Influence of development, estrogens, and food intake on apolipoprotein A-I, A-II, and E mRNA in rat liver and intestine

Bart Staels, Johan Auwerx, Lawrence Chan,* Arie van Tol,† Maryvonne Rosseneu, and Guido Verhoeven**

Laboratorium voor Experimentele Geneeskunde en Endocrinologie, Gasthuisberg, Katholieke Universiteit Leuven, Belgium; Departments of Cell Biology and Medicine,* Baylor College of Medicine, Houston, TX 77030; Department of Biochemistry I, Medical Faculty,† Erasmus University Rotterdam, Rotterdam, The Netherlands; and Department of Clinical Biochemistry, A.z. St. Jan,** B-8000 Brugge, Belgium

Abstract The influence of development and ethinylestradiol (EE) on apolipoprotein (apo) A-I, A-II, and E mRNA in rat liver and intestine was studied by dot blot hybridization and Northern blot analysis. ApoA-I mRNA levels were maximal in the perinatal period and declined after day 15. An opposite trend was noted for the apoA-II mRNA levels, whereas apoE mRNA remained fairly constant. Liver apoA-I mRNA levels increased after ovariectomy (OVX). A further rise was observed when EE was given at 2000 μg/day. When the influence of OVX and EE was controlled for food intake by pair-feeding, OVX still increased hepatic apoA-I mRNA. The rise in liver apoA-I mRNA after EE, however, was no longer significant. Under the same conditions OVX slightly increased intestinal apoA-I mRNA. EE (2000 μg/day) decreased intestinal apoA-I mRNA to 80% of the pair-fed controls. Liver apoA-II mRNA levels did not change after OVX when the animals were fed ad libitum, but decreased slightly when the rats were pair-fed. EE caused a dose-dependent decrease in liver apoA-II mRNA, irrespective of food intake. None of these treatments caused any change in liver apoE mRNA levels. Serum apoA-I levels increased upon OVX, while serum apoE did not change. EE provoked a dose-dependent decrease of both apolipoproteins in serum. In conclusion: *1*) Changes in food intake play an important role in the in vivo effects of estrogens on apolipoprotein mRNA levels. *2*)The stimulatory effect of OVX on hepatic apoA-I mRNA as well as the inhibitory effect of EE on hepatic apoA-II mRNA are independent of food intake. *3*)After OVX the changes in serum apolipoprotein levels parallel the changes in the corresponding hepatic and intestinal mRNAs. The changes in the serum levels observed after EE treatment, however, cannot be explained by the changes in the hepatic mRNA levels.—**Staels, B., J. Auwerx, L. Chan, A. van Tol, M. Rosseneu, and G. Verhoeven.** Influence of development, estrogens, and food intake on apolipoprotein A-I, A-II, and E mRNA in rat liver and intestine. *J. Lipid Res.* 1989. **30:** 1137–1145.

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Apolipoprotein (apo) A-I and A-II are the major apolipoproteins of the high density lipoproteins (HDL), which have been linked to reverse cholesterol transport and which are inversely correlated with the development of coronary atherosclerosis (1,2). ApoA-I is synthesized in the liver as well as in the intestine; apoA-II, on the other hand, is produced mainly by the liver (3,4). ApoE, a constituent of various plasma lipoproteins, is synthesized in many tissues, but again the liver is the major site of production (5). ApoE plays a central role in the transport and removal of cholesterol-loaded lipoproteins from the circulation (6).

The cloning of apoA-I (7–11), apoA-II (4,12,13), and apoE (14–18) and the availability of both human and rat cDNA probes have provided insight in the structure of the corresponding proteins and have opened the possibility to study the regulation of the respective mRNAs.

Lipoprotein metabolism is influenced by a variety of factors including diet, exercise, hormones, and development. Recently, two groups provided evidence that gene expression for apoA-I, apoE, apoC-III, and apoA-IV in rat liver follows a specific temporal pattern during development (3,16). Furthermore, it has been demonstrated that sex steroids, added in vitro, influence apoA-I and apoE mRNA levels in a human hepatocarcinoma cell-line (19). In the present investigations we studied the changes of apoA-I, apoA-II, and apoE mRNA levels during early postnatal development and the effects of sex hormones administered to gonadectomized animals in vivo.

