Influence of nicotinic acid on metabolism of cholesterol and triglycerides in man[1]

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Abstract The mechanisms for the hypolipidemic action of nicotinic acid were examined in 12 patients with hyperlipidemia. Most patients were studied in the hospital on a metabolic ward. The first month was a control period followed by 1 month on nicotinic acid. During treatment with nicotinic acid, the triglycerides (TG) decreased in total plasma by an average of 52% and in very low density lipoproteins (VLDL) by 36%. Transport rates of VLDL-TG were determined by multicompartmental analysis following injection of [^{3}H]glycerol as a precursor. Nicotinic acid decreased transport (synthesis) of VLDL-TG by an average of 21%. Kinetic modeling of the VLDL-TG data suggested that the TG reduction was due to a decrease in TG content of VLDL and hence a reduction in lipoprotein size more than number. For the whole group, plasma cholesterol fell during nicotinic acid therapy by a mean of 22%. The drug produced no detectable changes in fecal excretions of cholesterol (neutral steroids) or bile acids. However, it induced a small but significant increment in hepatic secretion of biliary cholesterol that might have led to a net loss of cholesterol from the body even though this loss could not be detected by sterol balance. Despite this increase in outputs of biliary cholesterol, there was not a significant increase in molar % cholesterol or in % saturation of gallbladder bile. Therefore, it is doubtful that nicotinic acid enhances the risk for cholesterol gallstones.—**Grundy, S. M., H. Y. I. Mok, L. Zech, and M. Berman.** Influence of nicotinic acid on metabolism of cholesterol and triglycerides in man. *J. Lipid Res.* 1981. **22:** 24–36.

Supplementary key words very low density lipoprotein · sterol balance · biliary lipids

The hypocholesterolemic effect of large doses of nicotinic acid was reported first by Altschul, Hoffer, and Stephen in 1955 (1) and was examined in considerable detail by several workers over the next few years (2–9). In several studies, it was observed that the drug also lowered plasma triglycerides (TG). Although the magnitude of plasma lipid lowering by nicotinic acid can be appreciable, its usefulness has been limited by certain disagreeable side effects such as flushing of the face and other skin reactions. Nevertheless, as its potential usefulness has become recognized, the drug has received renewed interest. The Coronary Drug Project (CDP) (10) showed that nicotinic acid can be taken for prolonged periods without serious toxicity; furthermore, in this study it caused an average lowering of plasma cholesterol of 9% and reduced the recurrence rate of myocardial infarction by 29%. Other recent studies have indicated that the drug can also add to the lipid-lowering action of other drugs, particularly clofibrate (11) and bile acid-binding resins (12–14).

Despite its potential usefulness in treatment of hyperlipidemia, the mechanisms by which nicotinic acid reduce TG and cholesterol in plasma have not been elucidated completely. For lowering of TG, both decreased influx and increased removal have been postulated (7–9, 15–17). Decreased cholesterol synthesis has been reported which might contribute to cholesterol lowering (18–20). The present study was undertaken to examine these possible mechanisms further; to do so we have carried out simultaneous measurements of metabolism of TG and cholesterol in hyperlipidemic patients treated with nicotinic acid.

METHODS

Patients

This study was performed in 12 male patients on the Special Diagnostic and Treatment Unit (metabolic unit) of the Veterans Administration Medical Center, San Diego, CA. The body habitus, plasma cholesterol, and TG at time of admission, and diagnosis of each patient are presented in **Table 1.** At admission, all patients had hyperlipidemia and most had ele-

Abbreviations: TG, triglyceride; GLC, gas–liquid chromatography; NA, nicotinic acid; IW, ideal weight; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein.

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TABLE 1. Clinical data

Patient	Age	Height	Weight	Ideal Weight	Cholesterol	TG	Clinical Diagnosis[a]
	yr	*cm*	*kg*	*%*	*mg/dl*	*mg/dl*	
1	54	183	78	105	233	242	Normal
2	42	175	80	119	389	750	Normal
3	50	169	65	101	418	734	CHD; PVD
4	59	186	94	127	298	232	Normal
5	54	181	95	133	362	259	CHD
6	56	168	72	116	324	825	CHD
7	52	178	73	109	273	322	Normal
8	49	169	77	122	343	570	CHD
9	60	174	72	109	274	110	CHD
10	51	171	81	128	519	1251	CHD; PVD
11	64	168	70	112	308	175	Normal
12	59	173	75	111	280	186	Normal

[a] CHD = Coronary heart disease; PVD = peripheral vascular disease.

vated TG. Following admission and ingestion of a low-cholesterol diet, levels of plasma lipids frequently decreased as we have observed commonly in patients under metabolic ward conditions. Several patients had mild abnormalities in glucose tolerance as is characteristic of patients with hypertriglyceridemia (21), but none had fasting hyperglycemia or required hypoglycemic agents. A detailed genetic classification of the type of hyperglyceridemia according to Goldstein et al. (22, 23) was not carried out. Some patients had clinical atherosclerotic disease, but none had congestive heart failure or evidence of liver or gastrointestinal disease. None had previous cholecystectomy. All patients gave informed consent for the investigation.

Experimental design

Patients were studied during two periods, each of approximately 5-weeks duration. The first period was for control studies; in the second, nicotinic acid was given at a dosage of 1 g three times daily. Nine patients (Nos. 1–9) were hospitalized on the metabolic unit throughout the entire study. Three patients (Nos. 10, 11, and 12) were studied as outpatients being admitted to the hospital only for tests. Those patients remaining on the metabolic ward were fed a diet of solid food and liquid formula, which was identical each day, throughout both periods. Plasma cholesterol and triglycerides were estimated twice weekly. An additional series of metabolic studies was carried out on as many patients as possible. For a variety of reasons some of the patients were unable to undergo all of the studies. The particular patients receiving each test are designated in the tables.

