

Effects of thyroid status and fasting on hepatic metabolism of apolipoprotein A-I

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Abstract Metabolism of apolipoprotein (apo)A-I was studied in normal and chow-fed hyperthyroid rats, in 24-h fasted untreated male rats, and in rats after thyroparathyroidectomy (TXPTX). Rats were made hyperthyroid by administration of T₃ (9.6 µg/day) or T₄ (30 µg/day) with an Alzet osmotic minipump. Hyperthyroidism produced a similar two- to threefold elevation in plasma levels of apoA-I in male or female animals. During treatment with T₃, plasma levels of T₃ ranged from 200 to 400 ng/dl and did not correlate with plasma apoA-I levels. The net mass secretion and synthesis ([³H]leucine incorporation) of apoA-I by perfused livers from male hyperthyroid rats was elevated, while secretion of albumin was not different than that of euthyroid rats. Furthermore, the incorporation of [³H]leucine into total perfusate and hepatic protein was not altered by hyperthyroidism. The effect of thyroid hormone on apoA-I concentrations remained elevated. Plasma T₃ decreased from 100 ng/dl to 40 ng/dl, in the hypothyroid rat resulting from TXPTX, but the plasma concentration of apoA-I did not change from that observed after 7 or 14 days of treatment, yet plasma apoA-I concentrations remained elevated. Plasma T₃ decreased from 100 ng/dl to 40 ng/dl, in the hypothyroid rat resulting from TXPTX, but the plasma concentration of apoA-I did not change during the 2-week experimental period. The net secretion of apoA-I by livers from hypothyroid animals was depressed and albumin was unaffected compared to the euthyroid. Overnight fasting of euthyroid rats did not alter hepatic apoA-I secretion or plasma apoA-I levels, although under fasting conditions we had reported that hepatic output of apoB and E of VLDL is depressed. The addition of oleic acid to the perfusion medium, sufficient to stimulate VLDL production, did not affect net hepatic secretion of apoA-I by livers from euthyroid, hyperthyroid, or hypothyroid rats. **■** In summary, hepatic synthesis of apoA-I appears to be controlled independently of other apolipoproteins and secretory proteins (albumin). Hepatic apoA-I synthesis is sensitive to thyroid status, increased in the hyperthyroid and decreased in the hypothyroid state. The specific stimulation of hepatic synthesis and secretion of apoA-I in the hyperthyroid state, however, tends to normalize over an extended period, perhaps from compensatory effects of a hormonal nature. —Wilcox, H. G., R. A. Frank, and M. Heimberg. Effects of thyroid status and fasting on hepatic metabolism of apolipoprotein A-I. *J. Lipid Res.* 1991. 32: 395–405.

Supplementary key words hyperalphalipoproteinemia • perfused rat liver • VLDL • apolipoproteins

The thyroid status of the animal or human is an important factor in the regulation of plasma lipoproteins and

hepatic lipid metabolism (1, 2). Plasma cholesterol concentration is reduced in hyperthyroidism, resulting, in part, from decreased formation and secretion of the VLDL and from increased bile acid synthesis and secretion, although rates of hepatic cholesterol biosynthesis are increased (1). In hyperthyroid patients, both LDL and HDL cholesterol concentrations are generally below normal. In contrast, hypercholesterolemia is common in hypothyroid patients and experimental animals, associated with increases in both LDL and HDL cholesterol.

It has been reported that, in humans, plasma concentrations of apoA-I decrease when hypothyroid patients are treated with T₄ (3), while another study has indicated that plasma apoA-I levels increased when the hyperthyroid patients were converted to the euthyroid state (4). Less attention has been directed toward effects of thyroid hormone on metabolism of apoA-I in animals. It was reported from our laboratory, in apparent contradiction to the reports in humans, that plasma apoA-I concentrations increase in normal rats treated with T₃, while apoA-I levels are unaffected in rats made hypothyroid by treatment with propylthiouracil (5). Furthermore, secretion of apoA-I by isolated perfused livers from hyperthyroid rats increased relative to euthyroid controls, while secretion was reduced considerably by livers from PTU-treated animals (5). Because of the unexpected nature of these observations, we studied effects of thyroid status on the temporal sequence of changes in apoA-I metabolism (hepatic synthesis, secretion, and plasma concentration of apoA-I). We also examined uptake of apoA-I by the isolated liver, and plasma clearance and turnover of apoA-I in the intact rat. Preliminary reports of part of this work have appeared (5).

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; TXPTX, thyroparathyroidectomy; apo, apolipoprotein; T₃, triiodothyronine; T₄, L-thyroxine; PTU, propylthiouracil; αGPD, α-glycerophosphate dehydrogenase; EU, euthyroid; HT, hyperthyroid.

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METHODS

Male and female rats (150–200 g) obtained from Harlan, Inc. were housed for 1–4 weeks and were allowed free access to Purina Rodent Chow and water before being used in experimental procedures. T_3 was administered to animals by osmotic minipump (6). Eight mg of T_3 was solubilized by ultrasonic treatment in 500 μ l n-butanol, and propylene glycol was added to a final volume of 10 ml (7). Dilutions were made with the propylene glycol-n-butanol vehicle as needed. A fresh solution was prepared for each new experimental set (weekly). Osmotic minipumps (Models 2001 or 2002, delivering 1.0 or 0.5 μ l/h, respectively, obtained from Alzet Corp., Palo Alto, CA), were loaded with the T_3 solution or with the vehicle alone. Delivery of T_3 was 9.6 μ g/day unless otherwise indicated. In some experiments, 25 mg of T_4 was solubilized similarly and delivered 30 μ g/day using the Model 2002 Alzet osmotic minipump. Minipumps containing T_3 or vehicle were stored overnight in 0.9% NaCl at 4°C before implantation. Surgical implantation of the minipumps was carried out under light anesthesia with diethyl ether between 0900 and 1100 h. Intraperitoneal placement of the minipumps was more convenient than subcutaneous placement, although similar results were obtained with either technique. In addition, untreated normal fed or fasted (24 h) animals were studied.

Hypothyroidism was induced by thyroparathyroidectomy (TXPTX). Surgery was carried out in male and female rats anesthetized with diethyl ether. A sham operation was carried out on control (euthyroid) rats. Hyperthyroidism was induced by administration of T_3 as in unoperated rats. For maintenance of TXPTX animals, the ground Purina Rodent Chow diet was fortified with Ca gluconate, $MgSO_4$, and Na_2HPO_4 . Calcium and magnesium were also added to the drinking water, as was vitamin D_2 (Drisdol, Winthrop Laboratories, NY). Concentrations were adjusted (8) such that each rat received 4500 U vitamin D_2 , 38 mg Ca, 0.8 mg Mg, and 12 mg phosphorus in 20 ml water or 10 g food. Rats were placed on this dietary supplement 2 days prior to surgery and throughout the ensuing treatment period.

