Iron-ascorbate alters the efficiency of Caco-2 cells to assemble and secrete lipoproteins

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Received 30 July 1999; accepted in final form 4 January 2000

Courtois, F., I. Suc, C. Garofalo, M. Ledoux, E. Seidman, and E. Levy. Iron-ascorbate alters the efficiency of Caco-2 cells to assemble and secrete lipoproteins. Am J Physiol Gastrointest Liver Physiol 279: G12–G19, 2000.—Although oxidative stress has been implicated in development of gut pathologies, its role in intestinal fat transport has not been investigated. We assessed the effect of Fe2+-ascorbate-mediated lipid peroxidation on lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly and secretion. Incubation of postconfluent Caco-2 cells with iron(II)-ascorbate (0.2 mM/2 mM) in the apical compartment significantly promoted malondialdehyde formation without affecting sucrase activity, transepithelial resistance, DNA and protein content, and cell viability. However, addition of the oxygen radical-generating system reduced 1) [14C]oleic acid incorporation into cellular triglycerides (15%, P < 0.0002) and phospholipids (16%, P < 0.0005); 2) de novo synthesis of cellular apolipoprotein A-I (apo A-I) (18%, P < 0.05), apo A-IV (38%, P < 0.05), and apo B-48 (45%, P < 0.003) after [35S]methionine addition; and 3) production of chylomicrons (50%), VLDL (40%), LDL (37%), and HDL (30%) (all P < 0.0001). In contrast, increased total cellular cholesterol formation (96%, P < 0.0001), assayed by [14C]acetate incorporation, was noted, attributable to marked elevation (70%, P < 0.04) in activity of ol-3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis. The ratio of Aoyl-CoA to cholesterol acyltransferase, the esterifying cholesterol enzyme, remained unchanged. Fe2+-ascorbate-mediated lipid peroxidation modifies intracellular fat absorption and may decrease enterocyte efficiency in assembling and transporting lipids during gut inflammation.

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the possible effects of the \( \text{Fe}^{2+} \)-ascorbate oxygen-radical generating system on lipid esterification and synthesis, apolipoprotein biogenesis, and lipoprotein assembly and secretion.

**MATERIALS AND METHODS**

Cell culture. Caco-2 cells (American Type Culture Collection, Rockville, MD) were grown at 37°C with 5% \( \text{CO}_2 \) in MEM (GIBCO BRL, Grand Island, NY) containing 1% penicillin/streptomycin and 1% MEM nonessential amino acids (GIBCO BRL) and supplemented with 10% decomplemented fetal bovine serum (FBS; Flow, McLean, VA). Caco-2 cells (passages 30–40) were maintained in T-75 cm² flasks (Corning Glass Works, Corning, NY). Cultures were split (1:6) when they reached 70–90% confluence, using 0.05% trypsin-0.5 mM EDTA (GIBCO BRL). For individual experiments, cells were plated at a density of \( 1 \times 10^5 \) cells/well on 24.5-mm polycarbonate Transwell filter inserts with 0.4-µm pores (Costar, Cambridge, MA), in MEM (as described above) supplemented with 5% FBS. The inserts were placed into six-well culture plates, permitting separate access to the upper and lower compartments of the monolayers. Cells were cultured for 20 days, a period at which the Caco-2 cells are highly differentiated and appropriate for lipid synthesis (31, 32). The medium was refreshed every second day.

Iron(II)-ascorbate (1:10, Sigma, Montreal, PQ, Canada) was then added to cells at different concentrations for 24 h. Suracte activity was measured as a marker of cell differentiation and transepithelial resistance as a marker of monolayer integrity (Millipore, Bedford, MA) (31, 32). Cell viability was assessed by trypan blue exclusion (32). Furthermore, DNA integrity (Millipore, Bedford, MA) (31, 32). Cell viability was assessed by trypan blue exclusion (32). Furthermore, DNA and protein content was evaluated as previously described (29, 32). All these parameters were tested in the presence or absence of \( \text{Fe}^{2+} \)-ascorbate.

