Apolipoprotein A-I Expression in Rats Is Not Altered by Troglitazone[1]

ARSHAG D. MOORADIAN,[2]* MICHAEL J. HAAS,* JOE CHEHADE,* AND NORMAN C.W. WONG†

The Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine, St. Louis University Medical School, St. Louis, MO 63104; and †the Division of Endocrinology, University of Calgary, Calgary, Alberta, Canada T2N4N1

Insulin is known to upregulate apolipoprotein A-I (apoA-I) promoter activity and to increase apoA1 gene expression *in vivo*. To determine if enhancement of insulin action with insulin sensitizers can also increase the apoA-I expression, we studied the *in vivo* effect of troglitazone, a potent insulin sensitizer, on the expression of rat hepatic and intestinal apoA-I mRNA using Northern blot analysis. The plasma, hepatic, and intestinal apoA-I content was also measured with immunoblot analysis using a specific anti-rat apoA-I antiserum. Troglitazone, given mixed with rat chow (0.2%) for 18 days, did not increase either plasma or tissue apoA-I mRNA or protein content. Intestinal apoA-I mRNA content relative to glyceraldehyde-3 phosphate dehydrogenase (G_3PDH) mRNA was significantly lower compared with hepatic tissue content in both control and troglitazone-treated rats. The effect of troglitazone on the rat apoA-I promoter was examined using transient transfection analysis in HepG2 cells transfected with the apoA-I-chloramphenicol acetyl transferase (CAT) reporter plasmid (pAI.474.CAT). CAT activity (percentage acetylation of chloramphenicol as means ± SEM) was not significantly different in ethanol (vehicle)-treated cells compared with cells treated with troglitazone (50.5% ± 2.5% in control cells vs 57.7% ± 8.2% and 53.5% ± 4.2% in cells treated with 10 and 100 m*M* troglitazone, respectively). It is concluded that troglitazone doses known to achieve insulin sensitization did not enhance rat apoA-I promoter activity sufficiently to result in an increased apoA-I mRNA or protein expression in the intact rat. However, peroxisome proliferator activator receptor (PPAR) agonists that have significant PPAR α activity in addition to their PPAR γ effects, may well be able to induce apoA-I expression. Exp Biol Med 227:1001–1005, 2002.

Key words: atherosclerosis; thiazolidinediones; high density lipoprotein; apolipoprotein A-I

Thiazolidinediones (TZDs) are antidiabetic agents that interact with peroxisome proliferator activator receptor (PPAR) γ and enhance insulin sensitivity (1). Because the PPAR subfamily of nuclear receptors is implicated in the modulation of apolipoprotein A-I (apoA-I) gene expression (2, 3), and because insulin is known to upregulate apoA-I promoter activity and enhance apoA-I expression (4, 5), we hypothesized that troglitazone, a potent insulin-sensitizing thiazolidinedione, would induce the expression of apoA-I *in vivo* and would upregulate apoA-I promoter activity *in vitro*. To test this hypothesis, rats were treated with troglitazone for 18 days and plasma apoA-I was measured along with hepatic and intestinal apoA-I mRNA content. These tissues were studied because they are the only source of plasma apoA-I in rats (6). In addition to study the effect of TZD's on rat apoA-I promoter *in vitro*, HepG2 cells transfected with the full-length rat apoA-I promoter were treated with troglitazone and the activity of the reporter gene was measured.

Materials and Methods

Materials Acetyl-coenzyme A was from Sigma Chemical Company (St. Louis, MO). Lipofectamine was purchased from Life Technologies (Gaithersburg, MD), and ^{14}C-chloramphenicol was from New England Nuclear (Boston, MA). Tissue culture media and fetal calf serum were from BioWittaker (Walkersville, MD). All other chemicals were of reagent grade and were purchased from either Sigma Chemical Company or Fisher Scientific Company (Pittsburgh, PA).

A rat insulin-specific radioimmunoassay kit was purchased from Linco Research, (St. Charles, MO). Troglitazone powder was a gift from Parke Davis (Ann Arbor, MI).