The plasma level of Ca^{2+} was similar in control and treated groups (both males and females; 11.5 vs. 11.2 mg/dl for TXPTX with or without T_3 administration, vs. sham-operated control rats, respectively). TXPTX animals consumed the same amount of chow per day as did sham-operated controls during the 14-day experimental period (6.0 ± 0.1 (n = 3); 7.0 ± 0.2 (n = 4); and 5.2 ± 0.2 (n = 4) g/day per 100 g body weight for sham-operated controls, hyperthyroid, and hypothyroid rats, respectively). Mean body weight gain tended to be lower in TXPTX rats than in controls, but statistical significance was not reached with the number of animals in these ex-

periments. In certain experiments, hypothyroidism was induced by treatment with propylthiouracil (PTU, 1 mg/day) (5), and hyperthyroidism by subcutaneous injections of T_3 (10 μ g) for 7 days (5). At the termination of each treatment period, blood was obtained from the abdominal aorta in anesthetized (see below) animals prior to removal of the liver for perfusion *in vitro* (between 0800 and 1000 h). Blood samples (0.2–0.3 ml) were obtained during the treatment period from tail veins.

Perfusion of livers

Rats were anesthetized with diethyl ether, and livers were isolated and perfused *in vitro* using apparatus and procedures described previously (9, 10). All rats had free access to food and water, unless otherwise stated, until initiation of experiments. Livers were perfused with a basal medium containing 30% (v/v) washed bovine erythrocytes, 6% (w/v) purified bovine serum albumin, and 100 mg glucose/dl of Krebs-Henseleit bicarbonate buffer (9). Generally, a complex of oleic acid and delipidated (11) bovine serum albumin, 6%, (molar ratio of oleate/BSA = 16) was infused at a rate of 11.7 mg/h (166 μ mol oleic acid/h). Because of dilution and hepatic fatty acid uptake, this ratio was reduced during steady-state perfusion conditions to 1.5–2.0. In certain perfusion experiments, the medium contained 8 mM leucine and a physiological mixture of amino acids (10). The high concentration of leucine was present when [4,5- 3H]leucine incorporation into protein was examined to compensate for any potential differences in leucine pool size in the livers of the various experimental groups.

Analysis of samples

Samples of perfusate were taken before, during, and after the perfusion. Liver samples were obtained at the termination of perfusion. Hepatic mitochondria were isolated by centrifugation from 2.0 g of tissue that had been homogenized by hand, using a Dounce homogenizer in ice-cold sucrose (0.25 M) and Tris buffer (0.05 M, pH 7.2). Seventy μ g of mitochondrial protein was analyzed for activity of α -glycerophosphate dehydrogenase (α -GPD) according to Lee and Lardy (12). The concentrations of plasma T_3 were measured by radioimmunoassay using commercial kits (Abbott Laboratories, Chicago, IL, or Micromedic, Horsham, PA). Calcium was measured by atomic absorption spectroscopy using the lanthanum-HCl method of Dalrymple and Kenner (13) on serum samples diluted 60-fold.

Radioimmunoassay of rat apoA-I

ApoA-I was obtained from HDL (d 1.063–1.210 g/ml) isolated by ultracentrifugation (24 h at 110,000 g) from pooled plasma of male Sprague-Dawley rats. HDL was floated at d 1.210 g/ml a second time after the initial isola-

tion. After exhaustive dialysis against 0.05 M NH_4HCO_3 -1 mM EDTA (pH 8.0), HDL was lyophilized and delipidated with ethanol-anhydrous diethyl ether 3:2 (v/v) at -20°C overnight. The apolipoprotein precipitate was dried under N_2 and solubilized in 6 M urea-0.2 M Tris buffer (pH 8.0). Ten to 20 mg of the solubilized HDL protein was applied to Sephacryl S200 and S300 columns connected in series (90×2.6 cm). The second peak eluting (14) was found to be homogeneous and greater than 99% apoA-I, as determined by mobility on SDS-PAGE and by isoelectric focusing. ApoA-I preparations were lyophilized and stored at -70°C . The apoA-I was used as antigen for antibody preparation, for standards in the assay, and for the lactoperoxidase iodinated (15) apoA-I standard.

Antisera to apoA-I was raised in male New Zealand rabbits by dorsal multiple intradermal injections of a 1:1 suspension of apoA-I (0.5-1.0 mg apoA-I, total) and complete Freund's adjuvant. After 1 month, an adequate titer was obtained. Serum was obtained, lyophilized, and stored at -70°C .

The double radioimmunoassay for apoA-I was carried out as described for apoE (16). All samples were diluted at least 1:10 with the RIA buffer (0.05 M barbital containing 0.02% NaN_3 , 3 g/dl bovine serum albumin, and 1% Triton-X100 (v/v), pH 8.6). Sample dilutions were made 24 h in advance of the assay. The standard curve ranged from 2 to 64 ng of apoA-I. The least mean square log-log plot had an average linear correlation of $r^2 = 0.996 \pm 0.001$ ($n = 10$ consecutive assays, over 4 weeks). In subsequent assays (several hundred) with different preparations of apoA-I, the linearity of the standard curve did not deviate. The intra-assay coefficient of variation was 2% while that for the inter-assay coefficient was 7%. The addition of 5 μg of pure apoA-I to plasma samples contained 3-10 μg of apoA-I gave a recovery of $100 \pm 5\%$ ($n = 6$), while the same amount added to 75-125 μg apoA-I in plasma gave a recovery of $92 \pm 11\%$ ($n = 6$). Analysis of different plasma samples at twofold dilutions gave a coefficient of variation of $14 \pm 5\%$ ($n = 6$). The radioimmunoassay for apoA-I was compared with that of radial immunodiffusion (RID). By RID (5) plasma apoA-I was 41.4 ± 2.0 mg dl and by RIA the value was 40.6 ± 2.5 ($n = 17$).

Preparations of apoE (200 ng), apoB (500 ng), or apoC (5000 ng) replaced less than 1% of the labeled apoA-I in the assay. Rat serum albumin (1000 ng), bovine serum albumin (Cohn Fraction V, 1000 ng, bovine HDL (1000 ng), and human serum (1:100, equivalent to about 2500 ng apoA-I) did not displace a significant amount of the rat radioligand.

An antiserum to apoA-I kindly provided by Dr. Gustav Schonfeld, (Washington University, St. Louis, MO) provided values indistinguishable for those with our own.

Similarly, rat apoA-I, prepared by Dr. Ladislav Dory (The University of Tennessee, Memphis, TN), when used as either standard or radioligand, yielded data indistinguishable from those obtained with our own preparations.

