Divergent effects of d-norgestrel on the metabolism of rat very low density and low density apolipoprotein B

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Abstract Rats treated with the contraceptive steroid d-norgestrel have lower plasma very low density lipoprotein (VLDL)-triglycerides and higher low density lipoprotein (LDL)-cholesterol than controls. To explain these results, the kinetics of VLDL and LDL turnover were studied by injecting ^{125}I-labeled rat-VLDL and ^{131}I-labeled rat-LDL simultaneously into rats treated with a small dose of d-norgestrel (4 μg per day per kg body weight$^{0.75}$ for 18 days, n = 22) and their untreated controls (n = 22). VLDL- and LDL-apoB specific activity-time curves obtained over 50 hr best conformed to a three-pool model. VLDL-apoB clearance expressed as irreversible catabolic rate (k_{01}) was markedly enhanced in the treated versus control rats (0.57 vs. 0.34 pools hr^{-1}), leading to a marked reduction in VLDL-apoB pool size (270 vs. 420 μg). However, VLDL-apoB production rates were similar in the two groups (160 vs. 140 μg/hr, respectively). The ^{125}I-labeled apoB specific activity-time curve derived from the catabolism of ^{125}I-labeled VLDL-apoB also showed enhanced clearance in d-norgestrel-treated rats. ^{125}I-Labeled IDL-apoB and ^{125}I-labeled LDL-apoB specific activity-time curves failed to intersect the VLDL-apoB curve at maximal heights, suggesting input of intermediate density lipoprotein (IDL) and LDL independent of VLDL catabolism in both groups. however, the extent of independent LDL-apoB production was similar in both groups. Clearance of ^{131}I-labeled LDL-apoB following injection of ^{131}I-labeled rat-LDL was delayed in the d-norgestrel-treated versus control rats. The irreversible catabolic rate of ^{131}I-labeled LDL-apoB was markedly slower with d-norgestrel (0.15 vs. 0.29 pools hr^{-1}), leading to an increased pool size (840 vs. 410 μg) of LDL-apoB. However, production rates of LDL-apoB were similar in d-norgestrel-treated and control rats (130 vs. 120 μg/hr). These divergent effects of d-norgestrel on apoprotein B metabolism in VLDL, IDL, and LDL in the rat are consistent with and explain both the triglyceride-lowering and cholesterol-elevating effects of d-norgestrel in this species. —**Khokha, R., M. W. Huff, and B. M. Wolfe.** Divergent effects of d-norgestrel on the metabolism of rat very low density and low density apolipoprotein B. *J. Lipid Res.* 1986. **27:** 699–705.

Supplementary key words B apoprotein • kinetics • very low density lipoproteins • low density lipoproteins • d-norgestrel

d-Norgestrel, a potent progestin, is widely used in oral contraceptive formulations in combination with estrogens (1). dl-Norgestrel (active isomer d-norgestrel) administered alone (2) or in combination with estrogens (3, 4) has been reported to lower plasma triglyceride levels, although some investigators have failed to observe this effect (5, 6). Studies in our laboratory show that d-norgestrel fed to female rats significantly lowers both plasma total and VLDL triglyceride levels. In contrast, d-norgestrel significantly elevates plasma total and LDL-cholesterol levels (7).

The hypotriglyceridemic effect of d-norgestrel could result from either reduced synthesis and secretion of VLDL triglycerides from the liver or enhanced clearance of VLDL particles from the plasma. On the other hand, the cholesterol-elevating effect of d-norgestrel could result from *a*) higher input of LDL via catabolism of VLDL (8), *b*) greater direct synthesis of LDL independent of VLDL catabolism (9), or *c*) impaired LDL receptor function (10).

In order to investigate the mechanisms underlying alterations in lipid levels induced by d-norgestrel, we examined the apoB kinetics of VLDL, IDL, and LDL after injecting isotopically labeled homologous lipoproteins (obtained from donor rats) into d-norgestrel-treated and control rats. Studies of apoB provide insight into apolipoprotein turnover because apoB stays with the VLDL particle during its sequential delipidation leading to IDL and finally ending with the formation of LDL (9). The effect of d-norgestrel on the kinetics of apoB metabolism and on the interrelationships with other apoB-containing lipoproteins is presented in this report.

