

# **Niacin Decreases Removal of High-Density Lipoprotein Apolipoprotein A-I But Not Cholesterol Ester by Hep G2 Cells**

## **Implication for Reverse Cholesterol Transport**

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## **Abstract**

 [Top](#)

*Abstract* Niacin (nicotinic acid) is the most potent clinically used agent for increasing plasma HDL and apolipoprotein (apo) A-I. The mechanism by which niacin increases apoA-I is not clearly understood. We have examined the effect of niacin on the hepatic production and removal of apoA-I using Hep G2 cells as an in vitro model. Incubation of Hep G2 cells with niacin resulted in increased accumulation of apoA-I in the medium in a dose-dependent manner. Incorporation of [<sup>3</sup>H]leucine and [<sup>35</sup>S]methionine into apoA-I and apoA-I mRNA expression was unchanged by niacin, suggesting that it did not affect apoA-I de novo synthesis. Uptake of radiolabeled HDL protein and HDL apoA-I by Hep G2 cells was significantly reduced to as much as 82.9±2.2% (*P*=.04) and 84.2±2.8% (*P*=.02), respectively, of the baseline with increasing concentrations of niacin (0 to 3.0 mmol/L). Specific <sup>125</sup>I-HDL protein uptake measured with a 50-fold excess of unlabeled HDL was reduced to as much as 78.3±4.8% (*P*=.005) in niacin-treated cells. The uptake of labeled cholesterol esters in HDL was unaffected by niacin. Niacin also effected a similar decrease in HDL protein uptake, but not cholesterol esters, from apoA-I-containing HDL particles isolated by immunoaffinity. The conditioned medium obtained from Hep G2 cells incubated with niacin significantly (*P*=.002) increased cholesterol efflux from cultured human fibroblasts. These data indicate a novel mechanism whereby niacin selectively decreases hepatic removal of HDL apoA-I but not cholesterol esters, thereby increasing the capacity of retained apoA-I to augment reverse cholesterol transport.

- [Abstract](#)
- ▼ [Introduction](#)
- ▼ [Methods](#)
- ▼ [Results](#)
- ▼ [Discussion](#)
- ▼ [References](#)

**Key Words:** atherosclerosis • coronary artery disease • hepatic HDL uptake • metabolism

## ► Introduction

Nicotinic acid (niacin), a water-soluble vitamin, has been used to regulate abnormalities in plasma lipid metabolism and in the treatment of atherosclerotic cardiovascular disease.<sup>1 2 3 4 5 6 7</sup> In pharmacological doses, niacin reduces total plasma cholesterol, triglyceride, VLDL, and LDL concentrations.<sup>8</sup> It is the most potent clinically used agent that increases circulating HDL cholesterol and apo A-I, the major protein of HDL.<sup>8 9 10 11 12</sup> Niacin in combination with bile acid binding resin also significantly decreases small, dense LDL particles in patients with low LDL.<sup>13</sup> Additionally, niacin treatment decreases plasma levels of lipoprotein(a) in hyperlipidemic patients.<sup>14</sup> Since HDL has been shown to inhibit LDL oxidation, promote reverse cholesterol transport, and be profibrinolytic, low levels of HDL in patients with coronary artery disease may result in increased cardiovascular events.<sup>15 16 17</sup> Furthermore, long-term follow-up of patients in the Coronary Drug Project,<sup>18</sup> coronary arteriographic studies including the Cholesterol Lowering Atherosclerosis Study,<sup>19</sup> and the Familial Atherosclerosis Treatment Study<sup>20</sup> indicated that treatment with niacin significantly reduced total mortality, reduced coronary events, and retarded progression and even induced regression of coronary atherosclerosis in some patients.

- ▲ [Top](#)
- ▲ [Abstract](#)
- [Introduction](#)
- ▼ [Methods](#)
- ▼ [Results](#)
- ▼ [Discussion](#)
- ▼ [References](#)

Although niacin has been widely used to increase plasma HDL levels, the mechanism by which niacin exerts its action is not clearly understood. On the basis of plasma kinetic studies, it was suggested that the rise in plasma apoA-I in patients treated with niacin was the result of decreased fractional clearance rates rather than alterations in synthetic rates.<sup>16 21</sup> These studies led us to examine in greater detail the direct effect of niacin on hepatic production and removal of HDL and its components. Because the liver is the major organ for the synthesis and removal of HDL in humans, we used Hep G2 cells, a human hepatoblastoma cell line that has been shown to be a useful model for

studying hepatic lipoprotein metabolism<sup>23 24</sup> and has helped in gaining concepts about mechanisms of action of hormones and drugs on HDL at the cellular level in contrast to in vivo kinetic studies. In this investigation, we examined the effect of niacin on cultured Hep G2 cells on: (1) synthesis and secretion of apoA-I; (2) steady state mRNA expression for apoA-I; (3) uptake of radiolabeled HDL protein, specifically HDL apoA-I, and HDL CEs; and (4) properties of secreted material to functionally efflux cellular cholesterol. This study demonstrated a selective decrease in hepatic HDL protein and apoA-I uptake by niacin. Part of this work has been presented in abstract form.<sup>25</sup>

## ► **Methods**

Tissue culture materials, medium, FBA, pure human apoA-I, and niacin were obtained from Sigma Chemical Company unless otherwise noted. FBS was obtained from Hyclone Laboratories. l-[4,5-<sup>3</sup>H]Leucine, [<sup>3</sup>H]cholesterol, and <sup>32</sup>P nucleotides were purchased from Amersham Corporation. The human hepatoblastoma cell line (Hep G2), human fibroblast cells, and human apoA-I DNA probe were obtained from American Type Culture Collection. The polyclonal antibody for human apoA-I was obtained from Boehringer Mannheim Biochemicals. All other chemicals used were of analytical grade.

▲	<a href="#">Top</a>
▲	<a href="#">Abstract</a>
▲	<a href="#">Introduction</a>
■	<a href="#">Methods</a>
▼	<a href="#">Results</a>
▼	<a href="#">Discussion</a>
▼	<a href="#">References</a>

### **HDL Isolation**

Blood samples were collected from fasting healthy human volunteers, and serum was isolated by centrifugation and pooled for lipoprotein isolation. HDL was isolated by sequential density ultracentrifugation at a final density of 1.210 g/mL.<sup>26</sup> The purity of lipoprotein was monitored by agarose gel electrophoresis, and protein content was measured by the method of Lowry et al<sup>27</sup> using bovine serum albumin as a standard.

