Induction of the Apolipoprotein AI Gene by Fasting: A Relationship With Ketosis But Not With Ketone Bodies

Michael J. Haas, Deanna Reinacher, Kristoffer Pun, Norman C.W. Wong, and Arshag D. Mooradian

Apolipoprotein AI (apoAI) expression is inversely related to the incidence of atherosclerosis. ApoAI expression is also influenced by the nutritional state and diabetes. We used both cell culture and animal models to examine the effect of fasting and ketoacidosis on apoAI gene expression. Two days of food deprivation in rats increased hepatic and intestinal apoAI mRNA by 2.6- and 2.3-fold, respectively ($P < .05$). The absolute concentration of plasma apoAI did not change. However, the plasma apoAI concentration relative to the plasma concentration of serum proteins was increased 23% ($P < .05$). In fasting rats, there was a significant positive correlation between the serum β-hydroxybutyrate concentration and hepatic or intestinal apoAI mRNA level. Despite this correlation, changes in apoAI mRNA are probably not mediated by ketone bodies, since neither hepatic nor intestinal apoAI mRNA levels were altered in rats maintained on a ketogenic diet for 10 days or treated with isobutyramide, an orally active ketone analog. In addition, the activity of the rat apoAI promoter was not altered in Hep G2 cells treated with isobutyramide or fatty acids or exposed to hypoglycemic conditions, while dexamethasone increased promoter activity 1.9-fold ($P < .05$). These data indicate that metabolic changes other than ketone bodies, such as an increase in plasma glucocorticoids, may account for starvation-induced expression of apoAI.

APOLIPOPROTEIN AI (apoAI), a major component of the high-density lipoprotein (HDL) particle, is necessary for the efficient transport and clearance of cholesterol from peripheral tissues to the liver for metabolism and secretion through a process called reverse cholesterol transport.[1,2] Epidemiologic studies suggest that low levels of this protein are associated with an increased risk of heart disease and atherosclerosis.[3] Due to the strength of this correlation, enhancing the expression of the apoAI gene in vivo may reduce this risk. This goal has proven elusive, presumably because the apoAI gene responds to many hormonal and dietary signals through a combination of negative- and positive-acting transcription factors.[4-10]

Our laboratory is investigating the biochemical basis for modulation of the apoAI gene in response to hormonal and dietary stimuli.[9-15] Short-term fasting increases hepatic apoAI mRNA levels,[16,17] and dietary restriction has been shown to elevate serum HDL levels.[18] In the following experiments, we determined whether fasting-induced ketoacidosis influences the expression of the apoAI gene. Short-chain fatty acids such as sodium butyrate have been shown to have profound effects on the expression of several genes, through inducing alterations in chromatin structure[19] or modulating the activity of phosphatases.[20] To determine whether some of the molecular pathways altered by starvation affect apoAI gene expression, we examined the effect of ketosis and ketone bodies on hepatic and intestinal apoAI expression during starvation, feeding of a ketogenic diet, or treatment with the sodium butyrate analog isobutyramide.

From the Division of Endocrinology, Diabetes, and Metabolism, Department of Internal Medicine, Saint Louis University Medical School, St. Louis, MO; and the Endocrine Research Group, Department of Medicine and Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada.

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Address reprint requests to Arshag D. Mooradian, MD, Division of Endocrinology, Saint Louis University Medical School, 1402 S Grand Blvd, St. Louis, MO 63104.

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MATERIALS AND METHODS

Animal Feeding and Dietary Manipulation

Four groups of 10 male 4-month-old Fischer 344 rats were used in these studies. One group was subjected to fasting for 48 hours but with access to water ad libitum. The second group of 10 rats were placed on a high-fat diet for 10 days. The high-fat diet (diet 96355; Harlan Teklad, Madison, WI) provided 90.7%, 9.0%, and 0.3% of the daily calories as unsaturated fat, protein, and carbohydrate, respectively. This diet induces a moderate state of ketoacidosis in adult rats in 1 week.[21]

To determine if ketone bodies influence apoAI gene expression, a third group of 10 rats received isobutyramide (500 mg/kg) by gavage and were killed 24 hours later. This orally bioavailable analog of sodium butyrate has a long plasma half-life and enhances the expression of butyrate-inducible genes in vivo.[22] The gavage was performed under light ether anesthesia to minimize stress. Using these experimental conditions, gavaging rats with saline does not alter hepatic apoAI mRNA levels. A fourth group of 10 rats were killed to provide a basis for comparison. The control rats were kept on standard laboratory rat chow (Harlan Teklad) and had access to food and water ad libitum. The mean daily intake in this group was 17.8 ± 1.8 g/rat/d. All animals were killed by exsanguination under pentobarbital anesthesia, and the liver and small intestine were excised and snap-frozen in liquid nitrogen. Blood samples were obtained for analysis of serum apoAI and ketone (β-hydroxybutyrate) levels.