The general design of the study was as follows. In most of those patients who underwent long-term hospitalization, cholesterol balance measurements were carried out; this was done during the first 4 wk of each period. Immediately thereafter estimations of hepatic secretions of biliary lipids and of pool sizes of bile acids were made. From the combined measurements of fecal steroids and hepatic secretions of biliary cholesterol, it was possible to estimate cholesterol absorption in several of the patients. During the time of cholesterol balance, multiple determinations of lipid composition of gallbladder bile were carried out. Finally, at the end of each period after completion of biliary secretion studies, transport rates of TG in very low density lipoproteins were estimated.

None of the three outpatients underwent cholesterol balance measurements; they were admitted only for studies of plasma lipids, TG kinetics, and biliary lipid metabolism. They were on an ad lib diet at home but maintained constant weight throughout the study.

During treatment with nicotinic acid, after an initial period of flushing of the face, as typically occurs with this drug, all patients tolerated the drug well and had no significant side effects. Liver function tests and plasma glucose and uric acid concentrations were measured at the beginning and end of the control period and after treatment with nicotinic acid. All values were in the normal range after completion of drug treatment.

Diets

The metabolic diet consisted of mixed solid food and liquid formula containing 40% of calories as fat. The basic composition and pattern of this diet has been described previously in detail (24). The patients were given three liquid meals and two solid-food meals per day; calories were divided approximately equally between the feedings. Liquid formulas were given at 8:30 AM, 1:00 PM, and 7:00 PM; they contained 15% of calories as milk protein, 45% as dextrose, and

40% as fat, mostly in the form of lard. These diets were prepared by Hospital Diet Products, Organon Corp., Buena Park, CA (courtesy of Mr. Alfred Teixeira). One solid-food meal was given at 11:00 AM and it contained dry cereal (corn flakes), nonfat bread, skim milk, added fat, and sugar for coffee. At 4:00 PM, the second meal was given which consisted of chicken that was stripped of fat, nonfat bread, potatoes, fat, and carbonated beverage (cola). Fat comprised approximately 40% of calories in solid-food meals. Cholesterol intakes ranged from 168 to 219 mg/day. Vitamin and mineral supplements were given daily. Each patient was weighed daily and caloric intake was adjusted to maintain total body weight at a constant level throughout the study.

Plasma concentrations of cholesterol and TG

Plasma concentrations of cholesterol and TG were estimated twice weekly using a Technicon Auto-Analyzer (model II, Technicon Instruments, Corp., Tarrytown, NY) (25, 26). Lipoprotein electrophoresis was carried out according to the method of Noble (27).

Cholesterol balance studies

Cholesterol balance studies were carried out in the hospital on seven patients as described previously (28–31). These patients were hospitalized throughout the balance study. Stools were collected throughout both dietary periods and were usually combined into 4-day pools. Fecal neutral and acidic steroids were isolated separately, and their masses were determined by gas–liquid chromatography (GLC) (28, 29). GLC analysis of neutral steroids distinguished between cholesterol and plant sterols and their steroid conversion products. Analyses were carried out entirely by chemical procedures. To correct for losses of neutral steroids, β-sitosterol was given as capsules (225 mg twice daily) (30), and excretion of acidic steroids was corrected for variations in fecal flow by use of chromic oxide (31).

Lipid composition of gallbladder bile

In ten patients, fasting gallbladder bile was obtained three times during each of the two study periods for lipid analysis. Samples were aspirated from a single-lumen tube positioned by X-ray guidance in the second portion of the duodenum. Gallbladder contraction was stimulated by intraduodenal injection of an emulsion of safflower oil, which was free of cholesterol and phospholipids. Gallbladder bile-rich duodenal fluid was then collected by slow suction over a period of 20 min. The collected bile (30–50 ml) was thoroughly mixed and a 10-ml sample was retained for analysis; the remainder was returned to the patient via the tube. Samples were added immediately to 30 ml of chloroform–methanol 2:1. Cholesterol and phospholipids were partitioned into the chloroform phase, and conjugated bile acids were in the water–methanol phase (32, 33). The phases were separated, and appropriate washings were carried out for quantitative isolation of individual constituents. After evaporation of the chloroform phase, cholesterol was measured directly on GLC as the trimethylsilyl (TMS) ether (34); 5-cholestane was used as an internal standard for GLC. Phospholipids were measured by the method of Rouser, Sidney, and Akira (35), and bile acids were determined by a standard enzymatic procedure (36). Bile lipid composition was expressed as molar % for each lipid component according to Admirand and Small (37). Percent saturation was calculated by the criteria of Carey and Small (38). These workers found that saturation of bile is a function of total solids present. For calculation of % saturation we have assumed that gallbladder bile contained 10% solids.

Outputs of biliary lipids

Hourly outputs of biliary cholesterol, bile acids, and phospholipids during constant feeding of a formula diet were determined in six patients by the marker-dilution technique of Grundy and Metzger (39). After an overnight fast, a three-lumen tube was positioned in the duodenum with the two most proximal outlets adjacent to the ampulla of Vater and the third outlet 10 cm distally. The tube was placed in the correct position with X-ray guidance. The same liquid formula as used throughout the study was infused continuously through one proximal lumen; β-sitosterol was also infused as a dilution marker. After allowing 4 hr for gallbladder contraction and for stabilization of hepatic bile secretion, hourly samples were obtained for 6 hr from the second proximal and distal outlets by slow and continuous aspiration. Less than 5% of intestinal contents passing these ports was removed. Since the input of β-sitosterol was known with precision, measurements of β-sitosterol and cholesterol recovery at the distal outlet gave the rate of cholesterol secretion. These data, combined with measurements of concentrations of bile acids and phospholipids relative to cholesterol at the proximal outlet, permitted calculation of the hourly outputs of bile acids and phospholipids. Equations used in these calculations have been presented previously along with corrections for cholesterol contents of formula diets (39).