Estimation of lipid peroxidation. Caco-2 cells were cultured in the presence or absence of \( \text{Fe}^{2+} \)-ascorbate. The reaction was terminated by the addition of 0.2% butylated hydroxytoluene (2,6-di-t-butyl-p-cresol, BHT, Sigma) to measure malondialdehyde (MDA), as an index of lipid peroxidation. The amount of free MDA formed during the reaction was determined by HPLC as described previously (6). Proteins were first precipitated with 10% sodium tungstate (Sigma) (Aldrich Chemical) solution, and protein-free supernatant was then reacted with an isovolume of 0.5% thiobarbituric acid (Sigma) solution at 90°C for 30 min. After cooling to room temperature, chromogen was extracted with 1-butanol and dried over a stream of nitrogen at 37°C. The dry extract was then resuspended in \( \text{K}_2\text{HPO}_4 \)/methanol (70:30, \( \text{pH} 7.0 \)) mobile phase before MDA detection by HPLC.

Measurement of lipid synthesis and secretion. Lipid synthetase and secretion were assayed as previously described (32, 42). Briefly, radiolabeled [\( ^{14} \text{C} \)]oleic acid (sp act, 53 mCi/mmol; Amersham, Oakville, ON, Canada) was added to unlabeled oleic acid and then solubilized in fatty acid-free BSA (BSA/oleic acid, 1.5:1 mol/mol). The final oleic acid concentration was 0.7 mM (0.45 µCi/well). Cells were first washed with PBS (GIBCO), and the [\( ^{14} \text{C} \)]oleic acid-containing medium was added to the upper compartment. \( \text{Fe}^{2+} \)-ascorbate (0.2 mM, 0.2 mM) was added to the upper chamber in serum-free MEM. At the end of a 24-h incubation period, cells were washed, then scraped with a rubber policeman in a PBS solution containing antiproteases (phenylmethylsulfonyl fluoride, pepstatin, EDTA, aminocaproic acid, chloramphenicol, leupeptin, glutathione, benzamidine, dithiothreitol, sodium azide, and Trasylol, all at a final concentration of 1 mM). An aliquot was taken for lipid extraction by standard methods (27) in the presence of unlabeled carrier (phospholipids (PL), monoglycerides, diglycerides, triglycerides (TG), free fatty acids, free cholesterol (FC), and cholesteryl ester (CE)).

The various lipid classes synthesized from [\( ^{14} \text{C} \)]oleic acid were then separated by TLC using the solvent mixture of hexane, ether, and acetic acid (80:20:3, vol/vol/vol), as previously described (27, 28). The area corresponding to each lipid was scraped off the TLC plates, and the silica powder was placed in a scintillation vial with Ready Safe counting fluid (Beckman, Fullerton, CA). Radioactivity was then measured by scintillation counting (LS 5000 TD, Beckman). Cell protein was quantified by the Bradford method, and results were expressed as dpm per milligram of cell protein. Lipid secreted in the basolateral compartment was analyzed and quantified, as described above, after centrifugation (2,000 rpm for 30 min at 4°C) to remove cell debris.

Cholesterol biogenesis was evaluated employing [\( ^{14} \text{C} \)]acetate as precursor (53.9 Ci/mmol) after a 24-h incubation period. Separation of FC and CE was performed by TLC.

Lipid carrier. Blood was drawn 2 h after the oral intake of a fat meal by human volunteers, and postprandial plasma was prepared to serve as a carrier for the lipoproteins synthesized by Caco-2 cells. The TG-enriched plasma was incubated at 56°C for 1 h to inactivate enzymatic activity in the presence of antiproteases.