A rat plasma albumin radioimmunoassay was developed and used to estimate the concentration of rat albumin in the liver perfusates. A standard curve using purified rat albumin (Sigma Chemical Co., St. Louis, MO) in the range 2 to 64 ng was found convenient. Radiiodination (15) of the rat albumin was carried out as described for apoA-I. Rabbit anti-rat albumin (Cappel Labs, Inc.) was used in the assay. The actual RIA procedure was identical to that with apoA-I, described here, or apoE (16).

Determination of radioactivity

Trichloroacetic acid-precipitable radioactivity was determined on the whole cell-free perfusate, on the perfused liver after washing and homogenization, and on the d 1.006-1.210 g/ml fraction of the terminal perfusate. Protein precipitation was carried out in 6% TCA and 1% leucine. All precipitates were washed twice with the same mixture, dissolved in either hyamine hydroxide (Research Products International, Mt. Prospect, IL) or TS-1 solubilizer (New England Nuclear, Boston, MA) (10), and neutralized with acetic acid. Radioactivity was determined, after addition of 10 ml Biocount scintillation fluid, in a Beckman model LS3501 liquid scintillation counter (10). A random coincidence monitor provided information on artifactual disintegrations. A reading of $<10\%$ was obtained before data were accepted.

For quantitation of total perfusate apoA-I radioactivity, 0.5 ml fresh rat plasma was added to each ultracentrifugal $d > 1.006$ g/ml infranatant fraction from 10 ml perfusate, and the density was adjusted to 1.210 g/ml with solid NaBr. The d 1.006-1.210 g/ml fraction was isolated by centrifugation for 24 h at 39,000 rpm in a Beckman 50.2 rotor. After exhaustive dialysis, the d 1.006-1.210 g/ml and $d < 1.006$ g/ml fractions were subjected to SDS-PAGE (10). The apoA-I band was dissolved in TS-1 after decolorizing with H_2O_2 (10). After neutralization the radioactivity was determined as described above.

Statistics

All data are expressed as means \pm SEM. Statistical differences between means were established by Student's t test (two-tailed) with $P < 0.05$ accepted as an indication of significance.

RESULTS

We reported previously that rats treated daily for 7 days with T_3 had higher plasma concentrations of apoA-I than

TABLE 1. Effects of triiodothyronine on hepatic secretion and synthesis of nascent HDL (d 1.006-1.210 g/ml)

Group	Protein Secreted	[³ H]Leucine Incorporated
	μg/g liver	dpm/g liver
EU (3)	175 ± 8	28,200 ± 2,400
HT (3)	225 ± 14 ^a	46,500 ± 1,000 ^a

Male rats (250-300 g) were made hyperthyroid (HT) by treatment with 9.6 μg T₃/day for 7 days using peritoneally implanted Alzet osmotic minipumps. Livers were then perfused for 4 h as described in the text. A pulse of 17 μCi of [4,5-³H]leucine was added to the original perfusion medium and 166 μmol oleic acid and tracer amounts of [4,5-³H]leucine (56 μCi/h) were infused/h. Plasma levels of T₃ were 85 ± 12 and 370 ± 40 ng/dl for euthyroid (EU) and HT animals, respectively.

^aP < 0.05 compared to EU.

did euthyroid controls, and that hepatic secretion of apoA-I was increased by hyperthyroidism (5). These data, in general, suggested that livers from hyperthyroid animals produced and secreted a greater amount of HDL than livers from euthyroid rats. The d 1.006-1.210 g/ml lipoprotein fraction, isolated from the medium perfusing livers from hyperthyroid rats, indeed contained more protein and radioactivity from [³H]leucine than did the perfusate from euthyroid controls (Table 1). There is little, if any, LDL secreted by the rat liver. We found only a very small amount of radioactivity in protein in the d 1.006-1.050 g/ml fraction (10-12%) suggesting that the majority of this fraction was nascent HDL. Therefore, these two fractions were not routinely separated. These data, therefore, would suggest that more HDL protein is secreted by livers from hyperthyroid than euthyroid animals.

The extent to which apoA-I was present in the d 1.006-1.210 g/ml fraction was examined. The distribution of apoA-I mass, determined by radioimmunoassay, among the lipoprotein fractions isolated from the perfusion medium at the termination of the experiments, and

in rat plasma, is presented in Table 2. The major proportion of apoA-I isolated from the perfusate was present in the d > 1.210 g/ml fraction (67 and 80%, for the euthyroid and hyperthyroid, respectively). In comparison, 19 and 12% of the apoA-I was present in the d > 1.21 g/ml fraction of plasma of euthyroid and hyperthyroid, respectively. In perfusates of both groups, however, a similar amount of rat apoA-I was associated with the d 1.006-1.210 g/ml fraction (HDL). Less apoA-I was associated with the VLDL (d < 1.006 g/ml) from the medium perfusing livers from hyperthyroid rats than from euthyroid rats. These data suggest that much of the apoA-I may be secreted in lipid-free form. When carrier rat plasma was added to samples of the perfusion medium containing secreted proteins (labeled with [³H]leucine), the recovery of labeled apoA-I in the d 1.006-1.210 g/ml fraction increased from 20-30% to >85%. This was also shown using a tracer amount of ¹²⁵I-labeled apoA-I as a marker. For this reason, fresh carrier rat plasma was added to perfusate d < 1.006 g/ml infranant fractions when evaluating the incorporation of [4,5-³H]leucine into the total apoA-I of the perfusate.

We reported previously that the hyperthyroid state in male rats leads to hyperalphalipoproteinemia (an increase in apoA-I) concomitant with increased hepatic secretion of apoA-I. It was of interest to determine whether the female rat responded similarly after 1 week of treatment (5) with T₃ (Table 3). Within 24 h of treatment, the maximal concentration of T₃ in the plasma was obtained, which did not fluctuate significantly during the treatment period (data not shown). No differences between male and female animals were observed when 9.6 μg T₃ was administered daily for 7 days. When a larger dose of T₃ was administered to male rats (19.2 μg daily for 7 days) an increase in plasma apoA-I concentration and a doubling of the plasma T₃ concentration above that at the lower dose was observed.

TABLE 2. Distribution of immunoreactive rat apoA-I among lipoprotein fractions isolated from liver perfusates and rat plasma

Group	Lipoprotein Density Fraction (g/ml)			
	d < 1.006	d 1.006-1.210	d < 1.210	d > 1.210
	μg apoA-I/ml			
A. Perfusate				
EU (6)	0.33 ± 0.07	0.58 ± 0.08	0.91 ± 0.20	1.86 ± 0.15
HT (6)	0.10 ± 0.04 ^a	0.80 ± 0.22	0.90 ± 0.80	3.68 ± 0.32 ^a
B. Plasma				
EU (4)			245 ± 22	56 ± 1
HT (4)			520 ± 56 ^a	71 ± 3 ^a

Samples of liver perfusate, or plasma of liver donors, were derived from animals and perfusion experiments as described in Table 1, except that radioactivity was omitted. Lipoprotein fractions were obtained by ultracentrifugation as described in the text. Plasma carrier was not added to perfusate samples. The d < 1.210 g/ml fraction of the liver perfusate is the sum of the d < 1.006 and d 1.006-1.210 g/ml fractions.

