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Nicotinic acid induces apolipoprotein A-I gene expression in HepG2 and Caco-2 cell lines

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ABSTRACT

The objective was to test the effect of nicotinic acid on apolipoprotein A-I (apo A-I) gene expression in hepatic (HepG2) and intestinal (Caco-2) cell lines. HepG2 and Caco-2 cells were treated with 0.1, 0.3, 1.0, 3.0, and 10 mmol/L of nicotinic acid; and apo A-I concentrations in conditioned media were measured with Western blots. Relative apo A-I messenger RNA (mRNA) levels, normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA, were measured with quantitative real-time polymerase chain reaction method. The nicotinic acid response element in the apo A-I promoter was identified using a series of apo A-I reporter plasmids containing deletion constructs of the promoter. In other experiments, HepG2 cells were also transfected with the apo A-I reporter plasmid and the hepatocyte nuclear factors 3α and β expression plasmids. The apo A-I levels in conditioned media from HepG2 cells, apo A-I mRNA levels, and apo A-I promoter activity increased significantly following treatment with 1.0, 3.0, and 10 mmol/L nicotinic acid. Nicotinic acid–induced promoter activity required a region of the apo A-I gene located between –170 and –186 base pairs. Exogenous overexpression of the hepatocyte nuclear factors 3α and β had no additive effect on apo A-I promoter. Apolipoprotein A-I concentrations in conditioned media and the apo A-I promoter activity were also significantly increased in Caco-2 intestinal cells. Nicotinic acid may increase apo A-I protein synthesis in the liver and small intestine. Induction of apo A-I gene by nicotinic acid requires a nicotinic acid responsive element in the apo A-I promoter.

1. Introduction

The high-density lipoprotein (HDL) has several atheroprotective properties notably through its participation in the process of reverse cholesterol transport [1]. Apolipoprotein A-I (apo A-I), the primary protein component of HDL, is a ligand for adenosine triphosphate–binding cassette protein A1 and G1, which are expressed in hepatocytes and macrophage cells, and mediate efflux of cholesterol in a concentration gradient-independent manner.

At the present time, there is paucity of therapeutic agents that can raise plasma HDL and apo A-I levels [2]. Niacin is currently the most effective agent available for clinical use. The precise mechanisms by which nicotinic acid increases

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HDL levels are not completely understood. In older clinical studies, administration of 3 g/d of nicotinic acid raised plasma HDL cholesterol by 23% ($P < .05$) and the plasma concentration of apo A-I by 7% ($P < .05$). The rise was attributed to a decrease in its fractional catabolic rate [3]. These observations are consistent with data in human hepatoma cell line HepG2 where nicotinic acid was found to inhibit the removal of HDL and apo A-I by inhibiting the hepatocyte surface expression of β-chain adenosine triphosphate synthase, an HDL–apo A-I holoparticle receptor [4,5]. In HepG2 cells, nicotinic acid did not appear to increase de novo synthesis of apo A-I as measured with radiolabeled amino acid incorporation studies [4] and did not increase apo A-I messenger RNA (mRNA) levels [4,6].

In a more recent clinical study, 2 g/d of extended-release nicotinic acid significantly increased HDL cholesterol and apo A-I concentrations; and these changes were associated with a significant increase in apo A-I production rate without a change in its fractional catabolic rate [7]. The latter study suggests that nicotinic acid may be increasing the expression of apo A-I gene. To test this hypothesis, we examined the effects of various concentrations of nicotinic acid on apo A-I gene expression in HepG2 and Caco-2 cells, 2 cell lines representative of the tissues that normally secrete apo A-I.

2. Materials and methods

2.1. Materials

RapidHyb, Redi-Prime labeling kits, Hybond nitrocellulose, and nylon membrane for Western and Northern blot analysis, respectively, were from Amersham Pharmacia Biotech (Arlington Heights, IL). Nicotinic acid and acetyl-coenzyme A were purchased from Sigma Chemical Company (St Louis, MO), and Lipofectamine was obtained from Life Technologies (Gaithersburg, MD). The radionuclide ^{14}C-chloramphenicol was from New England Nuclear (Boston, MA). Tissue culture media and fetal calf serum were purchased from BioWhittaker (Walkersville, MD). SYBR-Green reagents were purchased from Bio-Rad (Hercules, CA). All other reagent-grade materials were from Sigma Chemical or Fisher Scientific (Pittsburgh, PA).

2.2. Cell culture

HepG2 hepatoblastoma cells were maintained in Dulbecco modified essential medium containing 5% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). Caco-2 cells were grown in Eagle minimal essential medium containing nonessential amino acids, sodium pyruvate, glutamine, 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were maintained in a humidified environment at 37°C and 5% CO_2. Cell viability was monitored by trypan blue exclusion and was greater than 95% in all experiments.

