by the method of Lowry et al. (25), using water-saturated, peroxide-free diethyl ether to remove interfering lipids.

All chemicals were reagent grade and solvents (Fisher, St. Louis, MO) were redistilled in glass before use. Oleic acid was obtained from Nu-Check-Prep (Elysian, MN) and was of greater than 99% purity. [1-^{14}C]Oleic acid (sp act 50 mCi/mmol) was purchased from New England Nuclear Corp., Boston, MA). Bovine serum albumin (Fraction V powder, Pentex Biochemicals, Kankakee, IL) was delipidated and purified before use (26). DEX was used in the water-soluble form as the disodium phosphate salt (Dexamethasone Sodium Phosphate for injection, Fellows Medical Division, Chromalloy Pharmaceuticals, Inc., Oak Park, MI).

Statistical analyses were performed using computerized routines (27). Values are given as means ± S.E.M. Statistical significance was determined by the two-tailed Student's "t" test between the treated group and its respective control group (28). In experiments where DEX was added directly to the perfusate, significance was determined by Kendall's tau test (29), to show dose-related activity of the drug, and Dunnett's "t" test (30) for multiple comparisons to a single control. Statistical significance is defined as $P < 0.05$ between treated groups and the respective controls.

RESULTS

In a first series of experiments, the effects of adrenalectomy were studied. Since ADX rats ate less food than intact rats, it was necessary to match-feed sham-operated control rats to ADX rats for the 7 days prior to perfusion of the liver. Under these experimental conditions, weight gain of the rats, liver weight at the time of perfusion, and liver weight/body weight ratios were similar for both groups.

During 3 hr of perfusion, ADX did not affect total hepatic uptake of FFA (53.4 ± 5.2 vs 54.3 ± 3.2 μmol/g liver for control and ADX, respectively), output of triglyceride (1.8 ± 0.1 vs 1.7 ± 0.2 μmol/g, for control and ADX, respectively), or rates of ketogenesis (125.5 ± 41.6 vs 122.1 ± 27.5 μmol/g, for control and ADX, respectively). However, output of glucose by livers from ADX rats was increased under these experimental conditions (30.9 ± 5.8 vs 63.2 ± 5.5 μmol/g, $P < 0.005$). At the termination of perfusion, the concentration of triglyceride in livers from control rats (3.4 ± 0.2 μmol/g) was the same as in the ADX group (3.2 ± 0.3). During the experiments, flow rates of perfusate through the livers of both groups of rats were not different. Livers from sham-operated control rats, however, secreted more bile than did livers from ADX rats (0.22 ± 0.01 vs 0.15 ± 0.02 ml/g, $P < 0.05$). The concentration of FFA in the cell-free perfusate was equal for both groups (0.28 ± 0.10 μmol/ml). Adrenalectomy did not affect concentrations of plasma FFA or blood glucose or ketone bodies, but plasma concentration of triglyceride was higher in the ADX rats than in match-fed controls (0.57 ± 0.13 μmol/ml plasma vs 0.25 ± 0.04, $P < 0.05$).

In a second series of experiments, the effects of treatment with dexamethasone were investigated. The effects of DEX on food intake and growth characteristics of the rats are shown in **Fig. 1.** DEX at doses of 0.5 mg/kg body weight or greater caused a dose-dependent reduction of weekly food intake from 196 g for normal control rats to approximately 140 g (Fig. 1, A). Treatment with DEX upset the normal growth pattern of the rats (Fig. 1, B). Untreated control rats given food ad libitum gained approximately 18% of their starting body weight during the 7-day treatment period. Rats treated with DEX remained at constant weight at low doses of drug and lost

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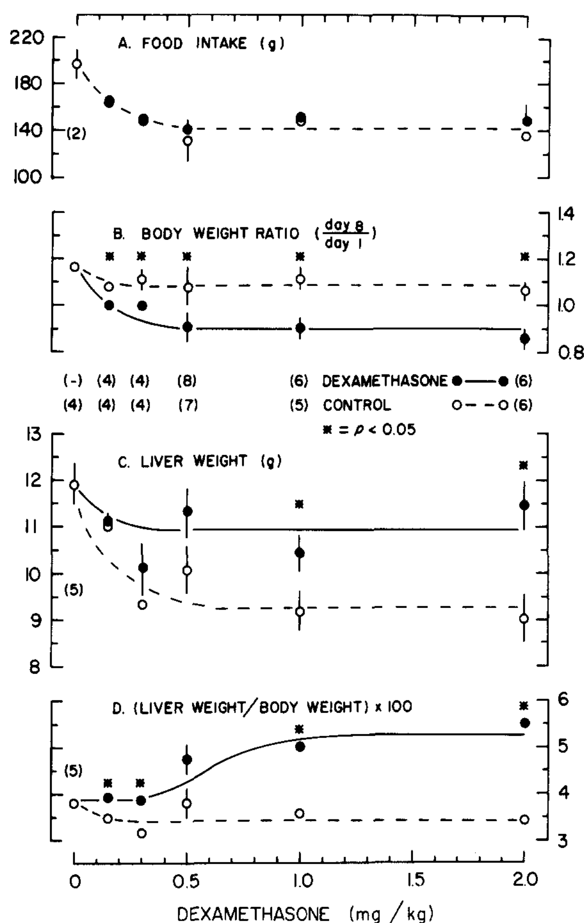

