Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8242–8246, December 1985
Medical Sciences

Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin

(apolipoprotein E/hormones/intestine/lipoprotein/liver)

NABIL A. ELSHOURBAGY*, MARK S. BOGUSKI†, WARREN S. L. LIAO*‡, LEONARD S. JEFFERSON§, JEFFREY I. GORDON†, AND JOHN M. TAYLOR*¶

*Gladstone Foundation Laboratories, Department of Physiology, Cardiovascular Research Institute, University of California, San Francisco, CA 94140-0608; †Departments of Biological Chemistry and Medicine, Washington University School of Medicine, St. Louis, MO 63110; and §Department of Physiology, M. S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033

Communicated by David M. Kipnis, August 7, 1985

ABSTRACT **Rat apolipoproteins (apo-) A-IV and A-I share many structural similarities, the most notable of which is a domain of repeated docosapeptides with amphipathic helical potential. Although the genes for apo-A-IV and apo-A-I probably diverged from a common ancestor, these proteins seem to have developed different functions in their evolution. In the present study, cloned cDNAs were used to characterize the expression of apo-A-IV and apo-A-I mRNAs in a wide variety of adult rat tissues, as well as in small intestine and liver obtained from fetal, suckling, and weanling animals; comparisons were made to the expression of apo-E mRNA. The apo-A-IV and apo-A-I mRNAs were most abundant in adult small intestine and liver, with trace amounts detected in other tissues. Substantial amounts of these mRNAs were detected in the yolk sac, suggesting that this fetal tissue plays an important role in lipid metabolism during gestation. Noncoordinate accumulation of apo-A-IV and apo-A-I mRNAs was observed within and between the liver and small intestine during neonatal development. The apo-A-IV mRNA levels in the developing small intestine and liver appeared to correlate with their triglyceride secretion rates, suggesting that this protein plays an important role in the metabolism of triglyceride-rich lipoproteins. When dexamethasone (0.1 μM), insulin (0.01 μM), or insulin and dexamethasone together were incubated with primary cultures of nonproliferating adult rat hepatocytes, apo-A-IV mRNA levels were 4-, 7-, and 11-fold higher, respectively, than in non-hormone-treated control hepatocytes. Hormone administration resulted in a 2-fold greater amount of apo-A-I mRNA in each case, with no significant change in the level of apo-E mRNA. The overall results suggest that these structurally related apolipoproteins are regulated in substantially different ways.**

Apolipoproteins (apo-) A-I and A-IV are components of chylomicrons, very low density lipoproteins, and high density lipoproteins in various mammalian species, including man and rat (see ref. 1 for review). We have recently isolated and determined the sequence of full-length cDNAs for the mRNAs encoding apo-A-IV and apo-A-I (2, 3). Their derived amino acid sequences exhibit striking similarities in sequence and structural organization. Both of their mature protein domains contain homologous tandemly arrayed repeated sequences of 22-residue-long units. Rat plasma apo-A-I (M_r, 27,369) contains 8 tandem repetitions of a 22-amino acid segment (3), whereas rat plasma apo-A-IV (M_r, 44,465) contains 13 tandemly repeated docosapeptides (2). In both proteins, the docosapeptide repeats are not exact duplications. However, amino acid substitutions are generally conservative in nature; thus, the physical and chemical properties of residues in corresponding positions of the repeat units are preserved. A variety of computational techniques have been used to show that these docosapeptides have the potential to form amphipathic α-helical structures. Amphipathic α-helices are thought to be the structural domains responsible for lipid binding (4) and, in some cases (5), activation of lecithin:cholesterol acyltransferase (LCAT). Human plasma apo-A-I is an important metabolic activator of LCAT (6), whereas rat apo-A-I may be a considerably less potent activator of LCAT because of deletion mutations in its repeated sequence domain (3). Human plasma apo-A-IV has been shown to be as active as apo-A-I in stimulating LCAT activity with artificial liposome systems (7). Nevertheless, the precise physiological role of apo-A-IV is unclear.