### **Separation of HDL Particles**

Serum from fasting (>12 hours) healthy volunteers was collected and LPAI (ie, all HDL particles bearing apoA-I, including apoA-II) particles were isolated by immunoaffinity column chromatography.<sup>28</sup> In brief, affinity columns specific for apoA-I were prepared by coupling polyclonal antibodies for human apoA-I to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's procedure. Aliquots of serum were loaded onto the apoA-I affinity column and incubated at 4°C for 16 to 18 hours to allow binding of HDL particles to specific antibody. The affinity column was then washed with 0.5 mol/L NaCl and retained apoA-I-containing HDL particles were eluted with 3 mol/L NaSCN, pH 6.0. LPAI HDL was tested by SDS-polyacrylamide gel electrophoresis for purity.

### **Cell Culture**

Hep G2 cells were grown in T-75 flasks with 15 mL of high-glucose DMEM containing 10% FBS, 1% glutamine-penicillin-streptomycin, and 1% fungizone. Cells were grown in a humidified incubator at 37°C in an atmosphere of 5% CO<sub>2</sub> and 95% air. Subcultures were made from confluent stock cultures by trypsinization with PBS containing 0.5 mmol/L ethylene diamine tetraacetic acid. Human fibroblast cells were cultured in T-75 flasks with 15 mL of DMEM at 37°C in a humidified incubator with 5% CO<sub>2</sub>. Subcultures were made as described above and passages between 18 and 20 were used for various experiments.

### **Studies on the Accumulation of ApoA-I Mass in Hep G2 Cells**

The cells were plated in 60-mm culture petri dishes at a concentration of  $4 \times 10^6$  cells per dish in 4 mL DMEM and grown for 3 to 4 days until they attained 75% to 80% confluence. The studies examining the dose response of niacin on culture medium concentration of apoA-I were performed by incubating Hep G2 cells with various amounts of niacin (0 to 3.0 mmol/L) at 37°C for 72 hours. At the termination of the incubation, culture medium from each flask were removed, and the cell monolayer was washed with PBS and collected for cellular protein measurement. A 50- $\mu$ L sample of culture medium was assayed for apoA-I by an enzyme-linked immunoassay using a human apoA-I-specific monoclonal antibody (HB-22) developed and characterized in our laboratory.<sup>29</sup> The concentration of apoA-I was expressed as micrograms per milligram of cellular protein.

### De Novo Synthesis of ApoA-I

Studies examining the effect of various doses of niacin on the de novo synthesis of apoA-I by Hep G2 cells were performed by measuring the incorporation of radiolabeled leucine into apoprotein secreted into the medium. Hep G2 cells ( $4 \times 10^6$ ) were incubated with varying concentrations of niacin (0 to 3.0 mmol/L) in high-glucose DMEM containing 10% FBS for 48 hours at 37°C in a humidified incubator. After the incubation, the medium was replaced with leucine-poor DMEM (5% leucine of normal medium) without FBS, containing the corresponding amounts of niacin and [<sup>3</sup>H]leucine (5  $\mu$ Ci/mL), and incubated for 18 hours at 37°C. At the end of the incubation, the medium was collected and used for immunoprecipitation. The cell monolayer was washed with PBS and collected for protein measurement. The incorporation of radiolabeled leucine into apoA-I was measured by immunoprecipitation using monospecific antibodies for apoA-I as described earlier.<sup>30</sup> The incorporation of [<sup>3</sup>H]leucine into total secreted protein was measured by trichloroacetic acid precipitation. The incorporation of [<sup>3</sup>H]leucine into apoA-I, or total trichloroacetic acid-precipitable protein, was expressed as counts per minute per milligram cellular protein.

In additional experiments, Hep G2 cells were incubated with varying concentrations of niacin (0 to 3.0 mmol/L) for 48 hours at 37°C. Medium was then changed to fresh methionine-free DMEM containing the respective concentrations of niacin and [<sup>35</sup>S]methionine (150  $\mu$ Ci/mL). After 15 minutes of pulsing, the medium was replaced with fresh DMEM containing 15  $\mu$ g/mL methionine and incubated for 1 hour at 37°C. At the end of the incubation period, culture media were collected and cells were harvested and lysed by lysis buffer. The radiolabeled apoA-I in medium and cell lysate were assayed by immunoprecipitation and were expressed in terms of total cellular protein.

### Uptake of HDL Protein or ApoA-I by Hep G2 Cells

Studies examining the uptake by Hep G2 cells were performed by using radiolabeled HDL total protein or apoA-I HDL. Radioiodination of HDL total protein was carried out by incubating freshly isolated HDL<sub>3</sub> (density, 1.12 to 1.21 g/mL) with carrier-free <sup>125</sup>I as described earlier by McFarlane.<sup>31</sup> After the iodination, unreacted <sup>125</sup>I was removed by gel filtration followed by exhaustive dialysis against PBS. Specific activity of <sup>125</sup>I-HDL protein was 186 cpm/ng protein. Radioiodination of apoA-I was performed by incubating apoA-I (350  $\mu$ g in 1.0 mol/L glycine, pH 7.4) with <sup>125</sup>I (1 mCi) and Iodogen (10  $\mu$ g in methylene chloride) for 30 minutes at room temperature. After the incubation, the <sup>125</sup>I-apoA-I was purified by passing through Sephadex G-25 columns and then dialyzed. <sup>125</sup>I-apoA-I was then incubated with HDL<sub>3</sub> for 1 hour at 37°C to reassociate apoA-I with HDL in vitro.<sup>32</sup> At the termination of the incubation, <sup>125</sup>I-apoA-I HDL was isolated by ultracentrifugation at a density of 1.21 g/mL. The radiolabeled apoA-I HDL was exhaustively dialyzed, and the specific activity was 62 cpm/ng protein. Radioiodination of apoA-I-containing HDL particles was carried out by the same method as for HDL total protein iodination described above. Specific activity of <sup>125</sup>I-apoA-I-containing HDL particles was 187.25 cpm/ng protein. Uptake studies were initiated by preincubating

Hep G2 cells with varying concentrations of niacin (0 to 3.0 mmol/L) for 48 hours at 37°C. The medium was replaced with fresh DMEM containing FBA (5 mg/mL), and either  $^{125}\text{I}$ -HDL or  $^{125}\text{I}$ -apoA-I HDL (50  $\mu\text{g}$  protein) was added. After 16 hours of incubation at 37°C, cell monolayers were washed thoroughly (4 to 5 times with PBS) and digested with 1N sodium hydroxide solution. An aliquot was used for radioactivity measurement. The uptake of radiolabeled HDL particles by Hep G2 cells was expressed in terms of cellular protein.

