Intestinal apolipoprotein A-I and B-48 metabolism: effects of sustained alterations in dietary triglyceride and mucosal cholesterol flux

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Abstract In recent studies (1985. *J. Lipid Res.* **26:** 368–379 and 1986. *J. Lipid Res.* **27:** 30–39) we characterized aspects of synthesis of rat intestinal apolipoproteins (apo) A-I and B-48 in vivo, and their short term regulation by dietary and biliary lipid flux. We now report studies extending these observations to the effects on intestinal apoA-I and apoB-48 metabolism of sustained (3 or 6 weeks) isocaloric intake of diets containing 0–30% (by weight) triglyceride, the latter as either butter fat (saturated) or corn oil (polyunsaturated). Additional studies were conducted to determine, separately, the effects of perturbations of intestinal mucosal cholesterol flux and hypothyroidism on intestinal apoA-I and apoB-48 metabolism. Intestinal synthesis (% total protein) of apoA-I and apoB-48 was not influenced by either dietary triglyceride quantity or quality (saturated vs. polyunsaturated fat); the values that were obtained were strictly comparable to those of both chow-fed animals and animals maintained for 3 weeks on fat-free chow. Intestinal apoA-I synthesis was not influenced by either acute or chronic perturbations of mucosal cholesterol flux. Hypothyroid rats demonstrated a 50% suppression of jejunal apoA-I synthesis. Intestinal synthesis of apoB-48, by contrast, appeared to undergo regulation by chronic (but not acute) perturbations of mucosal cholesterol flux. Maneuvers that augmented intestinal cholesterol uptake (particularly hypothyroidism) appeared to suppress intestinal apoB-48 synthesis by over 40%, while Surfomer (AOMA) administration reduced cholesterol absorption (control, 54 ± 7%; AOMA, 26 ± 8%; $P < 0.0005$) and resulted in a 24% increase in apoB-48 synthesis by jejunal enterocytes. Intracellular intestinal lipoproteins demonstrated marked cholesteryl ester enrichment of the triglyceride-rich lipoprotein fractions in hypercholesterolemic, hypothyroid rats. When all the groups were compared, cholesterol absorption (used as an index of mucosal cholesterol uptake) was negatively correlated with jejunal apoB-48 synthesis ($r = -0.92$, $P < 0.05$). The data suggest that regulation of rat intestinal apoA-I and apoB-48 metabolism is independent of triglyceride flux. It is further concluded that an important regulatory effect of mucosal cholesterol flux can be demonstrated on enterocyte apoB-48 synthesis. Finally, the data suggest the additional possibility that circulating levels of thyroid hormone may exert an independent effect on the expression of rat intestinal apolipoproteins A-I and B-48.—**Davidson, N. O., A. M. Magun, T. A. Brasitus, and R. M. Glickman. Intestinal apolipoprotein A-I and B-48 metabolism: effects of sustained alterations in dietary triglyceride and mucosal cholesterol flux.** *J. Lipid Res.* 1987. **28:** 388–402.

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A number of studies conducted in humans have suggested that consumption of a diet rich in saturated fat and cholesterol is associated with a variety of plasma lipoprotein abnormalities and increased susceptibility to coronary heart disease (reviewed in references 1 and 2). Consumption of polyunsaturated fat, by contrast, has been demonstrated in humans to be associated with the lowering of all major plasma lipoprotein fractions, including HDL (3). Data accumulated from kinetic studies suggest that this effect may be mediated, at least in part, by reduced synthesis of both apoA-I and apoB (4–7). Sustained intake of saturated fat has also been shown to affect rat mesenteric lymph lipoprotein morphology and composition (8). In addition to the effects on systemic lipoprotein metabolism, differences in saturation of dietary triglyceride have been reported to affect transport functions and enterocyte membrane fluidity in rat jejunum (9, 10).

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCA, trichloroacetic acid; PTU, propylthiouracil; TLC, thin-layer chromatography.

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Interpretation of the effects of dietary cholesterol augmentation upon intestinal lipoprotein metabolism is complicated by significant species differences (2). Studies from this laboratory (11) demonstrated the secretion of an abnormal lymph lipoprotein in the d 1.006–1.030 g/ml interval from rats chronically exposed to a high fat-1% cholesterol diet. Several independent groups have characterized a cholesteryl ester-rich beta-VLDL particle from secretory vesicles of both liver (12–14) and intestine (15) of hypercholesterolemic, hypothyroid rats. These abnormal particles appear to be newly synthesized as demonstrated by in vivo radiolabeled precursor incorporation studies. Although the combination of hypothyroidism and hypercholesterolemia has been extensively characterized in regard to the accumulation of abnormal plasma lipoproteins (16, 17), recent work has emphasized the need to differentiate the effects of augmented cholesterol flux from those of thyroid hormone deficiency (18–20). Studies in the rat, using a combination of hypothyroidism and cholesterol augmentation to produce marked hypercholesterolemia, have shown hepatic apoE synthesis to be increased twofold (21). Studies from the same laboratory suggested that rat intestinal synthesis of both apoA-I and apoE increased by a similar magnitude following the same regimen of dietary-induced hypercholesterolemia (22). By contrast, studies in the rabbit have produced conflicting data. One report suggested that dietary hypercholesterolemia was associated with an increase in hepatic apoE synthesis (23). Other workers, however, have shown that translatable mRNA values for both intestinal apoA-I and apoE, as well as hepatic apoE, were unchanged following prolonged intake of a high cholesterol diet (24). Thus, there appear to be both species- and organ-specific effects of dietary hypercholesterolemia on apolipoprotein metabolism.

We have recently studied aspects of the regulation of rat intestinal apoA-I and apoB-48 synthesis rates in vivo (25, 26). These studies showed that acute triglyceride flux failed to exert regulation of either apoA-I or apoB-48 synthesis. In view of the relative importance of the small intestine as a secretory source of apoA-I and apoB-48 (27), studies were undertaken to examine the importance of sustained alterations in dietary lipid flux in regulating intestinal apoA-I and apoB-48 metabolism.

MATERIALS AND METHODS

Chemicals and reagents

Protosol, Econofluor, [1,2-^{3}H (N)]cholesterol, and [4-^{14}C]cholesterol were all obtained from New England Nuclear (Boston, MA). L-[4,5-^{3}H]Leucine was obtained from Amersham (Arlington Heights, IL). Sodium taurocholate (> 99% pure) and heat-killed Cowan I strain *S. aureus* cells (Pansorbin) were obtained from Calbiochem (La Jolla, CA). Ultrafluor was obtained from National Diagnostics (Sommerville, NJ). Triton X-100 (Ultrograde) was obtained from LKB (Gaithersburg, MD). Propylthiouracil (2-thio-4-hydroxy-6-*n*-propyl-pyrimidine) and USP cholesterol were obtained from Sigma (St. Louis, MO). Surfomer (AOMA) was a kind gift from Dr. John Johnson, Monsanto Co. (St. Louis, MO).

The source and purity of all other materials used have been previously described (25, 26).

Animals and dietary protocol

Male Sprague-Dawley rats were obtained from Charles River, Wilmington, MA. Animals weighed approximately 150 g on receipt. Groups of rats were subsequently housed in gang cages, four rats per cage, and segregated according to specific dietary or treatment group.

Altered dietary triglyceride intake

Rats were fed pelleted chow containing 30–35% by weight triglyceride (57–61% caloric intake as long-chain triglyceride) either as butter fat or corn oil. These diets were commercially obtained (Diets #0845 and #0846 Bio-Serv, Frenchtown, NJ) and were administered ad libitum for 6 weeks. Both the saturated (butter fat) and polyunsaturated (corn oil) fat diets had comparable amounts of cholesterol (0.12% w/w). The fatty acid composition of both diets has been previously reported (10). Other groups of rats were maintained on a fat-free pelleted chow (Diet #0848, Bio-Serv, Frenchtown, NJ) containing 69% carbohydrate, 18% protein, and 5% fiber by weight. This diet was fed for 3 weeks. Vitamins, minerals, and trace amounts of essential fatty acids were supplemented by the manufacturer to ensure nutritional adequacy.