The striking similarities in the primary structures of apo-A-IV, apo-A-I, as well as apo-E (2, 3, 8) have important implications concerning the evolution and function of these proteins. We have proposed (3) that all three genes evolved by initial intragenic duplication of a common (33- or 66-nucleotide) segment followed by subsequent intergenic duplications, resulting in three separate genes. Because these duplications undoubtedly occurred prior to the evolutionary separation of rodents and humans, apo-A-I, apo-A-IV, and apo-E may be considered to be paralogous members of a gene family (3). Divergence of paralogous sequences usually results in the evolution of new functions and/or different patterns of expression (9).

To investigate the physiological role of apo-A-IV and to elucidate its potential functional relationship with that of its closest structural homolog, apo-A-I, the present report examines the distribution of their mRNAs in a variety of adult rat tissues that exhibit different capabilities for metabolizing lipids. The expression of these genes during development is characterized, and the changes in apo-A-I and apo-A-IV mRNA accumulation that were observed are correlated with the dramatic changes in lipid metabolism that occur in both the liver and small intestine during the fetal, suckling, and weanling periods. Finally, evidence that glucocorticoids and insulin have markedly different effects on the accumulation of apo-A-I and apo-A-IV mRNAs in primary cultures of hepatocytes is presented.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: apo-A-I, -A-IV, and -E, apolipoproteins A-I, A-IV, and E; LCAT, lecithin:cholesterol acyltransferase.

‡Present address: Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305
¶To whom reprint requests should be addressed.

EXPERIMENTAL PROCEDURES

Animals. All rats used were male, except in the developmental studies, and they were of the Sprague–Dawley strain. Rats (Simonsen Laboratories, Gilroy, CA) weighing 120–140, 190–210, or 400 g were allowed free access to Purina Rat Chow. Timed pregnant rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) for the collection of fetal, neonatal, and maternal tissues. Neonatal animals after parturition were suckling, and rat pups were allowed free access to maternal milk and rat chow at all times. Dexamethasone acetate (Decadron, Merck) was suspended in water and administered orally to adult male rats by stomach intubation (7.5 mg/kg once daily for 4 days).

Cultured Hepatocytes. Primary monolayer cultures of adult rat hepatocytes were prepared as reported (10), except the cells were allowed to attach to collagen-coated 6-cm culture dishes instead of nylon meshes. The cultures of hepatocytes were maintained for 96 hr in a defined serum-free medium (10), with the addition of either dexamethasone (0.1 μM), bovine insulin (0.01 μM) (Eli Lilly), or both dexamethasone and insulin. The medium was changed daily.

RNA Preparation. Tissues were excised, rinsed with cold 0.9% NaCl, and quickly frozen in liquid nitrogen. Total RNA was extracted from pulverized frozen tissue or cultured cells with guanidine thiocyanate followed by CsCl centrifugation (11).

RNA Dot Blot Analysis. Total cellular RNA was analyzed at four different concentrations (3, 2, 1, and 0.5 μg) and supplemented with yeast RNA to provide a final total amount of 3 μg of RNA per sample. The RNA samples were denatured, applied to nitrocellulose filters, and hybridized to ^{32}P-labeled cDNAs as described (12). The cDNA probes for rat apo-A-IV (2), apo-A-I (3), and apo-E (8) have been characterized previously. Each filter contained liver or intestinal RNA that was diluted over a 100-fold concentration range as reference standards. Autoradiograms of filters were analyzed by quantitative scanning densitometry in the linear range of film sensitivity. The hybridization conditions used (12) produced no detectable cross-hybridization as determined by RNA blot analysis (13). Control yeast RNA dots did not produce signals to any of the cDNAs used.

RESULTS

The relative amounts of apo-A-IV and apo-A-I mRNAs in 12 different adult male rat tissues were examined by RNA dot blot hybridization analysis using cloned cDNAs as probes. The results (Table 1) showed that both apo-A-IV and apo-A-I mRNAs were most abundant in the small intestine. The apo-A-IV and apo-A-I mRNA levels in liver were 12% and 36%, respectively, of that found in the small intestine; they were the same size (data not shown) as their intestinal counterparts as determined by RNA blot analysis (apo-A-IV, 1700 base pairs; apo-A-I, 1100 base pairs). The concentrations of apo-A-IV and apo-A-I mRNAs in the 10 other tissues examined were <4% of that found in the small intestine (Table 1). These observations contrast with the results from our previous studies of apo-E mRNA (12), which showed that in the adult rat, apo-E mRNA was relatively abundant in several extrahepatic tissues, including the brain and adrenal glands.

