

Butyrate Stimulates ApoA-IV-Containing Lipoprotein Secretion in Differentiated Caco-2 Cells: Role in Cholesterol Efflux

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Abstract The aim of this study was to determine: (1) whether the Short Chain Fatty Acids (SCFA) Acetate, Propionate, and Butyrate enhance the synthesis and secretion of intestinal apolipoprotein A-IV-containing lipoproteins and (2) if so, whether these particles are able to promote cholesterol efflux *in vitro*. For this purpose Caco-2 cells were used for their functional properties of differentiated enterocytes. They were incubated with the three SCFA (2, 4, and 8 mM) for 48 h. Only butyrate stimulated apoA-IV gene expression and this was associated with an increase in apoA-IV secretion. A non-denaturing 2D-PAGE (agarose gel was followed by PAGE) was used to identify apoA-IV-containing lipoproteins in various media, and showed that butyrate stimulated the secretion of two small HDL sized particles. The influence of these secreted particles on cholesterol efflux was investigated using incubation of media with ³H-cholesterol-labeled Fu5AH cells. The data indicate that conditioned media from Caco-2 cells treated with butyrate resulted in an increase of 20–30% in cholesterol efflux. We conclude that butyrate may regulate apoA-IV secretion and, therefore, modulate reverse cholesterol transport. *J. Cell. Biochem.* 83: 230–238, 2001. © 2001 Wiley-Liss, Inc.

Key words: short chain fatty acids; intestinal cells; apolipoprotein A-IV; HDL particles; cholesterol efflux

Apo A-IV is a major glycoprotein component of the lipoproteins synthesized and secreted by enterocytes into lymph fluid as a component of chylomicrons in the fed state [Uterman and Beisiegel, 1979; Weisgraber et al., 1978; Green et al., 1980; Bisgaier et al., 1985]. In fasting plasma, apoAIV is found in HDL and in lipoprotein-free fraction [Weinberg and Spector, 1985; Vesina et al., 1988; Lagrost et al., 1989; Nowicka et al., 1990; Duverger et al., 1993]. Based on previous *in vitro* observations, several functions have been ascribed to this apolipoprotein. For example, apoAIV has been shown to (1) promote cholesterol efflux from extrahepatic cells [Stein et al., 1986; Steinmetz et al., 1990; von Eckardstein et al., 1995], (2)

serve as a ligand for HDL binding to hepatocytes [Dvorin et al., 1986], (3) activate lecithin:cholesterol acyltransferase [Emmanuel et al., 1994] and cholesteryl ester transfer protein, (4) modulate the activation of lipoprotein lipase by apoCII [Goldberg et al., 1990], and (5) act in the brain and inhibit gastric secretion to reduce the severity of gastric ulceration [Okumura et al., 1995] and to regulate food intake [Fujimoto et al., 1993]. Overexpression of either human or mouse apoA-IV in transgenic mice confers significant protection against diet-induced atherosclerosis in cholesterol fed animals and apoE-deficient mice [Duverger et al., 1996; Cohen et al., 1997]. Recently, apoA-IV has been suggested to be a potent inhibitor of lipoprotein oxidation [Qin et al., 1998]. Expression, synthesis, and secretion of apoA-IV are regulated, by dietary fat, hormones, and hypolipidemic drugs [Apfelbaum et al., 1987; Apostopoulos et al., 1987; Staels et al., 1990]. Regulation of its synthesis and secretion by isolated long chain fatty acids has been demonstrated using differentiated Caco-2 cells [Stan

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et al., 1999]. Yet, to our knowledge no data is available regarding the potential role of short chain fatty acids (SCFA).

SCFA (acetate, propionate, and butyrate), are naturally occurring molecules produced by resident bacteria in the intestinal colon lumen [Cummings et al., 1987]. The concentration of these molecules was found to range from 1 to 13 mM (from jejunum to ileum respectively) [Cummings et al., 1987]. Many roles have been attributed to the SCFA. They act as a primary source of energy for colonic epithelial cells [Roediger, 1982] and they alter the rates of proliferation and differentiation of these cells [Whithead et al., 1986]. SCFA have also an effect on the proteins secreted by intestinal cells [Nishimura et al., 1998], modifying their rate and pattern. The goal of the present study was to determine whether the three main SCFA at concentrations found in the small intestine affect the secretion of one of the major apolipoproteins synthesized by intestine, apoA-IV. To evaluate the influence of the observed modifications on lipoprotein metabolism and cholesterol homeostasis, we tested the capabilities of the secreted lipoproteins to promote cholesterol efflux *in vitro* from Fu5AH, a well established cell model.