^aP < 0.05 compared to EU.

TABLE 3. Effects of treatment with T₃ on plasma concentrations of apoA-I and T₃ in male and female rats

Group	Plasma Concentrations	
	T ₃	ApoA-I
	ng/dl	mg/dl
Male		
EU (4)	122 ± 13	24.7 ± 4.3 (3)
HT (4)		
A. (9.6 µg T ₃ /day)	370 ± 37 ^a (3)	40.1 ± 3.8 ^a (3)
B. (19.2 µg T ₃ /day)	560 ± 15 ^{a,b}	63.3 ± 7.1 ^{a,b}
Female		
EU (8)	108 ± 3	25.3 ± 3.6
HT (7) (9.6 µg T ₃ /day)	358 ± 3 ^a	54.5 ± 6.1 ^a

Normal untreated male rats (240 ± 4 g) were divided into three groups; one group received vehicle alone (EU), and two groups were treated with T₃ (9.6 µg/day or 19.2 µg/day for 7 days) by osmotic minipump (HT). Female rats (184 ± 2 g) were treated for 7 days with vehicle alone (EU) or 9.6 µg T₃/day (HT).

^aP < 0.05 compared to EU.

^bP < 0.05 compared to HT (9.6 µg T₃/day).

The necessity for the high plasma level of T₃ to maintain the high plasma apoA-I levels is shown by the changes observed in plasma concentration of apoA-I during treatment with T₃ for a 2-week period, and the rapid decline in apoA-I levels when T₃ administration was discontinued (Fig. 1).

Although administration of T₃ to normal intact rats produced remarkable changes in apoA-I metabolism, it was of interest to determine whether similar results would be obtained in rats given T₃ after removal of the thyroid gland. TXPTX male rats were examined during a 2-week period to determine the temporal sequence of thyroid status on hepatic metabolism of apoA-I and to determine whether plasma levels of apoA-I and hepatic secretion of apoA-I were parallel events (Table 4). The maximal concentration of plasma T₃ was achieved 24 h after implantation of the minipump in TXPTX rats [also observed in T₃-treated intact rats (data not presented)], by which time the plasma apoA-I concentration was also elevated significantly. In the TXPTX rats given T₃, the plasma T₃ remained elevated and relatively constant over the 2-week period. In untreated TXPTX rats, the T₃ concentration decreased to a minimum by the third day, and remained at that level throughout the remainder of the experiment. In this group of rats, hepatic α-glycerophosphate dehydrogenase (αGPD) activity was also measured as additional confirmation of the hyperthyroid or hypothyroid state (12). After 2 weeks of treatment, hepatic mitochondrial αGPD activity for the hyperthyroid rats (n = 4) was 418 ± 29% (P < 0.05) of the sham-operated euthyroid animals, while in hypothyroid rats (n = 4), the activity was 50 ± 11% (P < 0.05) of control (n = 4). The αGPD activity was altered significantly by the third day in the

hyperthyroid rats (159 ± 9% of control), and by the fifth day in hypothyroid animals (58 ± 5% of controls).

The elevated plasma levels of apoA-I in the hyperthyroid rats were maintained over the 2-week period of the experiment (Table 4). Plasma concentrations of apoA-I were unaffected by hypothyroidism, and remained similar to euthyroid controls throughout the entire 2-week experiment. Hepatic output of apoA-I by livers from hyperthyroid rats exceeded that of livers from euthyroid animals by 2-3.5-fold (Table 4). Maximal output of apoA-I was reached 3 days after the start of treatment. In this study, hepatic output of apoA-I seemed to decrease gradually from the value at the third day, but remained elevated over controls at 2 weeks. Livers from hypothyroid (TXPTX) animals secreted less apoA-I 5 days after surgery, with a concomitant change in plasma T₃ concentration, and output remained at the low level during the 2-week treatment period.

Although not examined in the TXPTX animal, the net rate of apoA-I output by livers from intact rats after 28 days of treatment with T₃ (9.6 µg/day) was not different compared to control rats (Fig. 2), and significantly lower than observed at 7 days or 14 days (T₃-treated TXPTX rats) (Table 4). Nevertheless, the plasma apoA-I concentration was not different than the level after 7 days [58.2 ± 4.1 (n = 5) and 62.6 ± 3.1 (n = 14) mg apoA-I/dl, respectively, but remained elevated above control levels (34.4 ± 2.3 mg apoA-I/dl (n = 21)]. The plasma level of T₃ after the 28 days of treatment was similar to

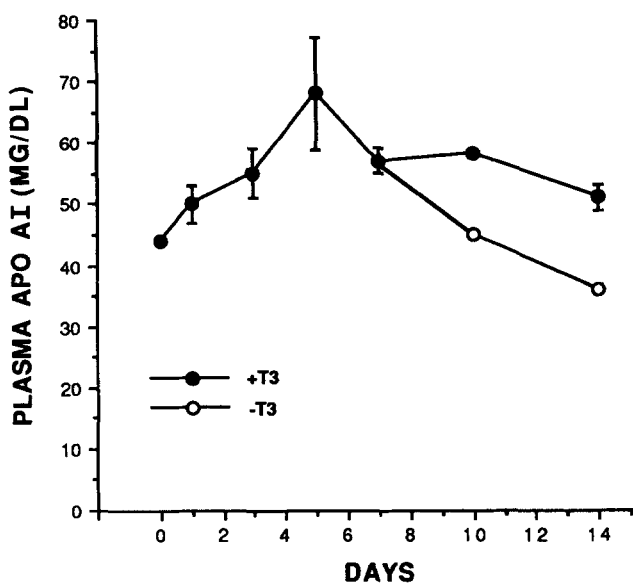


Fig. 1. Effects of treatment with T₃ on plasma apoA-I concentrations. Rats were made hyperthyroid by daily subcutaneous injections of 10 µg T₃ (5). In one group, injections were discontinued after 7 days. Standard error bars are indicated for observations where n = 3; for all other points, n = 2.