RNA Isolation and Northern Analysis Blot

Total RNA was isolated using the single-step acid guanidinium extraction procedure.[23] Northern blot analysis was performed as previously described[24] after fractionating the RNA samples on a 1% agarose gel containing 2.2 mol/L formaldehyde. The RNA was then transferred to a nylon membrane (Pharmacia Amersham Biotechnology, Piscataway, NJ) and hybridized to a ^{32}P-labeled cDNA probe specific for the human apoAI gene. After a high-stringency wash (0.1× standard saline citrate and 0.1% sodium dodecyl sulfate [SDS] for 30 minutes at 65°C) and exposure to film, the membrane was stripped and rehybridized a second time to a cDNA probe specific for the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) message. The hybridization signal was quantified with a scanning laser densitometer (Molecular Dynamics, Sunnyvale, CA) and the apoAI mRNA content was normalized to that for the G3PDH message. The G3PDH mRNA is constitutively expressed, and normalization of apoAI mRNA with G3PDH mRNA content yielded more consistent results compared with normalization against β-actin mRNA. Unlike G3PDH mRNA, β-actin mRNA levels were elevated significantly in the treated groups relative to the control.

ApoAI Western Blot Analysis

Serum and tissue apoAI protein content was determined by Western blot analysis as previously described[25] using an antiserum prepared and characterized in this laboratory.[11] Protein concentrations were determined using the Bradford assay,[26] with bovine serum albumin as the standard. Five micrograms of serum protein or 25 μg tissue protein were used in the analysis.

Serum Ketone Body Determination

Serum ketone body levels were determined using a commercially available kit (Sigma Chemical, St Louis, MO). β-Hydroxybutyrate dehydrogenase activity was determined by measuring the change in absorbance at 340 nm due to the conversion of NAD to NADH.

Cell Culture, Transfection, and Determination of Reporter Gene Activity

Hep G2 cells (hepatoma-derived) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 100 mg/dL glucose, 5% fetal bovine serum (FBS), and penicillin and streptomycin (100 U/mL and 100 μg/mL, respectively). Caco-2 intestinal cells were maintained in Earl's modified essential medium supplemented with 15% FBS, nonessential amino acids, 0.11 mg/mL sodium pyruvate, and penicillin and streptomycin. Both cell lines were maintained in a humidified incubator at 37°C and 5% CO_2/95% air.

Cells grown in 6-well plates and 80% confluent were transfected using Lipofectamine (Life Technologies, Gaithersburg, MD) with 1 μg of the plasmid pA1.474.CAT[27] and 1 μg pCMV.SPORT-β-Gal (Life Technologies). The plasmid pA1.474.CAT contains 467 base pairs (bp) of the promoter for the rat apoAI gene (−474 to −7) 5′-proximal to the transcriptional start site (+1). This promoter fragment includes most of the known *cis*-elements responsible for transcriptional control of the apoAI gene. The plasmid pCMV.SPORT-β-Gal, which contains the β-galactosidase gene driven by the cytomegalovirus (CMV) promoter, was used as an internal control to normalize for transfection efficiency. After 24 hours, the cells were either left alone or treated with the various effectors. When indicated, isobutyramide dissolved in dH_2O was added to a final concentration of 3 mmol/L. Stearic acid and oleic acid dissolved in ethanol were added to a final concentration of 3 mmol/L. Dexamethasone dissolved in dimethyl sulfoxide (DMSO) was added to 1 μmol/L. Control cultures received an equal amount of ethanol or DMSO depending on the solvent the treatment group received. To assess the effect of glucose on apoAI promoter activity, the cells were switched to glucose-deficient DMEM supplemented with 5% FBS and penicillin and streptomycin at the time of transfection. After 24 hours, the cells were either left alone or supplemented with 50, 75, or 100 mg/dL (final concentration) glucose. After another 24 hours, the cells were harvested and chloramphenicol acetyltransferase (CAT) activity was determined as previously described.[28] A portion of the extract was also assayed for β-galactosidase activity as described previously.[29]

Ethanol and DMSO are known to affect either apoAI gene expression or promoter activity.[30,31] Therefore, the effect of various chemicals dissolved in these solvents was always evaluated in comparison to vehicle-treated controls.