MATERIALS AND METHODS

Materials

DMEM, EMEM, glutamine, penicillin, streptomycin, trypsin-EDTA, sodium butyrate, propionate and acetate, agarose, acrylamide and bis (N,N'-methylene-bis-acrylamide), and PVDF Immobilon membranes were obtained from Sigma Chemicals Co. (Saint Quentin Fallavier, France). Fetal bovine serum was supplied by Gibco BRL (Karlsruhe, Germany). 1,2 α [N]³H-Cholesterol (1 μ Ci/ μ l) was obtained from Dupont-NEN England Nuclear (Boston, MA). ApoAIV specific oligonucleotide primers were synthesized by Genosys (Cambridge, UK).

Culture of Caco-2 Cells

Caco-2 cells constitute a cell line, derived from a human colon carcinoma, that differentiates spontaneously and possesses many characteristics of enterocytes [Pinto et al., 1983]. They were grown in 75 cm² flasks. When the cell layer became nearly confluent, cells were dissociated with 0.5% Trypsin and 0.2 g/L EDTA. Cells were

then plated at 4×10^4 onto a 6-well plates. Cells were grown in DME media containing 20% fetal bovine serum (FBS), 2 mM glutamine, 50 U/ml penicillin, and 50 mg/ml streptomycin, under humidified atmosphere at 37°C, 5% CO₂. The culture medium was changed every 2 days after the cells reached confluency. All experiments were conducted with 14 days post-confluence cells. At this time the differentiation-dependent expression of their functional properties typical of small intestinal enterocytes is maximal. At the start of each experiment, the maintenance medium was removed. Monolayers were washed three times with serum-free medium. To study the effect of SCFA on apolipoprotein secretion, differentiated cells were incubated with SCFA for 24 or 48 h at the following concentrations: 2, 4, and 8 mM.

Nondenaturing 2D-PAGE

The distribution of apoAI-containing lipoproteins in human serum and ApoA-IV-containing lipoproteins in Caco-2 cells media was determined by nondenaturing 2D-electrophoresis in which agarose gel was followed by polyacrylamide gradient gel electrophoresis [Asztalos et al., 1993]. Briefly, in the first dimension, 10 μ l of human serum or 20 μ g of secreted proteins by Caco-2 cells were separated by electrophoresis at 4°C on 0.7% agarose gel with a tris-tricine buffer (25 mM, pH 8.6), containing 3 mM calcium lactate and 0.05% sodium azide.

Samples were electrophoresed at constant voltage (250 V) until the bromophenol blue ran 3.5 cm and maintained at 4°C with a recirculating cooler. Agarose gel strips containing the pre-separated lipoproteins were then transferred to a 2 to 36% polyacrylamide gradient gel and sealed with the same agarose.

Separation in the second dimension was performed at 220 V for 24 h at a constant temperature of 10°C. Lipoproteins were transferred to PVDF immobilon paper using 20 mM Tris and 150 mM glycine buffer pH 8.4, at constant voltage and temperature (30 V at 10°C) for 24 h. ApoA-I-containing lipoproteins in human and ApoA-IV-containing lipoproteins in Caco-2 cells media were identified by the use of a rabbit anti-human apoA-I antibody and rabbit anti-human apoA-IV antibody respectively, and an anti-rabbit peroxidase conjugated IgG (H + L) (BIOSYS).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from cells using a TRIzol reagent (Life Technologies Cergy Pontoise). RT-PCR was carried out using the following primers: (ApoAIV) forward, 5' AGTC-ACACTGAGGAAGGAGG 3'; reverse, 5' CTCC-TTGGCATTGTTGCTCAGC 3' and (ApoAI) forward, 5' CTTGCTATCTCTTGCCT3'; reverse, ATTTCTGGCAGCAAGATG3': (semi-quantitative mRNA detection was performed as follows. Briefly, reverse transcription was carried out in 20 μ l containing 1 μ g of RNA, 1 μ l of hexanucleotides pd N6 (100 pM), 4 μ l of 5X reverse transcriptase buffer (Life Technologies), 1 μ l of desoxynucleotide triphosphate (10 mM each), 2 μ l dithiothreitol (0.1 M), 1 μ l murine Moloney leukemia virus (MMLV) reverse transcriptase (200 U) (Life Technologies). cDNA first-strand synthesis was performed at 40°C for 45 min.