Fig. 1. The effects of dexamethasone on food consumption and growth characteristics. Rats were injected daily with the indicated amounts of DEX for 7 days, while control rats received 0.9% NaCl. Controls were match-fed to the respective DEX rats. A. Control rats were maintained on a feeding schedule following the DEX rats by one day. B. The body weight ratio is that weight at the time of perfusion (day 8) divided by the body weight at the beginning of the treatment period (day 1). C. Liver weights were measured at the end of the perfusion. D. Ratio of liver weight to that of body weight at the time perfusion is presented. Data are means ± S.E.M. Statistical significance ($P < 0.05$) between the DEX and the control group is indicated by an asterisk. The number of observations is indicated in parentheses.

weight at higher doses. At a dose of 2 mg/kg, the animals lost 12% of their initial body weight. In contrast, each of match-fed control rats gained weight, although the gains were smaller for groups receiving less food. In the experiments with [1-^{14}C]oleate, DEX rats lost approximately 5% of their pretreatment weight after 7 days while the control rats gained 16%. Although the DEX rats lost weight during the treatment period, the livers from these rats at the time of perfusion were generally heavier than those of the respective control animals (Fig. 1, C). The livers from DEX rats at the 1 and 2 mg/kg doses were about 20% heavier than those of the control rats. Since treatment with DEX decreased body weight and increased liver weight, the ratio of liver weight to body weight of these rats was larger than that of the respective control rats at doses as low as 0.15 mg/kg (Fig. 1, D).

The uptake of FFA and the output of triglyceride, ketone bodies, and glucose by perfused livers from normal rats and from rats treated with 0.5 mg DEX/kg are shown in **Fig. 2.** In each case, the measured parameter was linear over the 3-hr period. Uptake of FFA was depressed in livers from DEX rats. The FFA taken up by the liver seemed to be preferentially directed to synthesis of triglyceride by DEX treatment, as suggested by the increase in the output of triglyceride by these livers. Moreover, the output of ketone bodies by livers from DEX rats was less than half that of the control rats. The output of glucose by livers from DEX rats was double that of livers from control rats. The effects of various doses of DEX on the uptake of FFA and the output of triglycerides, ketone bodies, and glucose by the perfused liver are shown in **Fig. 3.** DEX decreased uptake of FFA

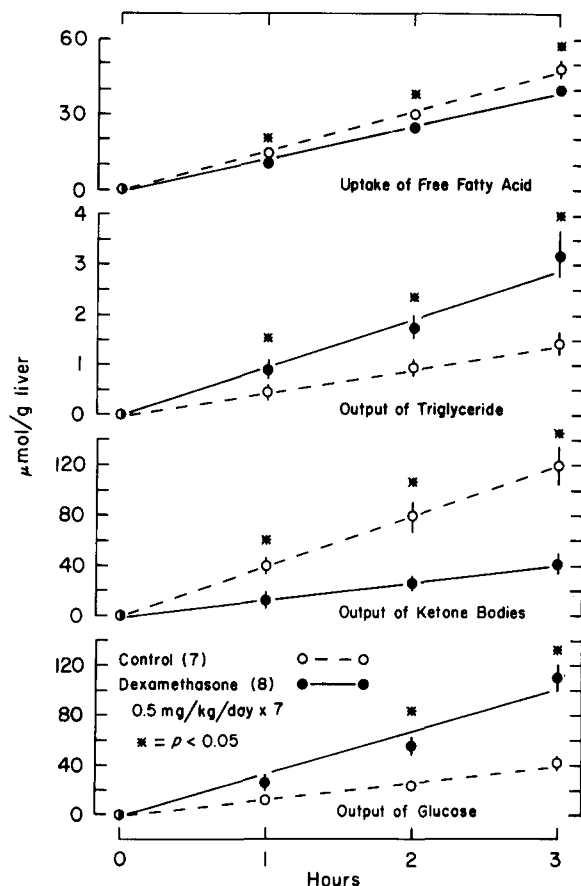

Fig. 2. Effects of treatment with dexamethasone on hepatic lipid metabolism. DEX rats were injected daily with 0.5 mg DEX/kg for 7 days, while control rats received 0.9% NaCl. Control rats were match-fed to the DEX rats. Livers were removed and perfused as described in Methods. Data are means ± S.E.M. Statistical significance ($P < 0.05$) between the DEX and the control groups is indicated by an asterisk.