### **Uptake of [ $^3\text{H}$ ]CE-Labeled HDL**

[ $1\alpha,2\alpha(n)$ - $^3\text{H}$ ] Cholesterol (4  $\mu\text{Ci}$ ) was added to the serum fraction, and the mixture was incubated for 18 hours at 37°C to allow CE formation via the lecithin:cholesterol acyltransferase enzyme reaction.<sup>33</sup> The [ $^3\text{H}$ ]CE HDL was isolated by ultracentrifugation at a density of 1.210 g/mL and dialyzed extensively against 0.15 mol/L NaCl. Uptake studies were performed by preincubating Hep G2 cells with or without niacin (1.5 mmol/L) or varying concentrations of niacin (0 to 3.0 mmol/L, experiment 2) for 48 hours. Medium was removed and cells were washed with PBS. Fresh DMEM containing 5 mg/mL FBA (fatty acid free) was added. Various amounts of [ $^3\text{H}$ ]CE-labeled HDL (0 to 100  $\mu\text{g}$  HDL protein per milliliter) at 1.5 mmol/L niacin or 50  $\mu\text{g}$  HDL protein per milliliter (experiment 2) was added at 0 to 3.0 mmol/L niacin. Cells were harvested 6 hours later, washed thoroughly, and digested with 1 mL of 1N NaOH.<sup>34</sup> Radioactivity was measured and expressed as counts per minute per milligram cellular protein. The same method was used for the [ $^3\text{H}$ ]CE of apoA-I-containing HDL particles isolated by immunoaffinity (ie, LPAI).

### **Measurement of Cholesterol Efflux**

Experimental protocols for these studies were exactly the same as described for medium accumulation of apoA-I. After the incubation of Hep G2 cells with niacin, the medium was collected and used for cholesterol efflux measurement. An aliquot of culture medium (5 mL) was concentrated to 1 mL by lyophilization and dialyzed against DMEM to remove excess salt present in the concentrated samples. The ability of these concentrated samples to efflux free cholesterol was measured by a previously described modified procedure of Fielding and Fielding<sup>35</sup> and Rothblatt et al<sup>36</sup> by using [ $^3\text{H}$ ]cholesterol-labeled human fibroblasts.<sup>37 38</sup> To ensure specific and homogenous incorporation of radiolabeled cholesterol throughout the cytoplasmic matrix of fibroblasts, we incubated fibroblasts with [ $^3\text{H}$ ]cholesterol for 72 hours at 37°C, washed with PBS, and then incubated with fresh DMEM containing 1% FBA for 16 hours at 37°C. The cholesterol efflux assay was initiated by incubating concentrated culture medium with [ $^3\text{H}$ ]cholesterol-labeled fibroblasts for 20 hours at 37°C in a humidified incubator. Quantitative analysis of the ability of Hep G2 cell culture medium (in presence or absence of niacin) was performed by measuring the radioactivity appearing in the medium per milliliter of incubation medium per milligram of fibroblast cellular protein.

### **Northern Blot Analysis**

Experimental protocols for Northern blot analysis were exactly the same as described in apo A-I accumulation studies. Total RNA was isolated from Hep G2 cells using the protocol of Chomczynski and Sacchi.<sup>39</sup> Twenty micrograms of total RNA was loaded into individual wells of a 1.0% agarose gel containing formaldehyde, and electrophoresis was performed.<sup>40</sup> The RNA from the gel was transferred onto MSI nylon membranes, UV cross-linked, and the membranes were hybridized overnight with the  $^{32}\text{P}$ -labeled cDNA probe for human apoA-I mRNA. The membranes were washed three times for 30 minutes: first in 2x SSC with 0.1% SDS at room temperature, second in 0.2x SSC with 0.1% SDS at room temperature, and third in 0.2x SSC with 0.1% SDS at 50°C. Autoradiography was performed by exposing the blots to X-ray films

with intensifying screens at  $-70^{\circ}\text{C}$ . Blots were then rehybridized with  $^{32}\text{P}$ -labeled human GAPDH (Clontech) cDNA probe as an internal control to assess RNA quantity and integrity. Quantification of mRNA signals was performed by densitometric scanning of autoradiographic bands and normalized with GAPDH mRNA signals, using the LKB laser densitometer (Pharmacia Biotech).

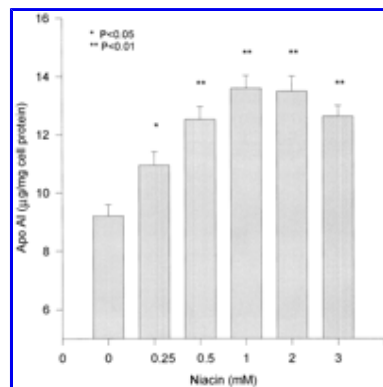
### Statistical Analysis

Mean data present average value for each set of experiments done in triplicate. Statistical significance was calculated by using the Student's *t* test, and  $P < .05$  was considered significant.

## Results

The preincubation of Hep G2 cells with varying concentrations of niacin (0 to 3.0 mmol/L) for 72 hours at  $37^{\circ}\text{C}$  resulted in a dose-dependent increase in apoA-I mass in the culture medium (Fig 1; 19% to 47% over control level). The treatment of Hep G2 cells with niacin, as low as 0.25 mmol/L concentration, significantly elevated apoA-I accumulation in the medium (19% over control level), and the maximum effect was observed at concentrations of 1.0 and 2.0 mmol/L (47% increase compared with control level).

- ▲ [Top](#)
- ▲ [Abstract](#)
- ▲ [Introduction](#)
- ▲ [Methods](#)
- [Results](#)
- ▼ [Discussion](#)
- ▼ [References](#)



**Figure 1.** Effect of niacin on apoA-I medium accumulation by Hep G2 cells. Cells were incubated with varying concentrations of niacin (0 to 3.0 mmol/L) for 72 hours at  $37^{\circ}\text{C}$ . At the end of the incubation, culture medium was assayed for apoA-I by ELISA using monoclonal antibody to apoA-I and expressed in terms of total cellular protein. Data are mean  $\pm$  SE of three separate experiments done in triplicate. Statistical significance was compared with results of control (\* $P < .05$ ; \*\* $P < .01$ ).

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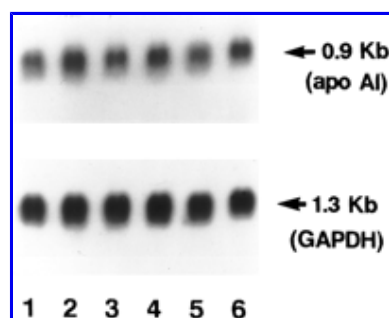
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Studies were designed to examine the de novo synthesis of apoA-I by measuring the incorporation of [ $^3\text{H}$ ]leucine into newly synthesized apoA-I secreted into the medium. The results from these studies indicated that the incubation of Hep G2 cells with niacin (0 to 3.0 mmol/L) for 72 hours did not alter the de novo synthesis of apoA-I (Table 1). Additional experiments were performed to confirm the de novo apoA-I synthesis data by using [ $^{35}\text{S}$ ]methionine pulse-chase protocols in Hep G2 cells. In these experiments, preincubating Hep G2 cells with niacin for 48 hours, pulsing with [ $^{35}\text{S}$ ]methionine for 15 minutes, and chasing for 1 hour caused no significant alteration in the incorporation of [ $^{35}\text{S}$ ]methionine into newly synthesized apoA-I in culture medium and cell lysate (Table 2). Northern blot analysis was performed to examine the effect of niacin on apoA-I gene expression by Hep G2 cells. Incubation of varying amounts of niacin (0 to 3.0 mmol/L) with Hep G2 cells had no effect on the steady state apoA-I mRNA levels compared with control level (Fig 2). Quantitative analysis performed by densitometric

scanning of Northern blot and normalization with GAPDH message indicated that the preincubation of Hep G2 cells with niacin (0.25 to 3.0 mmol/L) did not significantly alter apoA-I mRNA expression (data not shown).