The data presented show that apoA-I and apoA-II mRNA levels undergo opposite changes during development. Furthermore, it is demonstrated that apoA-I and apoA-II mRNA levels are also influenced in an opposite way by ethinylestradiol whereas apoE mRNA remains

Abbreviations: apo, apolipoprotein; EE, ethinylestradiol; OVX, ovariectomy; HDL, high density lipoprotein.

unaffected. Evidence is presented that some of the observed changes are indirect and may be provoked or enhanced by changes in food intake.

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats 5 to 80 days of age were used in the developmental study. Adult female Wistar rats were used in all the other experiments. Where indicated, ovariectomy was performed under ether anesthesia on day 90 (20). The animals were allowed to recover for 34 days. Treatment was started on day 125 and was continued for 7 days. Ethinylestradiol (0.02 up to 2000 μg/day) was administered either intragastrically or subcutaneously in 0.2 ml propyleneglycol. Sham-treated animals received the vehicle only. The animals were allowed free access to food except in the pair-fed studies, in which the food intake was restricted to the amount ingested by the animals with the lowest food intake (see Table 4). At the end of each experiment the animals were fasted overnight, weighed and killed by exsanguination under ether anesthesia. Blood was allowed to clot. The serum was stored at −20°C for determination of cholesterol, triglycerides, apoA-I, and apoE. The liver was removed immediately, rinsed with 0.9% NaCl and frozen in liquid nitrogen. The intestine was removed, rinsed with ice-cold 0.9% NaCl, and the epithelium was scraped off and frozen in liquid nitrogen.

Preparation of RNA

Total cellular RNA was prepared either from the liver and intestinal epithelium of individual animals (studies on the effects of estrogens) or from liver and intestinal fragments derived from several animals of the same age (developmental study). In the latter case, care was taken to take equal amounts of tissue from each animal. Total cellular RNA was prepared by the guanidine isothiocyanate/cesium chloride procedure (21).

Dot blot and Northern blot analysis

The apoA-I, apoA-II, and apoE mRNA content was quantified by dot blot hybridization using eight serial dilutions (1.5-fold) of RNA starting from an amount of 8 μg/dot. The RNA was denatured, applied to nitrocellulose filters, and hybridized for 72 h to ^{32}P-labeled cDNA as described previously (22). Labeling of the cDNA probes was performed using the nick translation kit provided by Amersham.

The rat apoA-I cDNA was prepared and comprised an 800-basepair fragment of apoA-I inserted into the Eco RI site of pGEM. The clone had been identified by cross-hybridization to cloned human apoA-I cDNA (8) and was confirmed by DNA sequencing. It comprises some 80% of the rat apoA-I mRNA starting from the 3' end. The rat apoA-II cDNA clone was identified in a rat liver cDNA library by cros-hybridization to human apoA-II cDNA. It contained the whole 3' untranslated region, mature peptide and propeptide coding regions, and part of the signal peptide region (14). The rat apoE cDNA clone was identified in the same library by cross-hybridization to a human apoE cDNA clone (23). It contained all the coding region of rat apoE sequence.

Routinely 10^7 cpm (150 ng) cDNA probe was allowed to hybridize with each filter. Filters were exposed to X-ray film (Curix RPII, Agfa-Gevaert or Hyperfilm-βmax, Amersham). Autoradiograms of filters were analyzed by quantitative scanning densitometry (LKB 2202 Ultrascan Laser Densitometer) in the linear range of film sensitivity. Curves were plotted on a log/log scale relating the densitometric measurements to the amounts of RNA in the corresponding dots. The relative amounts of mRNA were calculated using the parallel, linear parts of these curves. Northern blot hybridization of glyoxal-treated total cellular RNA was performed as described previously (22).

Estimation of the serum concentrations of cholesterol, triglyceride, apoA-I, and apoE

Serum total cholesterol was measured using the Monotest® Cholesterol kit provided by Boehringer Mannheim (24). Serum triglyceride concentrations were determined using a fully enzymatic kinetic UV-method (Automated Analysis Boehringer Mannheim Triglycerides kit) (25).

ApoA-I and apoE concentrations were measured by electroimmunoassay, exactly as described previously (26,27). Briefly, apolipoproteins present in sera and standards were precipitated with trichloroacetic acid in the presence of deoxycholate as a carrier. The resulting protein pellets were solubilized in 0.1 ml of 0.5 M NaOH and delipidated with tetramethylurea. Twenty mM Tris-HCl, containing 8 M urea, was added before the samples were analyzed by immunoelectrophoresis (27).