2.3. Western blot analysis

To measure apo A-I secretion, the cells were washed with serum free media (Dulbecco modified essential medium or Eagle minimal essential medium for HepG2 cells and Caco-2 cells, respectively) and maintained in these media for additional 24 hours. At the end of the incubation, conditioned medium was collected; and protein concentration was determined by the method of Lowry et al [8]. Five micrograms of protein from each sample was fractionated by electrophoresis on a 10% sodium dodecyl sulfate polyacrylamide gel as previously described [9] and transferred to nitrocellulose [10]. Nonspecific binding was blocked with 5% ovalbumin in phosphate-buffered saline (PBS) for 2 hours and incubated in 1% ovalbumin in PBS with the anti-human apo A-I primary antibody (1:750) (Calbiochem, San Diego, CA) overnight at 4°C. The blot was then incubated with a goat-anti-mouse IgG-horseradish peroxidase secondary antibody (1:5,000 in 1% ovalbumin in PBS) (Sigma) for 2 hours at room temperature. The membrane was then immersed in ECL (Amersham Pharmacia Biotech) and exposed to film. The amount of signal was quantified with a scanning laser densitometer (Molecular Dynamics, Sunnyvale, CA). To measure the albumin levels in conditioned media, the immunoblotting was repeated with anti-human albumin primary antibody (1:2500) (Accurate Chemical & Scientific, Westbury, NY).

2.4. mRNA measurement by quantitative real-time polymerase chain reaction

HepG2 cells were treated with either solvent or 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid for 24 hours; and total RNA was isolated as described previously [11]. Five micrograms of RNA was reverse-transcribed using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) and random primers. Each polymerase chain reaction contained 250 ng of complementary DNA and primers for apo A-I (forward, 5′-*AGC TTG CTG AAG GTG GAG GT*-3′; reverse, 5′-*ATC GAG TGA AGG ACC TGG C* -3′) [12] or glyceraldehyde-3-phosphate dehydrogenase (G_3PDH) (forward, 5′-*CTC TCT GCT CCT CCT GTT* CGA-3′; reverse, 5′-*TGA GCG ATG TGG CTC GGC T* -3′) [13]. Primer sequences were obtained from a database [14] and validated in the laboratory. Amplification was performed with the SYBR-Green reagents. For quantitative real-time polymerase chain reaction (qRT-PCR), the apo A-I and G_3PDH primer sets were amplified for 40 cycles at 95°C, 52°C, and 72°C, each for 1 minute. After amplification, a melt curve was performed to certify that only one product was amplified; and relative changes in apo A-I mRNA levels were calculated using the $\Delta(\Delta C_t)$ method and normalizing to G_3PDH mRNA levels. The qRT-PCR machine used was the MyiQ Real-Time PCR Detection System (Hercules, CA).

To measure the kinetics of apo A-I mRNA induction after nicotinic acid treatment, HepG2 cells were treated with 3 mmol/L nicotinic acid for 0, 1, 3, 6, 12, 24, 48, and 72-hours; and apo A-I and G_3PDH mRNA levels were measured in reverse-transcribed samples as described above.

2.5. Plasmids and transient transfection

The nicotinic acid response element in the apo A-I promoter was identified with a series of apo A-I deletion constructs.

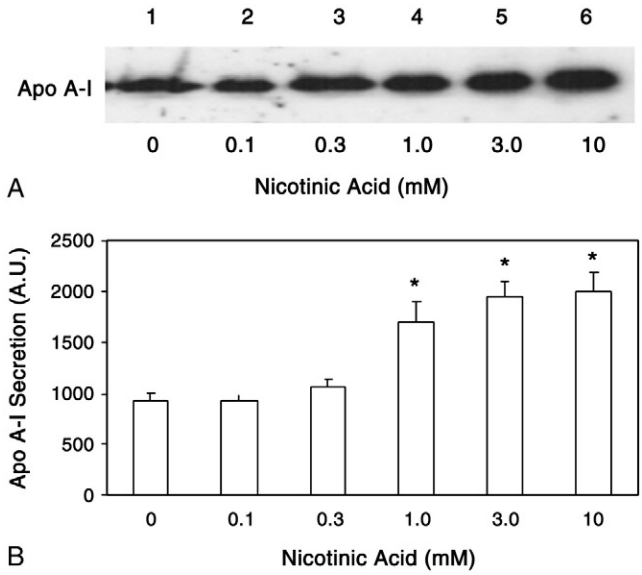

Fig. 1 – The effect of nicotinic acid on apo A-I expression in HepG2 cells. HepG2 cells were treated with 0, 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid for 24 hours; and apo A-I in the media was measured by Western blot (A). Band densities were measured by densitometry (arbitrary units) and are shown in panel B. Nicotinic acid concentrations greater than 1 mmol/L increased apo A-I levels significantly. *P < .01, treated vs control cells; n = 6.