Because morphological, biochemical, and functional gradients exist along the length of the small intestine, it might be expected that the abundance of apo-A-IV and apo-A-I mRNAs along the horizontal axis of the intestine could vary. Therefore, the distribution of apo-A-IV and apo-A-I mRNAs was measured among five anatomic domains of the small intestine: duodenum, proximal jejunum, middle jejunum, distal jejunum–proximal ileum, and ileum (14). Total cellular RNA was extracted from each segment and the relative amounts of these apolipoprotein mRNAs were determined by RNA gel blotting (12, 13), followed by hybridization, autoradiography, and densitometry. By taking into account the total yield of RNA from each segment, the relative content of apo-A-IV and apo-A-I mRNAs throughout the small intestine was determined (Fig. 1). Approximately 40% of the total apo-A-IV and apo-A-I mRNA in the small intestine was contained in the proximal jejunum. There was a progressive decrease in the content of both mRNAs toward the distal region of the small intestine. Despite these changes in mRNA content, the ratio of apo-A-IV/apo-A-I mRNA concentrations was approximately equal throughout the small intestine, suggesting that the expression of their corresponding genes might be coordinately regulated in this organ in adult animals.

To assess the potential for regulation, the expression of apo-A-IV and apo-A-I mRNAs during fetal and neonatal development was investigated, and the expression in the small intestine was compared to that found in the liver and the embryonic-related tissue, the yolk sac. Total cellular RNA was prepared from these tissues that had been collected at various times prior to and following parturition and was examined by dot blot analysis. Because the yield of RNA from individual tissues at early stages of development is relatively low, organs were pooled from groups of animals of the same age (n = 20). Thus, estimates of apo-A-IV and apo-A-I mRNA levels represent average values that would mask any differences that might exist between individual animals. Organs from both males and females were combined for each time point. The results of these analyses are shown in Fig. 2. Previously published data (12) concerning the abundance of apo-E mRNA in these samples have been included as well, to provide an integrated representation of the expression of the genes encoding the three principal rat apolipoproteins in high density lipoproteins.

The apo-A-I mRNA was detectable, in low amounts, in the fetal small intestine by the 17th day of gestation (Fig. 2 *Middle*). Its concentration increased abruptly within 24 hr of birth. The accumulation of this mRNA in the small intestine during the suckling period reached a peak (day 8) that was slightly greater than that observed in either weaned (day 35) or adult (400 g) rats. In comparison, apo-A-I mRNA was moderately abundant in the fetal liver and had a different pattern of accumulation than that observed in the small intestine. Adult liver levels of this mRNA were significantly lower than those encountered in the perinatal and postnatal

Table 1. Relative levels of apo-A-I and apo-A-IV mRNAs in adult rat tissue

Tissue	Relative level	
	apo-A-I	apo-A-IV
Small intestine	100	100
Large intestine	4	1
Liver	36	12
Stomach	3	<1
Testes	3	1
Spleen	1	1
Pancreas	2	1
Kidney	3	1
Lung	3	2
Brain	3	1
Adrenal gland	2	1
Heart	2	<1

Individual tissues were collected and pooled from 4–10 animals. Total cellular RNA was prepared from each tissue and examined by RNA dot blot analysis as described.

