

Metabolism of very low density lipoproteins in rats with isotretinoin (13-*cis* retinoic acid)-induced hyperlipidemia

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Abstract A significant rise in plasma triacylglycerols from the control level of 0.89 mmol/l to 1.88 mmol/l ($P < 0.001$) was observed in male Sprague-Dawley rats treated for 11 days with isotretinoin (oral dosing; 10 mg/day). This rise was due to an increased level of plasma very low density lipoproteins (VLDL). When VLDL from untreated rats were labeled with ^{125}I -labeled tyramine-cellobiose and injected intravenously into rats treated for 10 days with isotretinoin ($n = 6$) and in control rats ($n = 6$), it was found that the disappearance of radioactivity from the blood was dramatically retarded in the treated animals. The disappearance could be divided into two phases, a rapid (α) phase dominated the first 5 min and was followed by a slower (β) phase. The half-life of the β -phase increased significantly from 53 ± 7 min in the controls, to 120 ± 62 min after isotretinoin. VLDL prepared from isotretinoin-treated animals ($n = 6$) had about the same half-life in control animals (62 ± 8 min) as had ordinary VLDL. The elimination of tracer from the blood was mainly due to uptake by the liver. The amount of radioactivity in the liver after 30 min of circulation was significantly reduced from $34 \pm 7\%$ of injected dose in controls to $24 \pm 5\%$ in the isotretinoin group ($P = 0.013$). The uptake in other organs was $< 3\%$ per organ and was essentially unaffected by the treatment. When the different liver cell populations were analyzed for radioactivity after separation by Percoll®-centrifugation, it was found that the uptake by the parenchymal cells was significantly reduced from $32 \pm 7\%$ of the injected dose to $21 \pm 4\%$ ($P = 0.007$) by isotretinoin treatment, while the uptake in nonparenchymal cells was unaffected. Our results suggest that the hypertriglyceridemia seen after isotretinoin treatment is associated with reduced VLDL uptake by parenchymal cells of the liver. — Gustafson, S., C. Vahlquist, L. Sjöblom, A. Eklund, and A. Vahlquist. Metabolism of very low density lipoproteins in rats with isotretinoin (13-*cis* retinoic acid)-induced hyperlipidemia. *J. Lipid Res.* 1990. 31: 183–190.

Supplementary key words retinoids • lipoproteins • hyperlipidemia • plasma triacylglycerols • ^{125}I -labeled tyramine-cellobiose • kinetic studies • liver uptake

Isotretinoin (13-*cis* retinoic acid; Accutane®) is a synthetic retinoid widely used for oral treatment of recalcitrant nodulocystic acne and severe disorders of keratini-

zation. Unfortunately, hyperlipidemia is a common side-effect of retinoid therapy (for review see ref. 1). The associated lipoprotein changes are characterized by increased very low density lipoprotein (VLDL) triacylglycerol levels (2) and an increased low density lipoprotein (LDL) to high density lipoprotein (HDL) cholesterol ratio (3). Although these changes imply an increased risk for atherosclerosis (4–6), a detailed risk evaluation is not possible until the mechanisms underlying retinoid-induced hyperlipidemia are known. Hyperlipidemia may be the result of either an increased hepatic synthesis of lipoproteins or a decreased catabolism of circulating lipoprotein particles. Gerber and Erdman (7) have suggested that tretinoin (all-*trans* retinoic acid) enhances the synthesis of VLDL-triglycerides in rat liver. In the same study, however, they report decreased lipoprotein lipase activity in the gastrocnemius muscle during tretinoin treatment, suggesting a decreased catabolism of lipoproteins.

Studies on retinoid-induced hyperlipidemia in humans have indicated that isotretinoin and etretinate (the ethyl ester of an aromatic analog of retinoic acid) elevate the apoB-lipoprotein levels in serum (2, 8). In addition, there are several reports showing that both these retinoids, as well as acitretin (the free acid of etretinate), impede the rate at which intravenously injected fat is removed from the blood (9–11). This implies that the efferent (catabolic) pathway of human lipoprotein metabolism is affected by retinoids. In congruence to this, decreased muscle lipoprotein lipase activities have been recorded in retinoid-treated subjects (9).

Abbreviations: VLDL, very low density lipoproteins; ITVLDL, VLDL from isotretinoin-treated rats; LDL, low density lipoproteins; HDL, high density lipoproteins; TC, tyramine-cellobiose; PC, liver parenchymal cells; NPC, liver nonparenchymal cells; IT, isotretinoin; ^{125}I -TC, ^{125}I -labeled tyramine-cellobiose.

A new approach to the study of lipid removal from the blood is to inject labeled lipoproteins and to monitor the transfer of label from serum to tissues. When ^{125}I -labeled tyramine-cellobiose (^{125}I -TC) is used as labeling agent, the radioactivity will be trapped in the cells after the protein has been degraded. This permits quantitative analysis of the lipoprotein uptake in a particular tissue or organ, as well as more accurate measurement of its elimination from the blood (12).