Pool size of bile acids

The pool size of bile acids was measured simultaneously with hepatic secretion rates as described before (24). Briefly, 5 μCi of [24-^{14}C]cholic acid (New

England Nuclear Corp., Boston, MA) was given intraduodenally at the beginning of the formula infusion. After 4 hr, which allowed for equilibration, the ratio of isotope to total bile acids ("specific activity") became constant (24). A mean specific activity was determined on hourly samples over the next 6 hr, and the total pool of bile acids was determined by dividing the dose of radioactivity given by the mean specific activity.

Cholesterol absorption

Net absorption of cholesterol was measured in five patients as recently described (40). The basic equation for determining cholesterol absorption is as follows:

mass absorbed = (daily biliary secretion

+ exogenous intake) − daily fecal excretion.

Daily secretion rates of cholesterol were estimated by multiplying hourly secretion rates during continuous liquid formula infusion by 24; this conversion is based on our recent finding that hourly secretion of biliary cholesterol × 24 closely approximates its duodenal output throughout a 24-hour period measured in patients given three equal formula meals at 8 AM, 1 PM, and 6 PM (40).

When estimating cholesterol absorption by this method, the value obtained is the net absorption of cholesterol between the upper duodenum and the anus. If the intestine contributes cholesterol to the lumen during the measurement, the net absorption will be reduced correspondingly. However, as we have shown (40), values for percentage cholesterol absorption are as high or higher than those obtained previously by other methods, which should not occur if intestinal secretion of cholesterol had been appreciable.

Transport of very low density lipoprotein-triglycerides (VLDL-TG)

The protocol for estimating VLDL-TG transport was essentially that described recently from our laboratory (41). This was the last test done at the end of each period. Briefly, each patient was given a fat-free liquid diet every 3 hr around the clock beginning 36 hr before injection of [^{3}H]glycerol and for 48 hr following the injection. This fat-free diet contained 60% of the calories needed to maintain weight in the prestudy period. Removal of fat from the diet was necessary to prevent contamination of VLDL-TG with intestinal chylomicron-TG, and caloric intake was reduced to prevent carbohydrate-induced hypertriglyceridemia (21). On the other hand, maintenance of some caloric intake was needed to prevent a fall in VLDL-TG that would result from prolonged fasting (42). With the feeding schedule chosen, VLDL-TG concentrations were constant throughout the study.

After the 36-hr equilibration period, 300 μCi of [2-^{3}H]glycerol (New England Nuclear Corp.) was given intravenously, and blood samples were drawn at 15 min, 30 min, 1.0, 1.5, 2.0, 2.5, 4, 5, 6, 7, 9, 11, 13, and 15 hr, and then every 3 hr up to 48 hr. Blood samples containing EDTA were immediately centrifuged at 3000 rpm for 20 min at 5°C. The plasma was stored at 4°C. VLDL was isolated by preparative ultracentrifugation (43), and specific activities of VLDL-TG were estimated along with cholesterol content as described recently (25).

From plasma activities of VLDL-TG, several parameters of VLDL metabolism were determined. All simulation and data-fitting were performed using the SAAM simulator (on a Univac 1008) and the model of Zech et al. (41). Values calculated included fractional catabolic rates (FCR_C^{TG}) and transport (or synthesis) of VLDL-TG ($R_{gly,VLDL}^{TG}$ or R_{VLDL}^{TG}). Details of methodology and validation of this kinetic approach have been set forth in detail in the report of Zech et al. (41), and the notations used in the current study are the same as in that report.

In fitting the data to the model, some looseness of fit was noted when estimating the parameter values. Consequently, two null hypotheses concerning the comparison of VLDL-TG metabolism before and after nicotinic acid therapy were considered and tested. For the first, it was assumed that the residence time of VLDL particles in plasma (T_C^{VLDL} in Ref. 41) remained unchanged under nicotinic acid perturbation. This hypothesis was rejected because a reasonable fit of the data could not be obtained in the portion of the activity curve 6 hr after the peak in most of the subjects examined. The second hypothesis proposed that the fractional catabolic rate of delipidation at each of the delipidation steps ($L_{4,1}$ in Ref. 41) remained unchanged. This hypothesis provided as good a fit of the data as did the unconstrained model, with greater confidence in the parameter values. For this reason, the second hypothesis was adopted for analysis of the data.

From the parameters derived from these analyses, the particle size of VLDL relative to intermediate density lipoprotein (IDL) was estimated. Assuming IDL to be a unit size, the relative size of VLDL as it enters the plasma can be calculated as follows:

$$\frac{1}{\left(\frac{(4 - L_{4,1}) \times T_C^{VLDL}}{4}\right)^4}$$

where: T_C^{VLDL} = residence time of VLDL particles in the delipidation chain (41).