Animals Male Fischer 344 rats at 3 months of age were obtained from Harlan Industries (Indianapolis, IN). A group of rats (n = 11) were given troglitazone mixed with powdered rat chow (0.2%) for 18 days. The control rats (n = 11) were given powdered rat chow *ad libitum*. This dose was chosen because it has been previously shown to achieve insulin sensitization in rats (7). Body weight and food intake were monitored every 2 days during the experi-

[1] The study was partially supported by Harold Braun Memorial Fund and by a grant from Takeda Pharmaceutical.
[2] To whom requests for reprints should be addressed at Division of Endocrinology, St. Louis University Medical School, 1402 South Grand Boulevard, St. Louis, MO 63104. E-mail: mooradad@slu.edu

Received November 6, 2001.
Accepted July 5, 2002.

1535-3702/02/22711-1001$15.00

ment. The rats were sacrificed by exsanguination through the abdominal aorta under sodium pentobarbital anesthesia (45 mg/kg i.p.) and tissues of interest were collected and immediately frozen in liquid nitrogen. Plasma glucose levels on the day of death were measured with a glucose oxidase technique using a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was measured with a commercial rat-specific insulin radioimmunoassay kit (Linco Research).

apoA-I Purification. Purification of the rat apoA-I was performed to make suitable antibodies for Western blot analysis. High-density lipoprotein (HDL) was purified from rat serum following a procedure adapted from Jensen *et al.* (8). The pooled fractions were dialyzed against 0.9% NaCl and were identified as containing HDL/apoA-I by PAGE (9).

Protein in the HDL/apoA-I-containing fraction was acetone precipitated. After removal of the acetone, the protein was resuspended in 0.3 ml of sodium dodecyl sulfate (SDS) loading buffer (10). Purification of apoA-I was then performed using Prep Cell (model 491; Bio-Rad, Hercules, CA) as described previously (11, 12). The authenticity of rat apoA-I was validated by molecular size and by amino acid analysis of the purified protein excised directly from the gel, which showed 93% homology with the predicted amino acid composition.

Preparation of Rat ApoA-I Antibody. Purified apoA-I protein (20 μg dissolved in 3 ml of 0.9% saline) was resuspended in Freud's adjuvant (Sigma, St. Louis, MO) and was injected subcutaneously into the backs of two New Zealand rabbits. These rabbits were reinjected two more times over a 3-month period (11, 12). The serum was tested at 4 months from the time of initial injection and was used at a 1:10,000 dilution for Western blots.

Measurements of Plasma ApoA-I. Plasma or tissue proteins (25 μg) were electrophoresed in a denaturing SDS-12% polyacrylamide gel under reducing conditions (9). Proteins in the gels were electrophoretically transferred to a nitrocellulose membrane (10). The membrane was incubated with apoA-I antiserum at a final dilution of 1:10,000 for 2 hr at room temperature. Horseradish peroxidase-linked goat anti-rabbit immunoglobulin (IgG) was used at a final dilution of 1:10,000 for 1 hr at room temperature. Blots were developed using enhanced chemiluminescence (ECL) Western blotting reagents as described by the manufacturer (Amersham-Pharmacia Biotech, Arlington Heights, IL). Plasma apoA-I content was determined by densitometry using the personal densitometer from Molecular Dynamics (Sunnyvale, CA). The absorbance of bands was analyzed after background subtraction. The reproducibility of the ApoA-I quantitation was established with gels loaded with different amounts of authentic rat apoA-I (11, 12). The correlation coefficient between the amount of rat apoA-I applied to the gel and the optical density of the band found on immunoblot was 0.99. Interassay and intraassay coefficients of variation for the measurements were 12.8% and 6.5%, respectively (12).