METHODS AND MATERIALS

Preparation of animals

Female Sprague Dawley rats (250 g), 22 in each group (control and experimental) were individually caged in an

Abbreviations: LDL, low density lipoprotein(s); IDL, intermediate density lipoprotein(s); VLDL, very low density lipoprotein(s); apoB, apolipoprotein B.

animal room illuminated from 8:00 AM to 8:00 PM. Each rat in the experimental group received 4 μg of d-norgestrel/day · kg body $wt^{0.75}$ (average dose 1.4 μg/day) for 18 days along with rat chow. This dose is comparable to that used for human contraceptive purposes (2–6). The experimental diet was prepared by adding the d-norgestrel dissolved in absolute ethanol to rat chow and then the ethanol was allowed to evaporate. The control diet was treated with an equal amount of ethanol which was similarly removed by evaporation. Experimental and placebo portions of the diet were first fed to each rat between 6:00 and 11:00 PM, and the rats were observed until it was completely consumed. Thereafter, water and rat chow were available ad libitum. Potassium iodide (0.16 g/l) was added to the drinking water for 2 days prior to and after the administration of labeled lipoproteins.

Preparation of labeled lipoproteins

Lipoproteins for injection were obtained from female Sprague Dawley donor rats (250 g, n ≈ 44) that had been maintained on a synthetic diet containing 5% lard for 10 days prior to use. This was done to enhance the labeling of rat VLDL-protein by decreasing the polyunsaturated: saturated fatty acid ratio in the plasma lipids, leading to less incorporation of the label in the lipid moiety of the lipoproteins (11). However, a fat-free diet was fed for 12 hr prior to blood sampling of donor (as well as recipient) rats in order to eliminate chylomicrons from plasma.

Blood was collected by aortic puncture from the donor rats and pooled in tubes containing EDTA (1 mg/ml). The plasma was separated and subjected to ultracentrifugation in a Beckman 60 Ti rotor (16 hr, 40,000 rpm, 15°C) to isolate VLDL (d < 1.006 g/ml). The VLDL was washed twice through sterile saline in a 50 Ti rotor (16 hr, 40,000 rpm, 15°C). The LDL (d 1.02–1.05 g/ml) was isolated from the plasma infranatant remaining after separation of VLDL in a 60 Ti rotor (24 hr, 40,000 rpm, 15°C) and was washed once, through sterile saline solution of d 1.05 g/ml in a 50 Ti rotor (24 hr, 40,000 rpm, 15°C). The narrow density range (d 1.02–1.05 g/ml) was chosen to obtain an LDL fraction free of any contamination by VLDL or HDL. The VLDL and LDL were radiolabeled by a modification (12) of the McFarlane method (13). Briefly, the VLDL and LDL obtained were equilibrated to 0.4 M glycine–NaOH buffer, pH 10, and radioiodinated with ^{125}I and ^{131}I (Amersham, Oakville, Ontario), respectively. Free iodine was then removed by passage through a small Sephadex G-50 column equilibrated with saline. Isotopically labeled lipoproteins were sterilized by filtration through a 0.45-micron Millipore filter. Ninety-three percent of the radioisotope ^{125}I was bound to VLDL protein, 4% was associated with lipid, and 3% remained unbound; VLDL-apolipoprotein B (determined by isopropanol precipitation, ref. 14) accounted for 29% of the ^{125}I bound to VLDL protein. Ninety-four percent of the radioisotope ^{131}I was bound to LDL protein, less than 5% was associated with lipid, and 1% remained unbound; LDL-apolipoprotein B accounted for 73% of the ^{131}I bound to LDL protein. LDL-apoB accounted for 61% of the mass of LDL protein (d 1.02–1.05 g/ml).

Lipoprotein kinetic study

Rats were placed under mild ether anesthesia for the experiment. Each recipient rat received simultaneously 8 μCi of ^{125}I-labeled VLDL and 2 μCi of ^{131}I-labeled LDL in 0.5 ml of normal saline injected intravenously via a foot vein under mild ether anesthesia. At 5 min postinjection, a 50–70 μl sample of blood was collected from the tail vein into a heparinized microhematocrit tube in order to monitor the amount of radioactivity injected. At each subsequent time point (0.17, 0.33, 0.5, 1, 2, 4, 6, 12, 18, 24, and 50 hr), two rats from each of the d-norgestrel and control groups were anesthetized with ether and 9–10 ml of blood was drawn, by aortic puncture, into tubes containing EDTA (1 mg/ml). Plasma was pooled and lipoprotein fractions VLDL- (d < 1.006 g/ml), IDL (d 1.006–1.019 g/ml), and LDL (d 1.019–1.063 g/ml) were isolated at appropriate salt densities by the method of Havel, Eder, and Bragdon (15). All lipoprotein fractions were counted and concentrated by ultrafiltration using Amicon PM10 membranes. The B-apolipoprotein was isolated by isopropanol precipitation (14) and its specific activity was determined. Briefly, 250 μl of the lipoprotein (1 mg of protein/ml) was extracted with an equal volume of isopropanol and the apoB was pelleted by centrifugation. The pellet was washed with 250 μl of isopropanol–saline 1:1 (v/v). Lipids were then extracted with 1.0 ml of methanol–chloroform–diethyl ether 2:3:5 (v/v/v) and finally washed with diethyl ether alone. The apoB pellet was dried and dissolved in 0.1 N NaOH. ApoB was radioassayed for ^{125}I and ^{131}I and the protein content of the whole sample was determined by the method of Lowry et al. (16) to determine specific activity. The specific activity time curves of the VLDL- and LDL-apoB plotted semilogarithmically were best described by a three-pool model that provided a significantly better fit than a two-pool model when tested by residual error testing (17, 18). The curves were subjected to a computer program for the analysis of multicomponent exponential decay data based on a nonlinear least squares method (19). The parameters of the three-pool model, shown in **Fig. 1,** were calculated according to Goodman, Noble, and Dell (18) in accordance with Skinner et al. (20). Comparison of the specific activity curves of VLDL, IDL, and LDL allowed an examination of the precursor–product relationship between these fractions as previously discussed by Zilversmit (21).