Other groups of animals were maintained on Purina rat chow (Ralston-Purina, St. Louis, MO) that contained a minimum of 4.5% by weight triglyceride as determined by the manufacturer. Weight gain was comparable in all four dietary groups.

Altered mucosal cholesterol flux[3]

Rats were fed diets prepared by hand using Purina rat chow as the starting material. Five different dietary regimens were employed: *i)* chow + 5% (w/w) lard + 0.3% (w/w) Na taurocholate (lard/NaTc); *ii)* lard/NaTc + 1% (w/w) cholesterol (lard/NaTc/CH); *iii)* lard/NaTc/CH + 0.1% (w/w) propylthiouracil (lard/NaTc/CH/PTU); *iv)*

[3]A variety of regimens was used to produce alterations in luminal cholesterol load, mucosal absorption of cholesterol, and ambient serum cholesterol concentrations. These perturbations, together with the effects on intracellular cholesterol concentration and distribution previously documented by other workers (12–15), are grouped under the heading "altered mucosal cholesterol flux."

chow + 5% (w/w) lard + 0.1% PTU (lard/PTU); *v)* chow + 2% (w/w) AOMA.

Diets *i)–iv)* were prepared by melting lard in a glass beaker and pouring it over pellets of chow. Na taurocholate (with or without cholesterol, with or without PTU) was dissolved in hot ethanol and added to the chow/lard mixture and vigorously mixed. The ethanol was allowed to evaporate off under a fume hood for 24 hr. Diets were prepared weekly and stored in sealed containers at 4°C. Chow/AOMA diet was prepared by dissolving AOMA in warm acetone and mixing the solution with pelleted chow. Again, the solvent was allowed to evaporate off under a fume hood for 24 hr.

Diets were administered ad libitum for 2 weeks (chow/AOMA) or 3 weeks (other diets). Animals consuming diets containing PTU showed consistently less weight gain than respective controls (e.g., final weights lard/NaTc/CH/PTU, 251 ± 17 g (n = 8) versus lard/NaTc/CH, 288 ± 17 g (n = 8) $P < 0.001$). Serum, taken from representative numbers of animals in each group, was assayed for T_4 and T_3 levels by radioimmunoassay (Vet-Path Laboratories, Teterboro, NY). Animals receiving PTU (either with lard alone, group *iv),* or with lard, cholesterol, and Na taurocholate, group *iii),* had T_4 and T_3 levels at the lowest limit of detection, (e.g., group *iii)* T_4, 0.31 ± .02 μg/dl; T_3, 25 ± 0.7 ng/dl, n = 8) compared to the normal levels found in nine animals receiving the lard/cholesterol/Na taurocholate diet, (group *ii)* T_4, 2.6 ± 1.1 μg/dl; T_3, 107 ± 37 ng/dl).

In vivo intestinal apolipoprotein synthesis measurements

Details of the protocol used for measuring apolipoprotein A-I and B-48 synthesis rates in jejunal and ileal enterocytes have been described in detail (25, 26). Briefly, following a 14–16 hr fast, animals were anesthetized with sodium pentobarbital and 10-cm loops of jejunum (proximal end at ligament of Treitz) and ileum (distal end, 5 cm from ileocecal valve) were pulse-labeled with L-[4,5-^{3}H]leucine for 9 min. Following exsanguination of the rats, the loops were removed and enterocytes were isolated using citrate–EDTA chelation (25). All buffers contained 5–20 mM leucine to prevent isotope reutilization. The final cell pellet was homogenized in PBS–1% Triton–2 mM leucine–1 mM PMSF–1 mM benzamidine, pH 7.4, and a 105,000 *g* supernatant was prepared. Aliquots of homogenate were saved for measurement of total protein concentration (28) and trichloroacetic acid (TCA)-insoluble radioactivity. Aliquots of cytosolic supernatant were treated with excess anti-apoA-I or anti-apoB-100 antiserum and the immune complex was precipitated by addition of washed *S. aureus* cells (29). After extensive washing, the immune complex was characterized by SDS-PAGE and radioactivity incorporated into the specific apolipoprotein of interest was determined by liquid scintillation spectrometry. Characterization of antiserum specificity and parameters for determining antiserum excess stoichiometry have been previously provided (25, 26).

Apolipoprotein synthesis is expressed as a relative rate, % total protein synthesis. The value was derived by expressing [^{3}H]leucine incorporation into immunoprecipitable apolipoprotein per volume of supernatant as a fraction of its incorporation into the same volume of homogenate, following precipitation with hot 10% TCA. Each value represents the mean of two to four separate assays and reflects subtraction of nonspecific and background radioactivity.

Isolation of intracellular lipoproteins

The method employed was a modification of methods previously established in our laboratory (30) to isolate intracellular high density lipoproteins. Enterocytes were isolated from the entire small bowel of fasted rats five to eight per group) using citrate–EDTA chelation, and washed extensively. The cells were suspended in ice-cold hypotonic veronal buffer (37.6 mM Na diethylbarbiturate, 7.3 mM barbituric acid, 2 mM diethyl-*p*-nitrophenyl phosphate, pH 8.3) and dispersed by five strokes of a loose-fitting glass Dounce homogenizer. The solution was passed twice through a cold nitrogen cavitation apparatus at 2,000 psi for 15 min. The solution was then centrifuged for 1 hr at 100,000 *g* in an SW 28 rotor (Beckman). Chylomicrons were removed by pipette-aspiration from the surface and the infranatant lipoproteins were isolated by sequential ultracentrifugation (30) using NaBr to raise the interval density.

Each lipoprotein fraction was dialyzed into distilled water, pH 8.0, for 36 hr and analyzed for lipid composition following extraction with chloroform–methanol 2:1 (vol/vol). Lipid composition was determined by quantitative TLC as described (30) using silica gel G in a solvent system of hexane–diethyl ether–glacial acetic acid 70:30:1 (v/v/v).

Cholesterol absorption

A dual-isotope plasma ratio method was employed using the technique described and validated by Zilversmit and Hughes (31) and Zilversmit (32). Animals were fasted for 16–18 hr prior to study. The oral dose was prepared by adding 50 μCi [1,2-(^{3}H)N]cholesterol to 500 μl of corn oil containing approximately 20 mg of cholesterol. This was then sonicated with 9 ml of 5 mM Na taurocholate using a Branson sonifier at a setting of 50 watts for three 5-min bursts. Each animal then received 1 ml of this mixture by gavage. Within 2 min of this procedure each animal received an intravenous bolus of 2 μCi of [4-^{14}C]cholesterol in the form of a colloidal suspension (20 μl of ethanol in 1 ml of 0.154 M saline). Animals were fasted for an additional 2 hr and then allowed ad libitum access to the respective diets for 72–96 hr. Following exsanguination of the rats, aliquots of serum were saponified in KOH–methanol for

2 hr at 70°C and the neutral sterol fraction was extracted twice into 20 ml of petroleum ether and dried under N_2. Liquid scintillation spectrometry was performed using an LKB RackBeta (LKB, Gaithersburg, MD) with a dual channels-crossover program to minimize spillage (4% ^{14}C into ^{3}H). Calculations of cholesterol absorption were made by comparing $^{3}H/^{14}C$ ratios in the serum (at 72 or 96 hr) to the $^{3}H/^{14}C$ ratios in the administered dose. Biological reliability of the [^{3}H]cholesterol preparation used throughout this study (lot #2260-047) was determined (33). Four control animals received an intravenous bolus of [^{3}H]- and [^{14}C]cholesterol, mixed together at a $^{3}H/^{14}C$ ratio of 2.21 ± 0.03. Seventy-five hours later the animals were exsanguinated and the $^{3}H/^{14}C$ ratio of serum cholesterol was determined and found to be 2.20 ± 0.01 indicating that no loss of [^{3}H]radioactivity had occurred and that the biological characteristics of both isotopes were comparable (33).