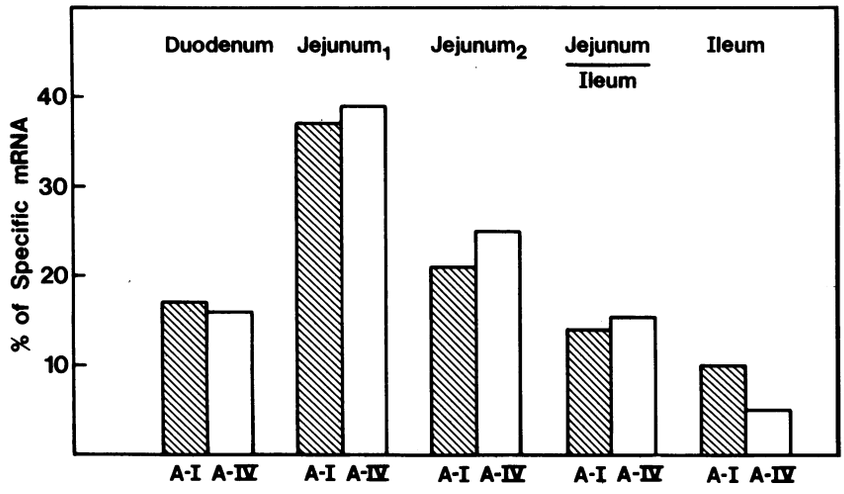

FIG. 1. Distribution of apo-A-I and apo-A-IV mRNAs in the small intestine. Entire small intestines from 10 rats were rinsed with cold 0.9% NaCl, cut into segments, and combined according to segment position along the length of the intestine. The first 10 cm was taken as duodenum, the next two segments (20 cm each) were taken as jejunum, the following segment (20 cm) was taken as the jejunum–ileum junction, and the remaining segment ranging from 18 to 24 cm long was taken as the ileum. For each sample, 40 μg of total cellular RNA was examined by RNA blot hybridization analysis, and the resulting autoradiograms were examined by quantitative scanning densitometry. The fractional amount of apo-A-I and apo-A-IV mRNAs in each intestine segment was calculated relative to the total amount on each specific mRNA in the whole small intestine.

period; in the intestine, adult and suckling levels were similar.

The induction of apo-A-IV mRNA during fetal and neonatal development was substantially different from that of apo-A-I. At parturition, there was a dramatic increase in the level of intestinal apo-A-IV mRNA to a level that was ≈4-fold greater than that of the adult intestine, followed by a gradual decrease in apo-A-IV mRNA levels over the next 14 days. These findings were in striking contrast to the expression of apo-A-IV mRNA in the developing liver. This mRNA was not detected in the fetal liver or in the neonatal liver until the time of weaning (after 14 days), at which time it appeared to increase gradually to adult levels.

In comparison, the developmental pattern of apo-E mRNA levels was quite different from that of apo-A-I and apo-A-IV (Fig. 2). In a previous study (12), only trace amounts of apo-E mRNA were detected in the small intestine at any time of development. Relatively low levels of apo-E mRNA were found in the fetal liver. There was an increase in the amount of apo-E mRNA at parturition to about one-half that of the adult concentration, with a subsequent increase to adult levels after weaning.

The detection of relatively large amounts of apo-A-IV and apo-A-I, as well as apo-E mRNAs, in the yolk sac, which is a tissue of fetal origin (15), was of particular interest. Two observations (data not shown) suggested that the apo-A-IV and apo-A-I mRNAs in the yolk sac were fully functional. First, RNA blot studies indicated that these mRNAs were the same sizes as their adult intestine and liver counterparts. Second, cell-free translation of yolk sac mRNA obtained at the 19th day of gestation yielded primary translation products, as shown by immunoprecipitation, of apo-A-IV and apo-A-I mRNA that were the same size as those produced by adult intestine mRNA. Because the yield of poly(A)-containing RNA from the yolk sac has been found to be about the same as that from the fetal liver (16), it seems likely that the fetus derives about one-half of its apo-A-I, and perhaps all of its apo-A-IV and apo-E, from the yolk sac during fetal development. These findings indicate that the yolk sac may have an important role in lipid metabolism during fetal development.

Alterations in maternal liver apolipoprotein mRNA levels at parturition were also observed, which may have been in response to maternal hormone changes that occur at this time. There was a substantial transient increase in apo-A-I mRNA levels 1 day prior to parturition (Fig. 2 *Lower*). A 2-fold transient increase in apo-E mRNA was detected also, but it occurred just after parturtion. In contrast, there was no significant change in the level of apo-A-IV mRNA in the maternal liver during the perinatal period.