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at doses of 0.5 mg/kg and higher, when expressed as μmol FFA/g liver. When the total mass of the liver was considered, this difference was observed at the 0.5 mg/kg dose (449.2 ± 2.9 μmol/liver per 3 hr for DEX vs 467.3 ± 3.6 for controls, $P < 0.002$) and the 2 mg/kg dose (453.8 ± 3.4 μmol/liver per 3 hr for DEX, vs 473.2 ± 4.0 for controls, $P < 0.005$). These differences, though statistically significant, are small and represent a variation of about 4% of the total FFA taken up by the whole liver during the experiment. However, based on the uptake/g liver, DEX depressed the uptake of FFA 16.8% ($P < 0.04$), 13.3% ($P < 0.05$), and 25.2% ($P < 0.001$) at the 0.5, 1.0, and 2.0 mg/kg doses, respectively, relative to the appropriate controls. In the [1-^{14}C]oleate experiments, there were no significant differences in uptake of oleate by the whole liver; on a g liver basis, uptake of oleate from the perfusate was 28.7 ± 1.2 μmol FFA/g per 3 hr for the DEX group and 31.0 ± 1.4 for controls. The specific activities of the oleate taken up by both groups of livers were equal, as were the concentrations of oleate in the cell-free perfusate.

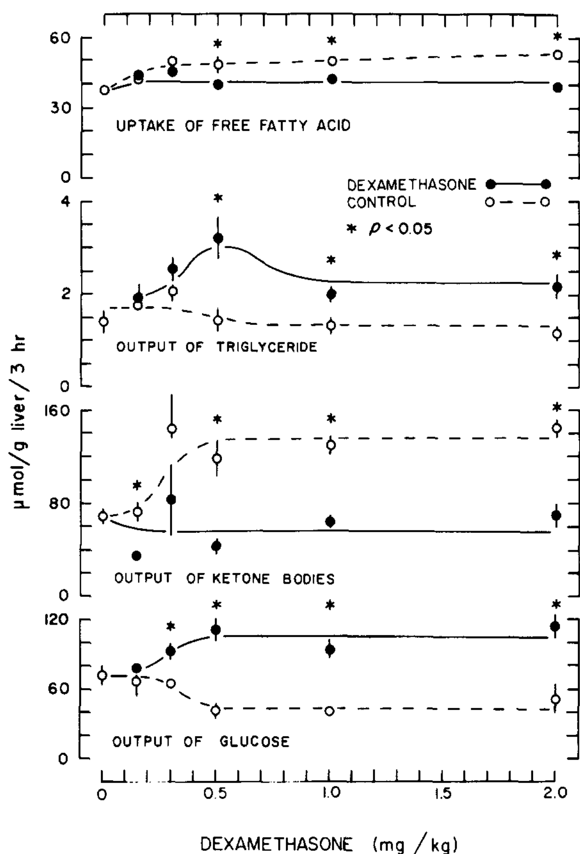

Fig. 3. The effects of various doses of dexamethasone on hepatic lipid metabolism. DEX rats were injected daily with the indicated amounts of DEX for 7 days, while control rats received 0.9% NaCl. Control rats were match-fed to the respective DEX rats. Livers were removed and perfused as described in Methods. Data are means ± S.E.M. of cumulative uptake or output during the 3-hr experiment. Statistical significance ($P < 0.05$) between the DEX and the respective control groups is indicated by an asterisk. The number of observations at each dose is indicated in Fig. 1.

The output of triglyceride by livers from DEX rats was stimulated at doses of 0.5 mg/kg and higher. A maximal effect of DEX on the output of triglyceride was seen at the 0.5 mg/kg dose (Fig. 3). The hepatic output of total perfusate triglyceride and VLDL triglyceride was also measured in experiments using [1-^{14}C]oleate; in those experiments, DEX stimulated the output of total perfusate triglyceride per g liver and per whole liver (data not shown). Output of VLDL triglyceride was stimulated when expressed per liver ($P < 0.025$) but was of borderline significance when expressed per g liver. The specific activities of the secreted triglyceride were equal. The output of VLDL triglyceride was correlated with the output of total triglyceride by the livers ($r = 0.77$, $P < 0.001$).

The output of ketone bodies by the liver was quite sensitive to the effects of DEX. Even at the dose of 0.15 mg/kg, ketogenesis was reduced (Fig. 3). In separate experiments with [1-^{14}C]oleate, rates of ketogenesis were 88 ± 10 μmol acetone/g liver per 3 hr and 118 ± 13 ($P < 0.1$) for DEX and control groups, respectively. The specific activities of secreted ketone bodies were equal in both groups. There were no differences in the production of $^{14}CO_2$ per g liver or per whole liver (2.7 ± 0.2 × 10^6 dpm/liver per 3 hr and 2.5 ± 0.2 × 10^6 for control and DEX groups, respectively).