Measurements of Hepatic and Intestinal ApoA-I mRNA Content. RNA was isolated by cesium chloride density gradient centrifugation (13). Recombinant plasmid pBR322 containing the rat apoA-I cDNA (NE-477) was kindly provided by Dr. J.I. Gorden (Washington University, St. Louis, MO). Amplification and preparation of plasmid DNA was accomplished using established procedures (14). The 412-bp NE-477 insert was cleaved from the purified recombinant plasmid DNA with *Eco*RI and *Sac*I using conditions specified by the supplier. The cleaved insert was separated from the vector DNA and was random primer-labeled with (α-^{32}P) dCTP (15). The isolated RNA was electrophoresed in a 1.5% agarose gel containing 2.2 mol/L formaldehyde (16), transferred to the nylon membrane by diffusion blotting, and finally hybridized with the apoA-I cDNA insert (17). The membrane was exposed to X-ray film for autoradiography. The blots were stripped and reprobed with a glyceraldehyde-3 phosphate dehydrogenase (G_3PDH) cDNA to normalize the changes in apoA-I mRNA.

Measurements of ApoA-I Promoter Activity. Cell Culture. HepG2 cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and penicillin and streptomycin (100 ng/ml and 100 units/ml, respectively). Cells were housed in a humidified incubator at 37°C with 5% CO_2 and 95% air.

Plasmids and Transient Transfection Analysis. The reporter plasmid pAI.474.CAT, containing the rat apoA-I gene promoter (18), was transfected into HepG2 cells with lipofectamine as described by the manufacturer. This reporter plasmid contains most of the *cis*-elements critical for regulation of apoA-I gene expression in the rat (4, 18, 19). Cells cultured to 80% confluency were transfected with 1 μg of the apoA-I reporter plasmid and 1 μg of the plasmid pCMV.SPORT-β-gal (Life Technologies). The latter plasmid, containing the β-galactosidase gene driven by the cytomegalovirus (CMV) promoter, was used to normalize CAT gene activity to transfection efficiency. After 24 hr, the cells were treated with either troglitazone dissolved in ethanol (10 and 100 m*M* final concentration), or with an equal volume of ethanol, and after another 24 hr, were harvested and assayed for CAT (20) and β-galactosidase (21) activity.

Statistical Analysis. All results are expressed as mean ± SEM. Two-way analysis of variance (ANOVA) followed by Tukey's test was performed to establish significance of the differences. Values were statistically different if $P < 0.05$.

Results

Table I summarizes the body weight, daily food intake, plasma glucose and insulin concentrations, and insulin-to-glucose ratios, as well as the retroperitoneal fat weight on the day the rats were sacrificed. Troglitazone-treated rats had lower insulin/glucose ratios compared with control rats

Table I. The Mean ± SEM Body Weight at Day 1 (Basal) and Day 19 of the Experiment, Daily Food Intake Plasma Glucose, and Insulin Concentrations, Insulin-to-Glucose Ratio, and the Weight of Retroperitoneal Fat

Rat group	Body weight (g)		Food intake (g/rat/day)	Plasma glucose (mg/dl)	Plasma insulin (ng/ml)	Insulin-to-glucose ratio	Retroperitoneal fat weight (g)
	Basal	Day 19					
Control (n = 11)	174.5 ± 2.5	255.2 ± 2.9	22.8 ± 0.7	133.0 ± 5.0	1.693 ± 0.329	0.0129 ± 0.0019	1.72 ± 0.12
Troglitazone treated (n = 11)	173.5 ± 1.9	246.8 ± 2.8	23.7 ± 0.9	125.3 ± 4.5	1.037 ± 0.167*	0.0081 ± 0.0011*	2.54 ± 0.19*

Note. *P < 0.05 compared with controls.

(0.0081 ± 0.0011 vs 0.0129 ± 0.0019; $P < 0.05$), suggesting that insulin sensitization was achieved in these rats. As expected, the retroperitoneal fat weight was significantly increased in troglitazone-treated rats relative to controls (2.54 ± 0.19 g vs 1.72 ± 0.12 g respectively; $P < 0.01$) Table I.