The effectors that modulate the levels of apo-A-IV, apo-A-I, and apo-E mRNAs during development have not been defined. However, the dietary changes that occur in the neonatal period have been shown to be accompanied by substantial alterations in the levels of various hormones (see ref. 17 for review), which might serve as potential regulators of apolipoprotein gene expression. Because a large increase in circulating corticosteroids is known to occur during weaning (17), a preliminary investigation into the effect of a glucocorticoid (dexamethasone) on apolipoprotein mRNA levels in the rat liver was conducted. The results in Fig. 3 show that oral administration of dexamethasone to Sprague–Dawley rats for 4 days resulted in a selective 4-fold increase in the level of apo-A-IV mRNA. In comparison, the level of apo-A-I mRNA was increased 2-fold, whereas the level of apo-E mRNA was not changed.

To determine whether glucocorticoids increase apo-A-IV mRNA concentrations as a result of a direct effect on the liver, primary cultures of nonproliferating hepatocytes prepared from normal adult male rats were studied. Incubation of these hepatocytes for 96 hr in a culture medium containing 0.1 μM dexamethasone produced an identical pattern of change in apolipoprotein mRNA levels as was observed *in vivo* (Fig. 4).

Because of its effects on plasma lipid and lipoprotein levels (18), insulin is another potential mediator of apolipoprotein gene expression. Therefore, the effect of insulin on the expression of the apo-A-IV, apo-A-I, and apo-E mRNA levels was examined (Fig. 4). A 96-hr incubation of the primary cultured hepatocytes in the presence of 0.01 μM insulin resulted in a 7-fold higher amount of apo-A-IV mRNA than in non-hormone-treated control cells. Insulin induced a 2-fold increase in apo-A-I mRNA levels, with no significant change in apo-E mRNA levels. Furthermore, incubation of the hepatocytes with both 0.1 μM dexamethasone and 0.01 μM insulin together resulted in an additive effect on the accumulation of apo-A-IV mRNA in these cells. This additive effect was not observed with apo-A-I mRNA, and there was no effect of the hormones on apo-E mRNA levels.

DISCUSSION

The expression of the rat apo-A-IV and apo-A-I genes during fetal and neonatal development has been characterized and compared to that of the apo-E gene. A complex pattern of changes in mRNA levels has been observed that may be related to changes in diet as well as changes in the metabolism of lipid by the liver. In this regard, Frost *et al.* (19) and Fernando-Warnakulasuriya *et al.* (20) recently have analyzed the rates of triglyceride secretion by suckling rats. In these neonates, nearly