Isolation of lipoproteins. For the determination of secreted lipoproteins, Caco-2 cells were incubated with the lipid substrate as described above, in the presence or absence of \( \text{Fe}^{2+} \)-ascorbate. The medium supplemented with antiproteases (as described above) was first mixed with a plasma lipid carrier (4:1, vol/vol) to efficiently isolate de novo lipoproteins synthesized. The lipoproteins were then isolated by sequential ultracentrifugation using a TL-100 ultracentrifuge (Beckman), as described previously (27, 28). Briefly, dylomicrons were isolated after ultracentrifugation (20,000 rpm for 20 min). Very low-density lipoprotein (VLDL) (1.006 g/ml) and low-density lipoprotein (LDL) (1.063 g/ml) were separated by spinning at 100,000 g for 2.26 h with a tabletop ultracentrifuge 100.4 rotor at 4°C. The high-density lipoprotein (HDL) fraction was obtained by adjusting the LDL infranatant to density at 1.21 g/ml and centrifuging for 6.5 h at 100,000 g. Each lipoprotein fraction was exhaustively dialyzed against 0.15 M NaCl and 0.001 M EDTA, \( \text{pH} 7.0 \), at 4°C for 24 h.

De novo apolipoprotein synthesis. The effect of \( \text{Fe}^{2+} \)-ascorbate on newly synthesized and secreted apolipoprotein A-I (apo A-I), apo A-IV, apo B-48, apo B-100, and apo E was assessed as described previously (25). To first induce apolipoprotein synthesis, cells were incubated apically with unlabeled oleic acid bound to albumin in serum-free medium, 24 h before \( [^{35} \text{S}] \)methionine incubation. The concentration of the unlabeled lipid was equivalent to the labeled substrate described above. During this time, \( \text{Fe}^{2+} \)-ascorbate was again added to the apical chamber. After a 24-h incubation, cells as well as the outer chambers were rinsed twice with PBS (GIBCO). The apical compartment was replaced with 1.5 ml of methionine-free medium containing the unlabeled substrate and \( [^{35} \text{S}] \)methionine (100 µCi/ml) (Amersham Life Sciences, 50 mCi/mmol). After incubation for 3 h at 37°C with 5% \( \text{CO}_2 \), the medium from the basolateral compartment was collected. Cells were scraped off the inserts in the cell lysis buffer, as described above. The medium and cell lysates were supplemented with the antiprotease cocktail. To assay a considerable amount of de novo apolipoprotein synthesis, the material from two wells was pooled.

Immunoprecipitation of apolipoproteins. The medium and cell lysates were first supplemented with unlabeled methionine to act as a carrier (final concentration, 0.1 mM). Immuno-
precipitation was performed in the presence of excess polyclonal antibodies to human apolipoproteins (Boehringer Mannheim) at 4°C overnight (25, 26). Samples were then washed with Nonidet P-40 (0.05%). They were subsequently centrifuged and resuspended in sample buffer (1.2% SDS, 12% glycerol, 60 mM Tris, pH 7.3, 1.2% β-mercaptoethanol, and 0.003% bromophenol blue) and analyzed by a linear 4–15% polyacrylamide gradient preceded by a 3% stacking gel, as previously described. Radioactive molecular weight standards (Amersham Life Sciences) were run in the same conditions. Gels were sectioned into 2-mm slices and counted after an overnight incubation with 1 ml of Beckman tissue solubilizer (0.5 N quaternary ammonium hydroxide in toluene) and 10 ml of liquid scintillation fluid (Ready Organic, Beckman). Results for each apolipoprotein studied were expressed as percent dpm/mg protein to assess the specific effect of Fe^{2+}-ascorbate on apolipoprotein synthesis and secretion.

Preparation of microsomes. Microsome fractions were prepared as previously described (22). Briefly, Caco-2 cells were incubated with Fe^{2+}-ascorbate for 24 h and then rinsed, homogenized, and centrifuged for 10 min at 10,000 g at 4°C. The supernatant fraction was centrifuged for 60 min at 100,000 g at 4°C. The washed microsomal pellets were quick frozen and stored at −80°C for later use.