Five microliters of the reverse transcriptase mix was added to 50 μ l of PCR buffer containing 5 μ l 10X PCR buffer, 1 μ l of dNTP (10 mM), 3 μ l of MgCl₂ (25 mM), 0.1 μ l of 0.5 U DNA Polymerase (Gold Star Red from Eurogentec) and 1 μ l of forward and reverse PCR primers (50 pmole). After a 3 min incubation at 95°C, PCR cycles consisted of 40 s at 95°C, 40 s at 58°C, and 50 s at 72°C. RT-PCR consisted of 35 cycles. Amplified DNA was separated through a 1.5% agarose gel and visualized by ethidium bromide staining. The size of the amplified fragment corresponded to an amplification of cDNA rather than genomic DNA.

Quantification of Lipids and Apolipoproteins

ApoA-IV was quantified by electroimmunodiffusion in 1% agarose gels, using a rabbit anti-human apoA-IV antiserum from the laboratoire de biochimie clinique et moléculaire, UFR de Pharmacie, Lille, France. ApoAI was quantified by the same method using a rabbit anti-human apoAI antibody from (Sebia, France). Briefly, the samples were subjected to electrophoresis at 100 V/plate for 4 h at room temperature. Immunoprecipitates were stained and the peaks were measured.

Phospholipids were measured by enzymatic procedure with kit purchased from BioMérieux, Lyon, France. Briefly, 10 μ l of medium from Caco-2 cells where subjected to hydrolysis by

phospholipase D and the liberated choline was measured by Trinder reaction.

Cholesterol Efflux

After the incubation of Caco-2 cells with SCFA, the medium was collected and used for cholesterol efflux measurement. Culture medium (6 ml) was concentrated to 1 ml by a concentrator device (Centricon, Amicon, USA). The ability of concentrated samples to efflux free cholesterol was measured by a procedure previously described by [De la Llera Moya et al., 1994] using ³H-cholesterol-labeled Fu5AH. Briefly, Fu5AH, were cultured in EMEM medium containing 10% FCS. Penicillin (100 IU/ml) streptomycin (100 μ g/ml), and glutamine (2 mM) were present in all media.

For efflux experiments, cells were plated in costar 24 plates and were grown in appropriate medium containing 10% FCS at 37°C in a humidified 5% CO₂ atmosphere.

When they were nearly confluent, cells were incubated for 24 h at 37°C with 1 μ ci/ml of [1,2-³H]cholesterol, 10% FCS in EMEM. To ensure the label was evenly distributed among cellular pools, the labeling medium was replaced with EMEM containing 1% BSA, and cells were incubated in albumin for 18–20 h prior to measuring cholesterol efflux.

The cells were then washed and incubated with the indicated acceptors prepared in EMEM and efflux was performed for 18 h.

Radioactivity was then measured in the medium plus the cells by liquid scintillation counting, and cholesterol efflux was expressed as the ratio of radioactivity in the medium to the total radioactivity in the medium and in the cells. Radioactivity in the cell fraction was determined after overnight extraction in isopropanol.

Other Procedures

Protein concentrations were measured according to Lowry's method [Lowry et al., 1951] using bovine serum albumin as the standard.

For statistical analyses, we used an analysis of variance (PROC. ANOVA) to compare several means. When the ANOVA demonstrated a significant difference between the means, we used Bonferoni *t*-test to compare each group with each other.

RESULTS

Butyrate Increases apoA-IV Gene Expression and Protein Secretion in Caco-2 Cells

To investigate the influence of SCFA on apoA-IV secretion, Caco-2 cells were treated for 48 h with butyrate, acetate, and propionate (2, 4, and 8 mM). ApoA-IV concentration was measured in the medium at the end of incubation times. Among the SCFA tested only butyrate significantly increased apoA-IV concentration (butyrate 2, 4, and 8 mM) (38 ± 5 , 37 ± 3 , and 45.8 ± 16 , respectively) (micrograms ApoA-IV/mg cell proteins, means \pm SD, $n = 6$ experiments on different days) vs. (Control 17 ± 4) (micrograms ApoA-IV/mg cell protein means \pm SD) $P < 0.05$ (Fig. 1A and B). No change was observed for apoAI when cells were incubated with the 3 SCFA concentration (Fig. 2). To determine if the increase of apoA-IV secretion when incubating cells with butyrate 8 mM was time dependent, we performed a kinetic study (Fig. 3) which demonstrated that 48 h was necessary to observe the effect of butyrate. With butyrate, there was an increase in the molar ratio between apoAIV and apoAI as shown in Table I. To determine whether the increase in apoA-IV concentration after butyrate treatment was due to an increased expression of apoA-IV gene, apoA-IV mRNA levels were analyzed by RT-PCR in parallel with apoAI mRNA. ApoA-IV mRNA levels increased significantly after 48 h of butyrate addition, while no effect was observed for ApoAI mRNA (Fig. 4).