**View this table:** [\[in this window\]](#) **Table 1.** Effect of Niacin on the Incorporation of [<sup>3</sup>H]Leucine Into Newly Synthesized ApoA-I by Hep G2 Cells [\[in a new window\]](#)

**View this table:** [\[in this window\]](#) **Table 2.** Effect of Niacin on the Incorporation of [<sup>35</sup>S]Methionine Into Newly Synthesized ApoA-I by Hep G2 Cells [\[in a new window\]](#)



**Figure 2.** Representative study examining the effect of niacin on the steady state apoA-I mRNA levels by Hep G2 cells. Cells were incubated at 37°C with varying concentrations of niacin (0 to 3.0 mmol/L) for 72 hours. Aliquots of total RNA (20 µg) isolated from Hep G2 cells were electrophoresed and Northern blot analysis was performed, using human apoA-I cDNA probe as described in "Methods". Lanes 1 through 6 correspond to niacin concentrations at 0, 0.25, 0.5, 1.0, 2.0, and 3.0 mmol/L, respectively. Top, ApoA-I mRNA; bottom, GAPDH mRNA (internal control).

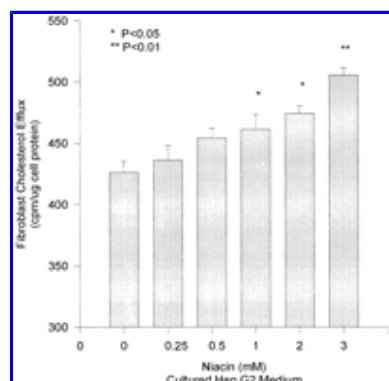
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The ability of niacin-induced secretion of apoA-I-containing lipoprotein particles to efflux cholesterol was examined by using [<sup>3</sup>H]cholesterol-labeled fibroblasts. Cholesterol efflux studies using conditioned medium obtained from Hep G2 cells treated with varying amounts of niacin (0 to 3.0 mmol/L) showed a dose-dependent increase in cholesterol efflux, as measured by the release of [<sup>3</sup>H]cholesterol from fibroblasts into the culture medium (Fig 3; 100%, 102%, 107%, 108%, 111%, and 119%, respectively). Additional studies were performed to determine whether niacin altered free cholesterol concentration in the cell medium, which may influence cholesterol efflux through equilibration. The concentration of free cholesterol in medium obtained from Hep G2 cells treated with niacin (used for cholesterol efflux studies) was not significantly different from control cell medium (free cholesterol concentrations in µmol/L: control medium, 2.71, and various doses of niacin [0.25 to 3.0 mmol/L]-treated cell medium, 2.6 to 2.73). Furthermore, no change in free cholesterol synthesis was noted between control and niacin-treated Hep G2 cells (30 hours), as determined by the incorporation of [<sup>14</sup>C]acetate into free cholesterol (data not shown). Cholesterol efflux studies performed at 4 hours, a shorter time period than reported in Fig 3 (16 hours), showed significant increase in cholesterol efflux using niacin-treated cell medium compared with control medium (data not shown). These results indicate that the increased cholesterol efflux shown in Fig 3 represents mainly unidirectional cholesterol efflux

induced by enhanced apoA-I accumulated in niacin-treated cell medium rather than equilibration of free cholesterol between cells and medium.



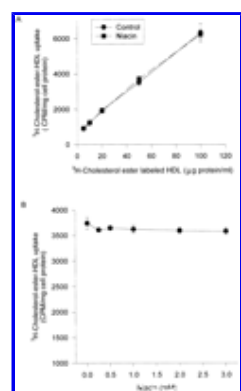
**Figure 3.** Effect of niacin-induced apoA-I-containing particles from Hep G2 cells on cholesterol efflux from cultured human fibroblasts. Hep G2 cells were incubated with varying concentrations of niacin (0 to 3.0 mmol/L) for 72 hours at 37°C. After the incubation, an aliquot of medium was concentrated (fivefold) and added to [<sup>3</sup>H]cholesterol-labeled human fibroblast cultures to measure its ability to efflux cholesterol, as described in "Methods." Radioactivity appearing in the culture medium (as a measure of cholesterol efflux) was expressed in terms of cellular protein. Data are mean±SE of four separate experiments done in triplicate (\**P*<.05; \*\**P*<.01).

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Since increased apoA-I mass accumulated in niacin-treated Hep G2 cell culture medium was not associated with apoA-I de novo synthesis, further experiments were performed to assess the effect of niacin on uptake of HDL and its components by Hep G2 cells. The uptake of the CE component of HDL (which was isolated by ultracentrifugation or by immunoaffinity techniques) by Hep G2 cells was measured by incubating with [<sup>3</sup>H]CE HDL particles for 6 hours at 37°C. As shown in Fig 4A, the preincubation of Hep G2 cells with niacin for 48 hours did not alter the uptake of [<sup>3</sup>H]CE-labeled HDL at varying concentrations in the incubation medium (0 to 100 μg HDL protein). Similarly, the uptake of [<sup>3</sup>H]CE HDL did not change in Hep G2 cells preincubated with niacin at varying concentrations (0 to 3.0 mmol/L, Fig 4B). To eliminate the possible effect of ultracentrifugation on HDL property, apoA-I-containing HDL particles were isolated by immunoaffinity columns. The CE component of HDL uptake was assessed by using the same method as described above. Hep G2 cells preincubated with niacin did not alter the uptake of [<sup>3</sup>H]CE HDL particles (Table 3).



**Figure 4.** Effect of niacin on [<sup>3</sup>H]CE-labeled HDL uptake by Hep G2 cells. Cells were preincubated with or without niacin (1.5 mmol/L) for 48 hours. Fresh DMEM containing 5 mg/mL FBA and various amounts of [<sup>3</sup>H]CE-labeled HDL (0 to 100 μg HDL protein per milliliter) was added. Cells were harvested 6 hours later, washed thoroughly, and digested with 1 mL of 1N NaOH. Radioactivity was measured and expressed as counts per minute per milligram cell protein (A). Another experiment was performed by incubating Hep G2 cells with varying concentrations of niacin (0 to 3.0 mmol/L) for 48 hours. Fresh DMEM containing 5 mg/mL FBA, niacin, and [<sup>3</sup>H]CE-labeled HDL (50 μg HDL protein per milliliter) was added. Cells were harvested 6 hours later, and radioactivity was counted and expressed in counts per minute per milligram cell protein (B). Data are mean±SE of three separate experiments done in triplicate.