Principal calculations and definitions

These were according to the appendix to Goodman et al. (18) with the following modifications according to

Skinner et al. (20):

$$d_1 = k_{22} + k_{33} = (\alpha_2 + \alpha_3) + [(\alpha_1 - \alpha_2)A_2 \div (A_1 + A_2 + A_3)] + [(\alpha_1 - \alpha_3)A_3 \div (A_1 + A_2 + A_3)]$$

where the specific activity of each compartment at t = 0 is normalized to 1.0 (20).

$$d_2 = k_{22}k_{33} = [(\alpha_2 - \alpha_1)(\alpha_2 - \alpha_3)A_2 \div (A_1 + A_2 + A_3)] - \alpha_2^2 + d_1\alpha_2$$

$$k_{22} = \tfrac{1}{2}(d_1 + \sqrt{d_1^2 - 4d_2})$$

$$k_{33} = \tfrac{1}{2}(d_1 - \sqrt{d_1^2 - 4d_2})$$

where A_1 = time zero intercept of rapid exponential
where A_2 = time zero intercept of intermediate exponential
where A_3 = time zero intercept of slow exponential
where α_1 = slope of rapid exponential
where α_2 = slope of intermediate exponential
where α_3 = slope of slow exponential
where k_{01} = rate constant (pools per hour) of apoB mass transfer out of the system to "pool zero" from pool 1. This corresponds to irreversible catabolic rate (18).

Analyses

Plasma lipids were extracted in chloroform–methanol 2:1 (v/v) and triglyceride and cholesterol concentrations were assayed by methods described previously (22–24). Variations in body weight and plasma lipids were assessed using the *t*-test for unpaired samples (25). Variation is expressed as the standard error of the mean.

RESULTS

Treatment with d-norgestrel significantly reduced plasma total triglycerides (49 ± 5 vs. 66 ± 7 mg/dl, $P < 0.05$) and significantly elevated the plasma total cholesterol (64 ± 2 vs. 54 ± 3 mg/dl, $P < 0.05$, n = 22 per group), as compared to untreated controls.

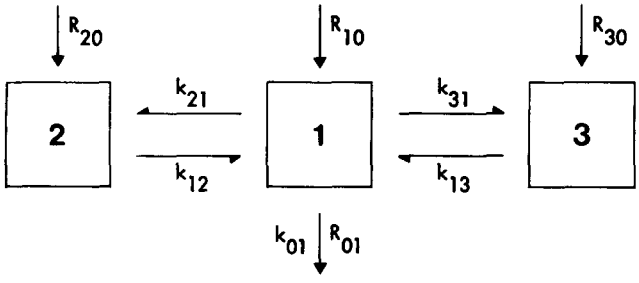

Fig. 1. Three-pool model of apolipoprotein B turnover of VLDL or LDL in rats. (See Tables 1 and 2 for values and references 18 and 20 for detailed explanation of symbols and assumptions).