The plasmids pAI.474.CAT, pAI.425.CAT, pAI.375.CAT, pAI.325.CAT, pAI.186.CAT, pAI.170.CAT, pAI.144.CAT, and pAI.46.CAT contain DNA from the apo A-I gene promoter from –474, –425, –375, –325, –186, –170, –144, and –46 to +7 base pairs (bp) (relative to the transcriptional start site), respectively, fused to the chloramphenicol acetyltransferase (CAT) gene [15,16]. HepG2 cells were transfected with 1 μg of apo A-I reporter plasmid and 1 μg of the plasmid pCMV.SPORT-β-gal using Lipofectamine. The plasmid pCMV.SPORT-β-gal (Life Technologies) expresses β-galactosidase under the control of the cytomegalovirus immediate-early promoter and is used to normalize transfection efficiency. In additional experiments, HepG2 cells were transfected with the apo A-I reporter plasmid pAI.474.CAT and the hepatocyte nuclear factors 3α and β (HNF3α and HNF3β, respectively) expression plasmids pCMV.HNF3α and pCMV.HNF3β. The cells also received the plasmid pCMV.SPORT.β-gal to determine transfection efficiency. After 24 hours, the cells were treated with 1 mmol/L nicotinic acid for 24 hours, at which time the cells were collected and assayed for CAT [17] and β-galactosidase activity [18].

2.6. *Statistics and data analysis*

All results are expressed as mean ± SEM. Analysis of variance followed by the Newman-Keuls procedure for subgroup analysis was carried out using the statistical package Statistica for Windows (Statsoft, Tulsa, OK). Significance was defined as a 2-tailed $P < .05$.

3. Results

3.1. *The effect of nicotinic acid on apo A-I protein in HepG2 cells*

The apo A-I levels (in arbitrary unit; AU) increased from 925 ± 79.6 AU in control cells to 916 ± 67, 1066 ± 74, 1698 ± 199, 1954 ± 149, and 2006 ± 188 AU in cells treated with 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid, respectively (not significant [NS], NS, $P < .003$, $P < .0005$, and $P < .0008$, respectively) (Fig. 1). Albumin levels did not change with nicotinic acid treatment (data not shown), suggesting that nicotinic acid specifically induces apo A-I protein secretion.

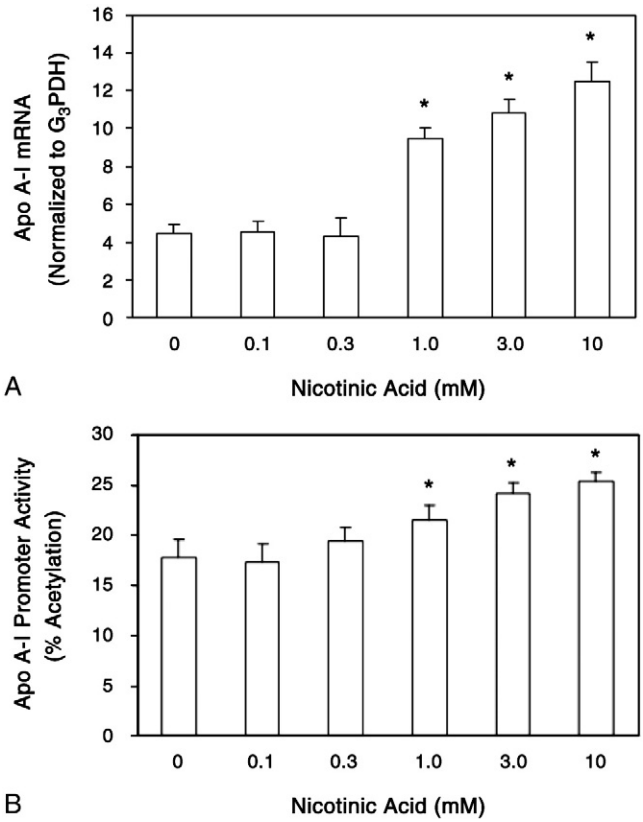

Fig. 2 – The effect of nicotinic acid on apo A-I mRNA expression and gene promoter activity. A, HepG2 cells were treated with the indicated concentrations of nicotinic acid for 24 hours, and apo A-I and G_3PDH mRNA levels were measured by qRT-PCR. Nicotinic acid concentrations greater than 1 mmol/L increased apo A-I mRNA levels significantly. *P < .01, treated vs control cells; n = 6. B. HepG2 cells were transfected with the apo A-I reporter plasmid pAI.474.CAT and the plasmid pCMV.SPORT.ß-gal (to determine transfection efficiency) and treated with the indicated concentration of nicotinic acid for 24 hours. Nicotinic acid concentrations greater than 1 mmol/L increased apo A-I promoter activity significantly. *P < .05, treated vs control cells; n = 6.