TABLE 2. Plasma lipid during control and nicotinic acid treatment

Patient	Period	Plasma Chol	Plasma TG
		mg/dl ± SD(n)[a]	
1	I	224 ± 9 (6)	263 ± 38
	II	204 ± 6 (7)[b]	123 ± 30[b]
2	I	266 ± 14 (7)	460 ± 81
	II	202 ± 15 (4)[b]	312 ± 56[b]
3	I	344 ± 34 (6)	777 ± 196
	II	276 ± 18 (6)[b]	222 ± 35[b]
4	I	237 ± 12 (7)	247 ± 37
	II	207 ± 6 (8)[b]	179 ± 17[b]
5	I	281 ± 27 (6)	220 ± 10
	II	218 ± 16 (7)[b]	160 ± 24[b]
6	I	276 ± 26 (6)	617 ± 87
	II	211 ± 16 (6)[b]	187 ± 46[b]
7	I	267 ± 15 (6)	604 ± 121
	II	246 ± 14 (6)[b]	396 ± 51[b]
8	I	260 ± 13 (6)	389 ± 74
	II	231 ± 16 (4)[b]	249 ± 33[b]
9	I	300 ± 18 (11)	110 ± 15
	II	259 ± 21 (8)[b]	93 ± 21
10	I	411 ± 122 (8)	914 ± 328
	II	232 ± 23 (6)	242 ± 64
11	I	319 ± 20 (6)	121 ± 33
	II	255 ± 22 (9)	95 ± 14
12	I	268 ± 13 (3)	224 ± 47
	II	169 (1)	118
Mean ± SEM	I	288 ± 15 (12)	412 ± 76
	II	226 ± 9 (12)[c]	198 ± 27

[a] n = Number of determinations of cholesterol and TG.
[b] Period II significantly lower than Period I by Student's *t*-test ($P < 0.05$ or less).
[c] Period II significantly lower than Period I by paired *t*-test ($P < 0.01$ or less).

The relative particle number also can be estimated as VLDL-TG transport (R^{TG}_{VLDL}) ÷ the relative particle size.

Since patients of this study were of different heights and weights, it was necessary to normalize the results so that a meaningful comparison among the various groups could be made. A common method for normalizing VLDL-TG transport data is to express them as mg/hr per kg of total body weight. However, as recently shown (44), calculation of transport rates per kg total body weight may be misleading. In obese subjects, it reduces transport to inordinately low values, as compared to absolute transports. In other words, dividing transport by a large mass of adipose tissue can obscure real increases in production of VLDL-TG, a process presumably confined to the liver and intestine. To overcome this problem, we proposed that it would be preferable to express transport rates as mg/hr per kg ideal weight (IW) (44). In the previous study (44), a tight correlation was noted between rates expressed in this way and absolute transport rates across a wide range of transport rates for subjects of all degrees of obesity. For this reason, transport rates were normalized to mg/hr per kg IW in the Results section.

RESULTS

Plasma lipids and lipoproteins

Mean values for concentrations of plasma cholesterol and TG during control and treatment periods are shown in **Table 2.** In all patients, nicotinic acid therapy produced a significant lowering of both cholesterol and TG. Mean reduction of cholesterol was 22% and for TG was 52%.

Table 3 shows plasma concentrations and transport rates of VLDL-TG for 8 patients treated with nicotinic acid and for 13 normal subjects studied by the same methods (44). Mean VLDL-TG concentration for normal subjects was 113 mg/dl, and for the eight patients before nicotinic acid, it was 247 mg/dl. In normal subjects, mean VLDL-TG transport (R^{TG}_{VLDL}) was 11.5 mg/hr per kg IW and fractional catabolic rate (FCR^{TG}) averaged 0.19 hr^{-1}. The causes of increased plasma VLDL-TG in the eight hyperlipidemic patients of this study were variable. Transport rates were essentially normal in five patients (Nos. 1, 3, 4, 6, and 8), and they had relatively low fractional catabolic rates (range = 0.10–0.14 hr^{-1}). The other three patients (Nos. 5, 7, and 10) had increased transport (synthesis rates) of VLDL-TG (range = 21–40 mg/hr/kg IW).

Nicotinic acid treatment produced a significant decrease in plasma VLDL-TG in all patients (mean decrease = 36%, $P < 0.005$). All except one (No. 8) also had a reduction in transport of VLDL-TG (mean reduction = 21%, $P < 0.05$). Typical plasma activity curves for one patient before and during nicotinic acid therapy are shown in **Fig. 1.** In addition, six of eight patients had an increase in fractional catabolic rate; in the remaining two it was unchanged. The mean increase in fractional catabolic rate was 21% ($P < 0.01$). This corresponded to a 27% decrease in chain residence time (T_C^{VLDL}). The fraction of VLDL-TG synthesis via the nonplasma path (i.e., the proportion of glycerol in VLDL-TG derived from sources other than plasma glycerol) was unchanged by drug treatment. However, nicotinic acid caused a reduction in the portion of VLDL-TG synthesis in the slow pathway ($P < 0.05$).

TABLE 3. VLDL-TG kinetics[a]

Patient	Period	Plasma Volume	VLDL Lipids		R_{VLDL}^{TG}	R_{VLDL}^{TG}	FCR_C^{TG}	T_C^{VLDL}	U_1^{TG}	Fast Path / Slow Path
			TG	Chol/TG						
		dl	*mg/dl*	*ratio*	*mg/hr*	*mg/hr/kgIW*	*hr^{-1}*	*hr*	*%*	*ratio*
1	I[b]	33.5	163	0.27	656	8.9	0.12	11.1	79	0.3
	II[b]		69	0.11	416	5.6	0.18	6.3	87	0.9
3	I	28.9	293	0.30	847	13.2	0.10	12.5	89	1.2
	II		193	0.22	725	11.3	0.13	8.3	89	2.7
4	I	35.6	170	0.18	847	11.5	0.14	9.1	96	1.0
	II		115	0.16	698	9.4	0.17	7.1	93	1.3
5	I	44.8	210	0.21	1505	21.2	0.16	14.3	89	1.2
	II		157	0.20	1125	15.9	0.16	14.3	92	1.0
6	I	29.9	289	0.23	864	13.9	0.10	16.7	95	4.0
	II		141	0.16	675	10.9	0.16	9.1	95	4.0
7	I	31.8	483	0.18	1843	26.3	0.12	12.5	92	1.0
	II		296	0.16	1412	20.2	0.15	9.1	94	2.0
8	I	30.1	248	0.30	821	13.0	0.11	14.3	92	0.3
	II		222	0.18	1002	15.9	0.15	8.3	94	0.5
10	I	31.3	348	0.34	2614	41.5	0.24	12.5	88	0.5
	II		242	0.29	1817	28.8	0.24	12.5	94	1.0
Means ± SEM	I		275 ± 37	0.25 ± 0.02	1250 ± 242	18.7 ± 3.8	0.14 ± 0.02	12.8 ± 1.0	90 ± 2	1.2 ± 0.4
	II		179 ± 26	0.18 ± 0.02	984 ± 160	14.7 ± 2.6	0.17 ± 0.01	9.3 ± 0.9	92 ± 1	1.7 ± 0.4
P value[c]			<0.005	<0.01	<0.05	<0.05	<0.01	<0.01	N.S.	<0.05
Normal (13 subjects)			113 ± 2.8	0.21 ± 0.004	806 ± 34	11.5 ± 0.5	0.19 ± 0.003	5.2 ± 0.1	82 ± 3	2.7 ± 0.1