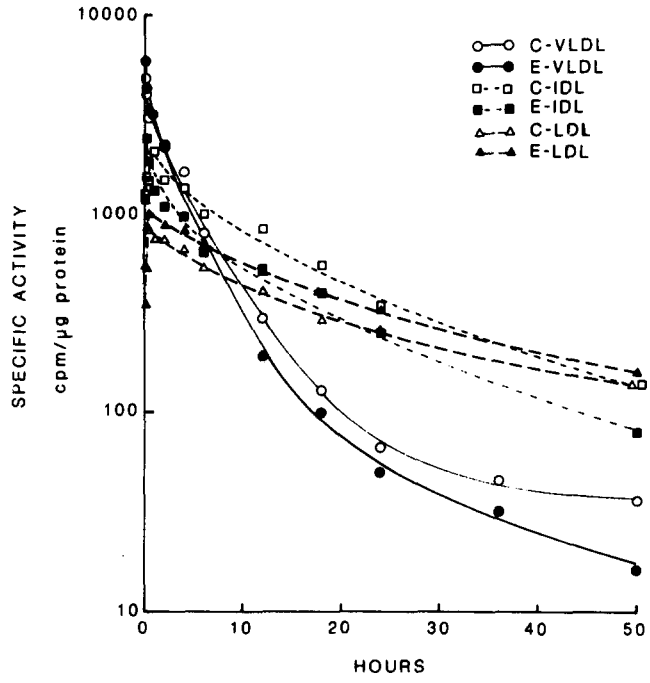

Fig. 2. Specific radioactivity of apolipoprotein B of VLDL, IDL, and LDL following intravenous injection of ^{125}I-labeled rat VLDL into control (C) and d-norgestrel-treated (E) rats. Each point represents the value obtained from the pooled plasma of two rats.

The values for specific activity of ^{125}I-labeled apoprotein B in VLDL, IDL, and LDL over 50 hr following injection of ^{125}I-labeled VLDL were plotted on a semilogarithmic scale (**Fig. 2**). VLDL-apoB clearance was enhanced in the treated versus control rats. The kinetics of VLDL turnover calculated from the ^{125}I-labeled VLDL-apoB decay curve are shown in **Table 1.** The higher initial specific activity of VLDL-apoB in d-norgestrel-treated rats as compared to controls (**Fig. 3**), reflects the smaller pool of apoB (270 vs. 420 μg). The irreversible catabolic rate of VLDL-apoB was markedly increased (0.57 vs. 0.34 hr^{-1}). However, the production rate was similar in the two groups (160 vs. 140 μg/hr).

For the purpose of clarity, the values for specific activity of ^{125}I-labeled apoB in VLDL, IDL, and LDL over the first 6 hr were plotted as a function of time in order to study the precursor–product relationships between the different lipoprotein fractions (Fig. 3). Faster clearance of VLDL-apoB in d-norgestrel-treated rats was accompanied by a rapid rise of IDL-apoB specific activity which peaked before the IDL-apoB specific activity curve for controls. The IDL-apoB specific activity curve declined faster in the treated versus control rats (Fig. 2). In both the groups, the IDL-apoB and LDL-apoB specific activity time curves reached maximal values well before intersecting VLDL-apoB curves, indicating direct input of these lipoproteins independent of VLDL decay.

TABLE 1. Kinetic parameters of rat ^{125}I-labeled VLDL-apoprotein B turnover

Group	Half-Life of Exponential			Rate Constants[a]					M_1[b]	PR[c]
	I	II	III	k_{12}	k_{21}	k_{13}	k_{31}	k_{01}		
		hr				*hr*$^{-1}$			*μg*	*μg/hr*
Control	0.16	3.0	30	2.48	1.85	0.030	0.073	0.34	420	140
d-Norgestrel[d]	0.11	2.6	16	2.59	3.54	0.051	0.096	0.57	270	160

[a]According to Goodman et al. (18), k_{01} denotes irreversible catabolic rate.
[b]Size of pool 1.
[c]Production rate = R_{01}.
[d]Dose was 4 μg d-norgestrel per day per kg body wt$^{0.75}$ for 18 days.

Fig. 4 and **Fig. 5** show, respectively, the LDL-apoB absolute specific activity curves and the LDL-apoB relative specific activities expressed as percent of the highest initial value (respective 10 min postinjection value for each group) following the injection of ^{131}I-labeled LDL in treated and control rats. Clearance of LDL-apoB was definitely delayed in the d-norgestrel-treated rats compared to controls. The kinetic parameters of LDL-apoB turnover (**Table 2**), calculated from data in Fig. 4, indicated a longer half-life and lower irreversible catabolic rate (0.15 vs. 0.29 pools hr^{-1}) with a markedly larger pool size (840 vs. 410 μg) of apoB in the treated versus control rats. However, the production rate of apoB was similar in both groups (130 vs. 120 μg/hr).

DISCUSSION

The present studies provide new insights into the mechanisms underlying alterations of serum lipid levels that occur during treatment with d-norgestrel. The significantly lower mean plasma triglyceride level in the treated group versus controls (49 ± 5 vs. 66 ± 7 mg/dl) was consistent with the lower VLDL-apoB pool (270 vs. 420 μg). The decreased pool size was related mainly to an increased irreversible catabolic rate of VLDL-apoB, since the production rate was essentially unchanged by d-norgestrel.