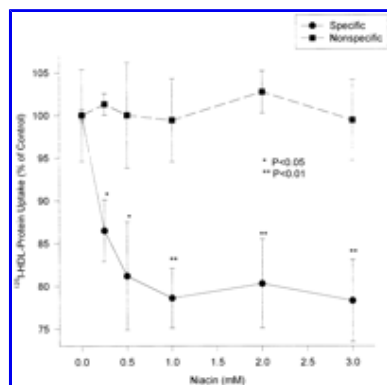
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**View this table:** **Table 3.** Effect of Niacin on [ $^3\text{H}$ ]CE-Labeled LPAI HDL Particle (Isolated by Immunoaffinity) Uptake by Hep G2 Cells  
[\[in this window\]](#)  
[\[in a new window\]](#)

To determine the differential uptake of HDL total protein or apoA-I and HDL cholesterol by Hep G2 cells, additional uptake experiments were performed by using  $^{125}\text{I}$ -HDL protein or  $^{125}\text{I}$ -apoA-I HDL. The incubation of Hep G2 cells with niacin for 48 hours significantly inhibited the uptake of  $^{125}\text{I}$ -HDL protein and  $^{125}\text{I}$ -apoA-I HDL particles similarly (up to 17.1% and 15.8%, respectively compared with control; Figs 5 and 6). Since total uptake may comprise both specific and nonspecific uptake, we further examined the effect of niacin on specific uptake of HDL protein by Hep G2 cells. Using a 50-fold excess of unlabeled HDL to inhibit specific uptake, nonspecific HDL protein uptake contributed  $21.6\pm 0.4\%$  of total uptake, and niacin at various doses did not affect nonspecific HDL protein uptake by Hep G2 cells. As shown in Fig 5, specific  $^{125}\text{I}$ -HDL protein uptake was significantly reduced to as much as  $21.7\pm 0.8\%$  in Hep G2 cells treated with niacin (1 to 3 mmol/L).

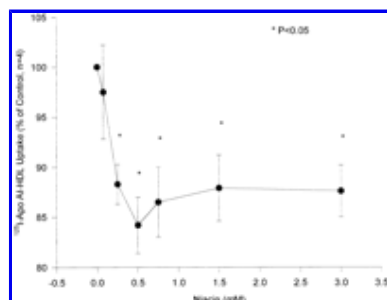


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**Figure 5.** Effect of niacin on  $^{125}\text{I}$ -HDL protein specific and nonspecific uptake by Hep G2 cells. Cells were preincubated with varying concentrations of niacin (0 to 3.0 mmol/L) for 48 hours. Fresh DMEM containing 5 mg/mL FBA, niacin, and  $^{125}\text{I}$ -HDL protein (100  $\mu\text{g}$  protein per milliliter) was added. Cells were harvested 16 hours later, washed thoroughly with PBS, and digested with 1 mL of 1N NaOH. Radioactivity was measured as total uptake and expressed in terms of total cellular protein. Nonspecific uptake studies were performed by adding a 50-fold excesses of cold HDL with  $^{125}\text{I}$ -HDL protein (100  $\mu\text{g}$  protein per milliliter) into culture medium. Specific HDL protein uptake was calculated by subtracting nonspecific from total uptake. Data are mean $\pm$ SE of six determinations.

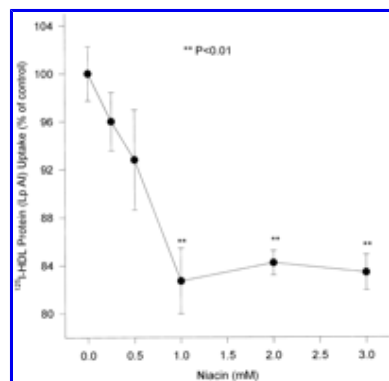


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**Figure 6.** Effect of niacin on  $^{125}\text{I}$ -apoA-I-labeled HDL uptake by Hep G2 cells. Cells were preincubated with varying concentrations of niacin (0 to 3.0 mmol/L) for 48 hours. Fresh DMEM containing 5 mg/mL FBA, niacin, and  $^{125}\text{I}$ -apoA-I-HDL (100  $\mu\text{g}$  protein per milliliter) was added. Cells were harvested 16 hours later, washed thoroughly with PBS, and digested with 1 mL of 1N NaOH. Radioactivity was measured and expressed in terms of total cellular protein. Data are mean $\pm$ SE of three separate experiments done in triplicate.

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To determine the uptake of apoA-I-containing HDL particles by Hep G2 cells, additional uptake experiments were performed by using immunoaffinity-isolated  $^{125}\text{I}$ -LPAI particles. The incubation of Hep G2 cells with niacin (0 to 3.0 mmol/L) for 48 hours significantly inhibited the uptake of  $^{125}\text{I}$ -LPAI protein (up to 17.3% compared with control; Fig 7<sup>↗</sup>).



**Figure 7.** Effect of niacin on  $^{125}\text{I}$ -apoA-I-containing HDL particle protein uptake by Hep G2 cells. Cells were preincubated with varying concentrations of niacin (0 to 3.0 mmol/L) for 48 hours. Fresh DMEM containing 5 mg/mL FBA, niacin, and immunoaffinity-isolated  $^{125}\text{I}$ -apoA-I-containing HDL particles (100  $\mu\text{g}$  protein per milliliter) was added. Cells were harvested 16 hours later, washed thoroughly with PBS, and digested with 1 mL of 1N NaOH. Radioactivity was measured and expressed in terms of total cellular protein. Data are mean  $\pm$  SE of three separate experiments done in triplicate.