Statistical Analysis

Statistical significance for the Northern and Western blot analyses, serum ketone determinations, and expression of reporter gene activity were assessed using Student's *t* test. The mean ± SD is presented. Correlations between apoAI gene expression and serum β-hydroxybutyrate levels were generated by linear regression analysis. The results were considered significant at a *P* level less than .05.

RESULTS

ApoAI mRNA Expression in the Liver and Intestine

To determine if fasting induces apoAI gene expression, Northern blot analysis was performed with total RNA isolated from both the liver and small intestine of control rats and rats that fasted for 48 hours. Representative Northern blots of hepatic and intestinal apoAI mRNA are shown in Fig 1A and B; the expected single 0.9-kb band is evident.

The mean value for apoAI mRNA in the various treatment groups is summarized in Fig 2 after normalization the relative apoAI mRNA level for each sample to its G3PDH content. Fasting for 48 hours increased the expression of the apoAI gene 2.6-fold in the liver (Fig 2A; $P < .05$) and 2.3-fold in the small intestine (Fig 2B; $P < .05$). ApoAI mRNA levels in both the liver and small intestine were unaffected in animals placed on a high-fat, ketogenic diet for 10 days and in those fed the long-acting butyrate analog, isobutyramide (Fig 2A and B).

Effect of Fasting and Ketoacidosis on Serum and Tissue ApoAI Protein Levels

The effect of fasting and ketoacidosis on serum apoAI protein levels was assessed by Western blot. The results indicate that apoAI protein levels did not change with fasting (Fig 3A, upper panel). Serum apoAI levels also were unaltered in the animals maintained on the high-fat diet for 10 days (Fig 3A, lower panel), as well as those treated with isobutyramide (data not shown).

Relative to control ad libitum–fed rats, fasting and the high-fat diet reduced total serum protein levels 20% ($P < .003$) and 13% ($P < .02$), respectively. When apoAI levels were normalized to the total serum protein concentration, a 23% increase ($P < .05$) in apoAI protein relative to total serum protein was evident in the animals fasted for 48 hours (Fig 3B).

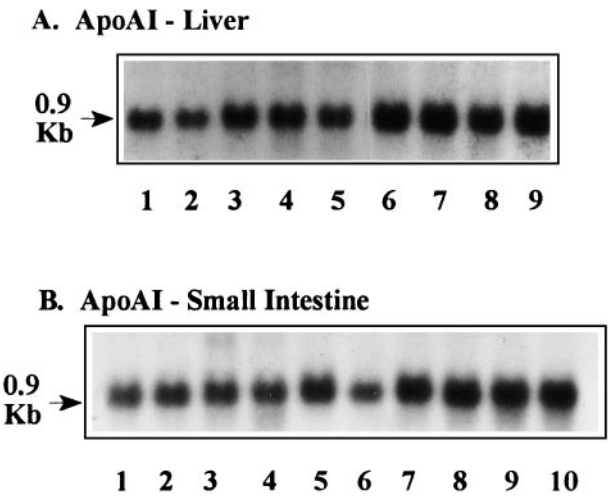

Fig 1. Representative Northern blots of RNA isolated from (A) liver and (B) small intestine. Control rats, lanes 1-5; rats fasted for 48 hours, lanes 6-9 for liver and 6-10 for intestine. The blots were hybridized with apoAI cDNA. The expected 0.9-kb band of apoAI mRNA is shown. The increased apoAI mRNA of (A) hepatic and (B) intestinal tissue in starved rats is evident. One lane between lanes 5 and 6 in A was deleted from the figure because of degradation of the RNA sample prior to analysis.

A 25% increase ($P < .05$) in apoAI protein levels was observed in animals fed a high-fat diet (Fig 3B). Although possible, it is unlikely that the changes in the total serum protein concentration could be the sole result of the reduced serum albumin concentration. The albumin content of the samples, estimated from the Coomassie blue–stained SDS-polyacrylamide gel, was not significantly altered. However, this staining method is not very precise.