In this report we show that oral intake of isotretinoin, within a few days, markedly elevates the triacylglycerol and VLDL levels in rat plasma and impedes the clearance of injected ^{125}I -TC-VLDL particles. The reduced clearance is mainly due to a decreased fractional removal of lipoproteins by the liver parenchymal cells.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats were purchased from Alab, Stockholm, Sweden, at the age of 6 weeks. They were fed with ordinary pellets, containing, inter alia, 5% crude fat and 12,000 IU of vitamin A / kg diet (R3-EWOS-ALAB Brood Stock Feed for Rats and Mice) and water ad libitum. The animals, weighing about 200 g, were allowed to acclimatize for 2 weeks before starting the experiment. Ten mg isotretinoin (Hoffman-La Roche AG, Basel, Switzerland) dissolved in 0.5 ml of peanut oil was injected intragastrically through an infant feeding tube quickly inserted in the esophagus. The control animals received the same volume of peanut oil without retinoid. The weight gain during the study was usually between 50 and 60 g.

Plasma lipid study

Experiment 1. In this experiment, four isotretinoin-treated and four control rats were used. Eleven days after commencing the treatment, the animals were starved for 4 h, weighed, and anesthetized with barbital. Blood was collected from the abdominal aorta in centrifuge tubes containing 40 μmol ethylenediamine tetraacetic acid (EDTA), 1 nmol dithiobisnitrobenzoic acid, and 2000 KIE aprotinin (Trasylo[®]) in 410 μl saline. Plasma was separated at 500 g for 20 min.

VLDL were isolated by preparative ultracentrifugation as described previously (13). Different rat plasma lipoprotein fractions are classified and denoted in accordance with Oschry and Eisenberg (14). Agarose gel electrophoresis of lipoproteins was carried out at pH 7.0 as described elsewhere (15). Plasma samples and the VLDL fraction were analyzed for cholesterol and triacylglycerols by enzymatic methods using kits supplied by Boehringer Mannheim, West Germany (catalog nos: 237 574 and 240 052).

High density lipoprotein-(HDL₂)-cholesterol was measured after precipitation of other lipoproteins with dextran sulfate and magnesium chloride (16).

Experiment 2. In this experiment, four isotretinoin-treated and six control rats were studied. On days 0, 1, 2, 4, and 8, tail blood samples were collected in plastic capillaries containing heparin (CB 300, Sarsted AB, Malmö, Sweden). The samples were taken immediately before dosing the animals. On day 9, the last dose was given in the evening, the animals were fasted overnight, and tail blood samples were collected in the morning. The plasma triacylglycerol and cholesterol analyses were carried out as described above.

Labeling of VLDL with ^{125}I -TC

Ten-50 nmol of tyramine-cellobiose (TC) were labeled with ^{125}I and activated according to Pittman et al. (12). VLDL, prepared as described above from rats fed a casein-based semipurified diet containing 0.5% cholesterol (13) for 4 weeks or from rats fed this diet for 10 days and receiving 10 mg isotretinoin daily as in experiment 2 of the plasma lipid study (IT-VLDL), were labeled, essentially as previously described (17), by incubating ^{125}I -TC with the lipoproteins at equimolar concentrations (TC to VLDL protein) at pH 9.0-9.5 at 37°C for 30-40 min. The ^{125}I -TC-VLDL or ^{125}I -TC-IT-VLDL were then equilibrated with 0.1 M NH_4HCO_3 on a Sephadex G 25 column (PD 10, Pharmacia, Uppsala, Sweden) and dialyzed for at least 48 h with 0.15 M NaCl, 10 mM NaH_2PO_4 , 0.05 M EDTA, pH 7.4, in order to remove unreacted ^{125}I -TC. By extraction with ethanol ether 3:1 (v/v) it was found that 10-11% of the radioactivity was associated with lipids. When delipidated VLDL proteins were subjected to polyacrylamide gel electrophoresis in SDS followed by staining with Coomassie brilliant blue and autoradiography, protein bands corresponding to apolipoprotein B, E and C apolipoproteins, i.e., those normally present in pure preparations of VLDL, were visualized. The autoradiographic pattern was virtually identical to the pattern seen with protein staining, indicating that the different proteins were uniformly labeled. We have earlier shown that ^{125}I -TC-based VLDL behaves similarly to VLDL iodinated by ordinary methods when studied in vivo in the rat. The plasma disappearance rate for TC-labeled VLDL is similar to that for ordinary iodinated VLDL, but the rate levels off in the case of ordinary iodination as degradation products start to appear after about 20 min (17).

In in vitro experiments, performed in order to test the physiological properties of the labeled VLDL, the TC-labeled VLDL were taken up by cultured rat liver endothelial cells and J774 macrophages, cells known to take up VLDL by receptor-mediated endocytosis (18, 19). The uptake could be effectively inhibited by unlabeled VLDL (Fig. 1), showing that the TC-labeling does not alter the normal properties of the lipoproteins.