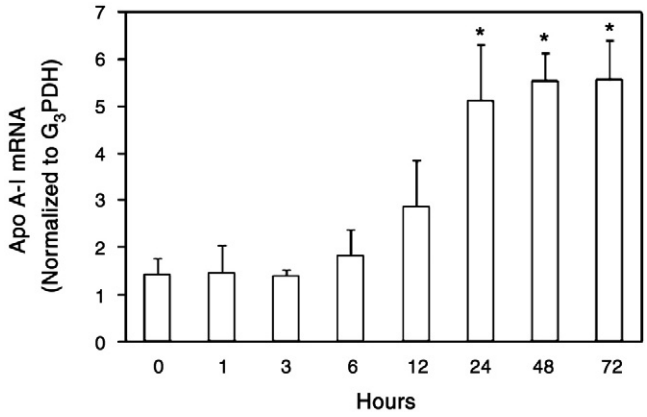

Fig. 3 – Kinetics of apo A-I mRNA induction in nicotinic acid–treated HepG2 cells. HepG2 cells were treated with 3 mmol/L nicotinic acid for 0, 1, 3, 6, 12, 24, 48, and 72 hours; and apo A-I and G3PDH levels were measured by qRT-PCR. Apolipoprotein A-I mRNA levels increased significantly at 24, 48, and 72 hours relative to untreated cells. *P < .007, at 24, 48, and 72 of hours treatment vs untreated cells; n = 6.

3.2. *The effect of nicotinic acid on apo A-I mRNA expression and apo A-I gene promoter activity in HepG2 cells*

Relative apo A-I mRNA, normalized to G_3PDH mRNA, increased from 4.45 ± 0.52 to 4.56 ± 0.54, 4.29 ± 0.94, 9.43 ± 0.59, 10.84 ± 0.67, and 12.49 ± 1.01 in cells treated with 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid, respectively (NS, NS, $P < .003$, $P < .002$, and $P < .002$, respectively) (Fig. 2A). The G_3PDH mRNA levels did not change significantly with treatment.

In HepG2 cells, nicotinic acid increased apo A-I promoter activity from 17.8% ± 1.1% acetylation in control cells to 17.3% ± 1.1%, 19.5% ± 0.7%, 21.5% ± 0.9%, 24.2% ± 0.6%, and 25.3% ± 0.5% acetylation in cells treated with 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid, respectively (NS, NS, $P < .05$, $P < .006$, and $P < .003$, respectively) (Fig. 2B). The β-galactosidase activity did not change significantly with increasing nicotinic acid concentration (data not shown.) These results suggest that the nicotinic acid–dependent increase in apo A-I mRNA and protein levels in HepG2 cells is due to an increase in promoter activity of apo A-I gene.

3.3. *Kinetics of apo A-I mRNA expression in nicotinic acid–treated cells*

To measure the kinetics of apo A-I mRNA induction following nicotinic acid treatment, HepG2 cells were treated with 3 mmol/L nicotinic acid for 0, 1, 3, 6, 12, 24, 48, and 72-hours (Fig. 3). Relative apo A-I mRNA, normalized to G_3PDH mRNA, increased from 1.42 ± 0.4 at baseline to 1.45 ± 0.6, 1.38 ± 0.1, 1.83 ± 0.5, 2.87 ± 1.0, 5.12 ± 1.2, 5.55 ± 0.6, and 5.57 ± 0.8 in cells treated for 1, 3, 6, 12, 24, 48, and 72-hours, respectively (NS, NS, NS, NS, $P < .0007$, $P < .0005$, and $P < .001$, respectively). Apolipoprotein A-I mRNA levels increased significantly and reached a steady state at 24 hours of treatment with nicotinic acid.

3.4. *Identification of a nicotinic acid response element in the apo A-I gene promoter*

To identify nicotinic acid responsive regions within the apo A-I promoter, HepG2 cells were transfected with a series of apo A-I reporter plasmid containing deletions within the gene promoter and treated with 3.0 mmol/L nicotinic acid for 24 hours. The cells were also transfected with pCMV. SPORT.β-gal to normalize transfection efficiency.

The CAT activity (Fig. 4A, B) and percentage change in apo A-I promoter activity (Fig. 4C) in cells transfected with the each plasmid after treatment with either nicotinic acid or the solvent dimethylsulfoxide (set at 100%) were calculated (Fig. 4).