Miscellaneous assays

Serum cholesterol and triglyceride concentrations were determined enzymatically using commercially available kits (Sigma). Protein was determined by the method of Lowry et al. (28) using bovine serum albumin as standard. Radioimmunoassay (RIA) of apoA-I and apoB was conducted as previously described (25, 26).

Data expression and statistical analysis

Apolipoprotein synthesis rates are expressed as % total protein (above). Apolipoprotein specific activity refers to cpm of immunoprecipitable apolipoprotein per ng of apolipoprotein mass (RIA). Apolipoprotein content is expressed as ng per mg of homogenate protein. Statistical comparisons of the effects of altered triglyceride intake were made by independent *t*-test. The overall level of significance of the various treatments producing altered cholesterol flux was determined using a two-way ANOVA. This identified significance of both treatment (diet and/or drug manipulation) and site (jejunum and/or ileum) upon apolipoprotein synthesis rates. Where appropriate, a one-way ANOVA was used (specifically in the AOMA group where no data for ileal apolipoprotein synthesis was generated). For apoA-I, two-way ANOVA yielded an F-value of 11.97 for treatment ($P < 0.00001$) and 8.48 for site ($P < 0.0058$). For apoB-48, two-way ANOVA yielded an F value of 10.59 ($P < 0.00001$) and 14.37 for site ($P < 0.0004$). There was no interaction between treatment and site for either apoA-I or apoB-48. Having established overall significance, post hoc independent *t*-test comparisons were made between the various treatment groups by site and compared to those of chow-fed control animals. These comparisons were made using pooled or separate variance analysis where appropriate.

RESULTS

Intestinal apolipoprotein metabolism: role of dietary triglyceride content

ApoA-I synthesis rates (% total protein) in jejunal enterocytes were comparable regardless of dietary triglyceride intake, either quality or quantity (**Table 1**). Ileal apoA-I synthesis rates were also unchanged from control values by augmented triglyceride intake (Table 1), suggesting that the polarity of apoA-I synthesis (jejunal > ileal) previously described in chow-fed rats (25) is not dependent upon triglyceride load. Jejunal apoA-I content was lower in saturated fat-fed animals than in the other three groups (Table 1). Correspondingly, apoA-I specific activity in jejunal enterocytes from this group was higher than in control chow-fed animals (Table 1). By contrast, jejunal apoA-I content was no different in animals fed polyunsaturated fat as compared to chow-fed controls (Table 1). The difference in jejunal apoA-I specific activity (Table 1) in this context, given similar apoA-I synthesis and content values, has no ready explanation. Possibly differences in turnover rates may account for the higher specific activity of jejunal apoA-I in polyunsaturated fat-fed animals. Ileal content and specific activity values for apoA-I showed no differences among the four groups (Table 1). [^{3}H]Leucine incorporation into newly synthesized protein was also comparable among the various dietary groups (Table 1).

Serum apoA-I values were higher in polyunsaturated fat-fed animals than in saturated fat-fed or control chow-fed rats (Table 1). This suggests an important species difference in the effects of polyunsaturated fat on the levels of serum HDL apolipoproteins since several studies have shown the opposite result (i.e., reduction of apoA-I levels) with this dietary manipulation in nonhuman primates and humans (1–3).

Enterocyte synthesis rates of apoB-48 were comparable to chow-fed animals regardless of dietary triglyceride consumption (**Table 2**). Despite similar synthesis rates of apoB-48, cellular content of apoB was elevated in both jejunum and ileum of polyunsaturated fat-fed animals when compared to either saturated fat-fed or control groups (Table 2). Jejunal apoB specific activity was higher in polyunsaturated fat-fed animals, a finding that again suggests the possibility that cellular turnover of apoB may be accelerated. This speculation might reconcile the paradox of a larger intracellular pool, at higher specific activity, in the setting of similar synthesis rates. Ileal apoB specific activity was lower in polyunsaturated fat-fed animals than chow-fed controls (Table 2). Jejunal apoB specific activity was also higher in saturated fat-fed than control animals, which, in the setting of similar pool sizes, again might suggest the

TABLE 1. ApoA-I metabolism in rat small intestine: effects of sustained alterations in dietary triglyceride intake

Diet	ApoA-I Synthesis		ApoA-I Content		ApoA-I Specific Activity		Total Protein Specific Activity		Serum ApoA-I
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	
	% total protein		*ng/mg protein*		*cpm/ng apoA-I*		*cpm/ng protein*		*mg/dl*
High saturated	1.58 ± 0.31 (8)	1.25 ± 0.36 (8)	214 ± 32[e,f] (12)	215 ± 62 (12)	116 ± 34[a] (8)	96 ± 35 (8)	1.60 ± 0.56 (8)	1.53 ± 0.36 (8)	50.7 ± 10.3 (8)
High polyunsaturated	1.75 ± 0.24 (8)	1.12 ± 0.50 (8)	305 ± 87 (12)	267 ± 83 (12)	117 ± 46[b] (8)	58 ± 35 (8)	1.73 ± 0.64 (8)	1.46 ± 0.34 (8)	70.5 ± 16.8[c,d] (8)
Fat-free	1.69 ± 0.16 (6)	ND	315 ± 57 (6)	ND	72 ± 37 (6)	ND	1.28 ± 0.46 (6)	ND	53.8 ± 11.4[b] (6)
Purina chow	1.98 ± 0.47 (6)	1.41 ± 0.30 (4)	305 ± 84 (6)	222 ± 53 (6)	66 ± 29 (6)	95 ± 24 (4)	1.61 ± 0.82 (6)	1.81 ± 0.65 (6)	41.9 ± 2.4 (6)
Difference	NS	NS		NS		NS	NS	NS	
From chow-fed animals			[e]$P < 0.005$		[a]$P < 0.02$ [b]$P < 0.05$				[c]$P < 0.002$ [b]$P < 0.05$
From high saturated-fed animals From high polyunsaturated-fed animals			[f]$P < 0.005$						[d]$P < 0.025$

Animals were fed pelleted rat chow ad libitum for 3 weeks (fat-free) or 6 weeks (other diets). Diets were obtained commercially and were of defined composition (Methods). Apolipoprotein synthesis was determined in vivo following a 14–16-hr fast (Methods). Data were derived from (n) animals, each value representing two to four separate assays, and are expressed as mean ± SD. Apolipoprotein content was determined by RIA (Methods). Data are expressed as ng per mg of protein, each value representing the mean ± SD for (n) animals. Apolipoprotein specific activity is expressed as ^{3}H-labeled immunoprecipitable apoA-I per volume of intestinal supernatant divided by apoA-I mass, determined by RIA (Methods), in the same volume. Data are expressed as means ± SD for (n) animals. Total protein specific activity is expressed as ^{3}H incorporation per ng TCA-precipitable protein (Methods). Data are expressed as means ± SD for (n) animals. Serum apoA-I concentration was determined by RIA at the conclusion of the study. Data are means ± SD for (n) animals. Differences were determined by Student's *t* test for independent observations. ND, not determined; NS, not significant.