TABLE 4. Temporal effects of thyroparathyroidectomy and triiodothyronine on plasma concentration of T₃ and apoA-I, and on hepatic secretion of apoA-I

Group	Days of Treatment			
	3	5	9	14
Concentration of T ₃ in plasma (ng/dl)				
A. Sham operated (3)	99 ± 11	90 ± 6	118 ± 13	108 ± 14
B. TXPTX + T ₃ (4)	343 ± 64 ^a	338 ± 34 ^a	341 ± 12 ^a	299 ± 39 ^a
C. TXPTX (3)	48 ± 18 ^a	32 ± 18 ^a	40 ± 11 ^a	60 ± 9 ^a
Concentration of apoA-I in plasma (mg/dl)				
A. Sham operated (3)	29.6 ± 0.6 (4)	34.3 ± 3.3 (4)	33.8 ± 3.6	33.8 ± 2.4
B. TXPTX + T ₃ (4)	59.5 ± 3.9 (3) ^a	69.2 ± 2.7 ^a	66.4 ± 2.0 ^a	68.5 ± 4.9 ^a
C. TXPTX (4)	32.9 ± 4.6	30.6 ± 3.3	31.2 ± 2.1	35.4 ± 1.3
Net hepatic secretion of apoA-I (μg/g liver/4 h)				
A. Sham operated (3)	21.4 ± 4.0	21.5; 22.1	15.6 ± 1.4	14.9; 14.3
B. TXPTX + T ₃ (4)	72.9 ± 10.6 ^a	70.0 ± 4.5 ^a	51.1 ± 3.0 (3) ^a	32.9 ± 5.4 (3)
C. TXPTX (4)	19.8 ± 5.0	6.5 ± 1.0 ^a	6.3 ± 1.0 ^a	5.9 ± 0.3 ^a

After TXPTX or sham operation, animals received either 0.9% NaCl or T₃ (9.6 μg/day) by osmotic minipump (see Methods section). All liver perfusions were carried out for 4 h during which 166 μmol oleic acid was infused/h, without added amino acids, as described in the Methods section.

^aP < 0.05 compared to euthyroid.

that at 7 days [336 ± 18 (n = 5) and 314 ± 21 (n = 14) μg/dl, respectively].

To compare effects of T₄ with those of T₃, thyroxine (30 μg/day) was administered by osmotic minipump to male rats for 7 days. The plasma concentration of apoA-I (50.4 ± 3.7 μg/dl) and hepatic output of apoA-I after treatment with T₄ were elevated above the euthyroid controls (Fig. 2), similar to that observed after 7 days of treatment with T₃. The 7-day treatment with T₄ resulted in an increase in plasma T₄ levels from 4.4 to 14.4 μg/dl (a value of 1.0 μg/dl was found for the hypothyroid rats). Treatment with T₄ also resulted in an elevation of the plasma T₃ levels to 227 ± 30 (15) ng/dl.

It is well established that free fatty acids are primary stimulants of the secretion of VLDL lipids and associated apolipoproteins by livers from normal rats. It was therefore of interest to determine whether secretion of apoA-I, the major apoprotein of HDL, might be affected similarly. However, hepatic secretion of apoA-I was unaffected by addition of oleic acid to the medium perfusing livers from fed euthyroid, hyperthyroid, or hypothyroid rats (Table 5). Furthermore, when either palmitate, stearate, eicosapentaenoate, or docosohexaenoate were provided to livers of control rats under similar conditions, apoA-I secretion was similar to that when fatty acid was not provided (data not shown). Additionally, fasting, which reduces VLDL apolipoprotein secretion, also failed to affect hepatic secretion of apoA-I (Table 5) in euthyroid animals. Overnight fasting also did not affect plasma concentration of apoA-I in this series of experiments (29.2 ± 0.8 (81) and 28.5 ± 1.4 (30) mg/dl for fed and fasted male rats, respectively).

To determine whether the increased net hepatic secretion of apoA-I by livers from hyperthyroid rats was due, at least in part, to an enhancement of the rate of biosynthesis, experiments were carried out to evaluate the effects of hormonal treatment on incorporation of radioactive

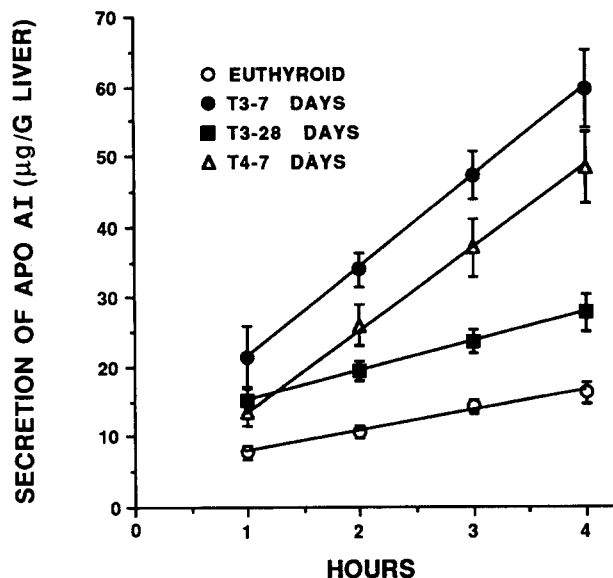


Fig. 2. Effects of treatment with T₃ or T₄ on hepatic secretion of apoA-I by the perfused liver. The cumulative net output of apoA-I is indicated. Normal rats were made hyperthyroid by constant infusion of 9.6 μg T₃ or 30 μg T₄ per day for 7 days or with 9.6 μg T₃ for 28 days. Perfusions were carried out with oleic acid, infused at a rate of 166 μmol/h. Leucine (8 mM) and a physiological mixture of amino acids were included in the perfusion medium (10). Slopes of the linear regression plots (μg/g liver per h) are 3.03 (euthyroid, n = 21); 12.77 (T₃-7 days, n = 17); 4.19 (T₃-28 days, n = 5) and 11.55 (T₄-7 days, n = 4); for all plots: r > 0.99.

TABLE 5. Lack of effect of oleic acid on hepatic synthesis and secretion of apoA-I

Group	Oleic acid infused ($\mu\text{mol/h}$)				
	0	55	110	166	332
	$\mu\text{g apoA-I/g liver/4 h}$				
Euthyroid	17.1 \pm 1.4 (35)	12.1 \pm 1.8 (4)	16.8 \pm 3.6 (4)	16.7 \pm 1.2 (35)	21.0 \pm 6.2 (3)
Fasting euthyroid (24 h)	21.7 \pm 3.0 (24)			17.7 \pm 1.7 (30)	
Hyperthyroid (4)	51.8 \pm 5.8		50.9 \pm 6.4	44.8 \pm 7.6	
Hypothyroid (4)	7.9 \pm 1.0		8.5 \pm 1.2	10.6 \pm 2.4	
	dpm/g liver/4 h				
EU	8360 \pm 640 (6)			8980 \pm 960 (7)	
EU (24 h fasting)	7800 \pm 1160 (7)			9600 \pm 1760 (5)	

Rats (200–250 g) were made hyperthyroid by subcutaneous injections of 10 $\mu\text{g T}_3$ /day per 100 g body weight for 7 days or were rendered hypothyroid by subcutaneous injection of 1 mg PTU/100 g body weight/day for 7 days (5). Unless indicated otherwise, rats had free access to food and water. Perfusions of livers from hyperthyroid and hypothyroid rats were carried out as described in the Methods section. The medium for perfusing livers from fed and fasting euthyroid rats contained 8 mM leucine and a mixture of amino acids (10). In certain of these perfusions, an initial pulse of 202 μCi of [4,5- ^3H]leucine was present and was infused at a rate of 42 $\mu\text{Ci/h}$.

leucine into this apolipoprotein. Incorporation of [4,5- ^3H]leucine into apoA-I with livers from either euthyroid or hyperthyroid rats (Fig. 3) was linear during the 4-h perfusion, after a short lag period. The rate of incorporation (calculated as the ratio of the slopes), after treatment with T_3 , was 2.5 times that of the euthyroid, somewhat lower than the increase in secretion of apoA-I mass (Fig.