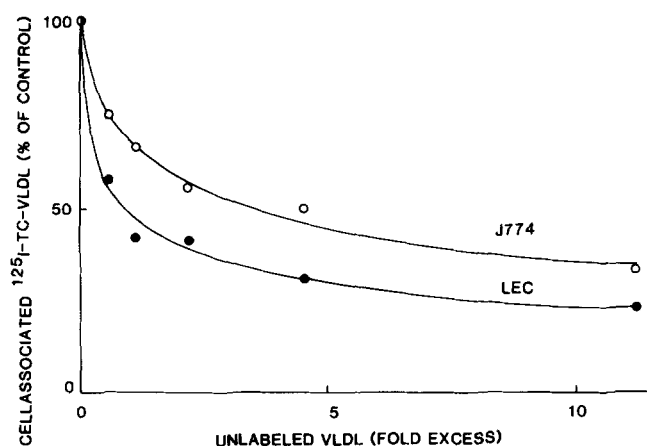


Fig. 1. The effect of unlabeled VLDL on the uptake of ^{125}I -TC-VLDL (10 $\mu\text{g}/\text{ml}$) by liver endothelial cells (LEC) and J 774 macrophages. After incubation for 1 h at 37°C , the cell-associated radioactivity was determined as earlier described (18) and expressed as a percentage of that found in the absence of unlabeled lipoproteins. Each point is the average of duplicate experiments.

Metabolism of ^{125}I -TC-VLDL from untreated animals

In these experiments, six isotretinoin-treated and six control rats were used. The animals received the same food and drug regimen as in experiment 2 of the plasma lipid study. At day 10, the animals were starved overnight and weighed before commencing the injections. The rats were anesthetized with pentobarbital (45 mg/kg body weight) and were injected in the tail vein with 30 μg ^{125}I -TC-VLDL protein ($0.6\text{--}0.7 \times 10^6$ cpm), in 0.9–1.0 ml 0.15 M NaCl, 10 mM NaH_2PO_4 , pH 7.4. Blood samples (50 μl) were repeatedly collected from the distal part of the tail during the 30-min circulation period. The amount of radioactivity present in the blood at 0 min was calculated by extrapolating backwards from the 1, 2, and 3 min determinations. In some cases, at the end of the experiment, a larger blood sample (300–400 μl) was collected. The serum from this sample was analyzed by agarose-gel electrophoresis (15) and autoradiography. After 30 min, the liver was subjected to collagenase perfusion as described by Öbrink (20) and the rat was subsequently killed. Blood, lungs, kidneys, and spleen were assayed for radioactivity.

The liver cells were separated according to Smedsrød and Pertoft (21) by centrifugation through Percoll® (Pharmacia, Uppsala, Sweden) and by selective adherence. Purity of nonparenchymal cell (NPC)-preparations was determined by observing the size of cells in a phase-contrast microscope. Contaminating parenchymal cells (PC) are easily observed due to their larger size. In the PC cultures, contaminating NPC were visualized by uptake of fluorescent beads (Kupffer cells) and uptake of fluorescent

ovalbumin (liver endothelial cells). The purity of all cell preparations was $>95\%$. The liver cells were cultured on 90-mm dishes (Flow Laboratories, Irvine, Scotland, U.K.) in RPMI 1640 medium containing penicillin (100 U/ml), streptomycin (100 mg/l), and gentamicin (50 $\mu\text{g}/\text{ml}$). The cells were cultured for 1.5 h and then scraped off from the dishes by a rubber policeman and assayed for radioactivity.

The calculations of liver uptake and the distribution between different liver cells are based on the fact (22) that a normal rat liver contains about 400×10^6 NPC and $1,200 \times 10^6$ PC. Total liver activity was calculated by counting a portion of the single cell suspension after collagenase perfusion, multiplying to get the activity of the whole volume, and adding the activity found in the noncollagenase degradable leftovers. The activities found in pure PC (activity/ 10^6 cells multiplied by 1,200) and NPC (activity/ 10^6 cells multiplied by 400) were added to give 100% (this value was generally within $\pm 10\%$ of the total liver value calculated as described above), and the distribution between NPC and PC was calculated in % of the total liver uptake. Cell number in suspension was determined by counting in a Bürcher chamber in a phase contrast microscope, and on dishes the cell number was determined using a microscope equipped with a grid.

Metabolism of ^{125}I -TC-VLDL from treated animals

Six control rats were starved overnight and weighed before the intravenous injections were started. The rats were anesthetized with pentobarbital (45 mg/kg body weight) and received an injection in the tail vein of 30 μg ^{125}I -TC-VLDL protein ($0.9\text{--}1.0 \times 10^6$ cpm), in 0.9–1.0 ml 0.15 M NaCl, 10 mM NaH_2PO_4 , pH 7.4. Assays of radioactivity in blood, organs, and liver cells were performed as described above.