TABLE 2. ApoB-48 metabolism in rat small intestine: effects of sustained alterations in dietary triglyceride intake

Diet	ApoB-48 Synthesis		ApoB Content		ApoB-48 Specific Activity		Serum ApoB
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	
	% total protein		*ng/mg protein*		*cpm/ng apoB*		*mg/dl*
High saturated	0.72 ± 0.13 (8)	0.50 ± 0.11 (8)	1358 ± 191 (12)	1125 ± 134 (12)	8.5 ± 3.3[a] (8)	6.8 ± 1.9 (8)	53.9 ± 7.0[c] (8)
High polyunsaturated	0.82 ± 0.15 (8)	0.40 ± 0.18 (8)	1697 ± 191[c,f] (12)	1318 ± 208[a,g] (12)	8.8 ± 3.7[b] (8)	4.4 ± 2.2[a] (8)	50.2 ± 7.8[c] (8)
Fat-free	0.77 ± 0.15 (6)	ND	1351 ± 134 (6)	ND	7.8 ± 4.2 (6)	ND	33.4 ± 8.4 (6)
Purina chow	0.74 ± 0.16 (6)	0.54 ± 0.12 (6)	1410 ± 282 (6)	965 ± 249 (6)	4.4 ± 1.3 (6)	8.4 ± 2.9 (6)	32.7 ± 3.5 (6)
Difference	NS	NS					
From chow-fed animals			[c]$P < 0.05$	[a]$P < 0.01$	[a]$P < 0.02$ [b]$P < 0.025$	[a]$P < 0.02$	[c]$P < 0.001$
From high saturated-fed animals			[f]$P < 0.001$	[g]$P < 0.02$			

Animals were fed pelleted rat chow ad libitum for 3 weeks (fat-free) or 6 weeks (other diets). Diets were obtained commercially and were of defined composition (Methods). Apolipoprotein synthesis was determined in vivo following a 14-16 hr fast. Data were derived from (n) animals, each value representing two to four separate assays, and are expressed as mean ± SD. Apolipoprotein content was determined by RIA. Data are expressed as ng per mg of protein, each value representing the mean ± SD for (n) animals. Apolipoprotein specific activity is expressed as ^3H in immunoprecipitable apoB-48 per volume intestinal supernatant divided by apoB mass, determined by RIA in the same volume. Data are expressed as means ± SD for (n) animals. Serum apoB concentration was determined by RIA at the conclusion of the study. Data are the means ± SD for (n) animals. Differences were determined by Student's *t*-test for independent observations. ND, not determined; NS, not significant.

possibility of increased turnover of apoB in this group. Of further interest was the demonstration that serum apoB concentrations were higher in both groups consuming the high fat diets with no differences attributable to the degree of triglyceride saturation. The pattern of molecular forms of serum apoB in this instance (i.e., B-100/B-48 ratio) was not specifically investigated.

Intestinal apolipoprotein metabolism: role of altered cholesterol flux

A variety of different dietary regimens was employed to produce alterations in mucosal cholesterol flux. Serum lipid concentrations in representative animals are presented in **Table 3.** Data on apolipoprotein metabolism from animals subjected to maneuvers that alter mucosal cholesterol flux were compared to a simultaneously studied control chow-fed group (Tables 1 and 2). The apolipoprotein synthesis data from this particular group were strictly comparable to previously published values for jejunal apoA-I and apoB-48 synthesis rates (25, 26). Furthermore, there being no differences in synthesis rates attributable to dietary fat content per se (Tables 1 and 2), it was concluded that the chow-fed group (consuming 4.5% by weight triglyceride) provided an adequate control for those animals receiving chow + 5% lard (equivalent to 9% by weight triglyceride).

Intestinal apoA-I synthesis rates were significantly suppressed in those groups rendered hypothyroid by PTU treatment (**Table 4**). The suppression was most marked in the group consuming PTU alone and appeared to affect both jejunal and ileal apoA-I synthesis (Table 4). Enterocyte apoA-I accumulation was observed in three of the four groups with enhanced cholesterol absorption (see below), the effect being most pronounced distally (Table 4). As a result of increased apoA-I content and reduced apoA-I synthesis in the lard/PTU group, specific activity of ileal apoA-I was significantly lower than in chow-fed controls (Table 4). Total protein synthesis among the five different groups was comparable in the jejunum, but animals consuming Na taurocholate were noted to have significantly reduced total protein synthesis by ileal enterocytes (Table 4). The mechanism and importance of this observation is unclear. Major changes in intestinal apoA-I metabolism, however, were observed in animals consuming the lard/PTU diet (Table 4) in which total protein synthesis in both jejunum and ileum was no different than synthesis in control animals. The complexity of the dietary interactions is fur-

TABLE 3. Serum cholesterol and triglyceride concentrations following sustained alterations in mucosal cholesterol flux

	Cholesterol	Triglyceride
	mg/dl	
Control-chow (5)	85 ± 5	112 ± 45
Chow/AOMA (5)	97 ± 19	94 ± 35
Lard/NaTc (9)	89 ± 11	60 ± 35
Lard/NaTc/CH (13)	294 ± 156[a]	54 ± 10
Lard/NaTc/CH/PTU (6)	505 ± 66[a]	58 ± 27
Lard/PTU (5)	142 ± 16[a]	63 ± 3

Animals were exsanguinated via the abdominal aorta while under anesthesia. Serum was assayed for cholesterol and triglyceride content and the data are expressed as mean ± SD for (n) animals per group. Identification of dietary groups is provided in the text (Methods).
[a]$P < 0.001$ versus lard/NaTc animals.

TABLE 4. ApoA-I metabolism in rat small intestine: effects of sustained alterations in mucosal cholesterol flux

Diet	ApoA-I Synthesis		ApoA-I Content		ApoA-I Specific Activity		Total Protein Specific Activity		Serum ApoA-I
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	
	% total protein		*ng/mg protein*		*cpm/ng apoA-I*		*cpm/ng protein*		*mg/dl*
Lard/NaTc	1.74 ± 0.20 (5)	1.45 ± 0.12 (5)	254 ± 24 (5)	265 ± 32 (5)	82 ± 29 (5)	39 ± 12[e] (5)	1.14 ± 0.33 (10)	0.94 ± 0.38[d] (10)	58.1 ± 12.5[a] (5)
Lard/NaTc/CH	1.42 ± 0.18 (4)	1.34 ± 0.30 (4)	436 ± 106 (4)	560 ± 157[e] (4)	48 ± 10 (4)	35 ± 15[d] (4)	1.20 ± 0.38 (9)	1.02 ± 0.50[f] (9)	32.5 ± 11.5 (9)
Lard/NaTc/CH/PTU	1.42 ± 0.19[a] (6)	1.28 ± 0.20 (6)	509 ± 141[f] (6)	635 ± 151[b] (6)	51 ± 17 (6)	24 ± 9[b] (6)	1.45 ± 0.57 (15)	1.01 ± 0.33[e] (15)	28.0 ± 2.4[b] (6)
Lard/PTU	0.93 ± 0.14[b] (5)	0.94 ± 0.35[a] (5)	487 ± 61[e] (5)	901 ± 297[b] (6)	36 ± 10[c] (5)	18 ± 12[b] (5)	1.89 ± 0.50 (5)	1.52 ± 0.51 (5)	71.0 ± 12.7[f] (5)
Chow/AOMA	1.49 ± 0.30 (6)	ND	313 ± 70 (6)	ND	83 ± 32 (6)	ND	1.70 ± 0.54 (6)	ND	46.9 ± 5.4 (6)
Difference from chow-fed animals (Table 1)	$^aP < 0.05$ $^bP < 0.001$	$^aP < 0.05$	$^fP < 0.02$ $^eP < 0.002$	$^eP < 0.002$ $^bP < 0.001$	$^cP < 0.05$	$^dP < 0.005$ $^eP < 0.002$ $^bP < 0.001$	NS	$^dP < 0.005$ $^eP < 0.002$ $^fP < 0.01$	$^aP < 0.05$ $^bP < 0.001$ $^fP < 0.02$

Diets were prepared by hand as detailed (Methods). Animals were allowed ad libitum access to food for 14 days (chow-AOMA) or 21 days (all other diets). See text for formulation of diets. Apolipoprotein synthesis was determined following a 14–16 hr fast as described in the text. Data are derived from (n) animals and are expressed as means ± SD. Apolipoprotein content was determined by RIA. Data are expressed as ng per mg of protein. Each value represents the mean ± SD for (n) animals. Apolipoprotein specific activity is expressed as detailed in Methods. Data are means ± SD for (n) animals. Total protein specific activity is expressed as ^3H incorporation per ng TCA-precipitable protein (Methods). Data are expressed as means ± SD for (n) animals. Serum apoA-I levels were determined by RIA. Data are means ± SD. Differences were determined by post hoc independent *t*-test comparison to chow-fed controls; ND, not determined; NS, not significant.