A representative Western blot of plasma apoA-I is shown in Figure 1. The expected single 28-kD apoA-I band was identified. When the antiserum preadsorbed with purified apoA-I was used, no bands could be seen on immunoblots, indicating the specificity of the antibody used (data not shown). The means (± SEM) for plasma apoA-I, hepatic, and intestinal apoA-I estimates from various immunoblots are summarized in Table II. The results are presented as a percentage of an internal control sample included in each assay. This normalization of data allows the pooling of the results from several immunoblots. Troglitazone treatment did not result in significant changes in plasma, hepatic, or intestinal apoA-I levels.

To determine if apoA-I mRNA was altered, Northern blot analysis was done. A representative northern blot of total hepatic RNA hybridized with the apoA-I cDNA is shown in Figure 2. The expected 0.9-kb band of apoA1 mRNA is indicated. To establish the specificity of the apoA-I mRNA changes, the G3PDH mRNA levels were measured in each experiment. Troglitazone treatment did not alter the apoA-I mRNA content relative to G3PDH mRNA in either the liver or small intestinal tissue (Table II).

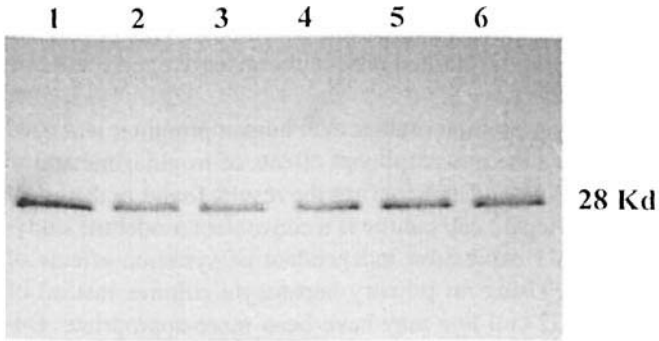

Figure 1. A representative Western blot of plasma proteins from control rats (lanes 1–3) and troglitazone-treated rats (lanes 4-6). Twenty-five micrograms of proteins was subjected to 12% SDS-PAGE, electrophoretically transferred to nitrocellulose membrane, and incubated with anti-rat apoA-I antiserum (1:10,000) for 2 hr. The single 28-kD apoA-I band is evident.

Table II. The Mean ± SEM of apoA-I Protein and mRNA in Various Tissues of Control Rats and Rats Treated with Troglitazone for 18 Days

	Control (n = 11)	Troglitazone treated (n = 10)
ApoA-I (percentage of control)		
Plasma	100.0 ± 11.6%	122.5 ± 16.6%
Liver	99.9 ± 8.1%	93.3 ± 10.1%
Intestine	100.1 ± 12.1%	92.8 ± 13%
ApoA-I mRNA-to-G_3PDH mRNA ratio		
Liver	0.998 ± 0.09	1.141 ± 0.08
Intestine	0.784 ± 0.05	0.693 ± 0.07

Note. The results of apoA-I protein are shown as a percentage of the apoA-I content of a control sample included as internal control in each assay. The ratio of apoA-I mRNA to G3PDH mRNA is shown. The differences between the two groups of rats did not achieve statistical significance. The intestinal apoA-I-to-G_3PDH mRNA ratio was significantly lower than that in the liver ($P < 0.01$) in both experimental groups.

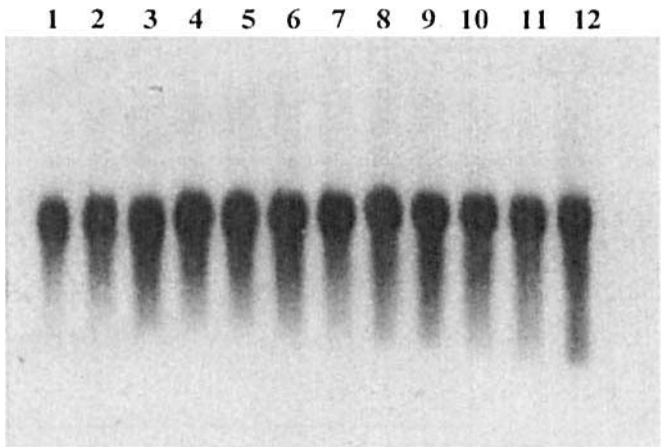

Figure 2. A representative Northern blot of hepatic RNA from control (lanes 1-6) and troglitazone-treated rats (lanes 7-12) hybridized with the 412-bp NE-477 apoA-I cDNA insert. A single 0.9-kb apoA-I mRNA band is evident.