ApoAI protein content in the liver and small intestine in all of the groups was not different from that observed in the control group. In each case, apoAI protein levels in the treated group were analyzed in parallel with samples from the control group so that direct comparisons could be made. Hepatic apoAI protein content in animals treated with isobutyramide was 256 ± 19.5 arbitrary units, while hepatic apoAI protein content in the control group was 221 ± 20.6 arbitrary units ($P < .70$). In the small intestine, apoAI protein content was 192 ± 8.1 arbitrary units for the treated group versus 171 ± 10.4 in the control animals ($P < .19$). In the fasted animals, hepatic apoAI protein content was 261 ± 11.5 arbitrary units, while in the controls, the value was 237 ± 22.4 arbitrary units ($P < .39$). ApoAI protein content in the small intestine of the fasted animals was 251 ± 11.1 arbitrary units versus the control value of 273 ± 13.2 arbitrary units ($P < .26$). Hepatic apoAI protein content in the animals maintained on the high-fat diet was 159 ± 9.2 arbitrary units versus 179 ± 7.8 arbitrary units in the control animals ($P < .70$). ApoAI protein content in the small intestine of the animals maintained on the high-fat diet was 210 ± 9.7 arbitrary units versus 229 ± 11.7 arbitrary units in the control animals ($P < .28$). In all cases, the differences between the experimental groups and the controls were not statistically significant ($P > .05$).

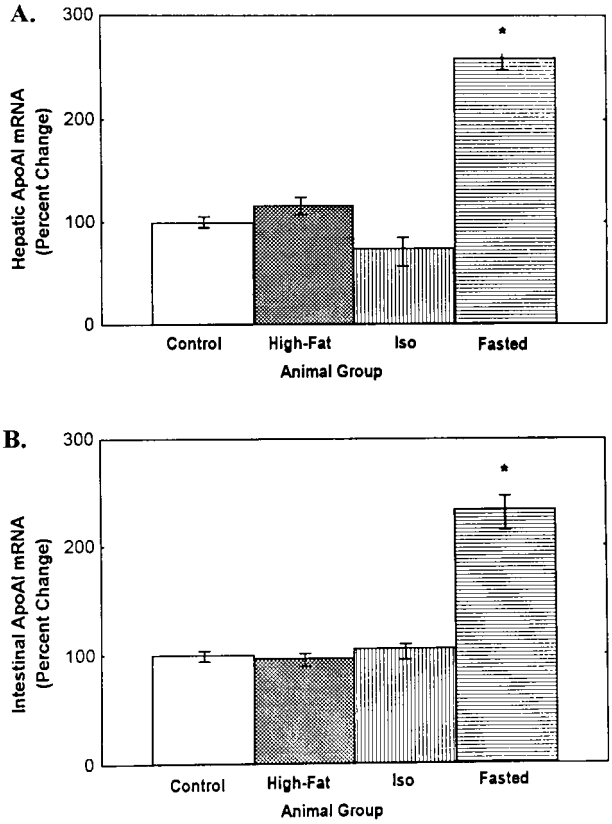

Fig 2. Mean ± SD (A) hepatic and (B) intestinal apoAI mRNA relative to G3PDH mRNA in control rats and in rats fasted for 48 hours, or fed a high-fat ketogenic diet for 10 days, or given isobutyramide (Iso) 500 mg/kg orally 24 hours prior to killing. *$P < .05$ *v* control rats.