2). The specific activity of the secreted apoA-I as determined by the incremental increase in apoA-I mass and radioactivity was essentially constant over the perfusion period and not different between euthyroid and hyperthyroid [604 \pm 53 and 686 \pm 62 dpm/ μg ($n = 12$ each; four experiments, three hourly time periods), respectively]. Furthermore, incorporation of [4,5- ^3H]leucine into apoA-I by livers from euthyroid rats was unaffected by fasting (Table 5), as was the net secretion of mass of this

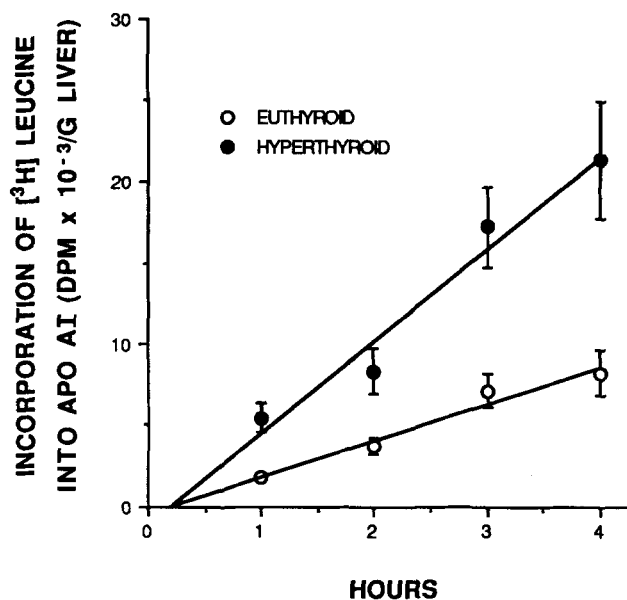


Fig. 3. Effects of hyperthyroidism on incorporation of [4,5- ^3H]leucine into apoA-I secreted by the isolated perfused liver. ApoA-I was isolated from the $d < 1.210$ g/ml ultracentrifugal fraction of the perfusate after the addition of fresh whole rat plasma as carrier (see Methods). Rats were made hyperthyroid by infusion of 9.6 $\mu\text{g T}_3$ /day for 7 days (osmotic minipumps). Oleate (166 $\mu\text{mol/h}$) was infused together with 53.5 μCi [4,5- ^3H]leucine/h (8 mM leucine). A pulse of 235 μCi [^3H]leucine was included in the initial medium (8 mM leucine) (see legend to Fig. 2). Data presented are cumulative. Three perfusions were carried out in each group. Slopes (dpm/g liver per h) were 2260 and 5640 for euthyroid and hyperthyroid, respectively ($r > 0.98$, both slopes).

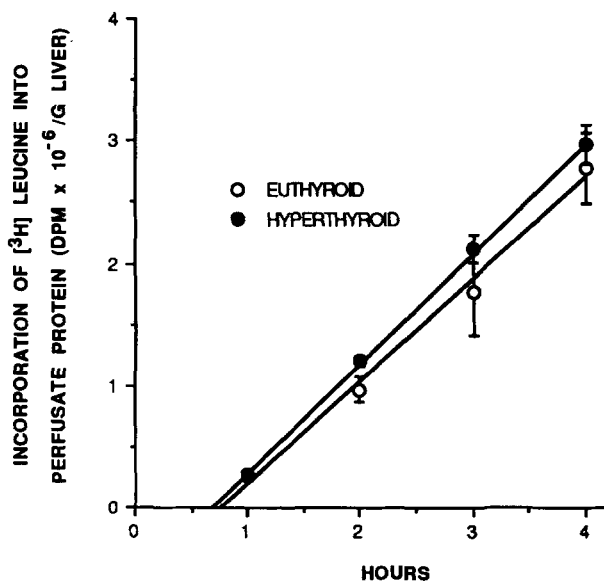


Fig. 4. Incorporation of [4,5- ^3H]leucine into total perfusate protein secreted by livers from euthyroid and hyperthyroid rats. Data represent cumulative TCA-precipitable radioactivity in the perfusate (See Figs. 2 and 3 for experimental details). Slopes (dpm/g liver per h) are 0.836×10^6 and 0.899×10^6 for euthyroid and hyperthyroid, respectively. Total TCA-precipitable radioactivity from [^3H]leucine in hepatic protein at the end of the perfusions (4 h) was 5.03 ± 0.28 and $5.42 \pm 0.33 \times 10^6$ dpm/g liver, for euthyroid and hyperthyroid, respectively. Six perfusions were carried out in each group. ($r > 0.99$ for both slopes).

apolipoprotein (Table 5). Although treatment of intact rats with T_3 increased the incorporation of leucine into secreted apoA-I, incorporation into total perfusate or hepatic protein was not affected. Incorporation of [3H]leucine into total perfusate protein was linear, after an apparent lag period, and was unaffected by thyroid status (Fig. 4). The secretion of albumin by livers from euthyroid and hyperthyroid animals was also unaffected (Fig. 5). The rates of secretion of albumin after either 7 or 28 days of treatment with $9.6 \mu\text{g } T_3/\text{day}$ were similar, and incorporation of [3H]leucine into total perfusate and hepatic protein was not affected by the longer period of treatment (data not shown).

After intravenous administration of a tracer ^{125}I -labeled apoA-I preparation, the plasma fractional clearance rates (FCR) were observed to be similar in fed male hyperthyroid and euthyroid rats (Table 6). The plasma half life of apoA-I calculated from the FCR was about 11 h for either group. However, because of the larger apoA-I pool in the hyperthyroid rats, the absolute turnover of plasma apoA-I (and rate of synthesis in the steady state) was nearly twice that of the euthyroid animals.