Statistical methods

Multiway analysis of variance was used to evaluate the results of the ethinylestradiol treatment and the route of administration. Values observed using different dosages and routes of administration were compared by contrast statements. A two-tailed unpaired Student's *t*-test was used to evaluate differences between means in the experiments on pair-fed animals.

RESULTS

Developmental changes in apolipoprotein levels in serum and apolipoprotein mRNAs in rat liver

The amount of mRNA for apoA-I, apoA-II, and apoE was compared in the livers of male rats 5–80 day old. A representative experiment is shown in **Fig. 1**, panel A.

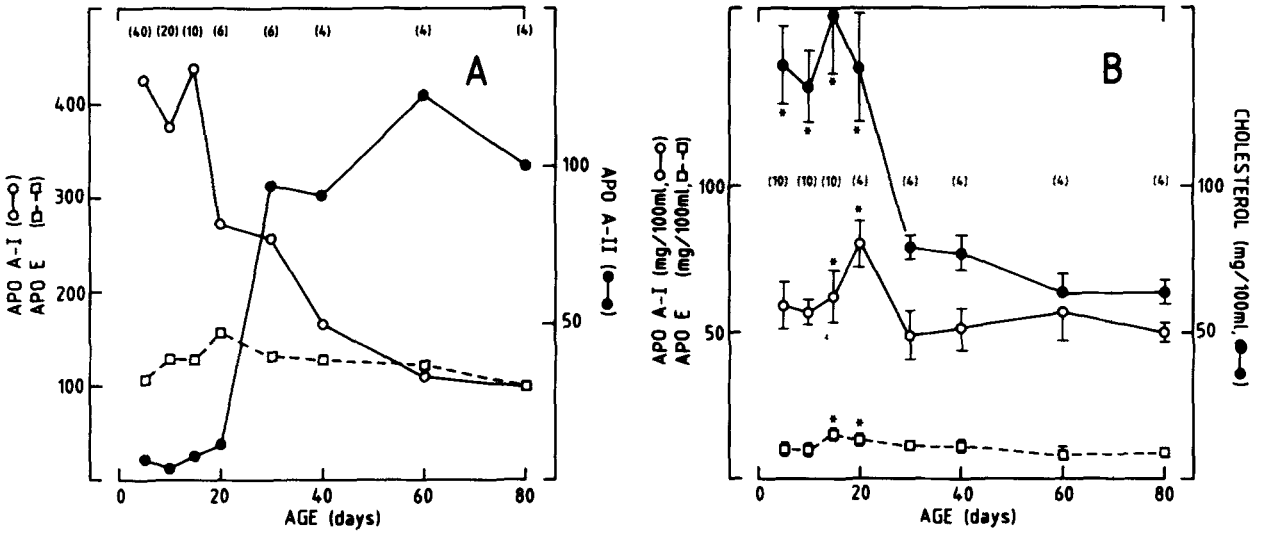

Fig. 1. Developmental changes in apolipoprotein mRNA levels in rat liver (panel A) and apolipoprotein levels in serum (panel B). A: RNA was prepared from the livers of male rats of different ages. The number of animals used for each preparation is indicated between brackets. mRNA levels for apoA-I, apoA-II, and apoE were measured using a dot blot analysis. Values are expressed in arbitrary units taking the amount of mRNA measured at day 80 as 100. B: Serum total cholesterol was measured using the Monotest® Cholesterol kit (Boehringer). ApoA-I and apoE concentrations were measured by electroimmunoassay. Values represent the mean ± SD. The number of animals used at each age is indicated between brackets. Points indicated by an asterisk are significantly ($P < 0.05$) different from the corresponding value observed at the age of 80 days by one-way analysis of variance and Duncan's Range test (panel B).

During this period apoE mRNA levels remained fairly stable whereas apoA-I mRNA levels decreased markedly. ApoA-II mRNA displayed a completely different pattern. It was barely detectable between days 5 and 20 but showed a dramatic increase between days 20 and 30. During the same period of development no changes were observed in intestinal apoA-I mRNA levels (data not shown). Plasma total cholesterol levels decreased about twofold between 20 and day 30. Only limited changes were observed in the serum levels of apoA-I and apoE. Both apolipoproteins displayed a small increase on day 15–20 and decreased to levels approximating those observed on day 5 thereafter (Fig. 1, panel B).