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## ► Discussion

On the basis of numerous epidemiological and case-controlled studies, it is generally accepted that an inverse correlation exists between the plasma concentrations of HDL and the severity of the development of atherosclerotic cardiovascular disease.<sup>41 42 43 44</sup> Although the mechanisms for this association are not clearly delineated, the cardioprotective effects of HDL have been largely attributed to the ability of apoA-I, the major protein of HDL, to initiate cholesterol efflux and thereby facilitate the removal of excess cholesterol from peripheral tissues (including arteries) and its delivery to the liver for degradation through reverse cholesterol transport pathways.<sup>35 45</sup> In recent years, additional direct support for the antiatherogenic properties of HDL has been provided by studies demonstrating that the direct infusion of high doses of HDL to cholesterol-fed rabbits could regress atherosclerotic lesion formation in these animals<sup>46</sup> and that cholesterol-fed transgenic mice engineered to produce high concentrations of apoA-I failed to develop atherosclerotic lesions.<sup>47</sup>

Using Hep G2 cells as an in vitro model, we and others have shown that gemfibrozil directly increased apoA-I synthesis without altering the uptake of apoA-I or HDL particles, an observation consistent with in vivo turnover studies previously reported by us indicating that gemfibrozil has a stimulatory effect on the transport rate of apoA-I without altering its fractional catabolic rate.<sup>48 49 50</sup> Taken together, these studies suggest that the in vitro Hep G2

▲ [Top](#)  
 ▲ [Abstract](#)  
 ▲ [Introduction](#)  
 ▲ [Methods](#)  
 ▲ [Results](#)  
 ▪ [Discussion](#)  
 ▼ [References](#)

cell model system may serve as an important cellular and molecular tool to determine the mechanisms of action of pharmacological agents and hormones. In this study, using Hep G2 cells as an *in vitro* model, we examined the effect of niacin on apoA-I synthesis and removal to delineate the hepatic cellular mechanism of niacin. The data indicate that niacin significantly increased apoA-I mass in the culture medium of Hep G2 cells without influencing apoA-I synthesis, as assessed by [<sup>3</sup>H]leucine or [<sup>35</sup>S]methionine incorporation into apoA-I and mRNA expression.

Since increased accumulation of apoA-I mass in the medium of Hep G2 cells treated with niacin was not associated with increased apoA-I synthesis, we hypothesized that niacin may influence the uptake or removal of HDL protein and/or apoA-I by Hep G2 cells. To test this hypothesis, we performed a series of experiments examining the uptake of HDL particles radiolabeled at different compartments (eg, <sup>125</sup>I-HDL total protein, <sup>125</sup>I-apoA-I HDL, or [<sup>3</sup>H]CE HDL) by Hep G2 cells. The results from these studies indicated that the incubation of Hep G2 cells with niacin for 48 hours significantly inhibited the uptake of HDL particles radiolabeled either at the total protein or apoA-I component. Competitive studies using excess unlabeled HDL indicate that 21.6±0.4% of total uptake is nonspecific, ie, cannot be inhibited. Niacin did not significantly affect nonspecific uptake, indicating that the actions of niacin are on a putative hepatic HDL receptor and perhaps on the very recently described "docking" HDL receptor for selectively removing HDL CEs.<sup>51</sup> We suggest this hypothesis as a distinct possibility that requires examination; however, it was beyond the scope of this investigation and remains a subject for future research.

In contrast to the effect of niacin on radiolabeled HDL protein or HDL apoA-I uptake, niacin did not alter the uptake of [<sup>3</sup>H]CE-labeled HDL by Hep G2 cells compared with control. Studies examining the interaction of HDL or its components with certain cell types (eg, primary human hepatocytes, adrenal cells, and Hep G2 cells) have indicated that HDL CEs are selectively taken up compared with HDL protein.<sup>52 53 54</sup> These observations have important implications regarding the effect of niacin on reverse cholesterol transport. Previously, we have hypothesized that agents that reduce the hepatic catabolism of HDL may not be as effective in reverse cholesterol transport as agents that stimulate synthesis.<sup>48</sup> However, the novel observation as discussed in this report indicates that if the selective hepatic removal of cholesterol remains intact, decreased catabolism of HDL protein or HDL apoA-I would result in a greater mass of circulating functional HDL, which could facilitate reverse cholesterol transport.

In the above HDL–Hep G2 interaction studies, HDL was obtained by ultracentrifugation. It is possible that the selective CE uptake may be an artifact of the drastic ultracentrifugal force and high salt solutions affecting HDL during its preparation from plasma. To address this question, we assessed the effect of niacin on HDL protein versus HDL CE Hep G2 cell uptake on HDL isolated by immunoaffinity, which would presumably result in "native" HDL particles. The data obtained confirmed the earlier observation. Niacin decreased uptake of LPAI protein but did not affect LPAI CE uptake (Fig 7<sup>✦</sup> and Table 3<sup>✦</sup>).

Our studies also show that accumulated apoA-I in Hep G2 cell culture medium in response to niacin treatment was functionally active in increasing cholesterol efflux from fibroblasts. These data indicate that niacin treatment may inhibit the removal of HDL apoA-I by Hep G2 cells, thus retaining excess apoA-I–containing HDL particles in the plasma that may ultimately retard atherosclerosis progression by augmenting reverse cholesterol transport pathway. The increased cholesterol efflux mediated by culture medium incubated with radiolabeled cholesterol is explained by the retention and increased concentration of apoA-I–containing particles in the medium. Although detailed characterization of the medium HDL particle composition was not available, it is possible that the cholesterol efflux was mediated by nascent HDL and possibly pre-β

migrating HDL, which has been shown to effect increased cholesterol efflux.<sup>55</sup> The data also indicate that niacin did not alter the culture medium concentration of free cholesterol, nor did it alter [<sup>14</sup>C]acetate incorporation into free cholesterol. Increased efflux was also observed at shorter time periods (4 hours) of incubation. Therefore, the measured increase in cholesterol efflux represents unidirectional flux.

Previous *in vivo* studies have shown that the treatment of patients with niacin markedly increased plasma HDL<sub>2</sub>:HDL<sub>3</sub> ratio.<sup>16 56</sup> Additionally, these studies have also indicated that the rise in plasma concentration of apoA-I in patients treated with niacin was primarily accomplished through a decrease in its fractional catabolic rate.<sup>16 22 56</sup> The observation made in Hep G2 cells regarding the effect of niacin on apoA-I removal is in line with *in vivo* HDL turnover studies in patients with hyperlipidemia, demonstrating that niacin treatment decreases the fractional catabolic rate of HDL but does not affect the HDL synthetic transport rate.<sup>16 22 56</sup> Previous investigators have suggested that the effects of niacin on the HDL subfraction distribution may be mediated via a net transfer of apoA-I from HDL<sub>3</sub> to HDL<sub>2</sub>.<sup>22 56</sup> Although these studies indicate the beneficial effects of niacin in raising the HDL<sub>2</sub> subfraction, the metabolic factors that regulate these cellular reactions are not clearly understood. In this regard, results presented in this investigation may provide a cellular mechanism by which niacin exerts its effects on increasing HDL size. For example, niacin, by decreasing the removal of HDL apoA-I by hepatocytes, would increase the residence time, *in vivo*, of HDL particles. Enrichment of these particles by cholesterol could occur through three possible mechanisms. First, a reduction in VLDL and LDL concentration after niacin treatment could result in decreased transfer of HDL CEs to VLDL and LDL, since these lipoproteins become rate limiting for CE transfer as their concentration drops. Second, increased peripheral cellular cholesterol efflux to increased apoA-I HDL could also result in a new steady state in which HDL CE content is increased, thereby resulting in larger HDL particles. Third, niacin could depress CE transfer protein, thereby resulting in lesser transfer of CEs to non-HDL lipoproteins. These possibilities need to be examined further.