Data processing

The kinetic data were processed according to the "area-method" of Nosslin (23) on a Macintosh Plus® or a Macintosh II® computer (Apple Computer Inc., Cupertino, CA). Briefly, the plasma disappearance of radioactivity over time is divided into its exponential constituents, each of which is characterized by a slope (b_i) and an intercept (c_i). The fractional catabolic rate is calculated as $[\sum 1 - n (c_i / b_i)]^{-1}$ as previously outlined (24). The graphs were constructed using the Cricket graph® program (version 1.0, Cricket Software, Philadelphia, PA).

Statistical analyses of significance of difference, mean values and standard deviation, were performed using the Student's t -test analysis on a Macintosh Plus® programmed with Statworks® (version 1.1, Cricket Software).

TABLE 1. Plasma levels of triacylglycerols, cholesterol, and lipoproteins in male rats fed isotretinoin for 11 days

Group	Plasma Triacylglycerols	Plasma Total Cholesterol	Plasma VLDL Cholesterol	Plasma HDL ₂ Cholesterol	Staining of Lipoproteins after Agarose Gel Electrophoresis		
					HDL ₂	LDL/HDL ₁	VLDL
					densitometric units		
	mmol/l	mmol/l	mmol/l ^a	mmol/l			
Isotretinoin-treated	1.88 ± 0.10 ^b	2.14 ± 0.25	0.21 - 0.21	1.07 ± 0.09	739 ± 79	154 ± 20	649 ± 78 ^b
Control	0.89 ± 0.17	2.09 ± 0.16	0.04 - 0.04	1.14 ± 0.09	694 ± 95	162 ± 23	245 ± 36

Unless otherwise specified, the values represent mean value and standard deviation of four animals.

^aRange of two samples (each containing plasma pooled from two rats).

^bSignificantly different from control ($P < 0.001$).

RESULTS

Plasma lipids

In experiment 1, the effects of isotretinoin (10 mg per day for 11 days) on the animals' physical condition and plasma lipid pattern were studied. There were no signs of retinoid toxicity; the weight gain and general condition of the isotretinoin-treated animals were indistinguishable from those of the control animals.

As is evident in **Table 1**, whereas the plasma triacylglycerol level was twice as high in the retinoid-treated animals compared with the controls, neither the total plasma cholesterol nor the HDL₂-cholesterol values were affected by the treatment with isotretinoin. Also, the VLDL-cholesterol level was several-fold higher in the retinoid-treated rats. In concord with these results, the VLDL-band, observed after agarose gel electrophoresis, was much more pronounced in plasma from isotretinoin-

treated rats compared with that from untreated rats as shown in **Table 1**.

In experiment 2, the changes in plasma triacylglycerol levels with time and the effects of a 12-h fasting period on isotretinoin-induced hyperlipidemia were studied. **Fig. 2** shows that the triacylglycerol level increased rapidly during the first days of isotretinoin treatment. Then the values plateaued at approximately 4 mmol/l, i.e., 2 times the pretreatment level. A smaller, but consistent increase in the triacylglycerol level was also observed in the control group; this effect is probably due to the stress imposed by tube feeding and blood sampling. The 12-h fasting period from day 9 to 10 caused a significant drop in the triacylglycerol level of the isotretinoin-treated animals. However, since a concomitant decline occurred in the control group, the difference between the two groups was still highly significant ($P = 0.001$). In a larger group of animals, a 12-h fast, after 10 days of treatment, resulted in a triacylglycerol level of 1.61 ± 0.77 mmol/l ($n = 11$) in controls and 2.76 ± 0.77 ($n = 8$) following isotretinoin ($P < 0.001$).

No variation of significance was observed with time or between different groups with regard to the total plasma cholesterol level.

Metabolism of ¹²⁵I-TC-VLDL from untreated animals

Using the experimental conditions established in experiment 2 of the plasma lipid study, we studied the metabolism of VLDL at day 10 after a 12-h fast. **Fig. 3** shows the disappearance of intravenously injected ¹²⁵I-TC-VLDL from the circulation of isotretinoin-treated rats and control rats. No radioactivity was found in LDL + HDL₁ and < 5% was found in HDL₂ after 30 min of circulation, as determined by agarose-gel electrophoresis and autoradiography (not shown).

When the elimination was studied in a semilogarithmic plot, it was found that the disappearance could be divided into two phases (**Fig. 4**). The rapid phase (α -phase) dominated the first 5 min after injection and was followed by a slower phase (β -phase) lasting throughout the experiment. The half-life of the α -phase was similar in both

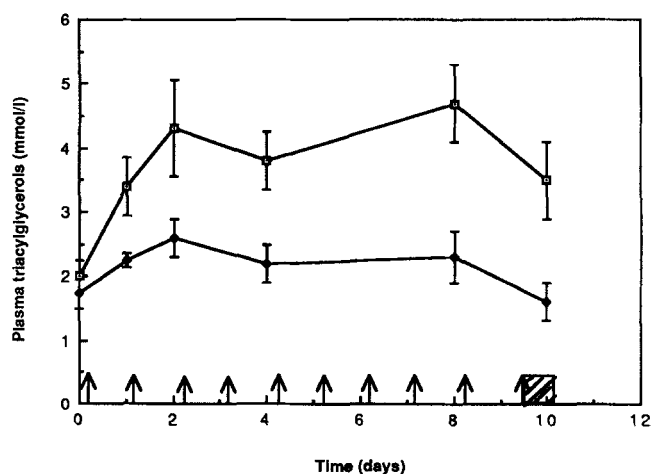


Fig. 2. Plasma triglyceride levels observed in male rats fed isotretinoin for 10 days (□) and control rats (◆). The values represent mean value ± standard deviation of four rats (isotretinoin-treated) and six rats (controls). The arrowheads show time of dosing. The hatched area denotes the fasting period.