Influence of dosage and route of administration of ethinylestradiol on serum lipids, apolipoprotein levels, and liver apolipoprotein mRNAs in ovariectomized female rats

In a series of preliminary experiments (not shown) we investigated the effects of castration and androgen treatment in adult male rats and the effects of ovariectomy and estrogen treatment in adult female rats. No consistent effects of castration and androgen treatment (testosterone propionate, 250 μg/day) on hepatic apolipoprotein mRNA levels were observed. Estrogens (estradiol benzoate, 20 μg/day) tended to increase apoA-I mRNA levels, although the extent of the increase was variable. Accordingly the effects of estrogens were studied in more detail. A wide range of estrogen doses (0.02–2000 μg ethinylestradiol) was investigated. Moreover, since there are indications that orally administered estrogens might have more pronounced effects on lipoprotein levels than parenterally administered hormones (28), half of the animals (n = 3) received ethinylestradiol intragastrically, whereas the other half was treated subcutaneously. Finally, in an attempt to reduce the variability of the estrogen effects observed in the preliminary experiments, hormone treatments were started systematically 34 days after ovariectomy and all animals were fasted 12 h before they were killed.

Two-way analysis of variance demonstrated that the route of administration of ethinylestradiol (oral/parenteral) had a significant influence on uterus weight and liver weight. For both of these parameters subcutaneous administration was more effective than oral treatment (**Table 1**). The route of administration had no effect on body weight (**Table 2**) or on any of the parameters of lipid metabolism studied (Table 2). Accordingly the results for these parameters were combined regardless of the route of administration. As expected, ovariectomy resulted in an increase and ethinylestradiol resulted in a dose-dependent decrease in body weight (Table 1). The latter effect is related to decreased food intake (29). Uterus weight dropped markedly after ovariectomy and was restored by treatment with ethinylestradiol. Liver weight (expressed per g body weight) decreased slightly after ovariectomy but increased markedly at high doses of ethinylestradiol.

Total serum cholesterol increased after ovariectomy and showed a dose-dependent decrease after estrogen administration (Table 2). A less marked decrease was observed in serum triglycerides (Table 2). In similar fashion serum apoA-I levels showed a slight increase after ovariectomy

TABLE 1. Influence of ovariectomy and ethinylestradiol on body, uterus, and liver weight

Treatment	Body Weight	Uterus Weight		Liver Weight	
	Oral + SC	Oral	SC	Oral	SC
	g	*mg/g*		*mg/g*	
Intact control	241 ± 24[d]	1.43 ± 0.06[a,*]	2.47 ± 0.87[a]	25.6 ± 3.0[b,*]	28.5 ± 3.9[b]
Ovariectomy (OVX)	300 ± 24[a]	0.41 ± 0.09[c]	0.36 ± 0.07[d]	23.6 ± 0.5[b]	24.1 ± 1.3[c]
OVX + 0.02 μg EE	292 ± 23[ab]	0.40 ± 0.10[c]	0.49 ± 0.05[d]	23.5 ± 2.1[b]	24.4 ± 1.2[c]
OVX + 0.2 μg EE	289 ± 14[ab]	0.39 ± 0.04[c,*]	1.16 ± 0.09[c]	23.9 ± 1.8[b]	24.1 ± 1.1[c]
OVX + 2.0 μg EE	274 ± 10[bc]	0.48 ± 0.03[c,*]	1.36 ± 0.06[c]	22.7 ± 0.4[b,*]	24.9 ± 2.2[c]
OVX + 20 μg EE	267 ± 10[c]	0.97 ± 0.19[b,*]	2.24 ± 0.40[ab]	25.8 ± 0.6[b,*]	29.1 ± 1.8[b]
OVX + 2000 μg EE	275 ± 10[bc]	1.80 ± 0.30[a]	1.91 ± 0.30[b]	39.8 ± 2.0[a,*]	43.2 ± 3.3[a]

Female animals (90 day old) were ovariectomized (OVX) and treated for 7 days with increasing doses of ethinylestradiol (EE) as indicated. Treatment was started 34 days after ovariectomy. In each experimental group half of the animals (six in the control group, three in all the other groups) received ethinylestradiol intragastrically (oral) and half received the same dose subcutaneously (SC). The results were combined when two-way analysis of variance demonstrated no effect of the route of estrogen administration. Organ weights are expressed per g body weight. Values represent the mean ± SD. Statistically significant effects of treatment ($P < 0.05$) are observed between values followed by different letters. Statistically significant effects of the route of administration are indicated by an asterisk.

and a marked decrease at high doses of ethinylestradiol (Table 2). ApoE levels were not influenced by ovariectomy, but became undetectable at doses of ethinylestradiol exceeding 2 μg/day.