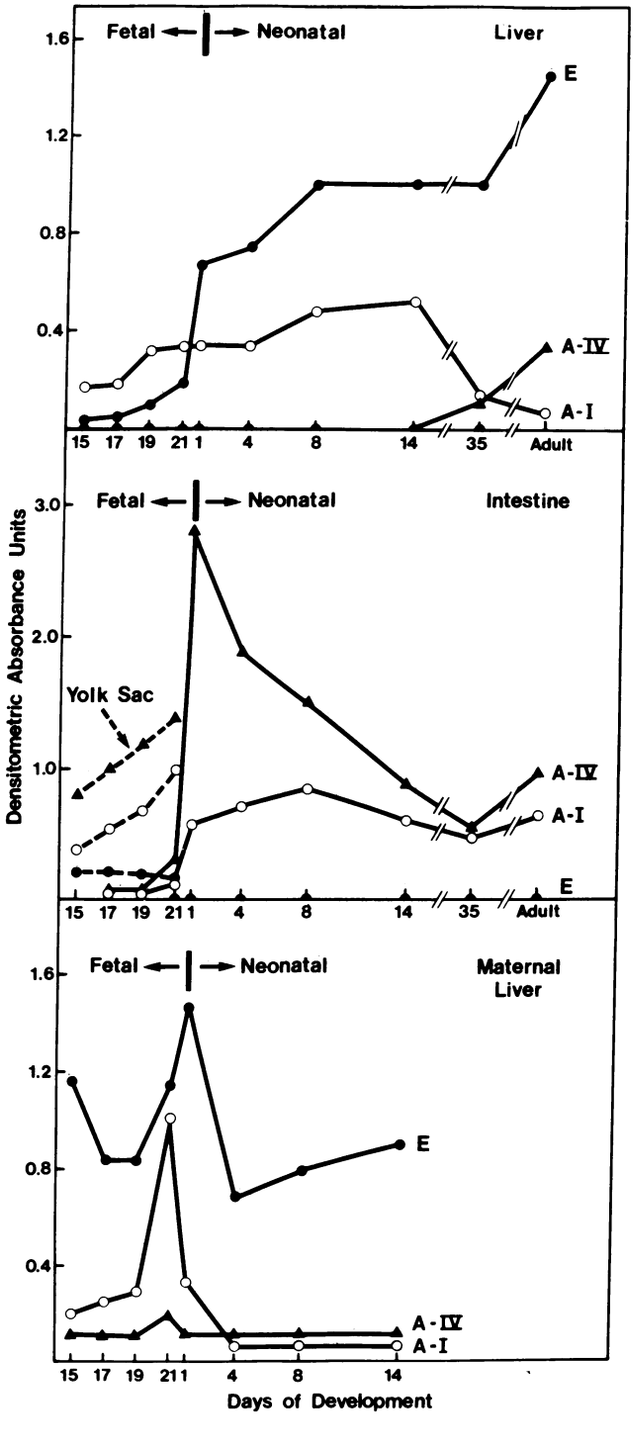

FIG. 2. Induction of apo-A-I and apo-A-IV mRNAs in tissues of the rat during fetal and neonatal development. Total cellular RNA from the tissues of 4–30 fetal, neonatal, or maternal rats were collected and examined by RNA dot blot analysis. Various exposures of the resulting autoradiograms were examined by quantitative densitometry, and the densitometric absorbances in the linear range of the exposures were compared to known standards of total liver RNA. The normalized densitometric absorbances are plotted to show the relative amounts of each mRNA in the different tissues.

all of the circulating plasma triglycerides are derived from the export of triglycerides (as chylomicrons or very low density lipoproteins) from the intestine. This circulating lipid reflects the high lipid content of the milk diet. In contrast, the liver of the suckling (i.e., 14-day-old) rat does not secrete triglycerides even though it has the capacity to synthesize these lipids.

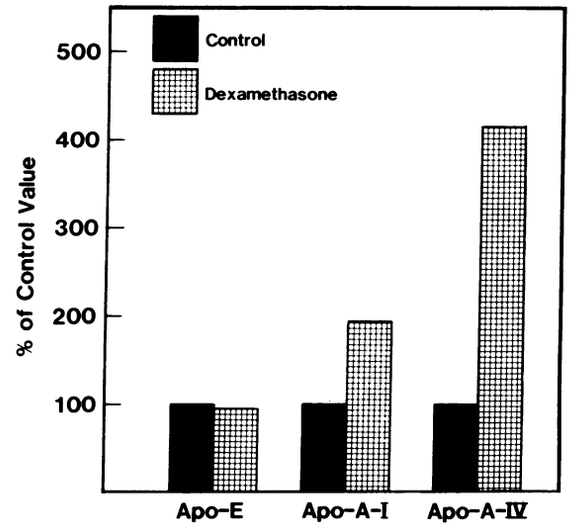

FIG. 3. Effect of dexamethasone on the accumulation of apo-E, apo-A-I, and apo-A-IV mRNAs in the adult rat liver. Total cellular RNA from livers (n = 4) of control or dexamethasone-treated rats was examined by the RNA dot blot method. Autoradiograms of the filters were analyzed by quantitative scanning densitometry to determine relative changes in the individual mRNA levels. The results from dexamethasone-treated animals are presented as a percentage of the control value.

The molecular basis of the apparent lack of triglyceride (or very low density lipoprotein) secretion by the liver of the suckling rat is not known. However, the lack of apo-A-IV synthesis by the liver during this time may be related to the deficiency in liver triglyceride export. No detectable hepatic apo-A-IV mRNA is present during the neonatal suckling period, despite the presence of substantial levels of apo-A-I and apo-E mRNAs. In contrast, apo-A-IV mRNA is the predominant mRNA species in the neonatal small intestine, where high levels of dietary triglycerides are reassembled and secreted. In addition, it should be noted that the availability of apo-B in the neonatal rat liver is probably a key determinant in very low density lipoprotein secretion, but knowledge of apo-B production during development is lacking.