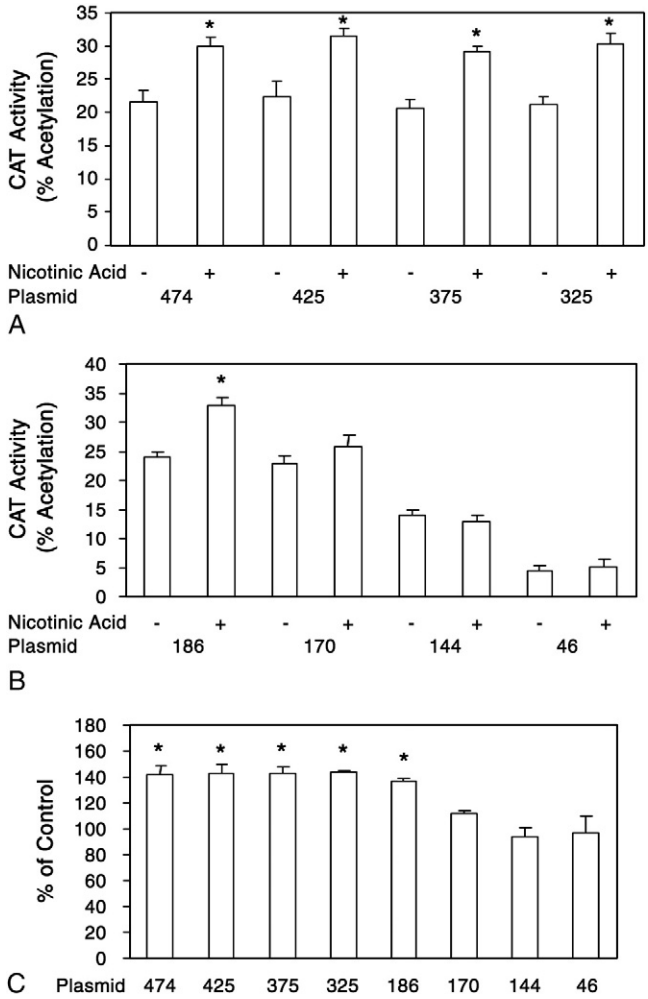

Fig. 4 – Identification of a nicotinic acid response element in the apo A-I gene promoter. HepG2 cells were transfected with plasmids containing successive deletions of the apo A-I gene promoter and treated with 3 mmol/L nicotinic acid for 24 hours. Apolipoprotein A-I promoter activity was measured and normalized for transfection efficiency (A and B), and percentage of control was calculated (C). Nicotinic acid treatment increased apo A-I promoter activity in cells transfected with 474, 425, 374, 325, and 186 bp of apo A-I flanking sequence, but activity was similar to control cells in cells transfected with 170, 144, and 46 bp of 5′-flanking sequence. *P < .05, treated vs control cells; n = 6.

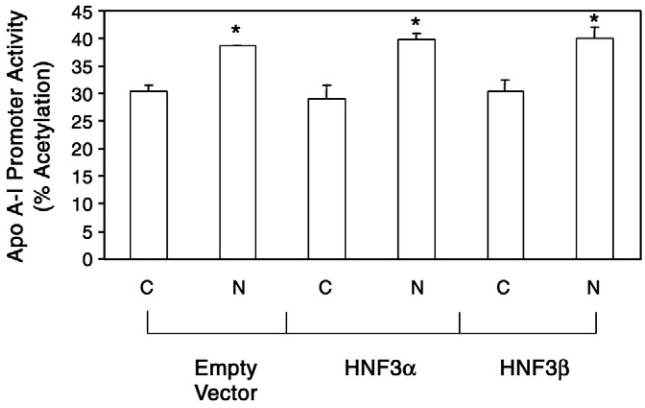

Fig. 5 – The effect of nicotinic acid in cells expressing exogenous HNF3α and HNF3β. HepG2 cells were transfected with the apo A-I reporter plasmid pAI.474.CAT and the HNF3α and HNF3β expression plasmids pCMV.HNF3α and pCMV.HNF3β. The cells also received the plasmid pCMV. SPORT.β-gal to determine transfection efficiency. After 24 hours, the cells were treated with 3 mmol/L nicotinic acid. After 24 hours, the cells were collected and assayed for CAT and β-galactosidase activity. Although nicotinic acid increased apo A-I promoter activity, there was no difference in apo A-I promoter activity in cells receiving empty vector compared with those transfected with the HFN3α and HNF3β expression constructs. *P < .05, treated vs control cells; n = 6.

Nicotinic acid treatment increased apo A-I promoter activity by 42.0% ± 7.2%, 43.3% ± 6.6%, 43.3% ± 4.7%, 43.7% ± 0.9%, and 36.7% ± 2.7% in cells transfected with 474, 425, 374, 325, and 186 bp of apo A-I flanking sequence ($P < .02$, $P < .03$, $P < .006$, $P < .009$, and $P < .005$, respectively); but activity was similar to controls in cells transfected with 170, 144, and 46 bp of 5′-flanking sequence. These results suggest that nicotinic acid–induced promoter activity required a region of the apo A-I gene located between –170 and –186 bp relative to the transcriptional start site at +1.

3.5. *The effect of nicotinic acid in cells expressing exogenous HNF3α and HNF3β*

Because the nicotinic acid responsive region of apo A-I promoter contains a binding site for HNF3, a key transcriptional modulator of apo A-I gene, HepG2 cells were transfected with the apo A-I reporter plasmid pAI.474.CAT and the HNF3α and HNF3β expression plasmids pCMV.HNF3α and pCMV. HNF3β. The cells also received the plasmid pCMV.SPORT.β-gal to determine transfection efficiency. After 24 hours, the cells were treated with 3 mmol/L nicotinic acid. After 24 hours, the cells were collected and assayed for CAT and β-galactosidase activity. Nicotinic acid increased apo A-I promoter activity in empty-vector transfected cells from 30.4% ± 0.7% acetylation to 38.7% ± 0.0% acetylation ($P < .002$). In cells transfected with the HNF3α expression vector, nicotinic acid treatment increased apo A-I promoter activity from 29.1% ± 1.4% acetylation to 39.8% ± 0.7% acetylation ($P < .006$), whereas in cells transfected with the HNF3β expression vector, nicotinic acid increased apo A-I promoter activity from 30.5% ± 1.1% acetylation to 40.0% ± 1.2% acetylation ($P < .005$). Although nicotinic acid increased apo A-I promoter activity, there was no difference in apo A-I promoter activity in cells receiving empty vector compared with those transfected with the HFN3α and HNF3β expression constructs. Thus, increased intracellular HNF3 levels had no additional effect on apo A-I promoter activity when combined with nicotinic acid treatment (Fig. 5).