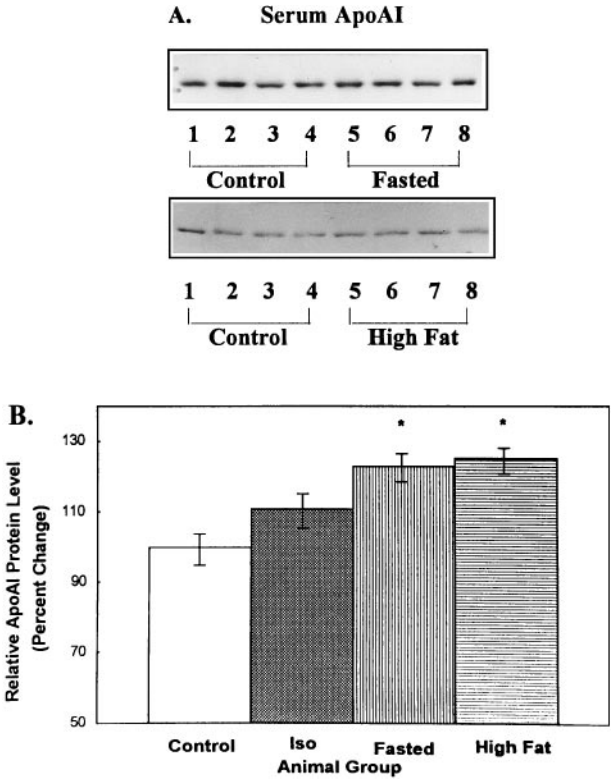

Fig 3. Serum apoAI protein levels determined by Western blotting. (A) Top, serum apoAI protein from controls (lanes 1-4) and fasted animals (lanes 5-8); bottom, serum apoAI protein from controls (lanes 1-4) and animals on the high-fat diet for 10 days (lanes 5-8). (B) Relative apoAI protein level determined by normalizing the serum apoAI protein concentration to the total serum protein concentration for each treatment group. Iso, isobutyramide-treated group. *$P < .05$ *v* control rats.

Correlations Between Serum β-Hydroxybutyrate Concentration and ApoAI mRNA Expression

To determine if starvation-induced ketosis may be responsible for the increase in apoAI mRNA levels, serum β-hydroxybutyrate levels were assessed and compared with apoAI mRNA levels in the liver and small intestine. The high-fat diet and the 48-hour fast increased β-hydroxybutyrate levels 2.7- and 3.5-fold, respectively ($P < .001$), when normalized to the control. Control animals had a mean serum β-hydroxybutyrate level of 5.0 ± 0.8 mg/dL, while animals maintained on a high-fat diet for 10 days and animals fasted for 48 hours, had mean β-hydroxybutyrate levels of 13.3 ± 3.6 and 17.5 ± 2.9 mg/dL, respectively.

When the serum β-hydroxybutyrate concentration in the fasted animals was plotted against their apoAI mRNA content, a linear relationship with a positive slope was evident in both the

liver (Fig 4A; $P < .003$) and the small intestine (Fig 4B; $P < .006$). The correlation coefficient (r) of this relationship was .46 in the liver and .42 in the small intestine. However, given the variability of the data, it is not possible to conclude with absolute certainty that the relationship is linear, even though there is a reasonably linear correlation coefficient.

ApoAI Gene Promoter Activity and Fasting

To determine whether the increased hepatic and intestinal apoAI mRNA content during starvation is secondary to increased apoAI gene promoter activity due to known hormonal or metabolic changes of the fasted animal, the effects of various factors altered with fasting were analyzed in cells transfected with the apoAI promoter-CAT constructs.

Effect of ketone bodies on apoAI promoter activity. To strengthen our impression that ketones do not have a significant role in regulating the expression of the apoAI gene, we examined the effect of isobutyramide on the apoAI gene promoter in both the Hep G2 liver cell line and Caco-2 intestinal cell line. In control Hep G2 cells transfected with the apoAI promoter construct, 23.0 ± 6.3% (mean ± SD) of the chloramphenicol was acetylated, while in cells treated with isobutyramide, 19.0 ± 0.4% of the chloramphenicol was acetylated (Fig 5). These differences were not statistically significant. There

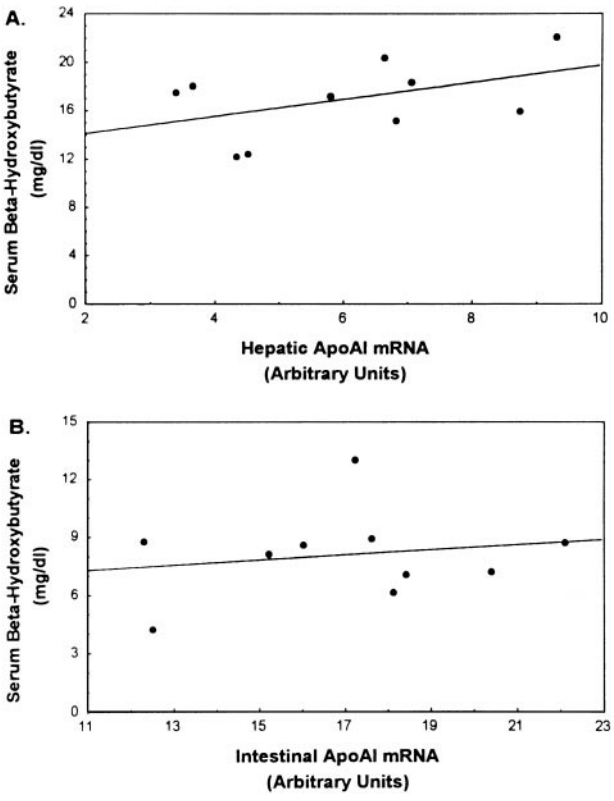

Fig 4. Correlations between (A) hepatic and (B) intestinal apoAI mRNA content and serum β-hydroxybutyrate concentration in fasted animals. Correlations between serum β-hydroxybutyrate levels and apoAI mRNA expression in fasted animals were analyzed and statistical significance was assessed by linear regression analysis. The correlation coefficient for the liver was .46 ($P < .003$) and for the small intestine, .42 ($P < .006$).