Assay of microsomal HMG-CoA reductase activity. Microsomal enzymatic activity was assayed as described previously (6, 22). The reaction mixture contained 100 mM potassium phosphate (pH 7.4), 150 µg microsomal protein, 20 mM glucose-6-phosphate, 12.5 mM dithiothreitol, 2.5 M NADP, and 1.2 U glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of [3-¹⁴C]-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), a labeled intermediate in the cholesterol synthesis pathway. After incubating for 30 min at 37°C, the reaction was terminated by adding chloroform/methanol (2:1, vol/vol) followed by [³H]cholesterol esterification into lactone, isolated by TLC, and counted using an internal standard to correct for incomplete recovery. HMG-CoA reductase activity was expressed as pimoles of mevalonate synthesized per milligram of protein per minute.

Microsomal ACAT activity. The standard acyl-CoA:cholesterol acyltransferase (ACAT) determination was based on our previous assay (6, 22). We added 5 nmol [¹⁴C]oleoyl CoA (specific activity, -10,000 dpm/nmol) to the mixture containing 150 µg microsomal protein to initiate the reaction in a buffer solution (pH 7.5) consisting of cholesterol, 0.04 mol/l KH₂PO₄, 50 mM NaF, 0.25 mol/l sucrose, and 1 mM EDTA. After incubating for 10 min at 37°C, the reaction was stopped by adding chloroform/methanol (2:1, vol/vol) followed by [³H]cholesterol esterification into an internal standard to estimate recovery.

Statistical analysis. All values were expressed as means ± SE. Data were analyzed by two-tailed Student’s t-test.

RESULTS

MDA generation after Fe^{2+}-ascorbate exposure. The effectiveness of Fe^{2+}-ascorbate in initiating lipid peroxidation was tested after incubation with Caco-2 cells. At the end of a 24-h culture period, the degree of lipid peroxidation was determined by measuring MDA in medium and cells. As illustrated in Fig. 1, Fe^{2+}-ascorbate promotes the production of peroxidation above baseline values, and the formation of MDA increased with rising Fe^{2+}-ascorbate concentrations in a dose-dependent manner. Neither Fe^{2+} nor ascorbate alone could induce lipid peroxidation (data not shown). The concentration-response relationship for intestinal epithelial peroxidation was 10-fold higher in the apical medium than in cells.

Effect of Fe^{2+}-ascorbate on Caco-2 cell functional integrity. Caco-2 cell integrity and viability were assessed by sucrose activity, cell monolayer transepithelial resistance, DNA and protein content, and trypan blue exclusion after an incubation of 24 h. As shown in Fig. 2, transepithelial resistance and sucrose activity remained unchanged with increasing concentrations of Fe^{2+}-ascorbate. Similarly, the cell DNA and protein content was unaffected by the addition of the oxygen-radical generating system. At all the Fe^{2+}-ascorbate concentrations tested, the cell protein-to-DNA ratio did not show any significant variability. Cell viability by trypan blue exclusion was also assessed and was uniformly >90% in the absence or presence of Fe^{2+}-ascorbate.

Effect of Fe^{2+}-ascorbate on lipid synthesis and secretion. Lipid synthesis and secretion were determined in the presence and absence of Fe^{2+}-ascorbate after a 24-h incubation period. When oleic acid bound to BSA was presented to confluent Caco-2 cell monolayers, the total fatty acid incorporation was decreased in cells with Fe^{2+}-ascorbate (596,415 ± 9,960 dpm/mg protein) compared with cells without Fe^{2+}-ascorbate (698,208 ± 15,084 dpm/mg protein). A similar trend was also noted...
Effect of Fe\textsuperscript{2+}-ascorbate on microsomal sterol enzymes. In view of the impact of lipid peroxidation on cholesterol biogenesis, we determined the effect of Fe\textsuperscript{2+}-ascorbate on HMG-CoA reductase (the rate-limiting enzyme in cholesterol synthesis) and ACAT (the enzyme responsible for the acylation of cholesterol into CE). The incubation of microsomal preparations from Caco-2 cells with Fe\textsuperscript{2+}-ascorbate caused a marked elevation in HMG-CoA reductase activity (70%, \( P < 0.04 \)) without altering ACAT activity (Fig. 5).