Stimulation of apoA-IV by butyrate was associated with a significant increase in phospholipid secretion (1.7-, 7-, and 16-fold, (Butyrate 2 mM, $P < 0.05$), (Butyrate 4 mM, $P < 0.001$) and (Butyrate 8 mM, $P < 0.001$) respectively vs. Control) (Fig. 5). Addition of propionate or acetate did not significantly alter phospholipid output (data not shown).

TABLE I.

Treatment	ApoA-IV/ ApoAI molar ratio
Control	0.52
But 2mM	1.4
But 4mM	2.34
But 8mM	2.74

Cells were incubated with varying concentrations of butyrate (2, 4 and 8 mM) for 48 hours at 37°C. At the end of the incubation, culture medium was concentrated and assayed for ApoA-IV and ApoAI by electroimmunodiffusion and molar ratios were calculated.

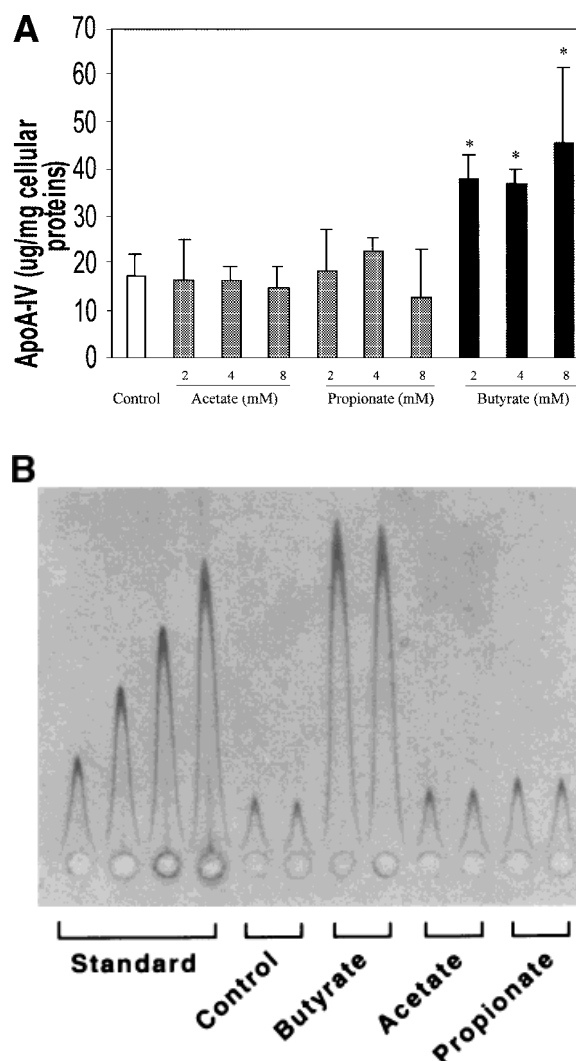


Fig. 1. Effect of SCFA on apoA-IV secretion by Caco-2 cells. **A:** Cells were incubated with varying concentrations of SCFA (acetate, propionate, or butyrate) (2, 4, and 8 mM) for 48 h at 37°C. At the end of the incubation, culture medium was concentrated and assayed for ApoA-IV by electroimmunodiffusion using a rabbit anti-human apoA-IV antiserum and expressed in terms of total cellular proteins. Statistical significance was tested against results from controls. Data are means \pm SD, $n = 6$ experiments. * $P < 0.05$. **B:** A 1% agarose gel picture showing the electroimmunodiffusion of apoA-IV secreted by Caco-2 cells incubated with SCFA (8 mM). After 4 h electrophoresis, immunoprecipitates were stained and peaks were measured. The concentration were determined using serum human apoAIV as a standard.