The effect of troglitazone on the apoA1 promoter was examined using transient transfection analysis in HepG2 cells. Cells transfected with the apoA1-CAT reporter plasmid were treated with troglitazone at either 10 or 100 m*M* for 24 hr. The results indicate that troglitazone treatment had no effect on transcription by the rat apoA-I promoter

(Fig. 3). CAT activity (percentage of acetylation of chloramphenicol) was not significantly different in ethanol (vehicle)-treated cells compared with cells treated with troglitazone (50.5% ± 2.5% in control cells vs 57.7% ± 8.2% and 53.5% ± 4.2% in cells treated with 10 and 100 m*M* troglitazone, respectively). Ethanol at the concentrations used as vehicle for troglitazone had no effect on apoA-I promoter activity.

Discussion

These results clearly indicate that troglitazone at doses known to achieve insulin sensitization does not increase apoA-I mRNA or protein expression in rats. This is consistent with the previously observed lack of an effect of yet another potent thiazolidinedione, BRL 49653, on rat plasma apoA-I or HDL cholesterol levels (2). The responsiveness of apoA-I system in this model has been established in previous studies where thyroid hormone treatment (22) or high carbohydrate feeding of rats (5) was associated with increased expression of apoA-I. Similarly, in the HepG2 cell cultures transfected with this apoA-I promoter construct, increased promoter activity could be shown in the presence of dexamethasone (23). The efficacy of the troglitazone treatment was demonstrated by the reduction in insulin-to-glucose ratio and by increased retroperitoneal fat weight (Table I). In addition, in a previously published study using

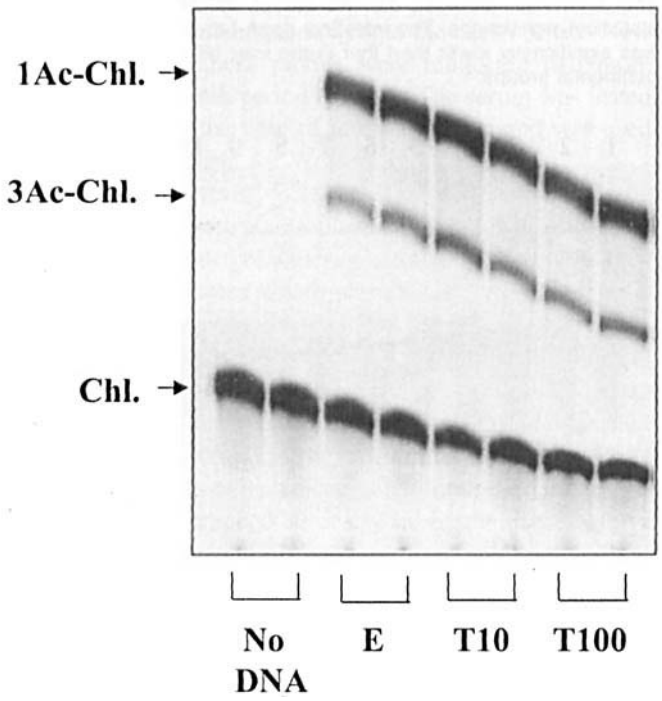

Figure 3. Effect of troglitazone on apoA-I promoter activity in HepG2 cells. HepG2 cells were transfected with the plasmid pA1.474.CAT and after 24 hr were treated with either ethanol (E) or troglitazone at final concentrations of 10 and 100 m*M* (T10 and T100, respectively). There was no difference in CAT activity in troglitazone-treated cells relative to the ethanol (vehicle of troglitazone)-treated controls. No DNA, Is mock-transfected cells; Chl, ^{14}C-chloramphenicol substrate; 1Ac-Chl, chloramphenicol acetylated at the 1 position; 3Ac-Chl,chloramphenicol acetylated 3-position.