The association between apo-A-IV synthesis and the secretion of triglycerides is supported by three additional observations. First, in suckling animals, the rate of triglyceride secretion by the small intestine is greater than that in adult animals (19). This augmented triglyceride secretion occurs at a time in the rat's development when intestinal apo-A-IV mRNA levels are at their maximum. Second, in adult rats maintained on a normal low fat/high carbohydrate chow diet, the liver is a major contributor to the plasma triglyceride pool through its export of very low density lipoproteins (21). In contrast to the suckling rat liver, the adult rat liver is a major site of apo-A-IV synthesis, with an apo-A-IV mRNA level nearly equivalent to that of the adult small intestine. Finally, acute triglyceride feeding results in a rapid increase in apo-A-IV mRNA levels in adult rat small intestine without a corresponding increase in the concentration of apo-A-I mRNA (22). This increase in apo-A-IV mRNA levels is also accompanied by increases in apo-A-IV and triglyceride secretion.

The mechanisms by which apo-A-IV may affect the metabolism of triglyceride-rich lipoproteins are not clear. The binding of apo-A-IV to lipids, which is mediated by its amphipathic helical structures, may serve to segregate and facilitate the intracellular assembly and secretion of its associated lipoprotein particles. The association of apo-A-IV with lipoproteins during circulation in plasma may be important with respect to its recently reported (7) LCAT activating

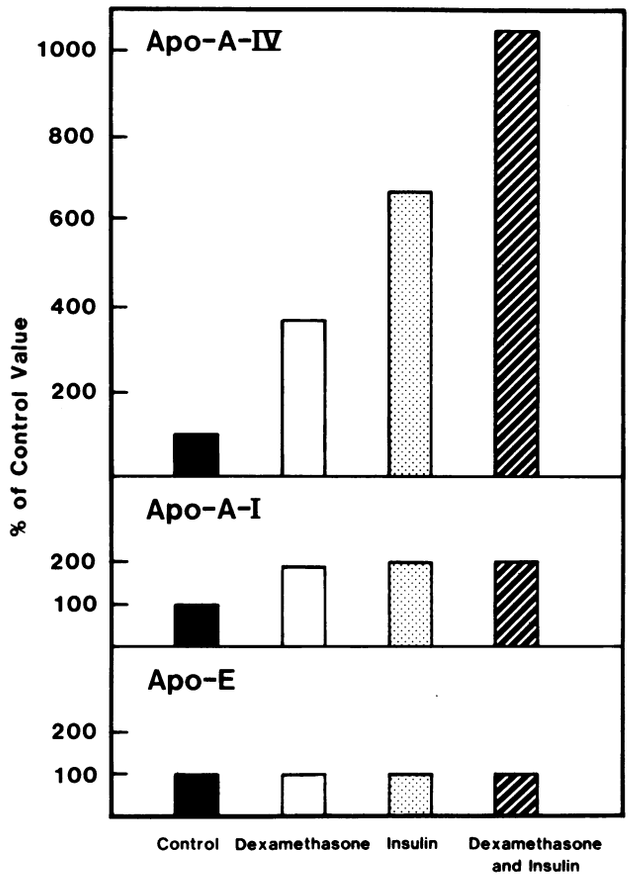

FIG. 4. Effect of dexamethasone and insulin on the accumulation of apo-E, apo-A-I, and apo-A-IV mRNAs in primary cultures of rat hepatocytes. Primary cultures of hepatocytes were incubated in defined medium as described, in the presence or absence of the indicated hormone. After 96 hr, the cells were harvested, and the total cellular RNA was prepared and analyzed by the dot blot method.

capability. In addition, the presence of apo-A-IV on lipoproteins may help direct them to tissue sites where metabolism of fatty acids is important. For example, endothelial cell surfaces of tissues active in fatty acid metabolism, such as adipose tissue, may bind triglyceride-rich lipoproteins through an interaction involving apo-A-IV. These various potential mechanisms of apo-A-IV action remain to be tested.

The factors that regulate the expression of apolipoprotein genes are largely unknown. The many physiological changes that occur during fetal and neonatal development make it difficult to identify specific modulators of apolipoprotein mRNAs. The noncoordinate alterations in mRNA levels that occur during this time suggest that transcription of these genes is controlled by more than one regulating element. Thus, certain hormones may be important in this regard.