3.6. *The effect of nicotinic acid on apo A-I expression in Caco-2 cells*

To extend the observation made in hepatocytes to intestinal cells, a second major site for apo A-I synthesis in vivo, Caco-2 cells were treated with various concentrations of nicotinic acid for 24 hours. The apo A-I concentrations increased from 1053 ± 67 AU to 965 ± 88, 1139 ± 63, 1227 ± 60, 1658 ± 50, and 1740 ± 152 AU in the media of cells treated with 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid, respectively (NS, NS, NS, $P < .01$, and $P < .01$; respectively) (Fig. 6A). In Caco-2cells, nicotinic acid increased apo A-I promoter activity from 22.5% ± 1.0% acetylation in control cells to 21.8% ± 1.2%, 21.5% ± 2.8%, 24.6% ± 0.6%, 28.4% ± 1.0%, and 28.0% ± 1.1% acetylation in

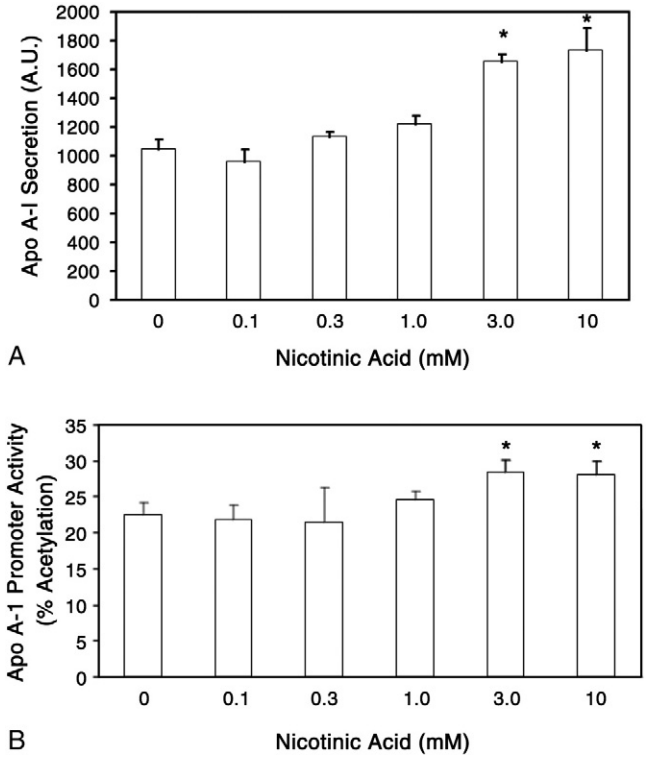

Fig. 6 – The effect of nicotinic acid on apo A-I gene expression in Caco-2 intestinal cells. Caco-2 cells were transfected with the plasmid pAI.474.CAT and treated with the indicated concentrations of nicotinic acid for 24 hours. Apolipoprotein A-I levels in the conditioned medium were measured by immunoblotting (A), and apo A-I promoter activity (B) was measured and normalized for transfection efficiency. Nicotinic acid treatment (3.0 and 10 mmol/L) increased both apo A-I secretion and apo A-I promoter activity. *P < .05, treated vs control cells; n = 6.

cells treated with 0.1, 0.3, 1.0, 3.0, and 10 mmol/L nicotinic acid, respectively (NS, NS, NS, $P < .02$, and $P < .02$, respectively) (Fig. 6B). The β-galactosidase activity did not change significantly with increasing nicotinic acid concentration (data not shown). There were no signs of toxicity with nicotinic acid treatment in this cell line, as assessed by trypan blue exclusion. These results suggest that nicotinic acid increases apo A-I secretion in intestinal cells similar to its effects in hepatocytes.

4. Discussion

The results of the experiments described here indicate that nicotinic acid at a concentration of 1 mmol/L or greater increases apo A-I secretion significantly in both HepG2 cells and Caco-2 cells. This is probably the result of increased production as the apo A-I mRNA concentrations increased, and the apo A-I promoter activity was enhanced by nicotinic acid. The nicotinic acid–induced promoter activity required a region of the promoter located between –170 and –186 bp relative to the transcriptional start site at +1. There are several transcription factors that are active at this site. One such factor known to be a potent modulator of apo A-I gene expression is HNF3 [19]. Preliminary experiments described here show that increased HNF3 expression by transiently transfecting cells with expression plasmids for both the α and β isoforms of HNF3 had no additional effect on apo A-I promoter activity when combined with nicotinic acid treatment. The likely reason for the lack of effect of HNF3 overexpression is that the HNF3α and 3β are abundant in HepG2 cells and it is possible that the promoter of apo A-I is already saturated with endogenous HNF3α and 3β. The transcription factors and signaling mechanisms that are essential for the nicotinic acid effects on apo A-I gene expression remain to be elucidated.