The catabolism of VLDL is a stepwise process involving initially the formation of remnants which may then be either catabolized to form LDL or directly removed from circulation without the formation of LDL (26, 27). This latter pathway is especially important in the rat. Eisenberg and Rachmilewitz (28, 29) have shown that, in rats, the major part of plasma VLDL-apoB removed from the circulation is degraded, presumably in the liver, without conversion to LDL. It is known that, in the rat, hepatic receptors bind both chylomicron and VLDL remnants generated through the action of lipoprotein lipase more efficiently as they become poor in C-apoproteins (10, 30). There is evidence that it is the chylomicron remnant receptor that removes VLDL remnants (31), rather than the LDL receptor which is thought to be responsible for VLDL remnant removal in rabbits (32) and probably other species. It is also known that VLDL remnants in the rat can bind to the LDL receptor of hepatic plasma membranes and that this binding can be enhanced by pretreatment of animals with pharmacological doses of ethinyl estradiol (33). The relative contribution of the hepatic chylomicron (apoE) receptor versus the LDL (apoB,E) receptor in the uptake of rat VLDL remnants is not known. Although we cannot ascertain the ultimate effects of d-norgestrel upon VLDL receptor function from the present studies, one possible mechanism underlying increased catabolism of VLDL and VLDL remnants

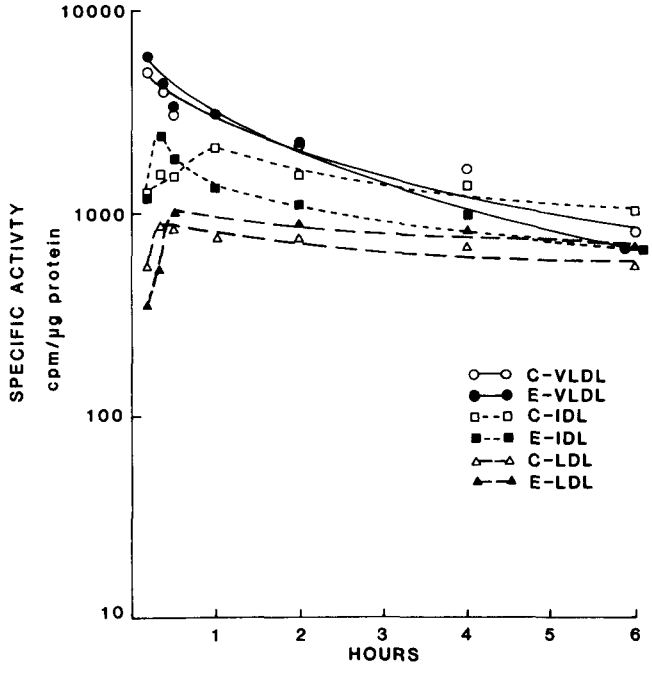

Fig. 3. Specific radioactivity of apolipoprotein B of VLDL, IDL, and LDL for first 6 hr following intravenous injection of ^{125}I-labeled rat VLDL into controls (C) and d-norgestrel-treated (E) rats. Each point represents the value obtained from pooled plasma of two rats.

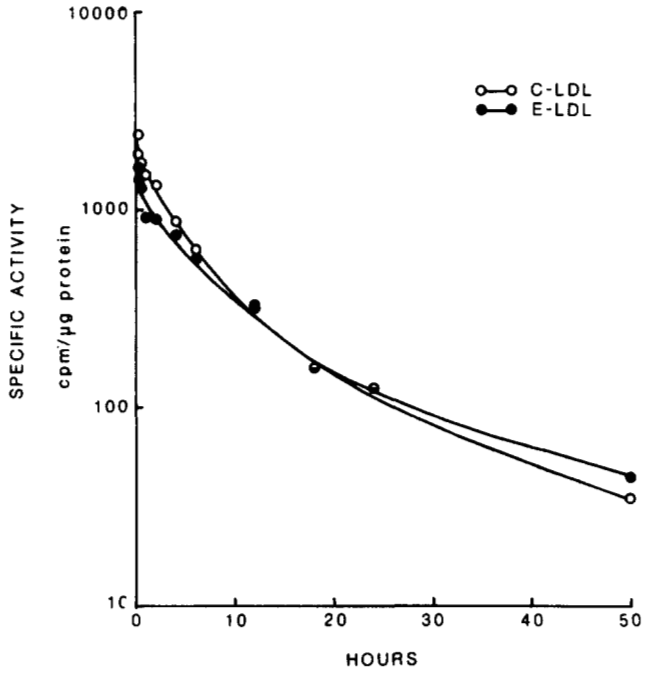

Fig. 4. Specific radioactivity of apolipoprotein B of LDL following intravenous injection of ^{131}I-labeled rat LDL into controls (C) and d-norgestrel-treated (E) rats. Each point represents the value obtained from the pooled plasma of two rats.

could include increased efficiency of uptake of these lipoproteins by the hepatic apoE receptor.