Characterization of Secreted apoA-IV-Containing Lipoproteins by 2D-PAGE

In order to determine whether butyrate affected the size of lipoprotein particles containing ApoA-IV, Caco-2-conditioned media were

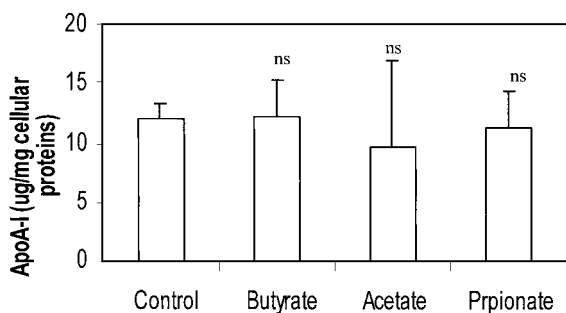


Fig. 2. Effect of SCFA on apoA-I secretion by Caco-2 cells. Cells were incubated with SCFA (acetate, propionate, or butyrate) (8 mM) for 48 h at 37°C. At the end of the incubation, culture medium was concentrated and assayed for ApoA-I by electroimmunodiffusion using a rabbit anti-human apoA-I antibody and expressed in terms of total cellular proteins. Statistical significance was tested against results from controls. Data are means \pm SD, n = 6 experiments. ns = non specific.

concentrated and 20 μ g of total protein were separated by 2D-PAGE.

Figure 6A shows the distribution of prebeta-HDL particles and α -HDL particles after non-denaturing 2D-PAGE of human plasma and immunoblot with antibody against human apoAI. Figure 6B presents the distribution of apoA-IV containing lipoproteins after non-denaturing 2D-PAGE of Caco-2 conditioned media (cells treated with 8 mM) (see Materials and Methods). Caco-2 cells secrete 2 HDL particles (LpA-IV_a and LpA-IV_b) having prebeta and slow alpha mobility respectively as compared to ApoAI-containing particles in human serum.

In basal conditions (0 mM butyrate), Caco-2 cells secrete a HDL particle (LpAIV_a) with pre β

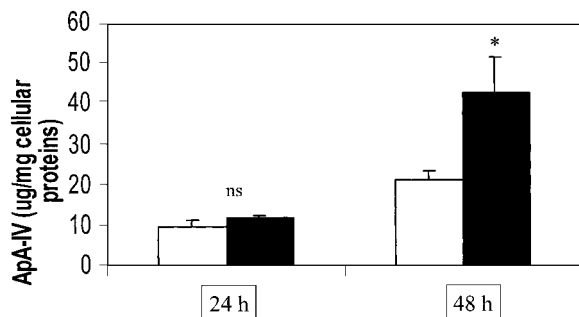


Fig. 3. Time effect of butyrate on apoA-IV secretion. Caco-2 cells were incubated for 24 and 48 h with butyrate (8 mM). At the end of the incubation, culture medium was concentrated and assayed for ApoA-IV by electroimmunodiffusion using a rabbit anti-human apoA-IV antibody and expressed in terms of total cellular proteins. Statistical significance was tested against results from controls. Data are means \pm SD, n = 6 experiments. ns = non specific. * P < 0.05.

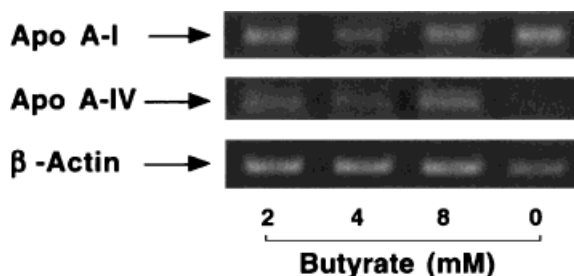


Fig. 4. Effect of butyrate on ApoA-I and ApoA-IV mRNA accumulation in Caco-2 cells. Cells were incubated with varying concentrations of butyrate (2, 4, and 8 mM) for 48 h at 37°C. At the end of the incubation total RNA was extracted from cells (see Materials and Methods) and RT-PCR was carried out on 1 μ g RNA. Amplified DNA was separated through a 1.5% agarose gel and visualized by ethidium bromide staining. The photogel is representative of three separate experiments.

mobility having a stoke's diameter of 7.5 nm, butyrate (2, 4, and 8 mM) treated cells secrete a large amount of this pre β particle. Moreover, Caco-2 cells treated with 8 mM butyrate, secrete an additional HDL particle (LpAIV_b) which migrates faster, with slow α -mobility. This particle is larger than LpAIV_a, with a stoke's diameter of 9.5 nm (Fig. 7).