The main concern with the present study is the discrepancy of the results with previously published studies [4,6] regarding the effects of nicotinic acid on apo A-I mRNA levels. The current observations are supported with the additional time course studies of apo A-I mRNA (Fig. 3). Furthermore, the observations are supported by the concomitant rise in apo A-I promoter activity by nicotinic acid in parallel to the rise in apo A-I mRNA levels. At the present time, there is no clear explanation of the discrepancy with previously published results on the effect of nicotinic acid on apo A-I mRNA [4,6] other than the potential differences in the responsiveness of cells from different sources.

The observations in this article are supportive of the most recent studies in humans where 2 g/d of extended-release nicotinic acid significantly increased HDL cholesterol and apo A-I concentrations, and these changes were associated with a significant increase in apo A-I production rate without a change in its fractional catabolic rate [7]. However, it is noteworthy that apo A-I synthesis rate in nicotinic acid–treated HepG2 cells was not increased in one study using the radiolabeled amino acid incorporation method [4]. The latter is a less sensitive tool to detect biologically significant changes in the apo A-I gene expression. It is noteworthy that our observations of nicotinic acid–related increased apo A-I secretion and increased apo A-I promoter activity were also demonstrated in the intestinal cell line Caco-2.

Physiological plasma concentrations of nicotinic acid normally range from 0.1 to 0.4 μmol/L [20,21]. During treatment with pharmacological doses of nicotinic acid, the plasma level depends on the pharmaceutical form ingested. Currently, there are 2 forms of nicotinic acid that are used clinically: a rapid-release and an extended-release form. Peak plasma levels at 30 to 60 minutes after ingesting 1 g of nicotinic acid are 15 to 30 μg/mL (122-244 μmol/L) [21]. These concentrations are substantially lower than the concentrations of nicotinic acid required to demonstrate biological activity in the present study (ie, 1.0 mmol/L or more). However, it is noteworthy that plasma levels of nicotinic acid may not reflect its bioavailability at the liver [22].

It is noteworthy that there are several mechanisms by which nicotinic acid can alter apo A-I levels and possibly enhance cardioprotection [20]. Decreased free fatty acid mobilization from adipose tissue has been suggested as a mechanism of the triglyceride (TG)-lowering effect of nicotinic acid. However, physiologically and clinically, this pathway may be only a minor factor in explaining the lipid effects of nicotinic acid [20]. More importantly, nicotinic acid directly inhibits hepatocyte diacylglycerol acyltransferase–2, a key enzyme for TG synthesis [23]. The inhibition of TG synthesis results in accelerated intracellular hepatic apo B degradation and the decreased secretion of very low-density lipoprotein and low density lipoprotein particles [20,23].

Additional effects of nicotinic acid include inhibiting the hepatocyte removal of HDL–apo A-I complex [24], increasing vascular endothelial cell redox state resulting in the inhibition of oxidative stress and vascular inflammatory genes [25], increasing the ability of HDL to promote net cholesterol efflux [26], and promoting cholesterol efflux from adipocytes to apo A-I via activation of the PPARγ-LXRα-adenosine triphosphate–binding cassette protein A1 pathway [27]. Indeed, recent studies have shown that nicotinic acid alters gene expression in tissues by various mechanisms [28].

The strengths of this study include the use of sensitive tools of qRT-PCR to measure the mRNA levels and the measurements of apo A-I promoter activity with promoter constructs of variable lengths identifying a region of the apo A-I gene located between –170 and –186 bp as the location of a novel nicotinic acid response element. However, the weakness of this observation is that site-directed mutagenesis studies should have been included to precisely define this response element.

In addition to these multiple salutary effects of nicotinic acid, the present study shows that nicotinic acid may increase HDL in part by increasing apo A-I protein synthesis in the liver and small intestine. Induction of apo A-I gene by nicotinic acid required a nicotinic acid responsive element in the apo A-I promoter. The translational implication of these observations is that future studies may be designed to identify analogs of nicotinic acid that have

enhanced activity at the apo A-I promoter without the known undesirable adverse effects of nicotinic acid.

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Conflict of Interest

Disclosure statement: Dr. Norman Wong is part time employee/consultant of Resverlogix Corp. The other coauthors do not have any conflict of interest to report.