the same group of animals, troglitazone-treated rats compared with control rats had significantly increased hepatic acetyl CoA carboxylase activity, the rate-limiting enzyme in fatty acid synthesis (24). It is possible that higher doses of troglitazone or for longer periods of time may have achieved induction of apoA-I expression. In addition, it is also possible that these observations in rats may not apply to humans because apoA1 regulation by PPAR is species specific (3).

The intestinal apoA-I mRNA content relative to G_3PDH mRNA was lower compared with hepatic apoA-I mRNA. This finding reflects previous reports showing that up to 55.7% of plasma apoA1 is synthesized in the intestine (6). This suggests that either total apoA-I mRNA content of the intestine is equivalent to the total hepatic apoA-I mRNA or that the apoA-I translation and export to the plasma is more efficient in rat intestinal tissue.

A recently published study found that apoA-I secretion in HepG2 cells is increased with pioglitazone and rosiglitazone, but not with troglitazone (25). Therefore, it is likely that the effects of various TZD's on apoA-I expression are variable. The precise underlying mechanisms for drug-specific effects are not known (26). The reason we did not include rosiglitazone or pioglitazone in these studies is because the aim of the study was to determine the effect of insulin sensitization on apoA-I expression, which is known to be an insulin responsive gene. We did not intend to establish agent-specific effects. Indeed, TZD's are known to differ in their effects on a host of parameters; however, they all share insulin-sensitization properties (26, 27).

The effect of troglitazone can be either direct (acting on hepatocytes PPAR γ) or indirect via insulin sensitization. This is why the effect of troglitazone was tested both *in vivo* and in cell cultures.

Studying rat models of insulin resistance would be of interest. However, the effect on apoA-I expression in these models would be difficult to interpret. We have shown in this same rat strain that insulin resistance either age-related or induced by high fructose feeding is associated with increased hepatic and plasma apoA-I levels (5, 11). Thus, the interplay of hyperinsulininemia and insulin resistance modulating apoA-I expression is complex. This is why we elected to study "normal rats" in the absence of confounding variables.

The rat promoter rather than human promoter was used to measure the transcriptional effects of troglitazone and to be able to extend and confirm the results found in the intact rat. The HepG2 cell culture is a convenient model for studying apoA-I expression independent of systemic effects of the drug. Using rat primary hepatocyte cultures instead of the HepG2 cell line may have been more appropriate. Despite these uncertainties, the present paper suggests that the insulin-sensitizing effects of TZD's are independent of any effect they may have on apoA-I expression. However, it is likely that PPAR γ agonists that have significant PPAR α activity, may well be able to induce apoA-I expression, and

may contribute to increased plasma apoA-I and HDL cholesterol levels (26, 27).

We authors thank Ms. Jianping Li and Ms. Deanna Reinacher for their excellent technical assistance.