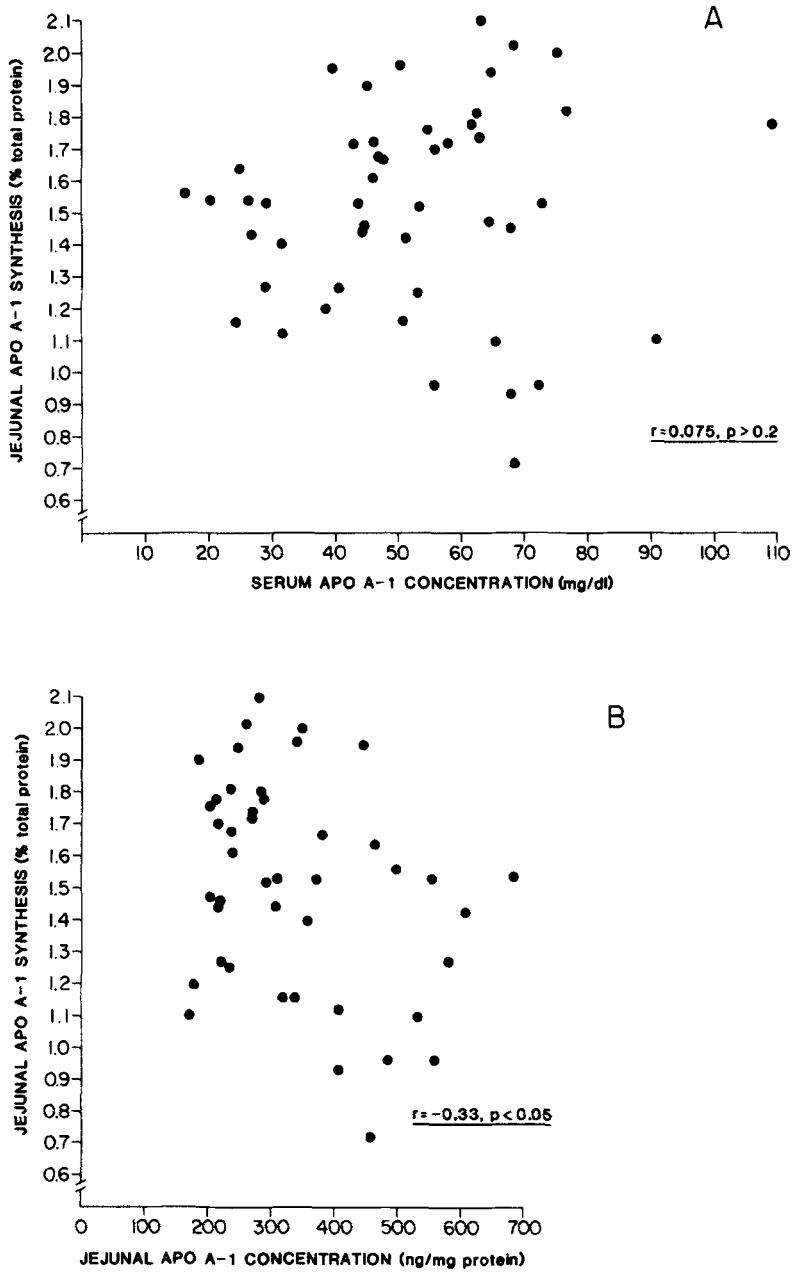

Fig. 1. A: Jejunal apoA-I synthesis: correlation with serum apoA-I concentration. ApoA-I synthesis rates and serum apoA-I concentration were simultaneously determined in 48 animals following a variety of dietary perturbations to both triglyceride intake and cholesterol flux (Methods). The equation for the regression is $y = 0.0013x + 1.45$. B: Jejunal apoA-I synthesis: correlation with enterocyte apoA-I concentration. ApoA-I synthesis and content determinations were simultaneously determined in 48 animals as above. The equation for the regression is $y = -0.0008x + 1.80$.

ther highlighted by changes observed in serum apoA-I levels, which showed an approximately 50% decrease from control levels ($P < 0.001$) in the lard/NaTc/cholesterol/PTU group, as compared to a 50% increase ($P < 0.02$) in animals fed lard/PTU alone (Table 4). There was no significant correlation between jejunal apoA-I synthesis rate and serum apoA-I concentration in 48 animals so examined (**Fig. 1A**). Jejunal apoA-I synthesis was negatively correlated with enterocyte apoA-I content ($r = -0.33$, $P < 0.05$, Fig. 1B), again suggesting that factors associated

TABLE 5. ApoB-48 metabolism in rat small intestine: effects of sustained alterations in mucosal cholesterol flux

Diet	ApoB-48 Synthesis		ApoB Content		ApoB-48 Specific Activity		Serum ApoB
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum	
	% total protein		*ng/mg protein*		*cpm/ng apoB*		*mg/dl*
Lard NaTc	0.60 ± 0.18 (5)	0.57 ± 0.19 (5)	1220 ± 155 (5)	1061 ± 34 (5)	5.4 ± 1.2 (5)	3.7 ± 1.5[e] (5)	46.5 ± 9.6[e] (5)
Lard/NaTc/CH	0.66 ± 0.16 (12)	0.47 ± 0.20 (12)	1227 ± 200 (12)	1099 ± 178 (12)	5.9 ± 2.6 (12)	3.7 ± 2.3[f] (12)	94.1 ± 27.0[b] (9)
Lard/NaTc/CH/PTU	0.42 ± 0.11[a] (6)	0.29 ± 0.07[c] (6)	984 ± 64[e] (6)	827 ± 130 (6)	7.2 ± 2.4[d] (6)	4.1 ± 1.5[e] (6)	131 ± 27.0[b] (6)
Lard/PTU	0.42 ± 0.02[a] (5)	0.23 ± 0.04[b] (5)	1382 ± 79 (5)	1396 ± 169[e] (5)	5.7 ± 14 (5)	2.6 ± 1.3[f] (5)	84.7 ± 19.4[b] (5)
Chow/AOMA	0.92 ± 0.20 (6)	ND	1184 ± 104 (6)	ND	13.0 ± 4.0[b] (6)	ND	37.7 ± 5.5 (6)
Difference from chow-fed animals (Table 2)	[a]$P < 0.005$	[c]$P < 0.002$ [b]$P < 0.001$	[e]$P < 0.02$	[e]$P < 0.02$	[d]$P < 0.05$ [b]$P < 0.001$	[e]$P < 0.02$ [f]$P < 0.002$	[e]$P < 0.02$ [b]$P < 0.001$

Diets were prepared by hand as detailed in Methods. Dietary regimen detailed in text. Apolipoprotein synthesis rates were determined and expressed as detailed in Methods. Data are derived from (n) animals and expressed as means ± SD. Apolipoprotein content was determined by RIA. Data are expressed as ng per mg protein, each value representing the mean ± SD for (n) animals. Apolipoprotein specific activity is expressed as detailed in the text. Data are means ± SD for (n) animals. Serum apoB concentration was determined by RIA. Data are means ± SD. Differences were determined by post hoc independent *t*-test comparison to chow-fed controls (Methods). ND, not determined.

with reduced apoA-I synthesis, notably hypothyroidism, are associated with accumulation of intracellular apoA-I.