The *in vitro* observations in this study do not completely answer the question of whether niacin favorably affects reverse cholesterol transport *in vivo* and thereby retards clinically documented atherogenesis. However, these findings indicate a novel concept and prompt the need for further studies. For example, the niacin effect described here also needs to be confirmed in primary human hepatocytes and in perfused livers. At the *in vivo* level, turnover studies that simultaneously assess niacin's effect on kinetic parameters of HDL apoA-I versus HDL CE need to be conducted to validate the concept. It is recognized that the isolated cell culture system is not totally representative of the dynamically changing humoral milieu of the liver *in vivo*.

In summary, in this study, niacin selectively decreased hepatic removal of HDL protein and apoA-I without altering the removal of HDL CEs. Thus, it increased the efficiency of selective hepatic removal of CEs from HDL. Niacin had no effect on apoA-I *de novo* synthesis and steady state mRNA expression by Hep G2 cells. The data indicate a new and previously undescribed concept for increasing HDL levels and possibly reverse cholesterol transport. Similar mechanisms may exist for other hormonal and pharmacological agents that alter HDL levels.

## ► Selected Abbreviations and Acronyms

apo	= apolipoprotein
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CE	= cholesterol ester
DMEM	= Dulbecco's modified Eagle's medium
FBA	= fetal bovine albumin
FBS	= fetal bovine serum
LPAI	= HDL particles containing apoA-I

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## ► References

- Altschul R, Hoffer A, Stephen JD. Influence of nicotinic acid on serum cholesterol in man. *Arch Biochem Biophys*. 1955;54:558-559.
- Parsons WB, Flinn JH. Reduction of serum cholesterol levels and beta-lipoprotein cholesterol levels by nicotinic acid. *Arch Intern Med*. 1959;103:783-790.
- Galbraith PA, Pevry WF, Beamish RE. Effect of nicotinic acid on serum lipids in normal and atherosclerotic subjects. *Lancet*. 1959;1:222-223. [[Medline](#)] [[Order article via Infotrieve](#)]
- The Coronary Drug Project Research Group. Clofibrate and niacin in coronary heart disease: the Coronary Drug Project. *JAMA*. 1975;231:360-381. [[Medline](#)] [[Order article via Infotrieve](#)]
- Carlson LA, Danielson M, Ekberg I, Klintemar B, Rosenhamer G. Reduction of myocardial reinfarction by combined treatment with clofibrate and nicotinic acid. *Atherosclerosis*. 1977;28:81-86. [[Medline](#)] [[Order article via Infotrieve](#)]
- Grundy SM, Mok HYI, Zech L, Berman M. Influence of nicotinic acid on metabolism of cholesterol and triglyceride in man. *J Lipid Res*. 1981;22:24-36. [[Abstract](#)]
- Figge HL, Figge J, Souney PF, Mutnick AH, Sacks F. Nicotinic acid: a review of its clinical use in the treatment of lipid disorders. *Pharmacotherapy*. 1988;8:287-294. [[Medline](#)] [[Order article via Infotrieve](#)]
- Carlson LA. The effect of nicotinic acid treatment on the chemical composition of plasma lipoprotein classes in man: drugs affecting lipid metabolism. *Adv Exp Med Biol*. 1969;4:327-338.
- Carlson LA, Olsson AG, Ballantyne D. On the rise in low density and high density lipoproteins in response to the treatment of hyperglyceridemia in type IV and type V hyperlipoproteinemias. *Atherosclerosis*. 1977;26:603-609. [[Medline](#)] [[Order article via Infotrieve](#)]
- Wahlberg G, Walldius G, Olsson AG, Kirstein P. Effect of nicotinic acid on serum cholesterol concentrations of high density lipoprotein subfractions HDL<sub>2</sub> and HDL<sub>3</sub> in hyperlipoproteinemia. *J Intern Med*. 1990;228:151-157. [[Medline](#)] [[Order article via Infotrieve](#)]
- Johansson J, Carlson LA. The effects of nicotinic acid treatment on high density lipoprotein particle size subclass levels in hyperlipidemic subjects. *Atherosclerosis*. 1990;83:207-216. [[Medline](#)] [[Order article via Infotrieve](#)]
- Gray DR, Morgan TR, Chretien SD, Kashyap ML. Efficacy and safety of controlled-release niacin in dyslipoproteinemic veterans. *Ann Intern Med*. 1994;121:252-258. [[Abstract/Free Full Text](#)]
- Superko HR, Williams PT, Alderman EL. Differential effect on HDL of niacin and resin in LDL subclass pattern A and B subjects. *Circulation*. 1993;88:2072A. Abstract.