DISCUSSION

Hyperalphalipoproteinemia and increased plasma concentrations of apoA-I have not been observed in the hyperthyroid human patient, as we have observed in rats. Indeed, hyperthyroid patients exhibit normal or slightly depressed plasma levels of apoA-I (4, 17). In contrast, plasma apoA-I concentrations in rats given T_3 are increased 2- to 3-fold (5). It was our purpose in these experiments to begin to explore potential factors that regulate plasma concentrations of apoA-I and nascent HDL in the animal model. Clearly, plasma concentrations of apoA-I and HDL are the resultant of rates of hepatic and gastrointestinal synthesis, and rates of clearance from the plasma. In this report, we examined regulation of apoA-I metabolism by thyroid hormone, and lack of control by nutritional state (fed vs. acute fasting) or substrate supply (free fatty acids).

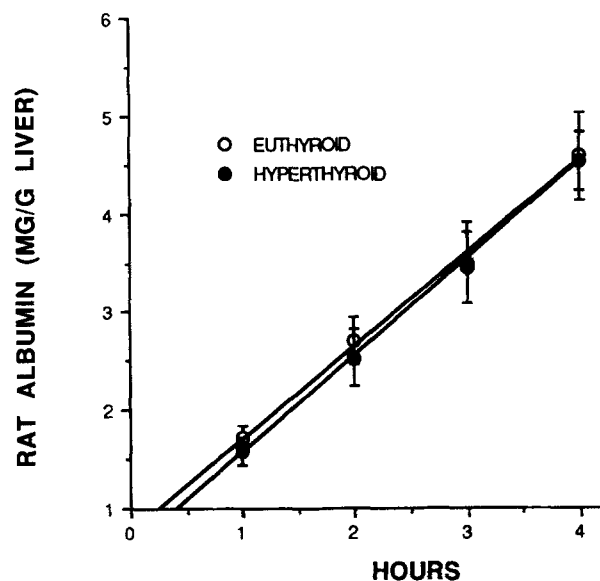


Fig. 5. Secretion of plasma albumin by livers from euthyroid and hyperthyroid rats. Perfusions are those described in the legend to Figs. 2 and 3. Cumulative data are presented. Slopes of least mean square plots are 0.94 and 0.98 mg/g liver per for euthyroid and hyperthyroid, respectively. Six perfusions were carried out in each group. ($r > 0.99$ for both slopes).

Our initial data on apoA-I metabolism were derived from experiments with normal rats given T_3 or propylthiouracil (5). We have now extended our studies to TXPTX animals, and to these rats treated with T_3 . The plasma of normal or TXPTX animals treated with T_3 contains higher levels of apoA-I than does that of euthyroid rats. A major factor accounting for these high concentrations is hypersecretion of apoA-I by the liver, due, in part, to an enhanced synthesis of the apolipoprotein. This increase in plasma apoA-I occurred within 1-2 days after administration of T_3 , and was accompanied by a concomitant increase in hepatic secretion which remained elevated above the euthyroid as long as treatment continued over a 2-week period. Both T_4 and T_3 induced the increase in plasma apoA-I concentration and hepatic secretion of apoA-I. We also observed previously that intestinal production of apoA-I is increased in the hyperthyroid rat and, therefore, must contribute to higher plasma levels of apoA-I in these animals (18).

TABLE 6. Effects of hyperthyroidism on plasma clearance of apoA-I

Group	Rat Weight	Plasma ApoA-I	FCR	Half-life	Absolute Turnover Rate
	<i>g</i>	<i>mg/dl</i>	<i>h⁻¹</i>	<i>h</i>	<i>mg apoA-I/h</i>
EU	299 ± 14 (3)	26.4 ± 1.3 (3)	0.058 ± 0.004 (7)	12.0	0.195 ± 0.026 (3)
HT	285 ± 4 (3)	41.7 ± 2.1 (3) ^a	0.066 ± 0.004 (7)	10.5	0.033 ± 0.040 (3) ^a

The fractional clearance rate (FCR) was determined from least mean square semilogarithmic plots at 3, 5, 22-24, and 28 h of TCA-precipitable counts after injection of tracer ^{125}I -labeled apoA-I prepared as described for the RIA of apoA-I (1.85×10^6 cpm/rat in 20 μl). Hyperthyroid (HT) rats had received $9.6 \mu\text{g } T_3/\text{day}$ by Alzet osmotic minipump for 14 days. A plasma volume of 4% of body weight was assumed for calculation of the absolute turnover rate.

^a $P < 0.05$ compared to euthyroid (EU).

Catabolism of apoA-I must be an important factor in regulation of plasma concentrations of apoA-I. As suggested by the absolute clearance of apoA-I, 2 weeks after initiation of treatment with T₃, increased catabolism may compensate partially for the increased hepatic production rates. The t_{1/2} value of 10.5 h determined for apoA-I in our studies agrees well with the value of 11.6 h reported by Sigurdsson, Noel, and Havel (19). Interestingly, we observed, in preliminary liver perfusion experiments, that uptake of apoA-I by livers from hyperthyroid animals exceeded that of euthyroid controls (Wilcox, H. G., and M. Heimberg, unpublished data), which suggests that thyroid hormone may affect apoA-I uptake by specific tissue beds. Glass et al. (20) determined that only about 25% of the catabolism of apoA-I occurred in the liver, whereas about 40% occurred in the kidney. If, indeed, uptake of apoA-I by livers from T₃-treated rats is increased, it would suggest that total hepatic secretion of apoA-I is even greater than that measured as net secretion under our experimental conditions in a recycling perfusion system. The elevated concentration of plasma apoA-I was maintained throughout a 28-day experimental period, although hepatic production of apoA-I, which remained elevated over that of controls, decreased after 2 weeks treatment, and after 1 month was similar to that of the euthyroid. Perhaps with longer periods of hyperthyroidism, plasma levels of apoA-I, balanced by increased clearance and less elevated production rates, might approach control values and, in this regard, be similar to those found in the hyperthyroid human.

The plasma apoA-I concentrations appeared to be similar in both male and female hyperthyroid rats suggesting that both sexes are similarly sensitive to this action of thyroid hormone on apoA-I metabolism.

When a hypothyroid state was induced, decreased hepatic production of apoA-I was observed. Plasma apoA-I concentrations, however, did not differ from those of the euthyroid, suggesting rates of clearance may be decreased in these animals.

Apostolopoulos, Howlett, and Fidge (21) observed a lower abundance of apoA-I-mRNA in livers from PTU-treated rats, and an increase in rats treated with T₄. These observations were essentially confirmed by Davidson et al. (22). Synthesis of apoA-I, moreover, paralleled qualitatively the levels of the specific mRNA (22). The constancy of the specific activity of apoA-I during perfusion, which we observed, and our finding of no difference between the euthyroid and the hyperthyroid conditions, would point to the fact that the differences in incorporation rate of [³H]leucine reflect differences in biosynthesis of this apolipoprotein. Our data are supported by the observations of Apostolopoulos et al. (21) and Davidson et al. (22), although quantitative correlations need to be made to establish the relationship between ambient levels of apoA-I-mRNA and apoA-I biogenesis.