[a] Abbreviations: $R_{VLDL}^{TG} = R_{gly,VLDL}^{TG}$ (Ref. 41), VLDL-TG synthesis; FCR_C^{TG}, fractional catabolic rate (chain); T_C^{VLDL}, chain residence time; U_1^{TG}, % VLDL-TG glycerol derived from nonplasma sources; IW, ideal weight; C, control period; NA, nicotinic acid period.

[b] Period I, control; Period II, nicotinic acid.

[c] Differences were compared by paired *t*-test.

For reasons indicated in the Methods section, the delipidation rate ($L_{4,1}$) was assumed to be the same for control and treatment periods. From the parameters shown in Table 2, it was possible to estimate a relative particle size and relative particle number of VLDL during the two periods. Although neither absolute sizes nor numbers can be determined, ratios of relative sizes and numbers during the two periods may be calculated from Equation 1. As shown in **Table 4,** the calculations indicate that the relative particle size was smaller during nicotinic acid (NA) treatment than in the control (C) state (NA/C = 0.79 ± 0.2, $P < 0.05$). In contrast, the relative particle number was unchanged by treatment (NA/C = 0.97 ± 0.28, N.S.). In spite of a reduction in particle size, nicotinic acid caused a decrease in cholesterol/TG ratios in VLDL; this suggests the formation of small, cholesterol-poor VLDL.

Cholesterol balance

Table 5 presents excretions of neutral steroids and acidic steroids as well as cholesterol balance for eight patients. Six of the eight had numerical increases in neutral steroid excretion on nicotinic acid. In only two, however, were the increases statistically significant, and changes were not significant for the whole group. Acidic steroid excretion was essentially unchanged, as was cholesterol balance. Thus, the only apparent change on the drug, if any, was a slightly increased excretion of neutral steroids.

Biliary lipids

Table 6 gives the lipid composition and saturation indices of gallbladder bile in ten patients before and during treatment with nicotinic acid. In eight of ten patients, gallbladder bile was supersaturated with cholesterol in the control period. Eight of the patients also had an increment in molar % cholesterol and saturation indices when nicotinic acid was started; however, the changes generally were small, and increments for the group were not statistically significant.

In **Table 7,** hourly outputs of biliary lipids are given for six patients along with lipid composition and pool sizes of bile acids. All six patients had in-

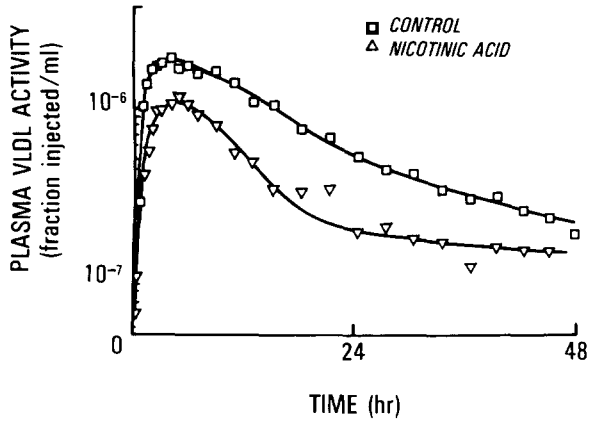

Fig. 1. Typical activity curve before and after nicotinic acid therapy. All fractional rate constants are the same as Fig. 2, reference 41, except the following: before nicotinic acid: $L_{1,14} = 1.5 \pm 0.18$; $L_{1,24} = 0.03 \pm 0.21$; $L_{1,1} = 0.31 \pm 0.12$; $L_{4,1} = 0.03 \pm 0.05$; $L_{10,4} = 0.10 \pm 0.08$; $L_{1,24} = 0.11 \pm 0.11$; delay 0.32 hr ± 0.08. After nicotinic acid: $L_{1,14} = 0.69 \pm 0.15$; $L_{1,24} = 0.01 \pm 0.56$; $L_{1,1} = 0.46 \pm 0.07$; $L_{4,1} = 0.03 \pm 0.05$; $L_{10,4} = 0.07 \pm 0.08$; $L_{1,24} = 0.14 \pm 0.33$; delay 0.32 hr ± 0.08.

creased outputs of cholesterol on the drug; the increase averaged 26% and was statistically significant. No significant changes were noted for outputs of bile acids or phospholipids, or for pool sizes of bile acids. Despite the consistent increase in hepatic secretion of cholesterol on nicotinic acid, the lipid composition and saturation indices of stimulated hepatic bile were not increased significantly.