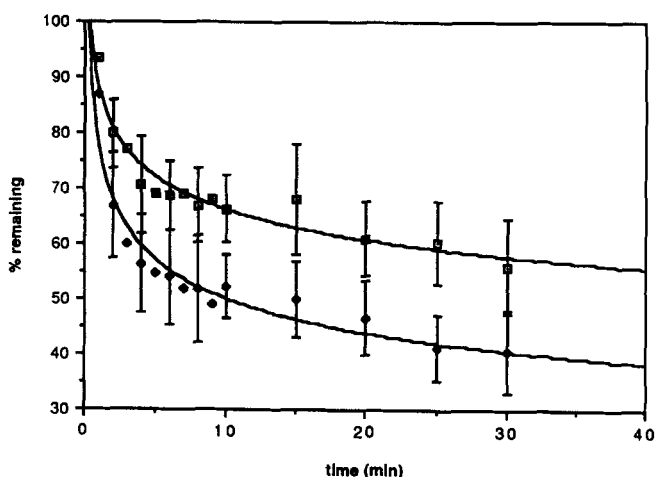


Fig. 3. The disappearance of intravenously injected $^{125}\text{I-TC-VLDL}$ in control rats (\blacklozenge , mean \pm SD, $n = 6$) and in rats treated with isotretinoin (\square , mean \pm SD, $n = 6$). Injection of labeled VLDL, blood sampling, and determination of radioactivity were as described in Methods.

groups with 0.80 ± 0.44 min in controls and 1.00 ± 0.35 min after isotretinoin treatment. However, the intercept with the ordinate showed that the α -phase accounted for $55.9 \pm 11.7\%$ of the injected tracer dose in the control group, significantly more than the value ($33.6 \pm 9.2\%$) in the group treated with isotretinoin ($P = 0.007$).

The half-life of the β -phase ($t_{1/2-\beta}$) was increased significantly from the control $t_{1/2-\beta}$ 53 ± 7 min to 120 ± 62 min ($P = 0.025$) in the treated animals. In the isotretinoin-treated rats, the overall fractional catabolic rate was decreased significantly from the control level of $0.192 \pm 0.026 \text{ h}^{-1}$ to $0.108 \pm 0.066 \text{ h}^{-1}$ ($P = 0.016$).

The distribution of $^{125}\text{I-TC-VLDL}$ in various organs was studied by measuring the amount of radioactivity associated with the liver, spleen, kidneys, and lungs (Table 2). The liver accounted for most of the uptake in both groups, but the uptake of radioactivity in the liver was significantly reduced from $34.1 \pm 7.3\%$ of injected dose in controls to $23.6 \pm 4.6\%$ of injected dose in the isotretinoin group ($P = 0.013$). Only the kidney showed any considerable uptake beside the liver with about 2.5%. The uptake in other organs assayed was $< 1\%$ /organ and was essentially unaffected by the treatment.

Table 2 also demonstrates that the uptake in PC of the liver decreased significantly from $31.8 \pm 6.9\%$ of the injected dose to $20.8 \pm 4.1\%$ ($P = 0.007$) by isotretinoin treatment, while the uptake in the liver NPC was unaffected.

Metabolism of VLDL from treated animals ($^{125}\text{I-TC-IT-VLDL}$)

To investigate the possibility that VLDL from isotretinoin-treated animals and control animals are biologically

different, we injected the $^{125}\text{I-TC-IT-VLDL}$ in control animals, using the same protocol as in previous experiments. The $t_{1/2-\beta}$ was found to be similar (62 ± 8 min) to the VLDL from untreated animals but significantly different from the $t_{1/2-\beta}$ of 120 ± 62 min observed in treated animals ($P = 0.037$). The hepatic uptake of $^{125}\text{I-TC-IT-VLDL}$ in the control animals (total uptake $32.2 \pm 3.1\%$ and uptake in PC $28.1 \pm 3.2\%$) and the fractional catabolic rate ($0.168 \pm 0.044 \text{ h}^{-1}$) were very similar to $^{125}\text{I-TC-VLDL}$ from untreated animals. The uptake in other organs tested did not differ from that of $^{125}\text{I-TC-VLDL}$ from untreated rats.