The output of glucose by livers from rats treated with DEX was increased over that of livers from control animals at doses of 0.3 mg/kg or higher (Fig. 3). The output of glucose by livers from control rats match-fed to rats receiving 0.5 mg/kg DEX was reduced approximately 40% compared to the output of ad libitum fed normal untreated rats ($P < 0.05$).

The observed differences between control and DEX-treated groups were actually diminished due to the increase in liver weight and the slightly reduced uptake of FFA by livers from DEX-treated rats. For these reasons, data on output of total triglyceride and ketone bodies were recalculated per μmol FFA taken up. When the data are expressed in this fashion, these differences are emphasized (**Fig. 4**). Even though less FFA was taken up by livers from DEX-treated rats, more triglyceride was synthesized and secreted into the perfusate. Similar results were obtained on measurement of VLDL triglyceride.

The hepatic concentration of triglyceride at the termination of the dose-response experiment is depicted in **Fig. 5**. The concentration of triglyceride in livers from DEX rats exceeded that in livers from control animals. Since livers from DEX rats were larger than those from the control rats, the total triglyceride content in these livers was also higher than in livers from control rats.

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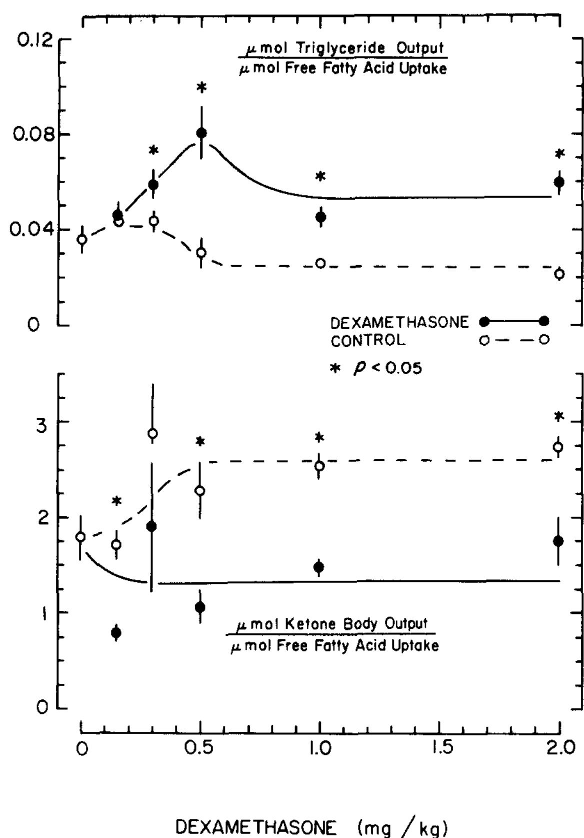

Fig. 4. The effects of dexamethasone on the output of triglyceride and ketone bodies relative to the uptake of free fatty acids. Data are means ± S.E.M. Statistical significance ($P < 0.05$) between the DEX and the respective control group is indicated by an asterisk. The experimental conditions, treatment of control and experimental groups, and number of observations are indicated in the Methods section and in the legend to Fig. 1.

In other experiments with [1-^{14}C]oleate, the concentration of hepatic triglyceride in livers from DEX-treated rats (4.6 ± 0.8 μmol triglyceride/g liver) exceeded that of the control group (2.5 ± 0.4). In separate experiments, nonperfused livers from rats treated with 0.5 mg DEX/kg (n = 7) contained 9.3 ± 1.5 μmol triglyceride/g liver while nonperfused livers from match-fed controls treated with 0.9% NaCl contained 4.3 ± 0.5 μmol triglyceride/g (n = 4, $P < 0.05$). These differences in hepatic triglyceride concentration, therefore, were due entirely to accumulation of triglyceride in vivo and not to accumulation under the conditions of these perfusion experiments. With the relatively small quantity of oleate infused, accumulation would not be expected (31). A comparison of the specific activities of the secreted perfusate and VLDL and hepatic triglyceride revealed no differences between DEX and control groups. However, the specific activity of the secreted triglyceride in each case was greater than that of the hepatic triglyceride, indicating that the total accumulated hepatic triglyceride could not have been the sole source of the secreted triglyceride; rather, the VLDL triglyceride is derived from a smaller metabolic pool that is not in rapid equilibrium with total hepatic stores (32).

In other experiments, a possible immediate rapid action of DEX directly on livers from normal fed rats was examined (**Fig. 6**). DEX did not affect uptake of FFA, or output of triglyceride, ketones, and glucose by the perfused liver at concentrations of 10^{-9} to 10^{-5} M DEX. At concentrations of 10^{-4} and 10^{-3} M, output of glucose was stimulated and triglyceride accumulated in the livers. Obviously, these concentrations of DEX are well above pharmacological concentrations in vivo; the effects observed may result from an hepatotoxic action of these very high concentrations of DEX, characterized by an inhibition of hepatic secretion of triglyceride.