The increase in serum apoA-I levels (Table 2), induced by ovariectomy, was accompanied by an increase in hepatic apoA-I mRNA levels (**Table 3**). Interestingly moderate doses of ethinylestradiol seemed to increase liver apoA-I mRNA levels further and a marked stimulation was observed at the highest dose of ethinylestradiol tested. The opposite effect was seen for apoA-II mRNA which showed a dose-dependent decrease at moderate doses of ethinylestradiol and a marked drop at the 2000-μg dose. ApoE mRNA levels, in contrast, were not significantly affected by any of the treatments studied.

Northern blot analysis confirmed the observed changes in the concentrations of apoA-I, apoA-II, and apoE mRNA and showed that the measured mRNAs had expected sizes of approximately 1100, 600, and 1250 bases, respectively (4,16,30) (**Fig. 2**).

Are the observed effects of ovariectomy and ethinylestradiol treatment related to changes in food intake?

Since it was observed that ovariectomy as well as ethinylestradiol treatment influenced body weight and food intake significantly, we determined the effects of ovariectomy and estrogen therapy in pair-fed animals. Food intake of the ovariectomized rats was restricted to the amount consumed by intact rats during the entire period (41 days). Similarly, the sham-injected ovariectomized controls received, during the 7 days of treatment, the amount of food consumed by the ethinylestradiol-treated animals. At the end of the treatment period there

TABLE 2. Influence of ovariectomy and ethinylestradiol on serum lipid and apolipoprotein levels

Treatment	n	Cholesterol	Triglycerides	ApoA-I	ApoE
			mg/dl		
Intact control	6	76 ± 15[b]	86 ± 31[a]	38.5 ± 3.8[b]	24.1 ± 2.4[a]
Ovariectomy (OVX)	12	96 ± 21[a]	70 ± 14[a]	49.0 ± 6.8[a]	23.3 ± 4.1[a]
OVX + 0.02 μg EE	6	93 ± 9[a]	86 ± 28[a]	43.7 ± 5.3[ab]	21.4 ± 2.4[a]
OVX + 0.2 μg EE	6	79 ± 15[b]	54 ± 13[a]	42.0 ± 3.7[b]	21.6 ± 2.8[a]
OVX + 2.0 μg EE	6	34 ± 9[c]	51 ± 17[a]	33.0 ± 7.4[c]	16.0 ± 1.1[b]
OVX + 20 μg EE	6	8 ± 5[d]	56 ± 16[a]	12.0 ± 4.6[d]	ND[c]
OVX + 2000 μg EE	6	7 ± 2[d]	22 ± 2[b]	12.3 ± 2.7[d]	ND[c]

Animals were treated and results are expressed as explained in Table 1. Cholesterol, triglycerides, and serum levels of apoA-I and apoE were measured as described in Materials and Methods; ND, not detectable.

TABLE 3. Influence of ovariectomy and ethinylestradiol on liver apolipoprotein mRNA levels

Treatment	n	ApoA-I	ApoA-II	ApoE
			units	
Intact control	6	60 ± 12^{a}	92 ± 12^{ab}	92 ± 7^{a}
Ovariectomy (OVX)	12	100 ± 14^{b}	100 ± 15^{a}	100 ± 15^{a}
OVX + 0.02 μg EE	6	110 ± 23^{bc}	90 ± 20^{abc}	94 ± 10^{a}
OVX + 0.2 μg EE	6	120 ± 35^{bc}	85 ± 12^{bc}	94 ± 9^{a}
OVX + 2.0 μg EE	6	128 ± 30^{c}	81 ± 16^{bc}	102 ± 12^{a}
OVX + 20 μg EE	6	109 ± 28^{bc}	74 ± 16^{c}	106 ± 20^{a}
OVX + 2000 μg EE	6	248 ± 37^{d}	23 ± 10^{d}	96 ± 18^{a}

Animals were treated and results are expressed as explained in Table 1. Apolipoprotein mRNA levels were measured in the livers of individual animals by a dot blot hybridization technique as described in Materials and Methods. Values are expressed in arbitrary units (U) taking the mean value of the ovariectomized animals as 100 U.

were no significant differences between the body weights of the treated animals and their respective controls (**Table 4**). Uterus weights, however, indicated that treatment was effective in each group. Ethinylestradiol treatment induced a dose-dependent increase in liver weight, which agreed with our previous results.