DISCUSSION

Our results show that rats respond with a prompt increase (+250%) in plasma triacylglycerol levels within the first few days after starting the administration of isotretinoin. This was exclusively due to a marked increase in VLDL. No effects on total plasma cholesterol, plasma HDL₂ cholesterol, or plasma combined LDL and HDL₁ cholesterol were noticed. To ensure a distinct and reproducible response in plasma lipids, we administered the isotretinoin (10 mg per day, i.e., 40–50 mg/kg body weight/day) enterally for 10 days. Previous investigators have used 300–315 μg of isotretinoin/g diet (approximately 5 mg per day) to produce significant increases in rat plasma triacylglycerols (25, 26). Although the isotretinoin dose was considerably higher than that used in humans (0.5–2 mg/kg per day), we observed no clinical signs of toxicity. We have also found that the plasma levels of isotretinoin 30 min after a 10-mg dose are 2–6 $\mu\text{g}/\text{ml}$ (A. Vahlquist, unpublished observations), i.e., less than 4 times higher than the usual peak values in humans (27). This indicates that the drug is much more rapidly metabolized (or less efficiently absorbed) in rats than in humans.

The animals were fasted for 12 h before the injection of $^{125}\text{I-TC-VLDL}$ for several reasons: *i*) the liver cells are more easily separated when not filled with glycogen; *ii*) fasting eliminates lipid transport from the gut; and *iii*) fasting reduces the risk for spurious lowering of the lipoprotein-elimination capacity secondary to overloading of the binding sites.

The retarded disappearance of $^{125}\text{I-TC-VLDL}$ from the blood of the treated animals, as compared to controls, can be explained by differences in elimination both via the rapid α -phase and the slower β -phase. For smaller plasma proteins, the α -phase usually represents the elimination of tracer from blood via diffusion into the interstitium. However, it has been clearly shown by agarose gel electrophoresis of human suction-blister fluid that VLDL are unable to pass into the interstitial fluid (28). The rapid α -phase elimination of labeled VLDL particles from the blood is

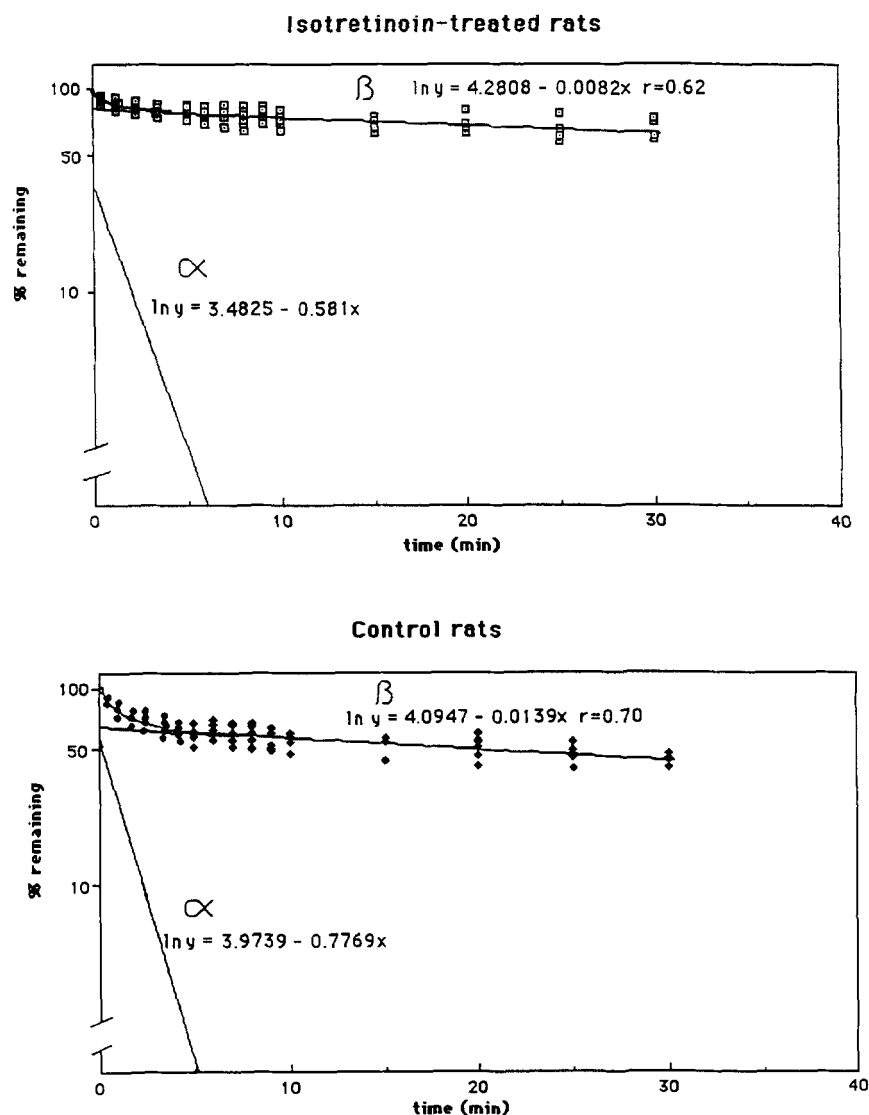


Fig. 4. Semilogarithmic plots of the elimination of label from the circulation (individual values). The α -phase was derived by curve peeling. For experimental conditions see Fig. 3.

therefore more likely due to their binding to receptors or enzymes lining the blood vessels. The α -phase constituted a larger proportion of the disappearance in the untreated animals, indicating that more labeled material can be bound initially to cellular receptors or enzymes. We have previously shown that VLDL readily bind to liver endothelial cells (17, 18), the only cell type known to carry hepatic lipase (29) an enzyme involved in the clearance of VLDL remnants (30). It is possible that the reduced initial disappearance of radioactivity from the blood of the treated animals is caused by decreased binding to liver endothelial cells, a deficiency of hepatic lipase, or a saturation of this enzyme with VLDL.