Jejunal apoB-48 synthesis showed a trend towards decline in animals consuming the lard/NaTc diet (**Table 5**); but this did not achieve statistical significance ($P > 0.05$). Animals consuming the lard/NaTc/cholesterol/PTU diet demonstrated a 43% decrease in jejunal apoB-48 synthesis and a 46% decrease in ileal apoB-48 synthesis (Table 5). Similar changes were encountered in animals consuming the lard/PTU diet (Table 5) suggesting that the hypothyroid state per se may be a potent regulatory factor in intestinal apoB-48 metabolism. In contrast to the effects of dietary maneuvers that *augment* intestinal cholesterol flux (see below), animals treated with 2% AOMA were found to have elevated apoB-48 synthesis rates ($P < 0.05$ versus lard/NaTc, $P < 0.01$ versus lard/NaTc/cholesterol) and an almost three-fold elevation in apoB-48 specific activity compared to chow-fed controls, $P < 0.001$ (Table 5). Serum apoB levels were unchanged in the AOMA group; values for other treatment groups were similar to previously reported values from other workers (34, 35). In 53 animals where simultaneous measurements were made, jejunal apoB-48 synthesis demonstrated a significant inverse correlation with serum apoB concentration ($r = -0.52$, $P < 0.0005$, **Fig. 2A**) and a positive correlation with jejunal enterocyte apoB content ($r = 0.44$, $P < 0.001$ Fig. 2B). Taken together this suggests that jejunal apoB-48 synthesis is subject to regulation by factors influencing both intracellular and systemic apoB metabolism.

To define the effects of a single high cholesterol challenge upon intestinal apolipoprotein metabolism, two groups of chow-fed animals were fasted for 18 hr and then challenged with either 1 g of corn oil alone or 1 g of corn oil containing 20 mg of cholesterol. Animals were studied 7 hr after challenge, a time course reported by other workers to be associated with increased serum apoB-48 accumulation following a single high cholesterol meal (36). As demonstrated in **Table 6**, there was no change in any parameter of intestinal apoA-I or apoB-48 metabolism attributable to a single high cholesterol bolus. Total protein synthesis rates (cpm/ng) were no different between the two groups (0.86 ± 0.19 and 1.10 ± 0.37, $P > 0.05$).

The lipid composition of intracellular intestinal lipoproteins was compared in animals consuming the lard/NaTc diet (control, **Table 7**) and the lard/NaTc/cholesterol/PTU diet (HC, Table 7). These groups were chosen as representative of altered cholesterol flux (see below) with minimally changed jejunal apoB-48 synthesis (control), and altered cholesterol flux with suppressed jejunal apoB-48 synthesis (HC), respectively. The major change encountered was a significant increase in cholesteryl ester mass, predominantly in the triglyceride-rich lipoprotein fraction (Table 7). These data support the earlier demonstration of cholesteryl ester-rich VLDL particles in intestinal Golgi from HC rats (15). The lipid composition of intracellular lipoproteins from the control group (Table 7) was not significantly different from fasted, chow-fed controls (Magun, A. M., N. O. Davidson, and R. M. Glickman, unpublished observations).

The suppression of intestinal apoB-48 synthesis in hypothyroid animals in combination with its relative induction

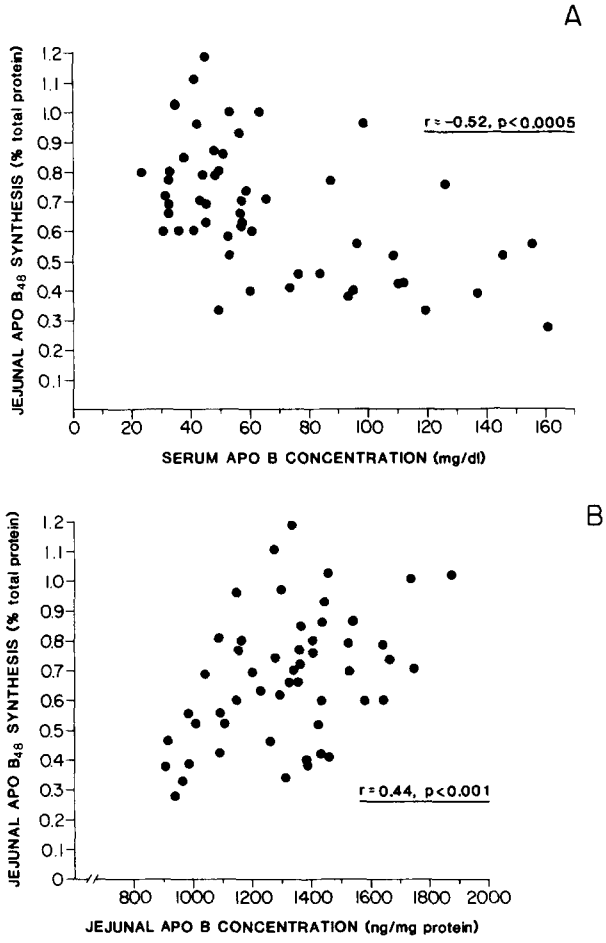

Fig. 2. A: Jejunal apoB-48 synthesis: correlation with serum apoB concentration. ApoB synthesis rates and serum apoB concentration were simultaneously determined in 53 animals following a variety of perturbations to both triglyceride intake and cholesterol flux (Methods). The equation for the regression is $y = -0.0032x + 0.89$. B: Jejunal apoB-48 synthesis: correlation with enterocyte apoB concentration. ApoB-48 synthesis and apoB content determinations were made simultaneously in 53 animals as above. The equation for the regression is $y = 0.0004x + 0.14$.

in animals treated with AOMA suggested a possible common link with intestinal cholesterol absorption. This hypothesis was tested by determining cholesterol absorption in the different groups, as illustrated in **Table 8.** Cholesterol absorption values in chow-fed controls (Table 8) were similar to previous reports (31, 32). Animals consuming lard/NaTc were found to have elevated cholesterol absorption (Table 8), presumably on the basis of an expanded bile salt pool. Animals consuming the lard/PTU diet were found to have a consistent increase in cholesterol absorption, an effect previously reported (37) with hypothyroid rats and whose only presumed basis is a decrease in intestinal motility. Animals consuming the lard/NaTc/cholesterol/PTU diet were also found to have elevated cholesterol absorption (Table 8). The apparent lack of synergism between bile salt augmentation and hypothyroidism may reflect sequestration of a portion of the administered label within an expanded intestinal microsomal cholesterol pool in this group (38). Administration of AOMA to lymph duct-cannulated rats has been shown to decrease recovery of administered luminal cholesterol (39). The present studies confirm these findings in free-living animals.

To determine whether a relationship could be proposed between mucosal cholesterol uptake and apoA-I or apoB-48 synthesis by jejunal enterocytes, the average group cholesterol absorption rate was compared to the average group apolipoprotein A-I and B-48 synthesis rate for each of the five groups studied. The result is shown in **Fig. 3.** Using % cholesterol absorption as an approximation to mucosal cholesterol uptake, apoA-I synthesis appears to be unrelated (Fig. 3A) while apoB-48 synthesis displays an inverse relationship to mucosal cholesterol absorption (Fig. 3B).

DISCUSSION

This study was undertaken to examine the effects of sustained alterations in dietary lipid intake upon intestinal apoA-I and apoB-48 metabolism. The experimental design was specifically aimed at discriminating the effects of altered triglyceride flux, both quantity and quality (saturated versus polyunsaturated) from altered mucosal cholesterol flux and, to the extent possible, from the effects of hypothyroidism.