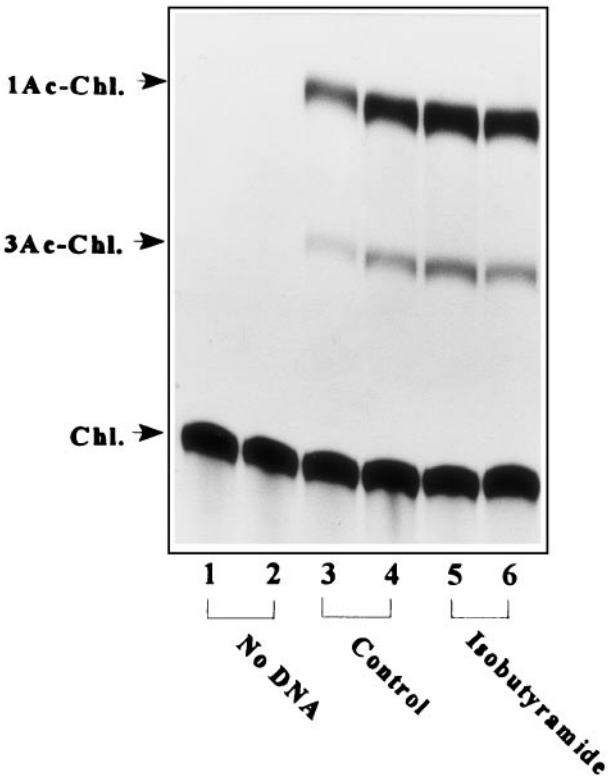

Fig 5. Effect of isobutyramide on the activity of the apoAI gene promoter. Hep G2 cells were transfected with the plasmids pA1.474.CAT and pCMV.SPORT-β-Gal, and after 24 hours, they were treated with isobutyramide (3 mmol/L). After 24 hours, the cells were harvested and assayed for CAT and β-galactosidase activity. The positions of the acetylated-chloramphenicol derivatives 1-acetyl-chloramphenicol (1Ac-Chl.) and 3Ac-Chl., as well as the unacetylated chloramphenicol substrate (Chl.), are indicated. CAT activity, normalized to β-galactosidase activity in control Hep G2 cells transfected with the apoAI construct, was 23.0% ± 6.3% (mean ± SD), while in cells treated with isobutyramide, 19.0% ± 0.4% of the chloramphenicol was acetylated. The difference was not statistically significant.

was also no significant difference in apoAI reporter gene activity in Caco-2 cells treated with isobutyramide, nor when both Hep G2 and Caco-2 cells were treated with sodium butyrate (data not shown).

Effect of hypoglycemia on the apoAI gene promoter. Since ketones have no demonstrable effect on apoAI gene expression in vitro or in vivo, we examined the role of other metabolites that are altered during fasting and which may modulate apoAI promoter activity. To simulate the moderate reduction in the mean plasma glucose concentration present in fasting animals, cells were cultured under varying degrees of hypoglycemia. Reducing the glucose concentration in the culture media from 100 mg/dL to 75 and 50 mg/dL did not elevate apoAI promoter activity. ApoAI promoter activity in the presence of 100 mg/dL glucose was 27.5% ± 0.8%, compared with 26.4% ± 1.8% and 26.8% ± 1.6% in the presence of 75 and 50 mg/dL glucose, respectively.

Effect of fatty acids on apoAI gene promoter activity. To determine if fatty acids, which are elevated during fasting, affect apoAI promoter activity, cells were treated with either

stearic acid (3 mmol/L) or oleic acid (3 mmol/L). Since ethanol induces apoAI gene expression,[30] the cells treated with various fatty acids were compared with cells treated with the ethanol vehicle. CAT activity was unaffected by either the saturated fatty acid (stearic acid) or the unsaturated fatty acid (oleic acid). CAT activity in the ethanol-treated control cells was 15% ± 2.3%, while cells treated with stearic acid and oleic acid acetylated 14.5% ± 2.5% and 10.5% ± 1.0% of the chloramphenicol substrate. The differences did not achieve statistical significance.