The increased size of the LDL-apoB pool and higher LDL cholesterol concentrations appear to be related to impaired removal, inasmuch as the LDL-apoB irreversible catabolic rate was decreased whereas the production rate was unchanged. A possible mechanism to account for delayed metabolic degradation could be impaired LDL receptor function in d-norgestrel-treated rats as compared to controls. Large pharmacological doses of ethinyl estradiol, which drastically lower plasma LDL, have been shown to increase the number of hepatic receptors for LDL in the rat (33, 34). Thus, by contrast, low doses of d-norgestrel could act by a converse mechanism by reducing LDL receptor activity in both nonhepatic and hepatic tissues. In addition, as discussed above, d-norgestrel might enhance the activity of the hepatic apoE receptor. The former would result in a decrease in LDL catabolic rate and an increase in plasma LDL concentrations, whereas the latter would explain the more efficient VLDL and VLDL remnant clearance and decrease in their concentrations. It is also known that the activity of hepatic LDL receptors can be easily modified by metabolic interventions that influence hepatic cholesterol metabolism (35). These perturbations had little effect on apoE receptor activity (suggesting that this receptor was resistant to metabolic regulation). However, a recent study in humans provides evidence that the hepatic apoE receptor can be regulated independently of the LDL receptor (36).

An alternate mechanism to explain the increase in VLDL catabolism by d-norgestrel could be increased enzymatic activity of lipoprotein lipase and/or hepatic triglyceride lipase, which are primarily responsible for the conversion of VLDL to IDL and to LDL. Although it has been suggested that, in humans, lipoprotein lipase is the rate-limiting enzyme of the VLDL catabolic pathway (37), it is possible that hepatic lipase may influence VLDL clearance under certain metabolic conditions. Hepatic triglyceride lipase in humans has been shown to be depressed by estrogen treatment (38) and elevated by norgestrel treatment (39). Human hepatic triglyceride lipase has been shown to have a high affinity for IDL (40) and therefore may play a role in its catabolism. Murase and Itakura (41) and Goldberg et al. (42) have suggested that blocking hepatic triglyceride lipase in rats or monkeys may impair the catabolism of IDL.

The fraction of IDL-apoB derived from catabolism of VLDL-apoB (calculated from the ratio of ^{125}I-labeled apoB specific activity of IDL to VLDL when IDL specific activity reached a maximal value, ref. 43) was similar in treated versus control rats (0.55 vs. 0.63). Likewise, the fraction of LDL-apoB derived from catabolism of IDL (calculated from the ratio of ^{125}I-labeled apoB specific activity of LDL to IDL when LDL specific activity reached a maximal value) was similar in treated versus control rats (0.54 vs. 0.56). Therefore, it is unlikely that the increase in VLDL catabolic rate is due to an increased conversion of VLDL-apoB to LDL-apoB. LDL-apoB specific activity time curves reached maximal values well

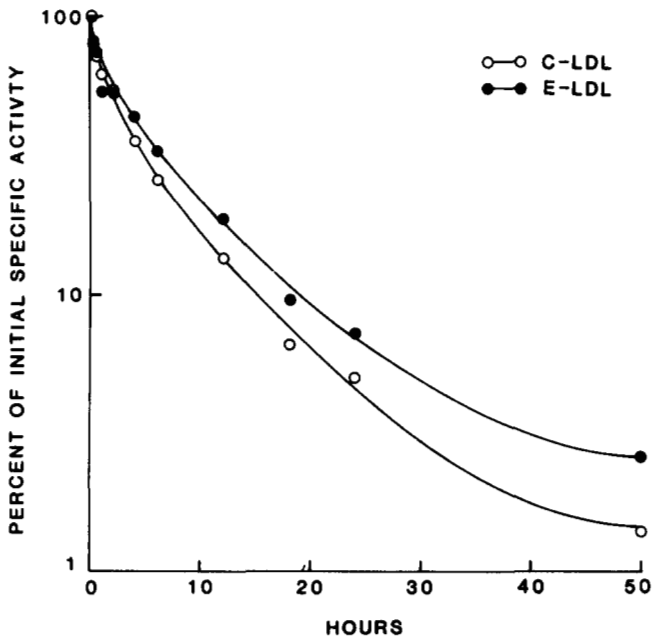

Fig. 5. Specific radioactivity of LDL-apolipoprotein B expressed as percent of the initial specific activity (10 min post-injection), following intravenous injection of ^{131}I-labeled rat LDL. Each point represents the value obtained from pooled plasma of two rats.