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14. Carlson LA, Hamsten A, Asplund A. Pronounced lowering of serum levels of lipoprotein Lp(a) in hyperlipidemic subjects treated with nicotinic acid. *J Intern Med.* 1989;226:271-276. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
15. Parthasarathy S, Barnett J, Fong LG. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta.* 1990;1044:275-283. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
16. Kashyap ML. Effects of drugs on HDL apoprotein metabolism. In Miller NE, ed. *High Density Lipoproteins and Atherosclerosis, II.* New York, NY: Elsevier Science Publishing Co; 1989.
17. Saku K, Ahmad M, Glas-Greenwalt P, Kashyap ML. Activation of fibrinolysis by apolipoproteins of high density lipoproteins in man. *Thromb Res.* 1985;39:1-8. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
18. Canner PL, Berge KG, Wanger NK. Fifteen-year mortality in coronary drug project patients: Long-term benefit with niacin. *J Am Coll Cardiol.* 1986;8:1245-1255. [\[Abstract\]](#)
19. Blankenhorn DH, Nessim SA, Johnson RL, Sanmarco ME, Azen SP, Carhin-Hemphill L. Beneficial effects of combined colestipol therapy on coronary atherosclerosis and coronary venous bypass grafts. *JAMA.* 1987;257:3233-3240. [\[Abstract\]](#)
20. Brown G, Albers JJ, Fisher LF. Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *N Engl J Med.* 1990;323:1289-1298. [\[Abstract\]](#)
21. Blum CB, Levy RI, Eisenberg S, Hall M III, Goebel RH, Berman M. High density lipoprotein metabolism in man. *J Clin Invest.* 1977;60:795-807.
22. Shepherd J, Packard CJ, Patsch JR, Gotto AM Jr, 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;63:858-867.
23. Zannis VI, Breslow JL, San Giacomo TR, Aden DP, Knowles BB. Characterization of the major apolipoproteins secreted by two human hepatoma cell lines. *Biochemistry.* 1981;20:2089-2096.
24. Thrift R, Forte TM, Cahoon BE, Shore VG. Characterization of lipoproteins produced by the human liver cell line, Hep G2, under defined conditions. *J Lipid Res.* 1986;27:236-250. [\[Abstract\]](#)
25. Jin FY, Kamanna VS, Dhillon KS, Kashyap ML. Effect of niacin on high density lipoprotein apoA-I metabolism by human hepatocytes (Hep G2). *J Invest Med.* 1995;43:301A. Abstract.
26. Havel RJ, Eder HA, Bragdon JH. The distribution and characterization of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest.* 1955;34:1345-1353.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193:265-275. [\[Free Full Text\]](#)
28. Cheung MC, Albers JJ. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. *J Biol Chem.* 1984;12201-12209.
29. Rothwell TC, Kamanna VS, Jin FY, Koren E, Foley T, Kashyap ML. Characterization of a monoclonal antibody (HB-22) and development of an enzyme-linked immunosorbent assay for human apolipoprotein AI. *Clin Chem.* 1995;41:1150-1158. [\[Abstract/Free Full Text\]](#)
30. Amarasuriya RN, Gupta AK, Given M, Horng YC, Maeda T, Kashyap ML. Ethanol stimulates apolipoprotein AI secretion by human hepatocytes: implications for a mechanism for atherosclerosis protection. *Metabolism.* 1992;41:827-832. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
31. McFarlane AS. Efficient trace-labelling of proteins with iodine. *Nature.* 1958;182:53. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
32. Shepherd J, Gotto AM, Taunton OD, Claslake MJ, Farish E. The in vitro interaction of human apolipoprotein AI and high density lipoproteins. *Biochim Biophys Acta.* 1977;489:486-501. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
33. Tollefson JH, Albers JJ. Isolation, characterization, and assay of plasma lipid transfer proteins. *Methods Enzymol.* 1986;129:797-816. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
34. Rinninger F, Pittman RC. Regulation of selective uptake of high-density lipoprotein-associated cholesteryl esters in human fibroblasts and Hep G2 hepatoma cells. *J Lipid Res.* 1988;29:1179-1194. [\[Abstract\]](#)
35. Fielding CJ, Fielding PE. Evidence for a lipoprotein carrier in human plasma catalyzing sterol efflux from cultured fibroblasts and its relationship to lecithin:cholesterol acyltransferase. *Proc Natl Acad Sci U S A.* 1981;78:3911-3914. [\[Abstract/Free Full Text\]](#)
36. Rothblatt GH, Bamberger M, Phillips MC. Reverse cholesterol transport. In: Colowich SP, Kaplan NO, eds.: *Methods in Enzymology.* Orlando, Fla: Academic Press; 1986:628-644.
37. Selam JL, Kashyap ML, Gupta AK, Turner D, Wong ND, Lozano JL, Charles MA. Alteration in reverse

- cholesterol transport associated with programmable implantable intraperitoneal insulin delivery. *Metabolism*. 1994;43:665-669. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
38. Gupta AK, Ross EA, Myers JN, Kashyap ML. Increased reverse cholesterol transport in athletes. *Metabolism*. 1993;42:684-690. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
39. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*. 1987;162:156-159. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
40. Brown T. Analysis of RNA by Northern and slot blot hybridization. In: Ausubel FM, eds. *Current Protocols in Molecular Biology*. Brooklyn, NY: John Wiley & Sons; 1993:4.9.1-4.9.14.
41. Miller GJ, Miller NE. Plasma high density lipoprotein concentration of ischemic heart disease. *Lancet*. 1975;1:16-19. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
42. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hully SB. HDL cholesterol and other lipids in coronary heart disease: the cooperative lipoprotein phenotyping study. *Circulation*. 1977;55:767-772. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
43. Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease: new perspective based on the Framingham Heart Study. *Ann Intern Med*. 1979;90:85-91.
44. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against heart disease: the Framingham Heart Study. *Am J Med*. 1977;62:707-714. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
45. Kashyap ML. Basic considerations in the reversal of atherosclerosis: significance of high density lipoproteins in stimulating reverse cholesterol transport. *Am J Cardiol*. 1989;63:56H-59H. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
46. Badimon JJ, Badimon L, Fuster V. Regression of atherosclerotic lesions by high density lipoprotein fraction in the cholesterol fed rabbit. *J Clin Invest*. 1990;85:1234-1241.
47. Rubin EM, Krauss RM, Spangler EA, Verstuyft JG, Clift SM. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature*. 1991;353:265-267. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
48. Saku K, Gartside PS, Hynd BA, Kashyap ML. Mechanism of action of gemfibrozil on lipoprotein metabolism. *J Clin Invest*. 1985;75:1702-1712.
49. Jin FY, Kamanna VS, Chuang MY, Morgan K, Kashyap ML. Gemfibrozil stimulates apolipoprotein A-I synthesis and secretion by stabilization of mRNA transcripts in human hepatoblastoma cell line (Hep G2). *Arterioscler Thromb Vasc Biol*. 1996;16:1052-1062. [\[Abstract/Free Full Text\]](#)
50. Tam S. Effects of gemfibrozil and ketoconazole on human apolipoprotein AI, B, and E levels in two hepatoma cell lines, Hep G2 and Hep 3B. *Atherosclerosis*. 1991;91:51-61. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
51. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*. 1996;271:518-520. [\[Abstract\]](#)
52. Glass C, Pittman RC, Civen M, Steinberg D. Uptake of high density lipoprotein-associated apoprotein AI and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J Biol Chem*. 1985;260:744-750. [\[Abstract/Free Full Text\]](#)
53. Stein O, Israeli A, Leitersdorf E, Halpesin G, Stein Y. Preferential uptake of cholesteryl ester HDL by cultured macrophage. *Atherosclerosis*. 1986;75:151-158.
54. Rinninger F, Brundert M, Jackle S, Galle PR, Busch C, Izbicki JR, Rogiers X, Henne-Bruns D, Kremer B, Broelsch CE, Greten H. Selective uptake of high-density lipoprotein-associated cholesterol esters in human hepatocytes in primary culture. *Hepatology*. 1993;19:1100-1114.
55. Castro GR, Fielding CJ. Early incorporation of cell-derived cholesterol into pre-beta-migrating high-density lipoprotein. *Biochemistry*. 1988;27:25-29. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)
56. Packard CJ, Steward JM, Third JLHC, Morgan HG, Lawrie TDV, Shepherd J. Effects of nicotinic acid therapy on high density lipoprotein metabolism in type II and type IV hyperlipoproteinaemia. *Biochim Biophys Acta*. 1980;618:53-62. [\[Medline\]](#) [\[Order article via Infotrieve\]](#)

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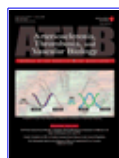
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