Effect of Fe\textsuperscript{2+}-ascorbate on apolipoprotein synthesis. One of the major objectives of these studies was to examine the modulation of apolipoprotein elaboration by Fe\textsuperscript{2+}-ascorbate. The treatment with the oxidant system resulted in a significantly reduced yield of the main apolipoproteins normally synthesized by the intestine: A-I (18%, \( P < 0.05 \)), A-IV (38%, \( P < 0.05 \)), and B-48 (45%, \( P < 0.03 \)) (Fig. 6). Fe\textsuperscript{2+}-ascorbate also affected the exocytosis of apo B-100 (35%, \( P < 0.02 \)), apo B-48 (36%, \( P < 0.003 \)), and apo A-IV (58%, \( P < 0.0008 \)).

Effect of Fe\textsuperscript{2+}-ascorbate on lipoprotein secretion. As expected from the inhibition of lipid synthesis and apolipoprotein biogenesis, Fe\textsuperscript{2+}-ascorbate affected the secretion of all four classes of lipoproteins studied (Fig. 7). A significant reduction was noted in chylomicrons (50%, \( P < 0.0001 \)), VLDL (40%, \( P < 0.0001 \)), LDL (37%, \( P < 0.0001 \)), and HDL (30%, \( P < 0.0001 \)) as shown in Fig. 7.

in the medium (18,436 ± 907 vs. 24,206 ± 580 dpm/mg protein for cells with or without Fe\textsuperscript{2+}-ascorbate, respectively). This decrease was essentially accounted for by a reduction in TG (15%, \( P < 0.0002 \) in cells; 26%, \( P < 0.0001 \) in medium) and PL (16%, \( P < 0.0005 \) in cells; 17%, \( P < 0.03 \) in medium) (Fig. 3). In the second step of our studies, the effect of Fe\textsuperscript{2+}-ascorbate on Caco-2 cell cholesterol biogenesis was evaluated using \(^{14}C\)acetate. As can be seen in Fig. 4, Fe\textsuperscript{2+}-ascorbate significantly increased the incorporation of the radiolabeled precursor into total cholesterol. The raised level of total cholesterol (96%, \( P < 0.0001 \) in cells; 142%, \( P < 0.03 \) in medium) was attributable to the increase of both fractions of FC (105%, \( P < 0.0001 \)) and CE (67%, \( P < 0.0001 \)) in Caco-2 cells and to FC (193%, \( P < 0.03 \)) only in the medium.
Effect of BHT. To uncover whether lipid peroxidation was fully behind the aforementioned lipid and lipoprotein alterations, Caco-2 cells were cultured in the presence of BHT before incubation with Fe$_2^+$/ascorbate. As noted in Table 1, the preincubation of Caco-2 cells with BHT led to a protection against Fe$_2^+$/ascorbate-mediated lipid peroxidation. Indeed, BHT in contrast to other antioxidants tested, such as vitamin E and glutathione (results not shown), was effective in reducing MDA generation. Concomitantly, BHT improved lipid esterification, cholesterol synthesis, and CM formation.