The plasma concentrations of FFA and triglyceride, blood ketones and glucose in response to treatment with DEX are shown in **Fig. 7.** Treatment with DEX increased the concentration of each blood component measured. Increases in the plasma concentration of triglyceride were observed at doses as low as 0.15 mg/kg. Concentrations of ketone bodies and glucose in whole blood of DEX rats were increased above respective controls at a dose of 0.3 mg/kg and higher.

The protein and lipid composition of the plasma and

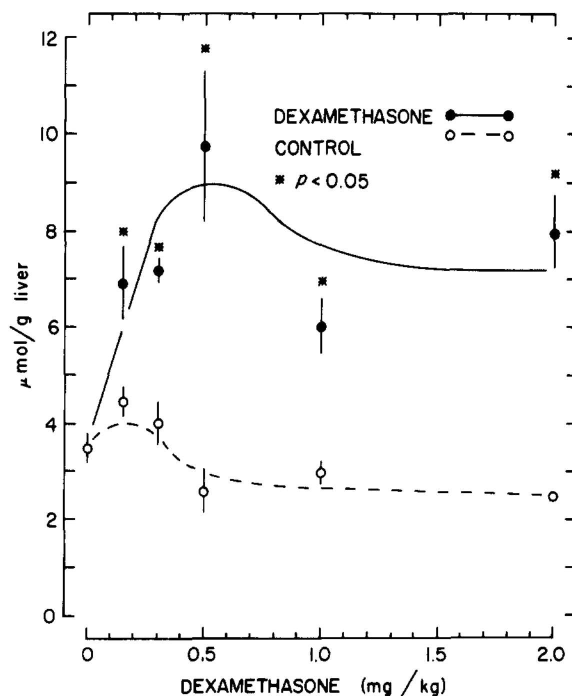

Fig. 5. The effects of dexamethasone on the concentration of hepatic triglyceride. Hepatic triglyceride was estimated at the termination of the perfusion experiments. Data are means ± S.E.M. Statistical significance ($P < 0.05$) between the DEX and the respective control group is indicated by an asterisk. The experimental conditions, treatment of control and experimental groups, and number of observations are indicated in the Methods section and in the legend to Fig. 1.

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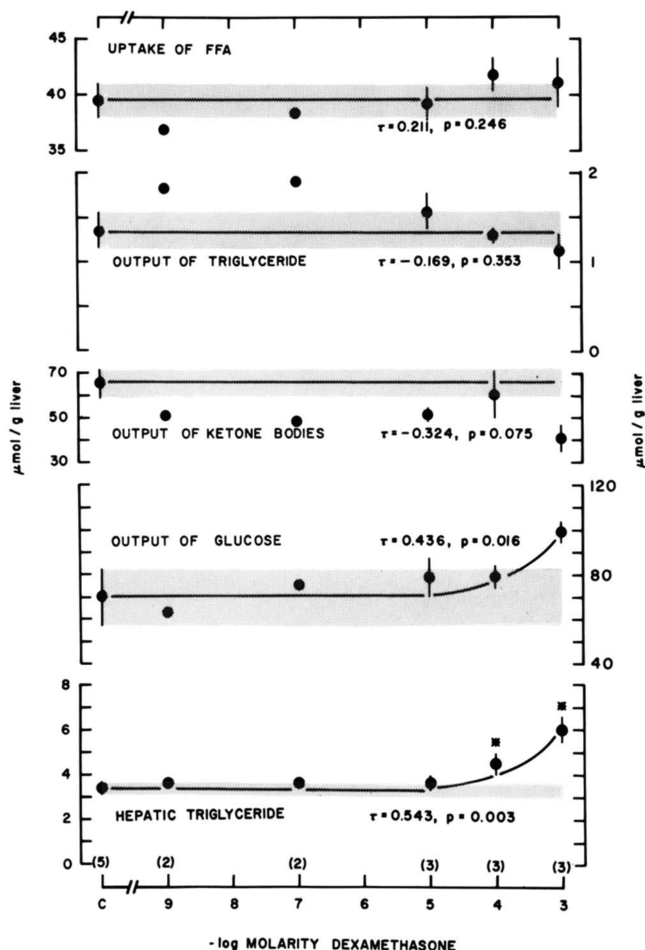

Fig. 6. Lack of effect of dexamethasone added to the medium perfusing the liver in vitro. Livers from normal fed rats were perfused as described in Methods. Data are means ± S.E.M. The shaded areas indicate control values. The Kendall's Tau was used to test for the correlation between the concentration of DEX and the effect on the measured parameter. The magnitude of τ indicates the strength of the correlation and the sign shows direct or inverse relationship. The τ values indicate whether the effect of the drug was dose related: values approaching 1.0 show a strong dose dependency. Dunnett's "t" test was used to compare values at each dose to the control value; significance at $P < 0.05$ is indicated by an asterisk. The number of observations at each concentration is shown in parentheses.