Cholesterol absorption

The net absorption of cholesterol for five patients before and during treatment with nicotinic acid is presented in **Table 8.** Absorption in the control period ranged from 54 to 78% (mean 63.8 ± 4.1%), and the percentage absorption was not changed significantly by drug therapy (68.8 ± 3.5%). It might be noted that the hepatic secretion of cholesterol was greater on nicotinic acid therapy as was the total mass absorption of cholesterol.

DISCUSSION

In accord with previous work, we have found that nicotinic acid reduces both cholesterol and TG in plasma. Plasma cholesterol fell an average of 22% and total TG was reduced by 52%. To our knowledge, no other single agent has such potential for lowering both cholesterol and TG. On the basis of current and previous observations, we might speculate about the mechanisms for reduction of both plasma lipids.

A decrease in TG could result from a lesser influx of VLDL-TG into plasma or an increase in its clearance. A reduced input might be anticipated from previous reports that nicotinic acid inhibits release of free fatty acids (FFA) by adipose tissue, both in vitro and in vivo (7–9, 45). Decreased availability of FFA in turn might retard hepatic synthesis of VLDL-TG, but this action has never before been documented for nicotinic acid.

A second possibility is that the drug could accelerate clearance of VLDL-TG. This mechanism was suggested by Carlson and Oro (15) who found that nicotinic acid is most effective in patients who probably have a decreased clearance of TG, i.e., in those with hyperlipidemias of Type V, severe Type IV, and Type III. Nevertheless, these workers with Boberg et al. (46) failed to observe an increase in heparin-releasable, lipoprotein lipase during treatment with nicotinic acid. This contrasts to clofibrate which augments lipoprotein lipase (47) as it promotes TG clearance (48). An alternative mechanism for increased clearance has been suggested by Carlson, Eriksson, and Walldius (17). They reported a case of massive hypertriglyceridemia associated with a defect in incorporation of fatty acids into adipose tissue glycerides; this defect was corrected by nicotinic acid which produced a marked reduction on plasma TG.

The present work suggests that the predominant mechanism for TG-lowering by nicotinic acid is a reduction in transport (or production) of VLDL-TG. The drug caused a reduction in total synthesis of VLDL-TG averaging 21%. It also reduced the proportion of VLDL-TG produced via the slow synthesis pathway. A decreased synthesis of VLDL-TG theoretically could be due to either of two mechanisms: *a*) a reduction in particle size with less TG per particle, or *b*) a decrease in the number of particles produced. The former seemed to predominate in most of our patients; that is, most produced smaller VLDL with

TABLE 4. Relative VLDL particle size and number

Patient	Delipidation Rate ($L_{4,1}$)	Relative Particle Size	Relative Particle Number
		NA/C[a]	*NA/C*[a]
1	0.07	0.68	0.87
3	0.05	0.79	1.06
4	0.07	0.89	0.93
5	0.08	1.00	0.72
6	0.03	0.78	1.00
7	0.08	0.72	1.05
8	0.08	0.49	2.50
10	0.24	1.00	0.75
Mean ± SEM	0.09 ± 0.02	0.79 ± 0.07	0.97 ± 0.10
P value[b]		<0.05	NS

[a] NA/C, ratio of nicotinic acid period (NA) to control period (C).
[b] Significance of difference was obtained using a paired *t*-test of the ratio of each patient against unity.

TABLE 5. Fecal steroid excretion during control and treatment periods

Patient	Period	Days:No[a] determ.	Cholesterol Intake	Total Neutral Steroids	Acidic Steroids	Total Fecal Steroids	Cholesterol Balance
				mg/day ± SD			
1	I	34:4	207	631 ± 55	414 ± 323	1045 ± 348	838 ± 348
	II	25:6	207	670 ± 43	295 ± 27	966 ± 49	759 ± 49
	Δ			+39[b]	−119	−79	−79
2	I	28:5	209	814 ± 49	338 ± 14	1152 ± 63	943 ± 63
	II	25:5	209	1068 ± 9	310 ± 72	1378 ± 97	1169 ± 97
	Δ			+254[c]	−28	+226[c]	+226[c]
5	I	30:6	219	701 ± 67	526 ± 6	1227 ± 226	1008 ± 226
	II	25:5	219	885 ± 125	313 ± 80	1198 ± 181	979 ± 181
	Δ			+184[c]	−213[d]	−29	−29
3	I	29:6	206	543 ± 56	616 ± 53	1159 ± 84	953 ± 84
	II	34:6	201	378 ± 96	664 ± 72	1042 ± 86	841 ± 117
	Δ			−165[d]	+48	−117[d]	−112
4	I	28:6	219	787 ± 424	385 ± 148	1172 ± 292	953 ± 293
	II	22:6	219	987 ± 304	404 ± 86	1392 ± 334	1173 ± 334
	Δ			+200	+19	+220	+220
8	I	33:7	207	365 ± 84	527 ± 81	892 ± 94	685 ± 94
	II	22:5	207	422 ± 97	496 ± 134	918 ± 197	711 ± 197
	Δ			+57	−31	+26	+26
9	I	28:6	168	541 ± 116	196 ± 24	737 ± 122	569 ± 122
	II	31:6	168	507 ± 82	159 ± 12	663 ± 86	495 ± 86
	Δ			−34	−37	−74	−74
Mean ± SEM	I		205 ± 6	626 ± 60	429 ± 53	1055 ± 68	850 ± 62
	II		204 ± 6	702 ± 106	377 ± 62	1080 ± 99	875 ± 94
	Δ		−1	+76	−52	+25	+25

[a] Days in each period:number of pools analyzed.
[b] Unless specifically designated (see footnotes *c* and *d*), the differences between periods I and II were not significantly different by Student's *t*-test, or paired *t*-test.
[c] Values for period II significantly higher than for period I ($P < 0.05$; Student's *t*-test).
[d] Values for period II significantly lower than for period I ($P < 0.05$; Student's *t*-test).

drug therapy than during control. In two patients (Nos. 5 and 10), however, relative particle sizes remained about the same, but relative particle number was reduced.