Cholesterol Efflux From Fu5AH Cells Into Caco-2 Conditioned Media

Cholesterol efflux studies using conditioned medium obtained from Caco-2 cells treated with varying amounts of butyrate showed an increase in cholesterol efflux (20% for butyrate 2 mM, P < 0.05 and 30% for butyrate 4 mM,

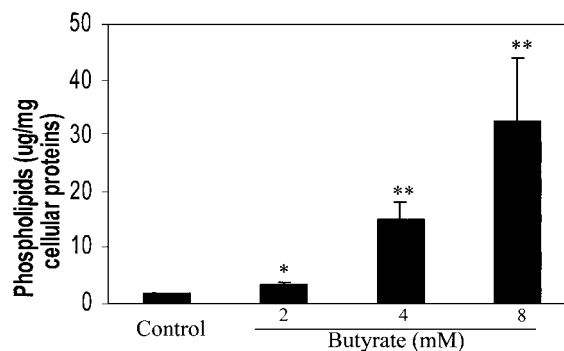


Fig. 5. Effect of SCFA on phospholipid secretion. Cells were incubated with varying concentrations of Butyrate (2, 4, and 8 mM) for 48 h at 37°C. At the end of the incubation, culture medium was concentrated and assayed for phospholipids. Statistical significance was tested against results from controls. Data are means \pm SD, n = 6 experiments. * P < 0.05, ** P < 0.001.

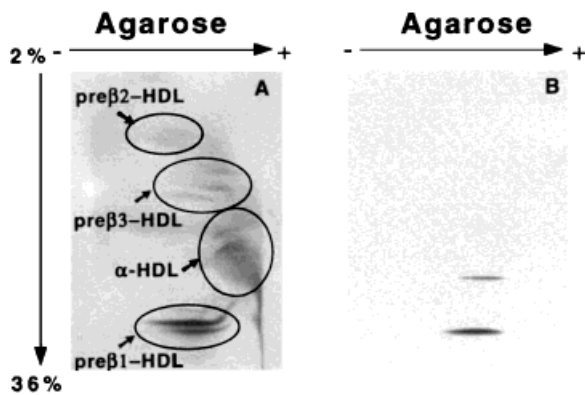


Fig. 6. Nondenaturing 2D electrophoresis of (A) 10 µl human serum and (B) 20 µg secreted proteins from caco-2 cells incubated with 8 mM butyrate. The migrations were performed in the sequence agarose gel electrophoresis → nondenaturing polyacrylamide gel electrophoresis (PAGE). After electroblotting to PVDF immobilon membranes, ApoA-I-containing HDL particles in human serum was detected using a rabbit anti-human apoA-I antibody, ApoA-IV-containing lipoproteins in Caco-2 cells medium were detected using a rabbit anti-human ApoA-IV antibody. LpA-IV_a and LpA-IV_b have prebeta and slow alpha HDL particles respectively as compared to ApoA-I-containing particles in human serum.

$P < 0.001$), as measured by the release of ³H-cholesterol from Fu5AH into the culture medium (Fig. 8). No significant difference was observed for cholesterol efflux for medium from Caco-2 cells pretreated with 8 mM of butyrate.

DISCUSSION

In this study using the Caco-2 cell system, we have delineated the effect of SCFA, naturally occurring molecules produced by intestinal resident bacteria, on various cellular processes

involved in HDL metabolism. To our knowledge, the current study is the first to demonstrate that Butyrate significantly increased the synthesis and secretion of ApoA-IV in the culture medium. The presence of SCFA in both colon and small intestine has been known for many years [Cummings et al., 1987] as well as the presence of the bacteria which produce polysaccharides degrading enzymes. Because total SCFA concentrations in jejunum were found to reach 1 mM, rising to 13 mM in the ileum [Cummings et al., 1987], it is physiologically relevant to assess their role in the synthesis and secretion of ApoA-IV in differentiated Caco-2 cells. However, the observed effect on apoA-IV secretion appears only after a 48 h incubation time which indicates that a long exposure to butyrate would probably be necessary to have an effect in-vivo. The question may be raised if its butyrate which plays a role or one of its metabolites. The Caco-2 cell model indeed is considered a useful model for enterocyte function [Pinto et al., 1983]. Several authors were unsuccessful in their attempt to detect apoA-IV in Caco-2 cells cultured on plastic dishes. By contrast, it was possible, in our experiments, to find apoA-IV in the medium of cells even in the absence of SCFA. The reasons for this discrepancy remain unclear. Several hypotheses could be raised: an influence of culture conditions or a better sensitivity of our detection methods. In our case, several lines of evidence were obtained: (1) detection of apoAIV in the medium by electroimmunodiffusion, (2) characterization of the secreted lipoprotein particles by 2D-PAGE, and (3) analysis of mRNA by RT-PCR. Since acetate