Another possibility would be that changes in extrahepatic lipoprotein lipase activities are the basis for reduced

liver uptake of VLDL in isotretinoin-treated animals. It has been shown previously that as triacylglycerols are removed from VLDL by lipoprotein lipase, the hepatic uptake of the particles is promoted (31). Since in our present study more than 95% of the radioactivity was still present in the VLDL fraction after 30 min of circulation, the modifications of the VLDL particles were probably not far reaching, but may have been large enough to influence the hepatic uptake of the particles. In this context it is of interest that, as mentioned above, decreased muscle lipoprotein lipase activities have been recorded in retinoid-treated subjects (9).

The β -phase of the ^{125}I -TC-VLDL disappearance is most probably due to catabolism or cellular internalization of the tracer. In fact, the fractional catabolic rate was

TABLE 2. Distribution of ^{125}I -TC-VLDL between different organs and liver cells

Source	Controls	Isotretinoin	Significance
	%	%	
Liver	34.1 ± 7.3	23.6 ± 4.6	0.013
PC	31.8 ± 6.9	20.8 ± 4.1	0.007
NPC	2.3 ± 0.6	2.8 ± 0.7	> 0.05
Kidneys	2.5 ± 1.2	2.9 ± 1.7	> 0.05
Spleen	0.40 ± 0.14	0.44 ± 0.27	> 0.05
Lungs	0.12 ± 0.08	0.14 ± 0.04	> 0.05

The distribution is the result of six different experiments in each group (percent of injected dose, mean ± SD). Injection of ^{125}I -TC-VLDL and determination of radioactivity were as described in Methods; PC, parenchymal cells; NPC, nonparenchymal cells.

more than 97% dependent on the elimination via the β -phase in both groups. The increased $t_{1/2-\beta}$ observed in the treated rats resulted in a significant reduction of the fractional catabolic rate by approximately 50%. This reduction can be explained by a reduced uptake of the tracer by liver PC. As the removal of ^{125}I -TC-VLDL, prepared from isotretinoin-treated animals, cannot be distinguished from that of control VLDL, isotretinoin-induced lowering of the VLDL clearance capacity is not due to intrinsic alterations of the VLDL particles, but must reside in a changed catabolic mechanism in the liver.

To conclude, our results indicate that the hypertriglyceridemia seen after medication with isotretinoin is caused mainly by a reduced uptake and degradation of VLDL by liver parenchymal cells. The mechanism behind this reduction is presently unknown. \square