TABLE 2. Kinetic parameters of rat ^{131}I-labeled LDL-apoprotein B turnover

Group	Half-Life of Exponential			Rate Constants[a]					M_1[b]	PR[c]
	I	II	III	k_{12}	k_{21}	k_{13}	k_{31}	k_{01}		
	hr					*hr*$^{-1}$			*μg*	*μg/hr*
Control	0.081	2.7	14	4.03	4.26	0.089	0.16	0.29	410	120
d-Norgestrel[d]	0.21	4.2	17	1.93	1.26	0.073	0.072	0.15	840	130

[a]According to Goodman et al. (18), k_{01} denotes irreversible catabolic rate.
[b]Size of pool 1.
[c]Production rate = R_{01}.
[d]Dose was 4 μg d-norgestrel per day per kg body wt$^{0.75}$ for 18 days.

before intersecting IDL-apoB or VLDL-apoB decay curves, indicating the presence of LDL-apoB production independent of VLDL-apoB catabolism. This has previously been demonstrated by others (9). The direct input of LDL into plasma did not seem to be affected substantially by d-norgestrel inasmuch as the fraction of LDL-apoB derived from catabolism of VLDL (calculated from the ratio of ^{125}I-labeled apoB specific activity of LDL to VLDL when LDL specific activity reached maximal value, ref. 43) was comparable in the two groups (0.30 vs. 0.22) and the total LDL-apoB synthesis was also unchanged.

In summary, the principal action of d-norgestrel appears to be on apolipoprotein B catabolism. d-Norgestrel enhances VLDL-apoB catabolism, whereas LDL-apoB catabolism is reduced. The effect may be on apoB catabolism directly by opposing effects on the hepatic apoE and LDL receptors. However, the effect on VLDL may be mediated through effects upon the lipolytic enyzmes modulated by d-norgestrel. These observations, of divergent effects of d-norgestrel on apoB metabolism in VLDL, IDL, and LDL, shed new light on the triglyceride-lowering and cholesterol-elevating effects of d-norgestrel.

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REFERENCES

1. Tyler, E. T. 1974. International aspects of the use of norgestrel. *In* The Second International Symposium. Excerpta Medica, Amsterdam, 1-8.
2. Spellacy, W. H., N. C. Buhi, and S. A. Birk. 1974. Norgestrel and carbohydrate-lipid metabolism: glucose, insulin and triglyceride changes during six months time of use. *Contraception.* **9:** 615-625.
3. Nielsen, F. H., E. Honore, K. Kristoffersen, N. J. Secher, and T. Pedersen. 1977. Changes in serum lipids during treatment with norgestrel, oestradiol-valerate and cycloprogynon. *Acta Obstet. Gynaecol. Scand. Suppl.* **56:** 367-370.
4. Robertson, D. N., F. Alvarez, I. Sivin, V. Brache, J. Stern. P. Leon, and A. Faundes. 1981. Lipoprotein patterns in women in Santo Domingo using levonorgestrel/estradiol contraceptive ring. *Contraception.* **24:** 469-480.
5. Rossner, S., O. Frankman, and L. Marsk. 1979. Effects of various low-dose contraceptive pills on serum lipoproteins. *In* Lipoprotein Metabolism and Endocrine Regulation. L. W. Hessel and H. H. J. Kraus, editors. Elsevier, Amsterdam. 91-98.
6. Silfverstolpe, G., A. Gustafson, G. Samsioe, and A. Swanborg. 1979. Lipid metabolic studies in oöphorectomized women. Effects of three different progestogens. *Acta Obstet. Gynaecol. Scand. Suppl.* **88:** 89-95.
7. Khokha, R., and B. Wolfe. 1984. Hypotriglyceridemic effects of d-norgestrel in rats. *Atherosclerosis.* **52:** 329-338.
8. Eisenberg, S. 1976. Mechanisms of formation of low density lipoproteins: metabolic pathways and their regulation. *In* Low Density Lipoproteins. C. E. Day and R. S. Levy, editors. Plenum Press, New York. 73-92.
9. Fidge, N. H., and P. Poulis. 1978. Metabolic heterogeneity in the formation of low density lipoprotein from very low density lipoprotein in the rat: evidence for the independent production of a low density lipoprotein subfraction. *J. Lipid Res.* **19:** 342-349.
10. Brown, M. S., P. T. Kovanen, and J. L. Goldstein. 1981. Regulation of plasma cholesterol by lipoprotein receptors. *Science.* **212:** 628-635.
11. Fidge, N., and P. Poulis. 1975. Studies on the metabolism of rat serum very low density apolipoprotein. *J. Lipid Res.* **16:** 367-378.
12. Fidge, N. H., and P. Poulis. 1974. Studies on the radioiodination of very low density lipoprotein obtained from different mammalian species. *Clin. Chim. Acta.* **52:** 15-26.
13. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **182:** 53-56.
14. Holmquist, L., and K. Carlson. 1977. Selective extraction of human serum very low density apolipoprotein with organic solvents. *Biochim. Biophys. Acta.* **493:** 400-409.
15. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345-1353.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265-275.
17. Mann, H. B. 1949. Analysis and Design of Experiments.