Effect of dexamethasone on apoAI promoter activity. Since starvation is associated with increased plasma glucocorticoid levels, the ability of the synthetic glucocorticoid dexamethasone (Dex) to regulate apoAI promoter activity was assessed in Hep G2 cells. Dex (1 μmol/L) increased apoAI promoter activity by 1.9-fold in Hep G2 cells (DMSO-treated, 35.3% ± 11.6% chloramphenicol acetylation *v* 66.5% ± 6.0% in Dex-treated cells, $P < .05$; Fig 6). All promoter activity experiments were replicated four times in either duplicate or triplicate measurements. It is noteworthy that CAT activity in the presence of the ethanol or DMSO vehicle alone varies from one experiment to the other by as much as 2-fold. This variability depends on the culture conditions and on the variability in the reagents used in the assay. Although interassay variability is large in such experiments, intraassay variability is small when normalized to the internal control, β-galactosidase activity.

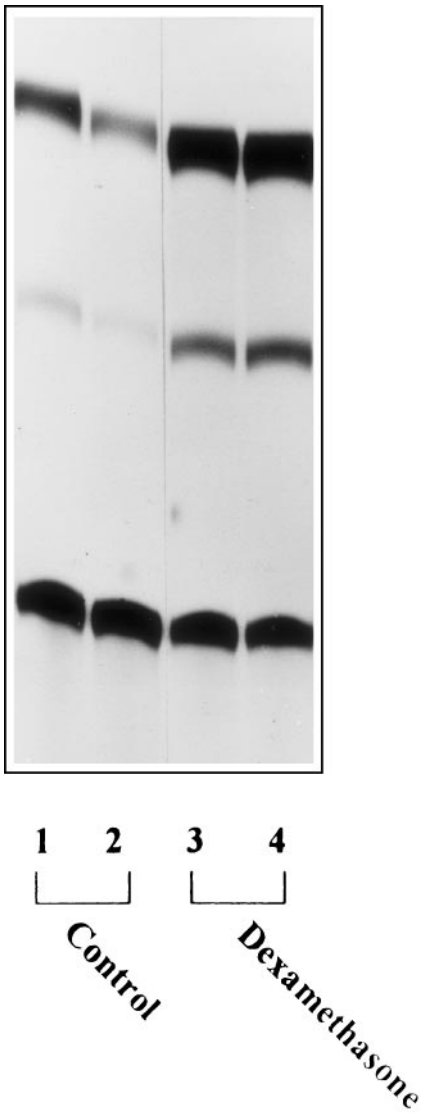

Fig 6. Effect of dexamethasone on the activity of the apoAI gene promoter. Hep G2 cells were transfected with the plasmids pA1.474.CAT and pCMV.SPORT-β-gal, and after 24 hours, they were treated with dexamethasone (1 μmol/L). After 24 hours, the cells were harvested and assayed for CAT and β-galactosidase activity. The differences in CAT activity normalized to β-galactosidase activity were statistically significant; dexamethasone increased CAT activity 1.9-fold ($P < .05$).

DISCUSSION

Expression of the apoAI gene is regulated in vivo by the metabolic and hormonal milieu. Metabolic abnormalities such as those frequently observed in diabetes and fasting result in changes in the availability of carbohydrates and fatty acids that fuel cellular respiration and protein synthesis. These changes also influence the hormonal signals that regulate gene expression, including the expression of transport proteins that are necessary for normal metabolic function.

A myriad of hormonal or metabolic changes occur during starvation and fasting that may have an affect on apoAI gene expression. Insulin expression is repressed while glucagon expression is upregulated in an attempt to normalize glucose levels, which initially plummet and then are maintained due to the catabolism of fatty acids and eventually protein. Fatty acid levels are elevated with fasting, as they are released from triglyceride storage within the hepatocyte and adipocyte.[32] Corticosteroid levels peak in response to fasting and then decrease but remain elevated throughout the fasting period.[32,33] This condition may be important for the upregulation of genes such as phosphoenolpyruvate carboxykinase and serine dehydratase that are essential for gluconeogenesis.[34,35] Thyroid hormone and growth hormone levels decrease in response to fasting.