The results of Figs. 3 and 4 indicate that glucocorticoids can affect the expression of apolipoprotein genes in a differential manner through a primary action on hepatocytes. The inducing effect on apo-A-IV mRNA levels suggests a specific involvement of glucocorticoids in the regulation of the corresponding gene. Whether the hormone response is due to a stimulation of apo-A-IV gene transcriptional activity by direct interaction with a glucocorticoid–receptor complex or by a secondary effect of intracellular action such as an alteration in apo-A-IV mRNA degradation rates is not known.

The striking selective increase in apo-A-IV mRNA levels in primary cultures of hepatocytes in response to insulin (Fig. 4) indicates that this polypeptide hormone may function as an important regulator of apo-A-IV mRNA. This effect may be related to insulin-associated modulations of intermediary (i.e., fatty acid) metabolism, an alteration in apo-A-IV mRNA turnover, or it may be the consequence of a primary action of the hormone response on the apo-A-IV gene. The additive nature of the insulin and glucocorticoid responses suggests that separate intracellular effectors are responsible for these hormonal effects. Furthermore, the modulation of apo-A-IV gene expression by insulin may be a dominant effect, which could provide an important tool for examining the mechanism of action of this hormone. The independent effects of insulin and dexamethasone, together with the diversity of the developmental changes, indicates a previously unsuspected complexity in the regulation of the apolipoprotein gene family.

We thank Robert Mahley for stimulating discussions as well as his continuing interest in these studies. The graphics assistance of James X. Warger, Norma Jean Gargasz, and Karen Leung, and the editorial assistance of Barbara Allen and Sally Gullatt Seehafer are appreciated.

1. Mahley, R. W., Innerarity, T. L., Rall, S. C., Jr., & Weisgraber, K. H. (1984) *J. Lipid Res.* **25,** 1277–1294.
2. Boguski, M. S., Elshourbagy, N., Taylor, J. M. & Gordon, J. I. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5021–5025.
3. Boguski, M. S., Elshourbagy, N., Taylor, J. M. & Gordon, J. I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 992–996.
4. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M. (1974) *FEBS Lett.* **38,** 247–253.
5. Kaiser, E. T. & Kezdy, F. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1137–1143.
6. Fielding, C. J., Shore, V. G. & Fielding, P. E. (1972) *Biochem. Biophys. Res. Commun.* **46,** 1493–1498.
7. Steinmetz, A. & Utermann, G. (1983) *Arteriosclerosis* **3,** 495 (abstr.).
8. McLean, J. W., Fukazawa, C. & Taylor, J. M. (1983) *J. Biol. Chem.* **258,** 8993–9000.
9. Fitch, W. M. & Margoliash, E. (1970) *Evol. Biol.* **4,** 67–109.
10. Flaim, K. E., Hutson, S. M., Lloyd, C. E., Taylor, J. M., Shiman, R. & Jefferson, L. S. (1985) *Am. J. Physiol.*, in press.
11. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
12. Elshourbagy, N. A., Liao, W. S. L., Mahley, R. W. & Taylor, J. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 203–207.
13. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
14. Hebel, R. & Stromberg, M. W. (1976) in *Anatomy of the Laboratory Rat* (Williams & Wilkins, Baltimore), pp. 48–50.
15. Gardner, R. L. (1983) *Int. Rev. Exp. Pathol.* **24,** 63–133.
16. Liao, W. S. L., Conn, A. R. & Taylor, J. M. (1980) *J. Biol. Chem.* **255,** 10036–10039.
17. Henning, S. J. (1981) *Am. J. Physiol.* **241,** G199–G214.
18. Pietri, A., Dunn, F. L. & Raskin, P. (1980) *Diabetes* **29,** 1001–1005.
19. Frost, S. C., Clark, W. A. & Wells, M. A. (1983) *J. Lipid Res.* **24,** 899–903.
20. Fernando-Warnakulasuriya, G. J. P., Eckerson, M. L., Clark, W. A. & Wells, M. A. (1983) *J. Lipid Res.* **24,** 1626–1638.
21. Risser, T. R., Reavan, G. M. & Reavan, E. P. (1978) *Am. J. Physiol.* **234,** E277–E281.
22. Gordon, J. I., Smith, D. P., Alpers, D. H. & Strauss, A. W. (1982) *Biochemistry* **21,** 5424–5431.