perfusate VLDL are compared in **Table 1.** Plasma from DEX-treated rats contained more VLDL triglyceride, phospholipid, cholesterol, and protein than did the plasma from control rats; these differences were not as dramatic in the perfusate VLDL, although DEX group values tended to be the largest in each case. The ratios phospholipid/triglyceride, cholesterol/triglyceride, and cholesteryl esters/triglyceride of plasma VLDL appeared to be similar in control and DEX groups. These same ratios tended to be smaller for VLDL secreted by perfused livers from DEX-treated rats compared to perfused controls, but the difference did not reach statistical significance. On the basis of previous work from this laboratory (26), these ratios would have been expected to become smaller with DEX treatment, since more VLDL was secreted and a larger particle might have been predicted. The DEX-stimulated output of VLDL may have been insufficient, however, to change the composition of the particle significantly under our experimental conditions.

No differences were observed in the plasma LDL protein, phospholipid, or cholesterol between DEX and control groups. The concentration of plasma HDL lipid and protein tended to be elevated following DEX treatment (**Table 2**). Furthermore, the ratio of total HDL cholesterol to total LDL cholesterol was larger in the DEX-treated rats than in control animals (4.1 ± 0.5 vs 2.0 ± 0.3, $P < 0.02$). The distribution of the apolipoproteins of the HDL isolated by isoelectric focusing are shown in **Table 3** and **Fig. 8.** The distribution of HDL apoproteins in control and DEX-treated animals could not be differentiated from one another with the small number of animals examined, although the apoE region tended to increase and apoC decrease with DEX treatment. Clearly, this point must be investigated carefully. In preliminary analyses, it was observed that the total apoA-I level was increased in the plasma of DEX-treated an-

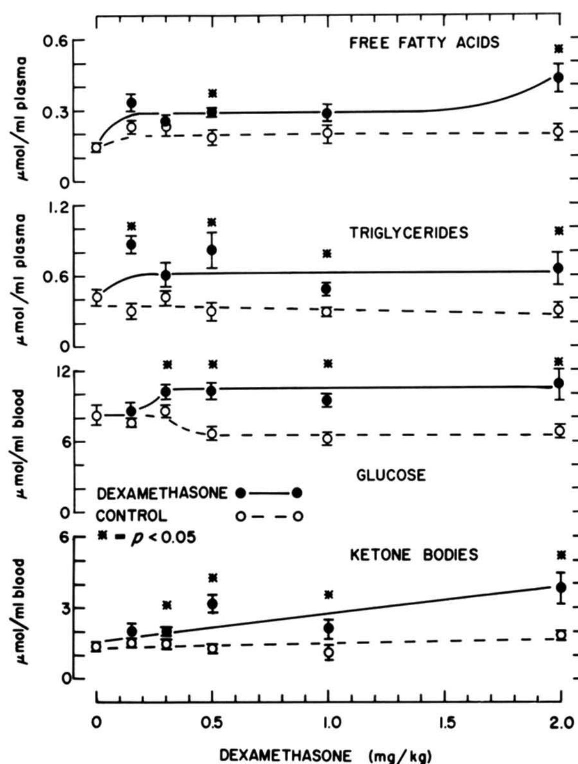

Fig. 7. The effects of dexamethasone on the concentration of plasma FFA and triglyceride and blood ketones and glucose. Blood samples were taken from the abdominal aorta at the time livers were removed for perfusion. Data are means ± S.E.M. Statistical significance ($P < 0.05$) between the DEX rats and respective control rats is indicated by an asterisk. The number of observations at each dose is as in Fig. 1.

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TABLE 1. Effects of dexamethasone on protein and lipid composition of perfusate and plasma VLDL

Class	Perfusate		Plasma	
	Control (4)	DEX (6)	Control (4)	DEX (5)
Protein	3.0 ± 0.1	4.2 ± 0.3[a]	2.9 ± 0.7	11.8 ± 2.4[a]
Triglyceride	20.4 ± 0.9	36.0 ± 4.1[b]	20.0 ± 5.3	102.6 ± 28.9[a]
Phospholipid	6.8 ± 0.9	9.4 ± 1.1	4.1 ± 1.1	22.4 ± 3.7[b]
Cholesterol	4.3 ± 0.7	5.2 ± 0.6	2.5 ± 0.3	13.4 ± 3.3[a]
Cholesteryl esters	1.0 ± 0.1	1.0 ± 0.3	3.0 ± 1.5	5.2 ± 1.7
Total cholesterol	5.3 ± 0.8	6.2 ± 0.7	5.5 ± 1.6	18.5 ± 4.8[a]
PL/TG	0.34 ± 0.06	0.27 ± 0.03	0.21 ± 0.02	0.24 ± 0.03
C/TG	0.22 ± 0.05	0.15 ± 0.02	0.15 ± 0.03	0.13 ± 0.01
CE/TG	0.05 ± 0.01	0.03 ± 0.01	0.12 ± 0.04	0.05 ± 0.04