Analysis of Variance and Analysis of Variance Design. Doner, New York.
18. Goodman, D. S., R. P. Noble, and R. B. Dell. 1973. Three-pool model of the long-term turnover of plasma cholesterol in man. *J. Lipid Res.* **14:** 178–188.
19. Bard, Y. 1974. Nonlinear Parameter Estimation. Academic Press, New York.
20. Skinner, S. M., R. E. Clark, N. Baker, and R. A. Shipley. 1959. Complete solution of the three-compartment model in steady state after single injection of radioactive tracer. *Am. J. Physiol.* **196:** 238–244.
21. Zilversmit, D. B. 1943. The design and analysis of isotope experiments. *Am. J. Med.* **29:** 832–848.
22. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
23. Carlson, L. A. 1963. Determination of serum triglycerides. *J. Atheroscler. Res.* **3:** 334–336.
24. Sperry, M. W., and M. A. Webb. 1956. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187:** 97–106.
25. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. 6th ed. Iowa State University Press, Ames, IA. 106 and 114.
26. Reardon, M. F., N. H. Fidge, and P. J. Nestel. 1978. Catabolism of very low density lipoprotein B apoprotein in man. *J. Clin. Invest.* **61:** 850–860.
27. Barter, P. J., and P. J. Nestel. 1972. Precursor product relationship between pools of plasma lipoprotein fractions in man. *J. Clin. Invest.* **51:** 174–180.
28. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. I. Fate in circulation of the whole lipoprotein. *Biochim. Biophys. Acta.* **326:** 378–390.
29. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. II. Fate in circulation of apoprotein subunits. *Biochim. Biophys. Acta.* **326:** 391–405.
30. Windler, E., Y. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. *J. Biol. Chem.* **255:** 8303–8307.
31. Cooper, A. D., M. A. Shrewsbury, and S. K. Erickson. 1982. Comparison of binding and removal of remnants of triglyceride-rich lipoproteins of intestinal and hepatic origin by rat liver in vitro. *Am. J. Physiol.* **243:** G389–395.
32. Kita, T., M. S. Brown, D. W. Bilheimer, and J. L. Goldstein. 1982. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoproteins in WHHL rabbits. *Proc. Natl. Acad. Sci. USA.* **79:** 5693–5697.
33. Windler, E. E. T., P. T. Kovanen, Y. S. Chao, M. S. Brown, R. J. Havel, and J. L. Goldstein. 1980. The estradiol-stimulated lipoprotein receptor of rat liver. *J. Biol. Chem.* **255:** 10464–10471.
34. Davis, R. A., and P. S. Roheim. 1978. Pharmacologically induced hypolipidemia: the ethinyl estradiol-treated rat. *Atherosclerosis.* **30:** 293–299.
35. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine and man. *J. Clin. Invest.* **68:** 1197–1206.
36. Hoeg, J. M., S. J. Demosky, Jr., R. E. Gregg, E. J. Schaefer, and H. B. Brewer. 1985. Distinct hepatic receptors for low density lipoprotein and apolipoprotein E in humans. *Science.* **277:** 759–761.
37. Reardon, M. F., H. Sakai, and G. Steiner. 1982. Roles of lipoprotein lipase and hepatic triglyceride lipase in the catabolism in vivo of triglyceride-rich lipoproteins. *Arteriosclerosis.* **2:** 396–402.
38. Applebaum, D. M., A. P. Goldberg, O. J. Pykalisto, J. D. Brunzell, and W. R. Hazzard. 1977. Effect of estrogen on post-heparin lipolytic activity: selective decline in hepatic triglyceride lipase. *J. Clin. Invest.* **59:** 601–608.
39. Tikkanen, M. J., E. A. Nikkila, T. Kuusi, and S. Sipinen. 1981. Different effects of two progestins on plasma high density lipoprotein (HDL_2) and post-heparin plasma hepatic lipase activity. *Atherosclerosis.* **40:** 365–369.
40. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism: in vivo and in vitro studies in man. *Eur. J. Clin. Invest.* **10:** 487–495.
41. Murase, T., and H. Itakura. 1981. Accumulation of intermediate density lipoprotein in plasma after intravenous admininstration of hepatic triglyceride lipase antibody in rats. *Atherosclerosis.* **39:** 293–300.
42. Goldberg, I. J., N-A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70:** 1184–1192.
43. Reardon, M. F., M. E. Poapst, and G. Steiner. 1982. The independent synthesis of intermediate density lipoproteins in type III hyperlipoproteinemia. *Metabolism.* **31:** 421–427.