Serum total cholesterol, triglyceride as well as apoA-I and apoE levels changed in the same fashion as in the first part of this study (**Table 5**). Pair-feeding of the animals did not influence the increase in liver apoA-I mRNA levels after ovariectomy as observed in the previous experiment. However, the increase in liver apoA-I mRNA provoked by the 2000-μg dose of ethinylestradiol was no longer statistically significant (**Table 6**). In pair-fed animals a small but significant decrease in hepatic apoA-II mRNA levels could be observed after ovariectomy and the dramatic fall in liver apoA-II mRNA levels at the highest dose of ethinylestradiol remained evident despite pair-feeding of the controls.

To rule out that the marked changes in serum apoA-I might be related to changes in the intestinal synthesis of the apolipoprotein rather than to changes in the hepatic synthesis, we finally investigated apoA-I mRNA in the intestines of the pair-fed animals (Table 6). Ovariectomy caused a limited but statistically significant increase in intestinal apoA-I mRNA whereas a high dose of estrogens provoked a small decrease in the same mRNA. These changes were not observed in experiments in which the food intake of the animals was not controlled (data not shown).

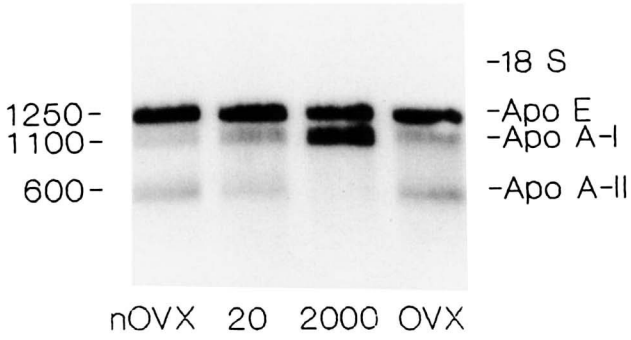

Fig. 2. Northern blot analysis of the influence of ovariectomy and estrogen treatment on mRNA levels for apoA-I, apoA-II, and apoE. RNA was prepared from the livers of intact, adult female rats (nOVX), ovariectomized animals (OVX), and ovariectomized rats treated with 20 and 2000 μg of ethinylestradiol for 7 days. Estrogens were administered from the 34th day after ovariectomy on. Twelve μg of each RNA preparation was subjected to electrophoresis in a 1% agarose gel. The RNA was transferred to a nylon membrane (Hybond-N, Amersham) and hybridized with a mixture of nick-translated cDNA probes for apoA-I, apoA-II, and apoE as described (22). The localization of the different apolipoprotein mRNAs and their corresponding sizes (in bases) are indicated on the autoradiograph.

DISCUSSION

These experiments confirm that apoA-I mRNA in rat liver shows a marked decrease during prepubertal life whereas liver apoE and intestinal apoA-I mRNA remain relatively stable (16). The serum level of apoE parellels that of the hepatic mRNA. The sharp drop in the hepatic apoA-I mRNA, however, is not observed in the serum. Furthermore, our data show that apoA-II mRNA, which has not been studied previously, shows exactly the opposite trend as apoA-I mRNA and increases dramatically between days 20 and 30 of life. It remains to be explored whether the observed changes are related to changes in food composition or to changes in hormone secretion and/or responsiveness during pubertal development.

Since sex hormones play an important function in development and gene expression during adulthood, we focused on the influence of sex hormones on apolipoprotein mRNA levels in the liver. It has been well documented that apoA-I serum levels are influenced by sex hormones in humans (31–35) as well as in the rat (29, 36–41), and

TABLE 4. Influence of ovariectomy and ethinylestradiol on body, uterus, and liver weight of pair-fed animals