[a] $P < 0.05$.
[b] $P < 0.01$.
Treatment of the animals and experimental conditions are explained in the legend to Fig. 4 and in the Methods section. Data are means ± SEM and are expressed as μmol/dl, except protein which is expressed as mg/dl. The number of observations in each group is shown in parentheses. Abbreviations: PL, phospholipid; C, cholesterol; TG, triglyceride; CE, cholesteryl esters. Significance of differences between control and DEX groups is indicated.

imals (54, 54 and 35, 33 mg apoA-I/dl plasma for DEX and control rats, respectively). The observation of a tendency for HDL protein and lipid components, and apoA-I, to be increased in plasma of DEX animals suggests an increase in total serum HDL resulting from the steroid treatment. Whether the apparent increase in HDL with DEX treatment results from increased formation or reduced catabolism of the HDL remains to be determined.

DISCUSSION

The lack of effect of adrenalectomy on the secretion of triglyceride and on ketogenesis by the perfused liver differs from earlier work reported from this laboratory (9). In those earlier studies, adrenalectomy was reported to decrease the hepatic output of triglyceride and to stimulate ketogenesis. Those experiments were carried out with perfused livers obtained from ADX and normal rats fed ad libitum. In retrospect, this comparison was inappropriate, because ADX rats consumed less food than did intact rats, and it is now well known that reduction in caloric intake itself will stimulate ketogenesis and reduce output of triglyceride (33). When this error was corrected by pair-feeding, as in the present work, no differences were observed in output of triglyceride or ketogenesis by the liver.

The results of the experiments reported here demonstrate clearly that the rat liver was stimulated by DEX to synthesize and secrete increased quantities of VLDL triglyceride. Even though the uptake of FFA by the livers from DEX rats was reduced slightly, the output of triglyceride by these livers was increased, especially when considered per μmol of FFA taken up. Concurrent with this effect, was the increased concentration of triglyceride in the livers from rats treated with DEX. Since under the conditions of these experiments livers did not gain or lose triglyceride during perfusion, and since the specific activity of the secreted triglyceride exceeded that of the hepatic triglyceride, the secretion of triglyceride must reflect the rate of synthesis and not the mobilization of previously accumulated triglyceride. Also, in agreement with previously published data, it is clear that only a fraction of the hepatic triglyceride is in the metabolic

TABLE 2. Effects of dexamethasone on protein and lipid composition of plasma HDL

Group	Protein	Cholesterol	Cholesteryl Esters	Phospholipid	Triglyceride	Total Lipids	Total
Control (3)	51.3 ± 8.9	2.3 ± 0.5	46.1 ± 2.5	21.4 ± 2.5	2.0 ± 1.2	71.8 ± 4.9	123.1 ± 13.2
DEX (5)	87.5 ± 11.4	3.3 ± 0.7	45.3 ± 4.0	37.0 ± 1.9	2.8 ± 1.6	88.3 ± 4.6	175.8 ± 14.3
P Value	<0.07			<0.05		<0.06	<0.05

Treatment of the animals and experimental conditions are explained in the legend to Fig. 4, and in the Methods section. Data are means ± SEM and are expressed as mg/dl. Number of observations is given in parentheses. The mass of cholesteryl esters, phospholipids, and triglycerides was calculated using molecular weights of 651, 775, and 890, respectively.

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TABLE 3. Effects of dexamethasone on apoprotein composition of rat plasma HDL

Apoprotein Class	Percent of Total Apoprotein: Control (2)	DEX (7)
Apo A-I	78.6, 70.6	77.8 ± 1.0
Apo E region	19.4, 23.7	14.2 ± 1.7
Apo C	2.0, 5.9	8.0 ± 1.1
Apo C-II / Apo C-III	0.67, 0.51	0.70 ± 0.06

Treatment of the animals and experimental conditions are explained in the legend to Fig. 4, and in the Methods section. Data are means ± SEM, and are expressed as percentage of total HDL apoprotein. The number of observations in each group is given in parentheses. Apoproteins were separated by isoelectric focusing, stained, and quantitated by densitometry.

pool from which perfusate triglyceride is derived. Based on these data, the rate of synthesis and the release of triglyceride are both increased by treatment of the donor rats with DEX; this effect is due to the steroid and not attributed to the feeding or growth patterns of the rats. The oxidation of FFA to ketone bodies was depressed by DEX. The reciprocal relationship between the hepatic synthesis of triglyceride and ketogenesis has been discussed previously (34). The complete oxidation of FFA to CO_2 was unaffected by treatment with DEX.