In addition, the fractional catabolic rate of VLDL-TG was greater in most patients during treatment with nicotinic acid, and the residence time of VLDL in the delipidation chain was decreased. This finding does not necessarily reflect an increased rate of removal of TG; since VLDL was probably smaller, absolute clearance rates of TG from plasma were actually lower during treatment. This is because smaller particles should pass through the delipidation chain more rapidly than larger ones.

The cause for the lowering of plasma cholesterol by nicotinic acid has not been worked out with certainty, but a significant portion of the decline occurs in LDL (14). Langer and Levy (49) have reported that the drug reduces the input of LDL-apoprotein B, and since LDL-cholesterol also is reduced, it seems likely that the number of particles in the LDL fraction is decreased. Since LDL is a catabolic product of VLDL (50), one possible mechanism for decreased LDL particle number could be a reduction in the number of VLDL secreted into plasma. We should note however that the relative particle size of VLDL was reduced to a greater extent than relative particle number. Thus, a decrease in VLDL-TG secretion without a reduction in VLDL particle number need not necessarily cause a reduction of LDL. Therefore, other causes of reduced LDL synthesis might be considered as well. For example, nicotinic acid might promote hepatic clearance of VLDL remnants which would prevent their conversion to LDL (51–54); or, direct synthesis of LDL (independent of VLDL), which has been reported for some patients (53, 55), might be inhibited by the drug.

The actions of nicotinic acid on plasma lipids and lipoproteins might or might not be associated with changes in overall cholesterol metabolism. Several investigators (12, 18, 19) have reported in vitro evidence for reduced synthesis of cholesterol in livers of

TABLE 6. Effects of nicotinic acid on lipid composition of gallbladder bile

Patient	Period	Cholesterol	Bile Acids	Lecithin	Bile Saturation (10% Solids)
			molar %		%
1	I	12.3 ± 1.6 (4)[a]	61.1 ± 3.1	26.6 ± 2.9	152
	II	6.2 ± 2.6 (8)	71.7 ± 6.3	22.0 ± 4.4	87
	Δ	−6.1	+10.6	−4.6	−65
2	I	10.1 ± 1.2 (4)	71.9 ± 3.3	17.9 ± 2.3	157
	II	13.1 ± 1.7 (4)	71.5 ± 2.8	15.3 ± 2.4	222
	Δ	+3.0	−0.4	−2.6	+65
3	I	10.1 ± 1.6 (3)	67.2 ± 1.6	22.6 ± 0.8	136
	II	11.0 ± 1.4 (5)	67.6 ± 2.2	21.3 ± 1.7	152
	Δ	+0.9	+0.4	−1.3	+16
4	I	10.6 ± 1.0 (4)	64.5 ± 1.9	24.9 ± 1.2	135
	II	12.4 ± 2.1 (3)	60.8 ± 1.6	26.8 ± 3.1	153
	Δ	+1.8	−3.7	−1.9	+18
5	I	13.8 ± 1.0 (4)	63.5 ± 1.9	22.7 ± 1.6	181
	II	15.3 ± 2.3 (3)	61.9 ± 3.7	22.8 ± 2.4	199
	Δ	+1.5	−1.6	+0.1	+18
7	I	7.4 ± 1.8 (5)	70.8 ± 2.6	21.8 ± 3.5	103
	II	9.6 ± 2.6 (3)	68.6 ± 2.5	21.7 ± 0.3	133
	Δ	+2.2	−2.2	−0.1	+30
8	I	10.9 ± 1.1 (3)	69.3 ± 2.4	19.8 ± 1.7	158
	II	10.2 ± 0.2 (3)	70.8 ± 2.4	19.0 ± 2.4	153
	Δ	−0.7	+1.5	−0.8	−05
10	I	5.2 ± 1.7 (3)	71.0 ± 2.0	23.8 ± 0.4	70
	II	5.6 ± 1.8 (6)	73.5 ± 3.9	20.9 ± 2.7	81
	Δ	+0.4	+2.5	−2.9	+11
11	I	9.1 ± 0.5 (3)	69.5 ± 2.7	21.3 ± 2.7	127
	II	10.6 ± 2.0 (6)	66.6 ± 1.7	22.7 ± 1.7	142
	Δ	+1.5	−2.9	−1.4	+15
12	I	4.5 ± 0.3 (3)	71.7 ± 0.6	23.7 ± 0.2	61
	II	7.4 ± 2.7 (4)	71.1 ± 6.8	21.5 ± 4.9	104
	Δ	+2.9	−0.6	−2.2	+43
Mean ± SEM	I	9.4 ± 0.9	68.1 ± 1.2	22.5 ± 0.8	128 ± 12
	II	10.1 ± 1.0[b]	68.4 ± 1.3[b]	21.4 ± 0.9[b]	143 ± 14[b]

[a] Mean ± SD (n, number of determinations).
[b] Period II not significantly different from Period I by paired *t*-test.

patients treated with the drug. Also, Moutafis and Myant (12) reported that changes in specific activity curves of plasma cholesterol, following injection of [^{14}C]cholesterol, were consistent with decreased synthesis of whole-body cholesterol; their studies were carried out in patients with homozygous familial hypercholesterolemia. A similar observation was made by Miettinen (20) for other hypercholesterolemic subjects. However, neither of these groups found a reduction in fecal excretion of either cholesterol or bile acid which should accompany any prolonged decrease in synthesis; in fact, Miettinen (20) found an increased fecal output of neutral steroids in several patients. Sterol balance studies and isotopic kinetic turnover of bile acids in hyperlipidemic patients treated with nicotinic acid also have been reported by Einarsson, Hellstrom, and Leijd (56). In their study, nicotinic acid produced no change in either net steroid balance or bile acid synthesis in nine patients with hypercholesterolemia (Type IIa hyperlipoproteinemia). In five hypertriglyceridemic subjects (Type IV), net steroid balance was unchanged but total bile acid formation was decreased by 25–30%. Also, Wollenweber, Kottke, and Owen (57) have examined effects of nicotinic acid on pool size and turnover of bile acids in six hypercholesterolemic patients. They reported that the combined turnover of cholic acid and chenodeoxycholic acid was not altered significantly by the drug.