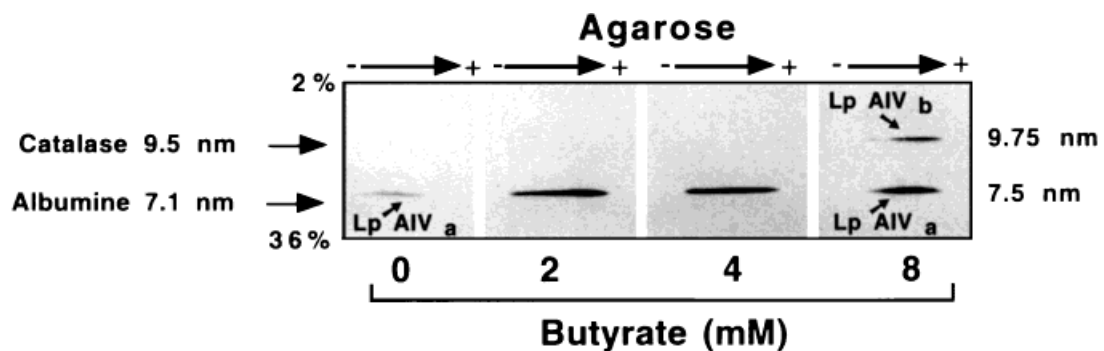


Fig. 7. 2D-electrophoresis and immunoblotting of ApoA-IV-containing lipoproteins in (0) control cells and (2, 4, and 8 mM) butyrate treated cells. Nondenaturing 2D electrophoresis of 20 µg secreted proteins was performed in the sequence agarose gel electrophoresis → nondenaturing PAGE. After electroblotting to PVDF immobilon membranes, ApoA-IV-containing

lipoproteins were detected using a rabbit anti-human ApoA-IV: LpA-IV_a was observed in control cells (at very low amount) and in butyrate treated cells 2, 4, and 8 mM (at high concentration) and LpA-IV_b only in butyrate (8 mM) treated cells. The photo gel is representative of two separate experiments.

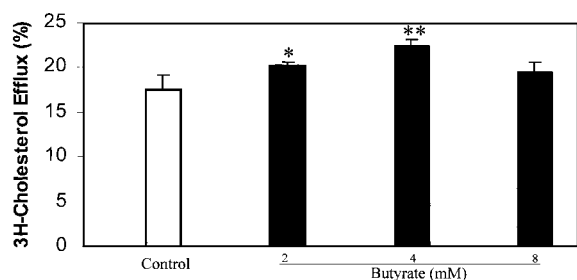


Fig. 8. [³H]-cholesterol efflux from fu5AH into the cell culture medium containing medium from Caco-2 cells treated with butyrate. Fu5AH cells were labeled with [³H]-cholesterol for 24 h, washed and incubated for another 24 h in the presence of BSA 1 mg/ml. Each monolayer received 0.5 ml containing 0.1 mg of proteins derived from non-treated cells (Control) and from butyrate treated Caco-2 cells (2, 4, and 8 mM). At the incubation time indicated, the cholesterol in the medium was measured as described under "experimental procedures". Data are means \pm SD, n=3 experiments in triplicates. $P < 0.05$, ** $P < 0.001$.

and propionate did not alter the secretion of ApoA-IV, the stimulatory effect of butyrate on ApoA-IV synthesis could be specific. However, as shown by RT-PCR, the effect of butyrate on ApoA-IV mRNA is much greater than its effect on the protein secretion. This would suggest that post-transcriptional events may be important for the enhancement of secretion rates. A potential mechanism could be a lack of full ApoA-IV secretion as a consequence of its intracellular degradation. One of the origin of this intracellular degradation could be the inability to pack ApoA-IV into lipoproteins due to the lack of lipids in sufficient quantities. Therefore, the inability of propionate and acetate to induce apoA-IV mRNA and protein secretion as well as apoAI mRNA and protein secretion, secretion may be due to their inability to sufficiently enhance lipid synthesis, specially phospholipids (data not shown). Butyrate leads to an increase in both phospholipids and apoA-IV synthesis. However, slight relative excess of ApoA-IV in comparison with lipids is most probable. Actually, phospholipid secretion increases in a dose dependent manner with butyrate, with levels almost double at 8 mM compared with 4 mM. In contrast apoAIV secretion reaches its maximum at 2 mM and does not increase thereafter. It may be hypothesized that this relative excess of apoAIV compared with lipids could lead to degradation of part of the protein molecules as it has been shown for other apolipoproteins [Fast et al., 1993; Kurokawa et al., 1995].

The mechanisms by which butyrate alters apoA-IV expression remains to be elucidated. However, butyrate affects the expression of several other genes and different possible mechanisms of its action have been proposed. They include (1) changes in chromatin structure [Candido et al., 1978], (2) alterations in enterocytic differentiation [Chung et al., 1985], and (3) induction of histone acetylation [Nishimura et al., 1998].