**DISCUSSION**

The Caco-2 cell line has been used to examine a variety of intestinal functions, including nutrient absorption (24). This intestinal model forms a highly polarized monolayer, exhibits many of the features of small intestinal cells, and displays vectorial transport.
Because Caco-2 cells reproduce several of the normal physiological responses to various modulatory agents, we examined their usefulness to study the effect of Fe$^{2+}$-ascorbate-induced lipid peroxidation on the intracellular phase of lipid absorption, i.e., intraenterocyte esterification and resynthesis of lipolytic products, the biogenesis of apolipoproteins required for TG-rich particles, and lipoprotein secretion. Our results show for the first time that Fe$^{2+}$-ascorbate can safely affect the efficiency of Caco-2 cells to assemble and transport lipids in lipoprotein forms.

Iron occupies a central role in oxygen-radical chemistry, because it can initiate oxygen radical formation (9). Not only is it a catalyst in the Haber-Weiss reaction, but it is also involved in the initiation and propagation of lipid peroxidation (14). Although the mechanisms underlying the cytotoxicity of iron in different organs are not completely understood, many reports have pointed to the participation of iron-mediated peroxidation in numerous pathological states, including atherosclerosis (38), cancer (16), ischemia-reperfusion injury (10), inflammatory bowel disease (35), and conditions of iron overload (4). Several laboratories (3, 5, 19) have shown the ability of Fe$^{2+}$ to initiate strong lipid peroxidation, whereas ascorbic acid can amplify the oxidative potential of iron by promoting metal ion-induced lipid peroxidation. The data presented here clearly indicate that the Fe$^{2+}$-ascorbate system functioned as a producer of lipid peroxidation and, at the same time, altered the integrity of intracellular fat transport. It is noteworthy that the iron dose used in the current study is comparable to normal iron concentration in the gut (2). The deteriorations resulting from the exposure of Caco-2 cells to Fe$^{2+}$-ascorbate are probably attributable to oxidative stress, because the addition of the BHT antioxidant simultaneously prevented the occurrence of lipid peroxidation and improved the cellular processes of lipid absorption. Previous studies (19) have addressed the issue of glucose transport under the influence of in vitro peroxidation, employing guinea pig brush-border membrane vesicles. The peroxidative attack, initiated by Fe$^{2+}$-ascorbate, resulted in the reduction of sodium-dependent glucose transport (19). All these data, taken together, strongly support the causative derangement of nutrient transport by oxidative stress.

Numerous studies have already emphasized that essential fatty acids are absolutely necessary for 1) the control of microviscosity and membrane fluidity of most cells (3), 2) the regulation of membrane protein (5), and 3) the synthesis of eicosanoids, such as prostaglandins, leukotrienes, and related substances that have a profound influence on many transport processes (23, 40). Our understanding of how lipid peroxidation influences the intestinal phase, i.e., the formation of lipid-carrying lipoproteins, remains sketchy. Nevertheless, it is tempting to assume that a peroxidative attack on membranes could deplete essential fatty acid content, thereby modifying intracellular fat transport. Accordingly, our previous work (23) stressed the critical role of essential fatty acid deficiency in the biophysical and biochemical events involved in fat absorption. The peroxidative modification of unsaturated phospholipids in the endoplasmic reticulum (the site of lipid esterification, apolipoprotein synthesis, and lipoprotein assembly) could conceivably hamper enterocyte lipid transport. Ongoing exploration of the mechanisms involved will have an important bearing on our understanding of disease states associated with peroxidative stress, such as inflammatory bowel diseases (35). In particular, Crohn's disease is known for its overproduction of harmful oxidants, which have been implicated in the pathophysiology of chronic inflammation and intestinal injury. Peroxidative damage to the absorptive epithelial cells is likely a major contributor to impaired transport of electrolytes, trace metals, and vitamins as well as other micronutrients. Given the negative influence of both cytokines (32) and essential fatty acid deficiency (23) on fat absorption, our data suggest that the combination of excessive proinflammatory cytokines and lipid peroxidation may give rise to disturbances in epithelial absorptive processes in inflammatory bowel diseases, such as impaired lipid transport. However, studies have not yet clearly identified whether essential fatty acid deficiency and lipid soluble vitamin depletion are the result of malabsorption vs. inadequate intake to meet needs.