In our study, nicotinic acid caused no consistent

TABLE 7. Effects of nicotinic acid in hourly outputs of biliary lipids, bile acid pool sizes, and composition of stimulated hepatic bile

Patient	Period	Biliary Lipid Outputs			Bile Acid Pool Size	Lipid Composition of Hepatic Bile			Bile Saturation (10% Solids)
		Cholesterol	Bile Acids	Lecithin		Cholesterol	Bile Acids	Lecithin	
		mg/hr ± SD[a]			*mg*		*molar %*		%
1	I	54 ± 5	1091 ± 179	325 ± 46	2362	5.1	79.7	15.2	92.2
	II	63 ± 4	1021 ± 101	401 ± 15	2940	5.9	75.1	18.8	91.5
2	I	93 ± 6	1039 ± 219	544 ± 77	2993	7.9	69.4	22.5	107.8
	II	109 ± 17	1068 ± 109	542 ± 81	1607	8.9	66.8	24.1	116.3
3	I	54 ± 11	918 ± 180	430 ± 90	2991	5.6	72.5	21.8	79.1
	II	66 ± 9	1351 ± 127	439 ± 50	4261	5.0	78.5	16.3	86.1
5	I	64 ± 7	989 ± 193	427 ± 56	3442	6.3	73.0	20.4	92.3
	II	112 ± 24	1539 ± 587	787 ± 301	2416	7.2	69.7	23.1	97.3
7	I	38 ± 4	702 ± 235	395 ± 276	4220	5.4	70.6	22.0	75.0
	II	46 ± 5	531 ± 83	265 ± 29	2686	7.8	69.5	22.5	106.5
8	I	62 ± 16	1012 ± 197	310 ± 102	1918	6.2	78.5	15.2	111.1
	II	66 ± 16	1200 ± 339	397 ± 136	1759	5.6	77.9	16.3	96.0
Mean ± SEM	I	61 ± 7	959 ± 56	405 ± 35	2988 ± 330	6.1 ± 1.0	73.9 ± 4.2	19.5 ± 3.4	92.9 ± 14.6
	II	77 ± 11[b]	1119 ± 34	472 ± 73	2537 ± 947	6.7 ± 1.5	72.9 ± 4.9	20.2 ± 3.5	98.9 ± 10.9
			N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[a] In all patients, hourly outputs were measured during a period of 6-hr formula infusion (steady state) after an initial infusion period of 4 hr (equilibration period). In each case, six hourly determinations were carried out.
[b] Period II significantly greater than Period I by paired *t*-test and by sign test at $P < 0.05$.

changes in excretion of either neutral or acidic steroids. A few patients had slight increases in neutral steroids, but increments were not marked. It is interesting to compare the actions of this drug with those of another hypotriglyceridemic agent, clofibrate. The latter drug clearly differs in its action on cholesterol metabolism by causing a marked and consistent increment in excretion of neutral steroids (58). This increase in neutral steroids probably reflects a mobilization of cholesterol from tissue pools. Although a similar marked increment was not observed with nicotinic acid, a decrease in tissue pools by means of a protracted but undetectable increase in fecal steroids remains a possibility. By the same token, if nicotinic acid partially inhibits cholesterol synthesis as suggested by others (12, 20), even an unchanged steroid excretion is compatible with a flux of cholesterol out of tissues. In this regard, Magide and

TABLE 8. Net cholesterol absorption

Patient	Period	Cholesterol Intake	Hepatic Secretion of Cholesterol	Fecal Neutral Steroids	Net Cholesterol Absorption	
		mg/day	*mg/day*	*mg/day*	*mg*	%
1	I	207	1296	631	872	58
	II	207	1512	670	1049	61
2	I	209	2232	814	1627	66
	II	209	2616	1068	1757	62
3	I	206	1296	543	959	63
	II	201	1584	378	1407	78
5	I	219	1536	701	835	54
	II	219	2688	885	1803	67
8	I	207	1488	365	1330	78
	II	207	1584	422	1369	76
Mean ± SEM	I	209	1570 ± 173	611 ± 76	1125 ± 153	63.8 ± 4.1
	II	208	1997 ± 268[a]	685 ± 132[b]	1477 ± 139[a]	68.8 ± 3.5[b]

[a] Period II significantly higher than Period I by paired *t*-test.
[b] Period II not significantly different from Period I by paired *t*-test.

Myant (59) reported a marked loss of cholesterol from muscle and skin of monkeys treated with nicotinic acid.

The rapid mobilization of body cholesterol by clofibrate appeared to cause supersaturation of bile along with increased risk for gallstones. Recently, Angelin, Einarsson, and Leijd (60) claimed that nicotinic acid likewise enhanced saturation of bile. In our study, the drug caused a small increment in biliary outputs of cholesterol (mean increase = 26%); however, no consistent reduction in pool sizes of bile acids were noted, and response in bile saturation was variable. Although several patients had greater saturation on nicotinic acid, the increases were minor compared to those induced by clofibrate. These findings are in accord with the results of the Coronary Drug Project (10); in this study an increase in gallstone disease was not detected during nicotinic acid therapy, but an increase was observed with clofibrate.

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