Treatment	Average Daily Food Consumption	Body Weight	Uterus Weight	Liver Weight
	g/day	*g*	*mg/g*	
Intact control	16.8 ± 4.9	226 ± 7	1.68 ± 0.40	25.1 ± 1.7
OVX Pair-fed		234 ± 11 (NS)	0.48 ± 0.04 ($P < 0.01$)	23.2 ± 1.0 (NS)
OVX + 20 μg EE	11.7 ± 2.1	227 ± 18	2.37 ± 0.42	32.2 ± 1.6
OVX Pair-fed		233 ± 27 (NS)	0.72 ± 0.33 ($P < 0.001$)	21.8 ± 1.1 ($P < 0.0001$)
OVX + 2000 μg EE	9.6 ± 4.1	233 ± 12	2.53 ± 0.39	43.4 ± 3.8
OVX Pair-fed		231 ± 23 (NS)	0.59 ± 0.06 ($P < 0.005$)	22.0 ± 0.8 ($P < 0.002$)

Female rats (90 day old) were ovariectomized (OVX) and treatment with the indicated dosage of ethinylestradiol (EE) or vehicle was started 34 days later and was continued for 7 days. Each group consisted of four rats and four pair-fed controls. Organ weights are expressed per g body weight. Values represent the mean ± SD. Statistical differences between the groups were analyzed by a two-tailed unpaired Student's *t*-test; NS, not significant.

studies on isolated hepatoma cells have suggested that changes in liver apolipoprotein production may contribute to the observed changes in lipoprotein metabolism (19,42,43).

In male rats we were unable to demonstrate any consistent effect of castration and of androgen replacement on apoA-I, apoA-II, and apoE mRNA levels in the liver (data not shown). These results corroborate previous studies that showed the absence of effects of gonadectomy and androgens on serum levels of apoA-I and apoE in normal Sprague-Dawley rats (38) and in normotensive Wistar Kyoto rats (37). Similarly, in HepG2 cells testosterone alone did not affect the rate of apolipoprotein secretion (19).

In female animals we observed a rise in liver apoA-I mRNA after ovariectomy and a further increase after administration of estrogens (Table 3). Pharmacological doses of ethinylestradiol were required, however, to produce at best a 2.5-fold increase in mRNA concentration. The oral route of hormone administration was not more effective than the parenteral route. Moreover, at these high doses of estrogens, the food intake of the animals diminished markedly and when the influence of this factor was eliminated by the use of pair-fed controls, the effect of estrogens on apoA-I mRNA was no longer statistically significant (Table 6). These data suggest that the observed effects of estrogens are due to dietary factors rather than to a direct action of these hormones on the liver. These in vivo data are discordant with earlier in vitro (19,43) and in vivo (29) observations. However, it should be kept in mind that high doses of ethinylestradiol cause a marked rise of the liver weight (Table 1). Accordingly, despite the absence of significant changes in apoA-I mRNA concentration, the total amount of this message in the liver increases substantially. This could possibly explain the apparent discrepancy between our data and those of Weinstein, Wilcox, and Heimberg (29) which showed an increased secretion of apoA-I by perfused livers from ethinylestradiol-treated female rats compared with pair-fed controls. On the other hand, it has been shown that treatment of HepG2 cells with estrogens results in a two-fold increase in the rate of apoA-I accumulation in the medium paralleled by a comparable increase in the corresponding mRNA (42,43). The reason for this discrepancy remains to be explored; species differences as well as the malignant nature of this cell line may be involved.

To our knowledge, the influence of estrogens on apoA-II mRNA levels has not been studied previously. The present experiments indicate that ovariectomy results in a small decrease in the hepatic level of apoA-II mRNA.

TABLE 5. Influence of ovariectomy and ethinylestradiol on serum lipid and apolipoprotein levels of pair-fed animals

Treatment	Cholesterol	Triglycerides	ApoA-I	ApoE
		mg/dl		
Intact control	82 ± 9	69 ± 21	45.1 ± 3.6	13.4 ± 0.9
OVX Pair-fed	124 ± 19 ($P < 0.01$)	59 ± 4 (NS)	64.6 ± 8.8 ($P < 0.01$)	17.1 ± 5.6 (NS)
OVX + 20 μg EE	6 ± 1	33 ± 11	6.3 ± 3.6	ND
OVX Pair-fed	82 ± 8 ($P < 0.0001$)	55 ± 6 ($P < 0.02$)	46.6 ± 5.0 ($P < 0.0001$)	11.6 ± 2.8
OVX + 2000 μg EE	8 ± 3	20 ± 6	8.2 ± 5.0	ND
OVX Pair-fed	76 ± 20 ($P < 0.01$)	54 ± 7 ($P < 0.0005$)	42.8 ± 9.5 ($P < 0.001$)	10.5 ± 2.0

Animals were treated and results are expressed as explained in Table 4. Cholesterol, triglycerides, and serum levels of apoA-I and apoE were measured as described in Materials and Methods; ND, not detectable; NS, not significant.