A major conclusion of this study is that intestinal synthesis rates of both apoA-I and apoB-48 were not influenced by the quantity (0–30%, w/w, triglyceride) or quality (saturated versus polyunsaturated) of dietary fat intake. There is little information concerning regulation of the intestinal contribution to plasma apoA-I and apoB-48 levels, although data in the rat suggest that approximately 50% of the daily input of apoA-I may originate from the small intestine (27). Previous studies in human subjects (4–7) had suggested that the hypolipoproteinemic effects of polyunsaturated fat may be mediated by decreased synthesis of apoA-I and apoB. Additional studies in African green monkeys demonstrated that the apoA-I/triglyceride ratio of lymph chylomicrons was significantly lower in animals fed polyunsaturated as compared to saturated fat (40). This suggested that altered intestinal secretion rates of apoHDL may in part account for the HDL-lowering effects of polyunsaturated fat. The findings of the present study suggest an important species departure in this regard. Specifically, serum apoA-I levels were elevated in polyunsaturated-fed animals (Table 1) while apoB levels were similarly elevated in both groups consuming high fat diets (Table 2). While potential mechanisms include altered systemic catabolism or augmented secretion rates of these apolipoproteins, the present studies demonstrate only that intestinal apoA-I and apoB-48 *synthesis rates* are unchanged by dietary triglyceride intake. Despite the apparent importance of the small intestine as a secretory source of apoA-I (27), there was no correlation

TABLE 6. Apolipoprotein metabolism in jejunal enterocytes: effects of a single high cholesterol challenge

Dietary Challenge	ApoA-I Synthesis	ApoA-I Content	ApoA-I Sp Act	Serum ApoA-I	ApoB-48 Synthesis	ApoB-48 Content	ApoB-48 Sp Act	Serum ApoB
	% total protein	*ng/mg*	*cpm/ng apoA-I*	*mg/dl*	*% total protein*	*ng/mg*	*cpm/ng apoB*	*mg/dl*
Triglyceride alone (n = 6)	1.48 ± 0.38	216 ± 46	76 ± 29	48.4 ± 7.5	0.80 ± 0.12	1233 ± 135	5.8 ± 2.5	87.8 ± 26.9
Triglyceride + 2% w/w cholesterol (n = 5)	1.61 ± 0.33	179 ± 33	79 ± 27	49.7 ± 11.1	0.69 ± 0.16	1270 ± 216	6.0 ± 2.3	58.4 ± 11.8
Difference	NS	NS	NS	NS	NS	NS	NS	NS

Chow-fed animals were fasted for 14–16 hr and susequently received, by gavage, either 1 g corn oil (triglyceride alone) or 1 g corn oil containing 2% w/w cholesterol (triglyceride + cholesterol). Animals were allowed ad libitum access to water but not food for 7 hr, whereupon they were individually studied as detailed (Methods). Jejunal enterocyte apolipoprotein synthesis, content, and specific activity measurements were made as detailed in the text. Data are expressed as means ± SD. Differences were determined by independent *t* test comparison to be insignificant, $P > 0.05$ (NS).

between circulating levels of apoA-I and jejunal apoA-I synthesis rates (Fig. 1A). By contrast, jejunal apoB-48 synthesis rates demonstrated a significant inverse correlation with serum apoB concentration (Fig. 2A). The importance of this latter observation remains speculative since the bulk of circulating plasma apoB is presumed to arise from hepatic synthesis (27) while chylomicron apoB-48 in the rat is not a precursor to circulating plasma LDL apoB (41).

In view of the demonstration that rat enterocytes can internalize significant amounts of both LDL and HDL via receptor-dependent and -independent mechanisms (42, 43), the relationship between plasma levels of apoA-I and apoB and intestinal apolipoprotein metabolism is of some importance. Fasting enterocyte contents of both apoA-I and apoB showed a significant inverse correlation with their respective serum concentrations (apoA-I, $r = -0.34$, $P < 0.02$; apoB, $r = -0.37$, $P < 0.01$, data not shown). The mechanism underlying this association, however, remains to be defined since it is unknown what proportion of the enterocyte apolipoprotein pool is locally synthesized. In terms of understanding the factors that regulate enterocyte apolipoprotein metabolism, the present studies demonstrate important differences between apoA-I and apoB-48. In particular, jejunal apoB synthesis rates and content were positively correlated (Fig. 2B) while a negative correlation was evident for apoA-I (Fig. 1B). Previous studies have demonstrated that jejunal apoA-I synthesis was not influenced by acute dietary triglyceride flux or diversion of biliary lipid (25), while apoB-48 synthesis appears to undergo regulation in response to changes in biliary lipid flux (26). Taken together with the present findings, this suggests that, unlike apoA-I, intestinal apoB-48 metabolism may be coordinately regulated by factors that influence systemic apoB metabolism.

A second major conclusion from this study is that intestinal apoB-48 synthesis was suppressed by maneuvers that increase mucosal cholesterol absorption (Table 5). Inhibition of mucosal cholesterol uptake, by contrast, was associated with increased apoB-48 synthesis (Table 5). A precise dose-response relationship cannot be postulated, however, since cholesterol intake and uptake by the mucosa were not specifically studied. The implications of this study are therefore limited to defining a relationship between percent cholesterol absorption and apolipoprotein synthesis. However, all the diets except *ii* and *iii)* (which had 1% cholesterol added) had comparable amounts of cholesterol, suggesting that *dietary* cholesterol intake was similar among the groups. Other workers have shown that *biliary* cholesterol secretion rates are similar following these various dietary maneuvers (38). This suggests that luminal cholesterol input (mg per day) is comparable in at least four of the five groups under comparison in Fig. 3. The contribution of hypothyroidism per se to the suppression of intestinal apoB-48 synthesis is difficult to address in isolation particularly because of the effects of hypothyroidism on

TABLE 7. Lipid composition of intracellular intestinal lipoproteins: effects of sustained alteration in mucosal cholesterol flux

Lipoprotein Fraction	n[a]	Percent Lipid Distribution (w/w) Following TLC[b]			
		CE	TG + (FA/MG)	FC	PL
Chylomicrons					
Control diet	4	4.0 ± 0.8	60.0 ± 20	13.5 ± 5.4	25.0 ± 12
HC diet	3	32.0 ± 6.1	44.7 ± 6.4	5.3 ± 3.2	18.3 ± 8.4
Difference		$P < 0.001$	NS	NS	NS
VLDL					
Control diet	4	6.8 ± 3.9	63.3 ± 15.4	10.0 ± 6.1	20.0 ± 10.7
HC diet	3	21.0 ± 6.6	50.7 ± 24	4.3 ± 1.2	17.0 ± 18
Difference		$P < 0.025$	NS	NS	NS
LDL					
Control diet	4	8.3 ± 4.6	53.0 ± 6.3	14.8 ± 5.3	24.0 ± 6.9
HC diet	2	22.5 (17, 28)	42.5 (45, 40)	9.5 (7, 12)	29.5 (39, 20)
Difference		$P < 0.05$	NS	NS	NS
HDL					
Control diet	4	7.5 ± 6.7	36.3 ± 13	9.0 ± 3.9	46.8 ± 15.6
HC diet	2	13.0 (3, 23)	40.5 (46, 35)	5.5 (3, 8)	42.5 (47, 38)
Difference		NS	NS	NS	NS

Intracellular intestinal lipoproteins were isolated from pooled enterocytes of fasted rats following nitrogen cavitation (Methods). Lipoproteins were prepared by sequential ultracentrifugation using NaBr to raise the density of each fraction. Animals were maintained on chow + 5% lard + 0.3% Na taurocholate (control) or the above diet supplemented with 1% cholesterol + 0.1% propylthiouracil (HC) for 21 days. Diets were prepared by hand (Methods) and administered ad libitum until 14–16 hr prior to study. Significance was determined by independent *t*-test.