Fasting is associated with a host of complex metabolic and hormonal changes that have conflicting effects on apoAI expression when assessed individually. Regulation of the apoAI gene by glucose requires an insulin-response core element located in the 5′-flanking region of the gene.[12] We have previously reported that hepatic apoAI mRNA content is increased in fructose-fed rats in which hyperinsulinemia and hyperglycemia occur concurrently.[11] In this experimental model, it appears that the signals generated by hyperinsulinemia outweigh the negative inhibitory signals elicited by hyperglycemia. However, the effect of relative hypoinsulinemia at the reduced plasma glucose concentrations commonly found during fasting is not predictable. As stated earlier, fasting is associated with a host of complex metabolic and hormonal changes, some of which have conflicting effects on apoAI expression when assessed individually. Because of this, several caveats in such studies should be kept in mind. There are significant interspe-

cies differences in the response of the apoAI promoter to various modulators.[7] In addition, caution should be exercised when extrapolating the results in transformed cell lines such as Hep G2 or Caco-2 cells to in vivo physiology. Nevertheless, cell culture studies are invaluable in examining the potential molecular mechanisms related to apoAI gene transcription.

In this study, we observed a significant increase in hepatic and intestinal apoAI mRNA in fasted rats (Figs 1 and 2). The increase in apoAI mRNA levels was directly proportional to the degree of ketosis (Fig 4) as determined by the steady-state serum β-hydroxybutyrate concentration. The serum apoAI protein concentration relative to total serum protein also increased significantly (Fig 3). However, this relative change in apoAI serum protein is out of proportion compared with the increase in apoAI mRNA we observed. It is likely that changes in the clearance kinetics of the apoAI protein may account for this discrepancy.

The precise cause of the increase in apoAI mRNA levels following fasting is not known. The potential role of ketosis was examined using multiple approaches. A direct measurement of the effect of ketosis in vivo was evaluated in rats rendered ketotic by feeding a high-fat diet and in rats treated with isobutyramide, an orally bioavailable analog of sodium butyrate. In addition, the effect of isobutyramide and sodium butyrate on apoAI promoter activity in cultured cells was also studied. These measurements failed to support a causal link between ketone bodies and the expression of apoAI during fasting. However, the possible correlations between the serum β-hydroxybutyrate concentration and the increase in apoAI mRNA suggest that cellular metabolic changes associated with ketosis, but not ketone bodies per se, may precipitate the increase in apoAI mRNA. Alternatively, the correlation between the serum β-hydroxybutyrate concentration and apoAI mRNA may merely reflect the degree to which individual rats respond to the stress of caloric deprivation. Suppression of insulin secretion, a decrease of insulin-like growth factor-I, or reduced thyroid hormone levels are unlikely to account for the increase in apoAI levels, since these hormones are known to upregulate apoAI gene expression.[9,12,36-38] On the other hand, the modest reduction in mean plasma glucose, the increased plasma concentration of free fatty acids, and increased glucocorticoid levels associated with fasting may contribute to the observed changes in apoAI mRNA, since these variables have been previously reported to be positive modulators of apoAI gene expression.[10,12,27,36,39,40] In the present study, reducing the ambient glucose concentration in the culture media or adding free fatty acids did not significantly alter apoAI promoter activity, while dexamethasone, a synthetic glucocorticoid, induced apoAI promoter activity. This finding is in agreement with previously published reports on the effect of glucocorticoids on apoAI gene expression.[10,15,36,39,40] Further studies should help to identify the precise molecular pathways necessary for elevating apoAI mRNA levels during starvation. In addition, since changes in mRNA levels may not reflect changes in the synthesis or turnover of apoAI, future studies should address the kinetics of apoAI production and clearance.

The human apoAI, apoCIII, and apoAIV genes are located within a few kbp of each other.[41,42] The apoAI gene is located 5′ to both the apoCIII and apoAIV genes. While the apoAI and apoAIV genes are transcribed in the same direction, the apoCIII gene, located between these two, is transcribed in the opposite direction.[41,42] This arrangement is conserved in both the mouse[43] and the rat.[44] Although coordinate regulation of the entire gene cluster has not been demonstrated, *cis*-elements within the human apoCIII promoter are capable of controlling expression of the apoAI gene in the small intestine.[45] Furthermore, several polymorphisms, as well as a *cis*-element located in the 5′-flanking region of the apoCIII gene, also effect intestinal expression of the apoAI gene.[46,47] These findings, as well as data showing that the apoCIII gene is negatively regulated by insulin,[48] indicate that the apoAI, apoCIII, and possibly apoAIV genes may be coordinately regulated by some signaling pathways. Further studies analyzing how starvation and ketoacidosis affect the expression of this gene cluster should indicate if coordinate regulation occurs, as well as to what degree and end.

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