TABLE 6. Influence of ovariectomy and ethinylestradiol on liver and intestinal apolipoprotein mRNA levels of pair-fed animals

Treatment	Liver ApoA-I	Liver ApoA-II	Liver ApoE	Intestinal ApoA-I
		units		
Intact control	100 ± 20	100 ± 7	100 ± 8	100 ± 3
OVX Pair-fed	156 ± 27 ($P < 0.02$)	79 ± 8 ($P < 0.01$)	106 ± 7 (NS)	132 ± 12 ($P < 0.01$)
OVX + 20 μg EE	84 ± 15	95 ± 9	102 ± 14	86 ± 12
OVX Pair-fed	100 ± 14 (NS)	100 ± 8 (NS)	100 ± 9 (NS)	100 ± 20 (NS)
OVX + 2000 μg EE	143 ± 55	35 ± 15	110 ± 18	84 ± 6
OVX Pair-fed	100 ± 20 (NS)	100 ± 19 ($P < 0.002$)	100 ± 4 (NS)	100 ± 4 ($P < 0.005$)

Animals were treated and results are expressed as explained in Table 4. Apolipoprotein mRNA levels were measured in the liver and the intestine of individual animals by a dot blot hybridization technique as described in Materials and Methods. Values are expressed in arbitrary units (U) taking the mean value of the control groups as 100 U.

This decrease is only evident, however, when the spontaneous increase in food intake normally observed after ovariectomy is prevented. Since fasting decreases the hepatic level of apoA-II mRNA (44) this effect again may be due to dietary factors. Estrogen administration, on the other hand, results in a dose-dependent decrease in hepatic apoA-II mRNA levels and at least at the 2000-μg/day dose of ethinylestradiol this decrease remains significant when the food intake of the control animals is restricted to the amount of food ingested by the estradiol-treated rats.

Under none of the conditions studied did we observe any effect of ovariectomy or estrogens on the hepatic level of apoE mRNA. This contrasts with the increase in apoE mRNA observed in HepG2 cells at high concentrations of estradiol (19). These data show again that care should be taken in the extrapolation of in vitro data based on a tumor cell line derived from one species to the in vivo situation in another species.

Nonetheless, it is obvious that the described changes in hepatic apolipoprotein mRNA levels cannot explain the changes observed in the serum. In fact, estrogen treatment is accompanied by a marked drop rather than an increase in apoA-I in the serum, a disappearance of apoE, a dramatic fall in total cholesterol, and an important reduction in triglycerides, changes that largely corroborate earlier observations (29,36,39,40,45). This discrepancy between hepatic apoE mRNA levels and serum apoE can be explained, at least in part, by the increase in hepatic LDL-receptors induced by estrogens (39,45,46). This discrepancy between hepatic apoA-I mRNA and the serum levels of this apolipoprotein is more difficult to explain. Several hypotheses should be considered. For instance, it is conceivable that the observed increase in hepatic apoA-I mRNA is neutralized by a much more pronounced decrease in apoA-I mRNA and apoA-I production in other tissues such as the intestine. To explore this possibility, we investigated the effects of ovariectomy and estrogen treatment on the intestinal concentration of apoA-I mRNA. In pair-fed animals we could show a slight increase in intestinal apoA-I mRNA after ovariectomy. Taking into account that the intestinal apoA-I mRNA levels are about fourfold higher than the hepatic levels, it is conceivable that the intestinal effect contributes to the small increase in serum apoA-I observed after ovariectomy. On the other hand, it is unlikely that the small decrease in intestinal apoA-I mRNA observed at the highest dose of ethinylestradiol tested explains the sharp drop in the serum level of this apolipoprotein. An alternative explanation that has to be considered is that estrogens might act at a posttranscriptional level to influence the hepatic and/or the extrahepatic synthesis of apoA-I. Indeed, it has recently been shown that translational regulation of intestinal as well as liver apoA-I and apoE synthesis occurs when male rats are fed an atherogenic diet containing cholesterol and propylthiouracil (47). Finally, the possibility should be considered that the major effects of estrogens are on the catabolism rather than on the synthesis of rat apoA-I (39,41,45).

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