[a] Refers to the number of pools of animals studied, each pool representing five to eight animals.

[b] Aliquots of each lipoprotein fraction were subjected to extraction in chloroform–methanol (2:1, v/v) and analysis by quantitative TLC (Methods). Data are means ± SD, except where n = 2 when both values are presented.

cholesterol absorption (Table 8). However, based on the relationship between cholesterol absorption and jejunal apoB-48 synthesis over a range of cholesterol absorption values in *euthyroid* rats, it appears reasonable to speculate that these two physiological parameters may be related.

The mechanism by which enhanced mucosal cholesterol flux might produce suppression of apoB-48 synthesis is unclear. Possibly the accumulation of cholesteryl ester-rich chylomicrons and VLDL (Table 7) may somehow alter the conformation and lipid-association properties of enterocyte apoB. Previous studies have shown that hypercholesterolemic hypothyroid rats accumulate cholesterol ester-rich VLDL particles within intestinal Golgi (15). The lipid composition of intracellular chylomicrons and VLDL was studied from a single pool of five rats maintained for 21 days on the lard/PTU diet (i.e., hypothyroid but not cholesterol-fed). In this group, cholesteryl ester comprised 10% of chylomicron and 10.5% of VLDL lipid, a distribution intermediate between lard/NaTc-fed and lard/NaTc/PTU/cholesterol-fed animals (see above, Table 7). The distribution of intracellular chylomicron and VLDL lipid into the other major classes (triglyceride + fatty acid/monoglyceride, free cholesterol and phospholipid) was indistinguishable from that in control animals (data not shown). It appears, therefore, that a characteristic alteration in intracellular lipoprotein lipid distribution accompanies the suppression of apoB-48 synthesis produced by augmentation of mucosal cholesterol flux. However, the data do not exclude the possibility that circulating levels of thyroid hormone may exert a direct effect on intestinal apoB-48 metabolism.

TABLE 8. Cholesterol absorption in free-living rats: dietary and pharmacologic manipulation

Treatment Group	n	Cholesterol Absorption (%)	Difference
Purina chow	5	54.1 ± 6.7	
Lard/NaTc	5	71.0 ± 9.5	$P < 0.01$
Lard/NaTc/CH/PTU	5	67.6 ± 9.4	$P < 0.05$
Lard/PTU	5	79.5 ± 3.5	$P < 0.005$
Chow/AOMA	5	25.9 ± 7.7	$P < 0.0005$

Animals were maintained on the respective diets for 14 days (chow/AOMA) or 21 days (other diets) prior to study. After a 14–16 hr fast, animals received an intragastric bolus of 10 μCi of [1,2-^{3}H]cholesterol as a sonicated emulsion containing 100 mg of triglyceride and 4 mg of cholesterol in 5 mM Na taurocholate. Each animal was simultaneously injected intravenously with 2 μCi of [4-^{14}C]cholesterol as a colloidal suspension in saline. Two hours later, the respective diets were readministered ad libitum for 72–96 hr, whereupon animals were exsanguinated via the abdominal aorta and plasma cholesterol specific radioactivity (^{3}H/^{14}C) ratios were determined (Methods). Data are expressed as means ± SD for (n) animals; the differences were determined from control, chow-fed animals by independent *t*-test. The legend to dietary groups is shown in the text (Methods).

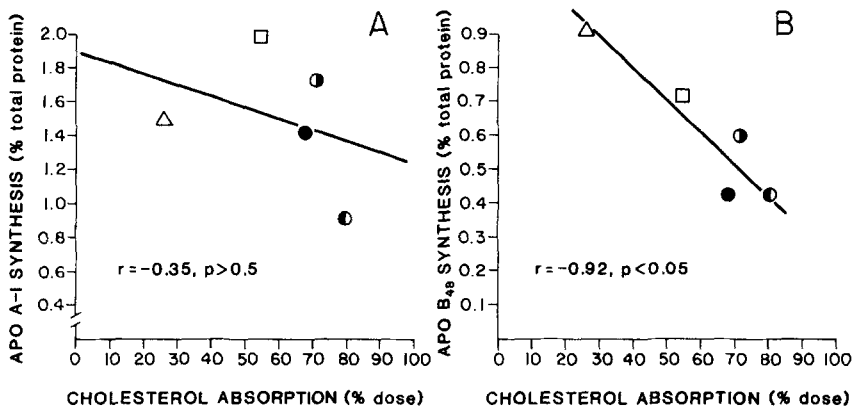

Fig. 3. Relationship between luminal cholesterol uptake and jejunal apolipoprotein A-I (A) and apoB-48 synthesis rates (B). Cholesterol absorption and jejunal apolipoprotein A-I and apoB-48 synthesis rates were measured under a variety of conditions of altered mucosal cholesterol flux (Methods). Each point represents the group mean of two to four separate determinations each of synthesis rate and the group mean cholesterol absorption rate. Data for these various parameters are presented in Tables 4, 5, and 8, respectively. Legend to groups: (◑), chow + 5% w/w lard + 0.3% w/w Na taurocholate; (●), above diet + 1% w/w cholesterol + 0.1% w/w propylthiouracil (PTU); (◐), chow + 5% w/w lard + 0.1% PTU; (△), chow + 2% w/w AOMA (Surfomer); (□), control-chow.

Jejunal apoA-I synthesis was suppressed almost 50% in hypothyroid rats compared to animals receiving the lard/NaTc diet (Table 4) and by 34% in comparison to hypothyroid rats consuming additional cholesterol and bile salt (Table 4). This observation, in conjunction with the divergent effects of these diets upon serum apoA-I concentration, suggests that hypothyroidism exerts a potent effect upon both intestinal and systemic apolipoprotein A-I metabolism which is then subject to modification by luminal sterol flux. The data do not support a primary role for altered mucosal cholesterol flux as a regulator of intestinal apoA-I synthesis. The results of the present study differ from those of Tanaka et al. (22) who found that rat intestinal apoA-I and apoE synthesis were both increased twofold by intake of a lard/PTU/cholesterol/bile salt diet. Those studies were conducted using in vitro incubations and translational analysis of mRNA activity, which makes the results not strictly comparable to those currently reported. However, this issue is presently unresolved.

External bile diversion was shown to suppress both jejunal and ileal apoB-48 synthesis rates, an effect that was fully reversible in jejunum by administration of 10 mM Na taurocholate (26). One potential mechanism for this effect is facilitated uptake of luminal sterol. Other studies have suggested a specific role for acute cholesterol flux in regulating aspects of apoB-48 metabolism in the rat (36). In the present report, however, animals studied 7 hr after a 20-mg cholesterol bolus was administered in corn oil were found to have values no different from those of matched controls for every parameter of both intestinal apoA-I and apoB-48 metabolism (Table 6). This suggests that *acute cholesterol* flux does not, in fact, regulate intestinal metabolism of either apolipoprotein.

The present studies provide further evidence that chylomicron apolipoprotein expression may be regulated independently with regard to lipid flux (44). Thus while neither intestinal apoA-I nor apoB-48 synthesis appears to be induced by acute triglyceride flux (25, 26), apoA-IV synthesis appears to undergo marked induction in response to bolus triglyceride administration (43) (Davidson, N. O., unpublished studies). In addition, circulating levels of thyroid hormone appear to mediate an effect on intestinal apoA-I and apoB-48 synthesis, the mechanism for which may be analagous to the tissue-specific effects recently demonstrated in regard to the myosin heavy chain multigene family (45). Further studies, directed at probing the relationships alluded to above, are currently in progress.

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