The exact metabolic steps at which FFA is partitioned between the pathways of esterification and oxidation are not known. McGarry, Takabayashi, and Foster (35) and McGarry (36) have proposed that malonyl CoA inhibits mitochondrial oxidation of FFA by inhibition of carnitine acyltransferase I, thereby increasing the availability of FFA for esterification. The activity of acetyl CoA carboxylase, the enzyme which synthesizes malonyl CoA, is stimulated by DEX (37) and this effect may partially account for the enhanced synthesis of triglyceride and the depression of ketogenesis. However, since ketogenesis by livers from fed animals is already at a minimal rate, depression of this rate by DEX would not be expected to provide significant FFA for esterification to account for the increased secretion of triglyceride by livers from DEX-treated rats. Stimulation of the esterification pathway itself may contribute to the depression of ketogenesis. Phosphatidate phosphohydrolase, an important regulatory enzyme of triglyceride synthesis, has been shown to be activated by glucocorticoids in vivo and in vitro (38–40). Ca^{2+} may also be involved in the effect of DEX on triglyceride synthesis. It is conceivable that glucocorticoid-induced hypocalcemia (41) may affect rates of glycerolipid synthesis, in that an inverse relationship between hepatic microsomal Ca^{2+} uptake and synthesis of glycerolipids has been observed (42, 43).

DEX stimulated the hepatic output of glucose. The glucose released by the liver in these experiments is considered to arise from glycogenolysis of preformed glycogen, since no gluconeogenic substrate was added to the perfusate. The enhanced deposition of hepatic glycogen in response to glucocorticoids is well known (44). The livers from DEX-treated rats would be expected to contain more glycogen than livers from control rats and, therefore, more glycogen would be available for release into the perfusate.

Hypertriglyceridemia was evident after 7 days of treatment with DEX. The regulation of the concentration of triglyceride is a function of both entry and removal of triglyceride from the plasma compartment. Apparently, the stimulated release of triglyceride by the liver contributed, at least in part, to the hypertriglyceridemia. The activity of adipose tissue LPL has been shown to be depressed in rats after treatment with glucocorticoids (11, 37) and, therefore, may contribute to hypertriglyceridemia, particularly if a minimal required threshold level for LPL activity has been crossed. It is of passing interest that the concentration of perfusate VLDL triglyceride in the DEX group was approximately twice that of the control (Table 1), while plasma VLDL triglyceride of the DEX-treated group was about five times

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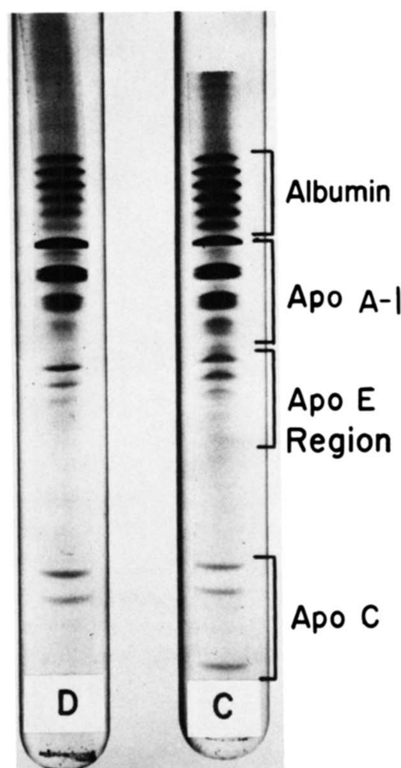

Fig. 8. Effect of dexamethasone on plasma HDL apoproteins. Apoproteins were isolated as described in Methods. The apo E region includes apo A-IV. Treatment of the animals and experimental conditions are explained in the Methods section. C indicates control group and D, the dexamethasone treated group.

that of the control. It is conceivable, therefore, that VLDL triglyceride is removed from the plasma at a slower rate in DEX-treated rats than in control groups (11), in addition to being secreted at an increased rate.

We observed no differences in the amount or composition of the LDL due to treatment with DEX, in contrast to the observations of Bagdade et al. (11) that DEX decreased the concentration of plasma LDL cholesterol. The reason for this difference is not obvious. The ratio of HDL cholesterol to LDL cholesterol was increased by DEX. Moreover, these data suggest that treatment with DEX increased the plasma concentration of HDL. This particular observation needs to be studied more carefully, specifically to determine whether glucocorticoids stimulate hepatic production of HDL.

The data reported in this study with DEX confirm and extend previous observations from this laboratory (8, 9) and others (10–13). Under conditions of availability of constant concentrations of exogeneous FFA, livers from corticosteroid-treated rats will direct more of FFA into formation and secretion of VLDL triglyceride and less to ketogenesis. The exact regulatory mechanisms are not defined by these experiments, but it may, in part, be control of glycerolipid synthesis. The stimulated output of triglyceride by the liver in response to treatment with glucocorticoids undoubtedly is a contributing factor to steroid-induced hypertriglyceridemia.

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