REFERENCES

[1] Hachem SB, Mooradian AD. Familial dyslipidaemias: an overview of genetics, pathophysiology and management. Drugs 2006;66:1949-69.
[2] Haas MJ, Mooradian AD. Therapeutic interventions to enhance apolipoprotein A-I–mediated cardioprotection. Drugs 2010;70:805-21.
[3] Shepherd J, Packard CJ, Patsch JR, Gotto Jr AM, Taunton OD. Effects of nicotinic acid therapy on plasma high density lipoprotein subfraction distribution and composition and on apolipoprotein A metabolism. J Clin Invest 1979;63:858-67.
[4] Jin FY, Kamanna VS, Kashyap ML. Niacin decreases removal of high-density lipoprotein apolipoprotein A-I but not cholesterol ester by HepG2 cells. Implication for reverse cholesterol transport. Arterioscler Thromb Vasc Biol 1997;17:2020-8.
[5] Zhang LH, Kamanna VS, Zhang MC, Kashyap ML. Niacin inhibits surface expression of ATP synthase beta chain in HepG2 cells: implications for raising HDL. J Lipid Res 2008;49: 1195-201.
[6] Siripurkpong P, Na-Bangchang K. Effects of niacin and chromium on the expression of ATP-binding cassette transporter A1 and apolipoprotein A-1 genes in HepG2 cells. J Nutr Biochem 2009;20:261-8.
[7] Lamon-Fava S, Diffenderfer MR, Barrett PH, Buchsbaum A, Nyaku M, Horvath KV, et al. Extended-release niacin alters the metabolism of plasma apolipoprotein (Apo) A-I and apo B–containing lipoproteins. Arterioscler Thromb Vasc Biol 2008;28:1672-8.
[8] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
[9] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227: 680-5.
[10] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350-4.
[11] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.
[12] Parolini C, Chiesa G, Zhu Y, Forte T, Caligari S, Gianazza E, et al. Targeted replacement of mouse apolipoprotein A-I with human apo A-I or the mutant apo A-I Milano. Evidence of APOA-IM impaired hepatic secretion. J Biol Chem 2003;278:4740-6.
[13] Carrano G, Albertin G, Forneris M, Nussdorfer GG. Similar sequence-free amplification of human glyceraldehydes-3-phosphate dehydrogenase for real time RT-PCR applications. Mol Cell Probes 2005;19:181-6.
[14] Lefever S, Vandesompele J, Speleman F, Pattyn F. RTPrimerDB; the portal for real-time PCR primers and probes. Nucleic Acids Res 2009;37(Database issue): D942-5.
[15] Haas MJ, Horani M, Mreyoud A, Plummer B, Wong NC, Mooradian AD. Suppression of apolipoprotein AI gene expression in HepG2 cells by TNF alpha and IL-1beta. Biochim Biophys Acta 2003;1623:120-8.
[16] Taylor AH, Wishart P, Lawless DE, Raymond J, Wong NC. Identification of functional positive and negative thyroid hormone-responsive elements in the rat apolipoprotein AI promoter. Biochemistry 1996;35:8281-8.
[17] Gorman CM, Moffat LF, Howard BH. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol Cell Biol 1982;2:1044-51.
[18] Herbomel P, Bourachot B, Yaniv M. Two distinct enhancers with different cell specificities coexist in the regulatory region of polyoma. Cell 1984;39:653-62.
[19] Widom RL, Ladias JA, Kouidou S, Karathanasis SK. Synergistic interactions between transcription factors control expression of the apolipoprotein AI gene in liver cells. Mol Cell Biol 1991;11:677-87.
[20] Kamanna VS, Kashyap ML. Mechanism of action of niacin. Am J Cardiol 2008;101(8A):20B-6B.
[21] Gille A, Bodor ET, Ahmed K, Offermanns S. Nicotinic acid: pharmacological effects and mechanisms of action. Annu Rev Pharmacol Toxicol 2008;48:79-106.
[22] Weiner M. Clinical pharmacology and pharmacokinetics of nicotinic acid. Drug Metab Rev 1979;9:99-106.
[23] Le Bloc'h J, Leray V, Chetiveaux M, Freuchet B, Magot T, Krempf M, et al. Nicotinic acid decreases apolipoprotein B100–containing lipoprotein levels by reducing hepatic very low density lipoprotein secretion through a possible diacylglycerol acyltransferase 2 inhibition in obese dogs. J Pharmacol Exp Ther 2010;334:583-9.
[24] Zhang LH, Kamanna VS, Zhang MC, Kashyap ML. Niacin inhibits surface expression of ATP synthase β chain in HepG2 cells: implications for raising HDL. J Lipid Res 2008;49: 1195-201.
[25] Ganji SH, Qin S, Zhang L, Kamanna VS, Kashyap ML. Niacin inhibits vascular oxidative stress, redo-sensitive genes, and monocyte adhesion to human aortic endothelial cells. Atherosclerosis 2009;202:68-75.
[26] Yvan-Charvet L, Kling J, Pagler T, Li H, Hubbard B, Fisher T, et al. Cholesterol efflux potential and antiinflammatory properties of high-density lipoprotein after treatment with niacin or anacetrapib. Arterioscler Thromb Vasc Biol 2010;30: 1430-8.
[27] Wu ZH, Zhao SP. Niacin promotes cholesterol efflux through stimulation of the PPARgamma-LXRalpha-ABCA1 pathway in 3T3-L1 adipocytes. Pharmacology 2009;84:282-7.
[28] Choi S, Yoon H, Oh KS, Oh YT, Kim YI, Kang I, Youn JH. Widespread effects of nicotinic acid on gene expression in insulin-sensitive tissues: implications for unwanted effects of nicotinic acid treatment. Metabolism 2011;60: 134-44.