1. Chehade JM, Mooradian AD. A rational approach to drug therapy of type 2 diabetes mellitus. Drugs **60:**95–113, 2000.
2. Lefebre AM, Peinado-Onsurke J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, Fievet C, Auwerx J, Staels B. Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. Arterioscler Thromb Vasc Biol **17:**1756–1764, 1997.
3. Vu-Dac N, Chopin-Delannoy S, Gervois P, Bonnelye E, Martin G, Fruchart J-C, Laudet V, Staels B. The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erb α mediate the species-specific regulation of apolipoprotein A_1 expression by fibrates. J Biol Chem **273:**25713–25720, 1998.
4. Murao K, Wada Y, Nakamura T, Taylor AH, Mooradian AD, Wong NCW. Effects of glucose and insulin on rat apolipoprotein A-I gene expression. J Biol Chem **273:**18959–18965, 1998.
5. Mooradian AD, Wong NCW, Shah GN. Apolipoprotein A1 expression in young and aged rats is modulated by dietary carbohydrates. Metabolism **46:**1132–1136, 1997.
6. Wu AL, Windmueller HG. Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. J Biol Chem **254:**7316–7322, 1979.
7. Miles PD, Higo K, Romeo OM, Lee MK, Rafast K, Olefsky JM. Troglitazone prevents hyperglycemia-induced but not glucosamine-induced insulin resistance. Diabetes **47:**395–400, 1998.
8. Jensen H, Schoonderwoerd G, Dallinga-Thie GM. Separation of rat plasma HDL subfractions by density gradient centrifugation and the effect of incubation on these fractions. Biochim Biophys Acta **754:**271–278, 1983.
9. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685, 1970.
10. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Proc Natl Acad Sci U S A **76:**4350–4354, 1979.
11. Shah GN, Wong NCW, Mooradian AD. Age-related changes in apolipoprotein A_1 expression. Biochim Biophys Acta **1259:**277–282, 1995.
12. Taylor AH, Raymond J, Dionne JM, Romney J, Chan J, Lawless DE, Wanke IE, Wang NC. Glucocorticoid increases rat apolipoprotein A-1 promoter activity. J Lipid Res **37:**2232–2243, 1996.
13. Goodman HM, McDonald RJ. Cloning of hormone genes from a mixture of cDNA molecules. Methods Enzymol **68:**75–90, 1979.
14. Clewell DB, Helsinki DR. Supercoil circular DNA-protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. Proc Natl Acad Sci U S A **63:**1159–1166, 1969.
15. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem **132:**6–13, 1983.
16. Lehrach H, Diamond D, Wozney JM, Boedtkel H. RNA molecular weight determinations by gel electrophoresis under denaturing conditions: A critical reexamination. Biochemistry **16:**4743–4751, 1977.
17. Thomas PS. Hybridization of denatured RNA and small RNA fragments transferred to nitrocellulose. Proc Natl Acad Sci U S A **77:**5201–5205, 1980.
18. Romney JS, Chan J, Carr FE, Mooradian AD, Wong NCW. Identification of the thyroid hormone-responsive messenger RNA spot 11 as apolipoprotein-A1 messenger RNA and effects of the hormone on the promoter. Mol Endocrinol **6:**943–950, 1992.
19. Taylor AH, Wishart P, Lawless DE, Raymond J, Wong NCW. Identification of functional positive and negative thyroid hormone-responsive elements in the rat apolipoprotein A-1 promoter. Biochemistry **35:**8281–8288, 1996.
20. Gorman CM, Moffat LF, Howard BH. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol **2:**1044–1051, 1982.
21. Herbomel P, Bourachot B, Yaniv M. Two distinct enhancers with different cell specificities coexist in the regulatory region of polyoma. Cell **39:**653–662, 1984.
22. Mooradian AD, Wong NCW, Shah GN. Age-related changes in the responsiveness of apolipoprotein A1 to thyroid hormone. Am J Physiol **271:**R1602–R1607, 1996.
23. Haas MJ, Reinacher D, Pun K, Wong NCW, Mooradian AD. Induction of the apolipoprotein AI gene by fasting: a relationship with ketosis but not with ketone bodies. Metabolism **49:**1572–1578, 2000.
24. Thampy GK, Haas MJ, Mooradian AD. Troglitazone stimulates acetyl-CoA carboxylase activity through a post-translational mechanism. Life Sci. **68:**699–708, 2000.
25. Sakamoto J, Kimura H, Moriyama S, Odaka H, Momose Y, Sugiyama Y, Sawada H. Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone. Biochem Biophys Res Commun **278:**704–711, 2000.
26. Mooradian AD, Chehade J, Thurman JE. The role of thiazolidenediones in the treatment of patients with type 2 diabetes mellitus. Treat Endocrinol **1:**13–20, 2002.
27. Willson TM, Brown PJ, Sternbach DD, Heake BR. The PPARS: from orphan receptors to drug discovery. J. Med. Chem. **43:**527–550, 2000.