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REFERENCES

- Marsden, J. 1986. Hyperlipidemia due to isotretinoin and etretinate treatment: possible mechanism and consequences. *Br. J. Dermatol.* **114**: 401-407.
- Vahlquist, C., G. Michaëlsson, A. Vahlquist, and B. Vessby. 1985. A sequential comparison of etretinate (Tigason[®]) and isotretinoin (Roaccutane[®]) with special regard to their effects on serum lipoproteins. *Br. J. Dermatol.* **112**: 69-76.
- Melnik, B., U. Bros, and G. Plewig. 1987. Evaluation of the atherogenic risk of isotretinoin-induced and etretinate-induced alterations of lipoprotein cholesterol metabolism. *J. Invest. Dermatol.* **88**: 39-43.
- Åberg, H., H. Lithell, I. Selinus, and H. Hedstrand. 1985. Serum triglycerides are a risk factor for myocardial infarction but not for angina pectoris: results from a 10-year follow-up of Uppsala Primary Preventive Study. *Atherosclerosis.* **54**: 89-97.
- Goldstein, J., and M. Brown. 1977. The low-density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46**: 897-930.
- Gordon, T., W. Castelli, M. Hjortland, W. Kannel, and T. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease: the Framingham study. *Am. J. Med.* **62**: 707-714.
- Gerber, L., and J. Erdman. 1981. Hyperlipidemia in rats fed retinoic acid. *Lipids.* **16**: 496-501.
- Gollnick, H. 1985. Retinoids and blood lipids; an update and review. In *Retinoids: New Trends in Research and Therapy*. J. Saurat, editor. Karger, Basel, Switzerland, 445-460.
- Vahlquist, C., H. Lithell, G. Michaëlsson, I. Selinus, A. Vahlquist, and B. Vessby. 1987. Plasma fat elimination, tissue lipoprotein lipase activity and plasma fatty acid composition during sequential treatment with etretinate and isotretinoin. *Acta Derm. Venereol. (Stockh.)* **67**: 12-15.
- Gollnick, H., W. Schwartzkopff, M. Schleising, and C. E. Orfanos. 1983. Verhalten der lipolytischen Aktivität des Serums vor und inunter 13-*cis*-Retinsäure bei parenteraler Fettbelastung. *Hautarzt.* **34 Suppl VI**: 268-270.
- Vahlquist, C., I. Selinus, and B. Vessby. 1988. Serum lipid changes during Acitretin (etretin) treatment of psoriasis and palmo-plantar pustulosis. *Acta Derm. Venereol. (Stockh.)* **68**: 300-305.
- Pittman, R. C., T. E. Carew, C. K. Glass, S. R. Green, C. A. Taylor, and A. D. Attie. 1983. A radioiodinated, intracellularly trapped ligand for determining the sites of plasma protein degradation in vivo. *Biochem. J.* **212**: 791-800.
- Eklund, A., L. Sjöblom, and A-M. Östlund-Lindqvist. 1986. Accumulation of cholesteryl esters and triglycerides in cultured macrophages incubated with plasma very low density lipoproteins from rats fed on casein or soybean protein diets containing moderate levels of cholesterol. *Atherosclerosis.* **62**: 129-136.
- Oschry, Y., and S. Eisenberg. 1982. Rat plasma lipoproteins: re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. *J. Lipid Res.* **23**: 1099-1106.
- Eklund, A., and L. Sjöblom. 1986. Improved banding pattern of rat plasma lipoproteins developed by agarose gel electrophoresis at pH 7.0. *Biochim. Biophys. Acta.* **877**: 135-140.
- Sjöblom, L., and A. Eklund. 1989. Determination of HDL₂-cholesterol by precipitation with dextran sulfate and magnesium chloride: establishing optimal conditions for rat plasma. *Lipids.* **24**: 532-534.
- Gustafson, S., H. Pertoft, and B. Vessby. 1989. Initial binding of very low density lipoproteins to non-parenchymal liver cells, predominantly liver endothelial cells, during in vivo circulation in rats. In *Cells of the Hepatic Sinusoid*. E. Wisse, D. L. Knook, and K. Decker, editors. Volume 2. The Kupffer Cell Foundation, Rijswijk, The Netherlands, 163-165.
- Gustafson, S., A-M. Östlund-Lindqvist, and B. Vessby. 1985. Uptake and degradation of human very low density lipoproteins by rat liver endothelial cells in culture. *Biochim. Biophys. Acta.* **843**: 308-315.
- Lindqvist, P., A-M. Östlund-Lindqvist, J. L. Witztum, D. Steinberg, and J. A. Little. 1983. The role of lipoprotein lipase in the metabolism of triglyceride-rich lipoproteins by macrophages. *J. Biol. Chem.* **258**: 9086-9092.
- Öbrink, B. 1982. Hepatocyte-collagen adhesion. *Methods Enzymol.* **82**: 513-529.

21. Smedsrød, B., and H. Pertoft. 1985. Preparation of pure hepatocytes and reticuloendothelial cells in high yield from a single rat liver by means of Percoll centrifugation and selective adherence. *J. Leukocyte Biol.* **38**: 213-230.
22. Knook, D. L., and C. Sleyster. 1976. Separation of Kupffer and endothelial cells of the rat liver by centrifugal elutriation. *Exp. Cell Res.* **99**: 444-449.
23. Nosslin, B. 1964. *Cited in Metabolism of Human Gammaglobulin*. S. B. Anderson, Blackwell Oxford.
24. Vahlquist, A., P. A. Peterson, and L. Wibell. 1973. Metabolism of the vitamin A transporting protein complex. I. Turnover studies in normal persons and in patients with chronic renal failure. *Eur. J. Clin. Invest.* **3**: 352-362.
25. Gerber, L., and J. Erdman. 1980. Comparative effects of all-*trans* and 13-*cis* retinoic acid administration on serum and liver lipids in rats. *J. Nutr.* **110**: 343-351.
26. Alam, B., and S. Alam. 1983. Excessive intake of 13-*cis* retinoic acid and fatty acid composition of tissues. *J. Nutr.* **113**: 64-69.
27. Vahlquist, A., and O. Rollman. 1987. Clinical pharmacology of three generations of retinoids. *Dermatologica.* **175 Suppl. I**: 20-27.
28. Vessby, B., S. Gustafson, M. J. Chapman, K. Hellsing, and H. Lithell. 1987. Lipoprotein composition of human suction-blister interstitial fluid. *J. Lipid Res.* **28**: 629-641.
29. Kuusi, T., E. A. Nikkilä, I. Virtanen, and P. K. J. Kinnunen. 1979. Localization of the heparin-releasable lipase in situ in the rat liver. *Biochem. J.* **181**: 245-246.
30. Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45**: 161-179.
31. Windler, E., Y-S. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255**: 5475-5480.