Because we found that butyrate has a dual effect on both lipid and apoA-IV secretion in Caco-2 cells, we assessed its effect on the secretion of Apo-AIV-containing lipoproteins, using two-dimensional electrophoresis based on electrophoresis mobility and size. This method has been used successfully to establish the presence of several distinct HDL subpopulations [Asztalos et al., 1993; Castro and Fielding, 1988]. Our 2D-PAGE and subsequent anti-apoA-IV immunoblotting showed an increase of apoA-IV in particles named LpAIV_a in Caco-2 cells treated with butyrate (2, 4, and 8 mM) and secretion of a new particle LpA-IV_b in Caco-2 cells treated with 8 mM. LpAIV_a and LpAIV_b are HDL-sized lipoproteins having a stoke's diameter of 7.5 and 9.5 nm respectively. These particles were detected into two subfractions with pre β -mobility as well as slow α -mobility. They form sharp bands, probably because they are homogeneous lipid-poor lipoproteins. To our knowledge, this is the first time that such particles are characterized in the medium culture of Caco-2 cells. However, our findings may be analyzed in view of the results obtained in human plasma by von Eckardstein et al. [1995]. Like in our case, this author found in human plasma three different LpA-IV particles exhibiting an HDL size. Some of them with an α -mobility and some of them with a slow α -mobility. The difference in the electrophoretic mobility may be explained by different metabolic events which may affect plasma lipoproteins while those found in our experiments are crude particles. Those particles could belong to a pool of lipoproteins directly in contact with peripheral cells and therefore involved in the very first step of the so called reverse cholesterol transport process, namely cholesterol efflux. To assess the capabilities of the selected LpA-IV particles in this process, we used a cell culture system based on Fu5AH. The choice of this cell line was motivated by several reasons, as (1) it specifically binds to HDL [Karlin et al., 1987]

and (2) most importantly, it exhibits the most rapid efflux of any cell line [Rothblat et al., 1986].

Incubation of medium from Caco-2 cells treated with butyrate removed significantly 20–30% of ^3H -Cholesterol from Fu5AH cells. It is likely that this uptake of ^3H -cholesterol is due to the presence of these two particles in the medium. Since these two particles are very poor in cholesterol and rich in phospholipids, any appearance of radioactivity in the presence of them indicates a specific movement of ^3H -cholesterol from the Fu5AH cells suggesting that these particles may serve as initial acceptors of cell derived cholesterol. Another line of evidence is that butyrate did not stimulate neither ApoAI mRNA nor ApoAI output compared to basal conditions after 48 h of incubation. As shown in Table I increasing butyrate concentration leads to an increase in the molar ratio apoAIV/ApoAI. ApoAI and apoA-IV are both synthesized by the intestine and play a major role in cholesterol efflux. Therefore, it may be concluded that butyrate, in concentration close to those expected in vivo, stimulates the secretion of lipoprotein particles enriched in ApoA-IV and exhibiting the capability to remove cholesterol from peripheral cells. However, it is difficult to draw on what would happen in vivo from our experiments. Actually the Caco2 model has its limitation, although this cell line present some differentiated features after confluence that have been considered characteristics of enterocytes. Nevertheless, we have experience done on human intestinal biopsy (data not shown) indicating that incubation of human cells with butyrate 8 mM leads to 3-fold increase in apoAIV secretion. This would argue in favor of a similar effect in vivo. However, our finding should lead to further studies in vivo to evaluate the effect of a supplementation with butyrate, either directly or through the effect of bacterial enzyme on naturally occurring polysaccharides in diet.

In summary, this study demonstrates for the first time that (1) butyrate stimulates apoA-IV synthesis and secretion by intestinal Caco-2 cells, (2) phospholipids secretion are linked to apoA-IV secretion, and (3) the conditioned media containing LpA-IV_a and LpA-IV_b secreted after stimulation by butyrate are acceptors of Fu5AH cells-derived cholesterol. These results make butyrate very attractive as a potential pharmacologic agent to raise ApoA-

IV-containing HDL, since these HDL particles play a role in cholesterol efflux, the first step in reverse cholesterol transport.

Table I ApoAIV/apoAI molar ratio. Cells were incubated with varying concentrations of butyrate (2, 4, and 8 mM) for 48 h at 37°C. At the end of the incubation, culture medium was concentrated and assayed for apoAIV and apoAI by electroimmunodiffusion and molar ratios were calculated.

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