We (6) previously determined the effect of iron overload on plasma lipid profile and lipoprotein composition in rats administered a diet enriched with 3% iron carbonyl. The latter dietary regimen provoked marked hyperlipidemia, evidenced by the concomitant increase in plasma TG and cholesterol. In addition, various chemical abnormalities characterized the composition and size of lipoproteins. These disturbances were correlated with lipid peroxidation as reflected by elevated MDA concentrations. However, in the current study, Caco-2 cells submitted to lipid peroxidation displayed defective lipoprotein production. In view of this compromised intestine lipoprotein secretion, it is therefore tempting to suggest that the in vivo hyperlipoproteinemia identified in rats with iron loading is attributable...
to liver hypersecretion only. Additional work is needed to clarify this important issue.

The experiments performed in the current study were aimed at defining whether iron-catalyzed lipid peroxidation could modify intestinal cholesterol metabolism. Our data revealed that de novo cholesterogenesis, assessed by the incorporation of [14C]acetate, was markedly increased in treated Caco-2 cells. This was associated with a significant enhancement of HMG-CoA reductase activity. At this time, we are not able to specify whether iron-catalyzed lipid peroxidation modulates the enzyme activity by altering its concentration through transcriptional or posttranscriptional modifications. Iron-catalyzed lipid peroxidation also may affect polyunsaturated fatty acids, resulting in changes in the physical properties of the fluidity of the membrane in which HMG-CoA reductase is embedded. Such indirect effects have been shown to alter the immediate environment of the enzyme, thereby affecting its function (11, 41).

Unlike HMG-CoA reductase, ACAT activity was resistant to iron-induced lipid peroxidation. Furthermore, concurrent iron-catalyzed peroxidation in the livers of rats with iron overload, the activities of the two enzymes were dissimilar; HMG-CoA reductase was suppressed while ACAT was activated (6). Apparently, the two enzymes respond to peroxidative stress differently.

Apolipoproteins are necessary for exogenous and endogenous lipid transport. Their biosynthesis is a principal determinant of plasma lipoprotein levels, and defects in their synthesis or function affect lipoprotein metabolism (21). Our study showed that iron-catalyzed lipid peroxidation resulted in diminished newly synthesized apolipoproteins. Even the biogenesis of apo B that is crucial for the assembly and exocytosis of TG-rich lipoprotein was markedly impaired. At present, the mechanisms involved in these abnormalities are not elucidated, including apo B elongation or translation, the rate of apo B translocation across the endoplasmic reticulum, and intracellular degradative pathway of apo B (18). Similarly, Murthy et al. (33) found that less newly synthesized apo B was secreted by cells incubated with 13-hydroxyoctadecadienoic acid, an oxidized lipid. Murthy et al. (33) suggested that defective apo B translocation was responsible for the reduced apo B secretion. Furthermore, the original observation that the diminished synthesis of apo A-IV, apo B, and lipoprotein caused by Fe$^{2+}$-ascorbate could be attributed to its direct effects on TG production is unlikely. The incubation of Caco-2 cells with Fe$^{2+}$-ascorbate in the absence of oleic acid, the main precursor of TG biogenesis, resulted in similar differences between control and treated cells (results not shown).

In summary, the Fe$^{2+}$-ascorbate system appeared to be very effective in promoting lipid peroxidation of Caco-2 cells in view of the markedly increased MDA levels. Concomitantly, it elicited a reduction in lipid esterification and synthesis, apolipoprotein biogenesis, and lipoprotein secretion. We conclude that long-lasting lipid peroxidation may overwhelm intraluminal antioxidant defense and impair intestinal fat transport.

We thank Danielle St-Cyr Huot for typing the manuscript. This study was supported by grants from the Medical Research Council of Canada (MT-10584) and the Canadian Foundation for Crohn’s and Colitis.

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