The duration of the effect of T₃ on plasma levels of apoA-I is short-lived. A persistent high plasma T₃ level seems to be required. The plasma concentration of apoA-I returned to euthyroid levels 3 days after withdrawal from treatment with T₃. Plasma levels of 200–400 μg T₃/dl were achieved in these studies when 9.6 μg T₃ was administered by osmotic minipump over a 24-h period. Similar effects were seen with administration of T₄ which produced a T₃ level within this range of T₃ values. The hormone was administered per rat and not per unit weight, which may have given rise to the somewhat variable T₃ concentrations in the various experimental groups. We did not observe any correlation in this range of T₃ plasma levels with either plasma apoA-I levels or hepatic secretion of apoA-I. However, when a higher dose was given (19.2 μg T₃/day), the plasma concentration of T₃ was 560 ng/dl, and the plasma apoA-I was elevated over that with the lower dose, suggesting that in a broader range some positive relationship might exist.

The extent to which apoA-I associates with lipid complexes synthesized and secreted by the liver is unclear. The work of Felker et al. (23) clearly demonstrates that apoA-I and E are secreted in ultracentrifugal defined lipoprotein fractions. In their work, >90% of the apoA-I of plasma was associated with lipid complexes, the major portion residing in a d 1.063–1.210 g/ml HDL-like fraction. The remainder was found in the d > 1.210 g/ml fraction, which, they suggested, resulted from ultracentrifugal stripping of the apolipoprotein. As reported here, only a small percentage of the total secreted apoA-I was associated with the d < 1.210 g/ml lipoprotein fraction. Other workers who have examined secretion of apoA-I by isolated perfused livers also observed a large percentage of apoA-I in the d > 1.210 g/ml fraction. Jones et al. (24) reported only 9–20% while Johnson, Babiak, and Rudel (25) found about 20% of the apoA-I secreted by livers from monkeys to be in the HDL fraction. Our study and those of these investigators (24, 25) used a perfusion medium containing bovine serum albumin. Most preparations of bovine serum albumin are known to be contaminated with bovine apoA-I (26), a finding that we have substantiated. It is possible that the bovine apoA-I displaced the comparatively small amounts of newly secreted rat apoA-I from the HDL complex. Fortunately, bovine apoA-I does not cross-react immunochemically with rat apoA-I (refer to Methods section) so that our measurements reflect the presence of rat apoA-I. However, it is certainly conceivable that apoA-I is secreted in free form, unassociated with lipid. The extent to which apoA-I is secreted into the blood in free form remains unclear. The newly secreted apoA-I probably later associates with lipids and other apolipoproteins in the vascular system through those processes involved in formation of mature plasma HDL. Hussain et al. (27) proposed that apoC-III and apoA-II, as well as apoA-I, are secreted by isolated

hepatocytes to a large extent in lipid-poor form, associating later with extracellular lipids.

In general, total hepatic protein synthesis (measured by incorporation of [³H]leucine) was not affected by the hyperthyroid state. Similarly, hepatic secretion of albumin was unaffected by treatment with T₃. Clearly, the stimulatory effect on apoA-I synthesis and secretion appears to be specific. Additionally, the synthesis of another specific export protein (e.g., α₂-globulin) has been shown to be enhanced by thyroid hormone (28). Thyroid hormone appears also to have another specific effect on protein synthesis. We observed that the synthesis of apoE and apoC, but not apoB, apolipoproteins, which are associated with VLDL, are depressed by thyroid hormone treatment (Wilcox, H. G., and M. Heimberg, unpublished data).

It appears, therefore, that in early stages of the hyperthyroid state very specific stimulatory effects on apoA-I synthesis and secretion are seen, but that over a more extended period compensatory effects, perhaps hormonal in nature, tend to normalize apoA-I metabolism. Undoubtedly, many different factors regulate plasma concentrations of apoA-I, in addition to those reported here. Windmueller and Spaeth (29) were unable to detect any differences in synthesis of apoA-I by perfused livers from normal fed or fasted rats, an observation confirmed by us. However, they reported that hepatic apoA-I synthesis was stimulated in rats on high carbohydrate diets or by livers from rats that had been fasted and refed, and claimed therefore, that a correlation existed between apoA-I synthesis and secretion of triglyceride.

In our experiments, stimulation of triglyceride synthesis and VLDL secretion by oleate did not alter synthesis or secretion of apoA-I by isolated perfused livers from normal fed or fasted (24 h) rats, or from hypothyroid or hyperthyroid animals. Furthermore, fasting, which per se decreases VLDL secretion, did not affect hepatic output of apoA-I; yet hyperthyroidism, which also depresses VLDL secretion, increased apoA-I output. Similarly, feeding of orotic acid to rats, which depresses formation of apoB and apoE and diminishes secretion of the VLDL, does not affect production of apoA-I by the liver (30). These observations suggest that apoA-I secretion is not directly dependent on, or correlated with, VLDL formation and secretion. In contrast to our data, Murata, Imazumi, and Sugano (31) reported that secretion of apoA-I by the perfused liver was stimulated by infusion of oleate, compared to controls without added fatty acid. A similar finding with HepG2 cells was reported by Dashti, and Wolfbauer (32), who later showed that hepatic levels of apoA-I mRNA were not changed by oleic acid-stimulated synthesis of triglyceride and VLDL secretion (33). The reason for this discrepancy of the oleic acid effect on apoA-I synthesis is not obvious. In the studies of Murata et al. (31), 10% soybean oil was included in the diet, com-

pared to rats fed standard chow, as used in our experiments. Dietary effects may be implicated further, since Sugano, Tanaka, and Ide (34) observed a 2.5-fold increase in secretion of apoA-I by perfused livers from rats fed a 20% casein diet for 4 weeks, compared to a diet containing an equal weight of soybean protein.

Furthermore, Triton WR1339 added in vitro (0.2–1.0 mg/ml of perfusion medium) did not affect the secretion of apoA-I (H. G. Wilcox and M. Heimberg, unpublished observations) although the administration of this nonionic detergent in vivo is known to cause a marked diminution of plasma apoA-I levels (35); this effect, therefore, is probably not the result of inhibition of hepatic apoA-I secretion, but must be a consequence of A-I catabolism. ApoA-I secretion has also been reported to be increased in livers from rats with experimentally induced nephrosis (36, 37) and in cholestatic animals (38), although the mechanism of these effects is obscure.

It is clear that our understanding of the various factors that may modulate the synthesis, secretion, and catabolism of apoA-I and nascent HDL by the liver is minimal. It is most probable, however, that synthesis and secretion of apoA-I is hormonally regulated (e.g., thyroid hormone), and is independent of many factors that modify synthesis and secretion of VLDL apolipoproteins. ■

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