Influence of thiol balance on micellar cholesterol handling by polarized Caco-2 intestinal cells

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Abstract **The in vitro thiol redox modulation of cholesterol homeostasis was investigated in polarized Caco-2 intestinal cells. Cells were pre-incubated with the pro-oxidant compound $CuSO_4$ or with the antioxidant *N*-acetylcysteine (NAC), to induce a mild shift of the intracellular redox potential toward, respectively, a more oxidizing or a more reducing equilibrium, via a manipulation of intracellular soluble thiols (glutathione). Then, monolayers were exposed to micellar cholesterol and both the cholesterol uptake and export, as well as the cholesteryl ester cycle, were analyzed. We found that pro-oxidizing conditions induced a significant cholesterol retention within the cells, particularly in the unesterified form (FC), as a result of an augmented sterol incorporation coupled with a reduced rate of FC esterification. A reduction in FC export was also evident. Furthermore, the combination of FC retention and the oxidative imbalance leads to significant alterations of the monolayer integrity, evidenced by both the enhanced tight junction permeability and the lactate dehydrogenase release into the basolateral medium. In contrast, a more reducing environment generated by NAC pre-treatment favors the limitation of the resident time of FC into the cells, via a reduced cholesterol uptake and a concomitant increased cholesterol esterification. In addition, a significant higher FC extrusion into the basolateral medium was also appreciable. Our results indicate that the thiol balance of intestinal cells may be critical for the regulation of cholesterol homeostasis at the intestinal level, influencing the lipid transport throughout the intestinal barrier.**

Key words: Caco-2; Redox balance; Glutathione; Cholesterol

1. Introduction

Cholesterol homeostasis within the cell is maintained by balancing cholesterol absorption and endogenous cholesterol synthesis with mechanisms able to favor the export of cholesterol excess and to prevent its accumulation.

Deregulation of cholesterol homeostasis may have important consequences, altering cell structure and function. There is a wide consensus that cholesterol accumulation in macrophages, a key event leading to the generation of foam cells during atherosclerosis [1], is associated with an increased plasma membrane rigidity, a widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway [2,3]. In addition, cholesterol overload has also been associated with the occurrence of oxidative imbalance due to the transformation of exceeding cholesterol in oxysterols [4].

It is well accepted that a plethora of genetic, dietary and physiological factors may interfere with cholesterol absorption at intestinal level, leading to alterations in blood cholesterolemia. Among them, it has been proposed that the redox profile of intestinal mucosa may have an important role [5]. From a general point of view, the maintenance of cellular thiol redox balance, a dynamic process achieved essentially by preservation of the reduced (GSH)/oxidized (GSSG) glutathione ratio, is critical to drive subcellular and metabolic functions [6], including those involving lipid metabolism [7–10]. Recently, by manipulating the thiol profile of Caco-2 intestinal cells by two compounds having opposite effects on the GSH/GSSG ratio we have demonstrated that the internal thiol balance is able per se to modulate the way in which enterocytes process dietary fatty acids [7].

In view of these findings and the above-mentioned association between oxidation and cholesterol accumulation in cells, it appears reasonable to speculate the existence of a reciprocal correlation between the oxidative state and cholesterol route within the cell. The present work was undertaken to investigate the effects of mild manipulations of the cell's thiol balance on Caco-2 cholesterol homeostasis, when cells were exposed to micellar cholesterol. Our results indicate that also slight but consistent modifications of the internal redox profile affect the cholesterol processing by Caco-2 cells, suggesting that oxidative stress may alter lipid transport throughout the intestinal monolayer via mechanisms involving cholesteryl ester (CE) cycle down-regulation and cytoskeleton alterations.

2. Materials and methods

2.1. Cells

The human colon carcinoma cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD, USA) and routinely subcultured at a density of 1.6×10^4 cells/cm^2 on 25-cm^2 flasks in Dulbecco's modified Eagle's medium (DMEM, HyClone, Europe Ltd.) supplemented with 0.2 mM L-glutamine, 1% non-essential amino acids (HyClone), 5 U/ml penicillin, 5 μg streptomycin and 10% (v/v) fetal bovine serum (HyClone), and maintained at 37°C in an atmo-

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Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferase; ALP, alkaline phosphatase; CE, cholesteryl ester; CEH, cholesteryl ester hydrolase; DMEM, Dulbecco's modified Eagle's medium; FC, free cholesterol; GSH, reduced glutathione; GSSG, oxidized glutathione; LDH, lactate dehydrogenase; NAC, *N*-acetylcysteine; SH, sulfhydryl groups; TEER, transepithelial electrical resistance

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sphere of 5% CO_2, 95% air. For the experiments, once the flasks reached 80% confluence, the cells were split and seeded onto Millicell tissue culture plate inserts (30 mm diameter, 0.4 μm pore size, 4.2 cm^2 effective membrane area: Millipore, Bedford, MA, USA) at a density of 8×10^4 cells/cm^2 and placed in six-well culture plates (35 mm diameter; Falcon), permitting a separate access to the luminal (apical) and lymphatic (basolateral) compartments of the monolayers [11]. The culture medium was regularly changed three times a week. It has been reported that Caco-2 cells grown in these permeable supports in bicameral chambers, under control conditions for more than 20 days, spontaneously differentiate into an ileal-like polarized cell monolayer which develops brush border microvilli, tight junctions, and both specific small intestinal enzymes and gene products [12–14]. For this reason, this cell line has been widely used as an in vitro model system in many studies concerning the evaluation of intestinal absorption of substances present in food, contaminants and drugs [11,15]. Then, cells were left in culture for 21–23 days, at which time a highly differentiated phenotype was clearly appreciable.

2.2. Morphological studies

Before any following treatments and in order to ascertain if Caco-2 cells have reached the typical fully differentiated enterocyte morphology, cells were processed for scanning electron microscopy (SEM). Filters were fixed in 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at room temperature for 20 min. Following post-fixation in 1% osmium tetroxide (wt/vol) for 30 min, filters were detached from the holder and were dehydrated through graded ethanols, critical point dried in CO_2 and gold coated by sputtering. The samples were observed with a Cambridge 360 scanning electron microscope.

2.3. Alkaline phosphatase (ALP) activity

To monitor the degree of biochemical differentiation of Caco-2 cells, the evaluation of ALP activity by a commercially available kit (Boehringer Mannheim, Milan, Italy) over the period of culture was carried out. Briefly, at different times in culture, cell monolayers were washed twice with phosphate-buffered saline (PBS), pH 7.4, and gently scraped from the filters. Total cell lysates were prepared by sonication in a Soniprep 150 (two impulses, 30 s each), and aliquots of 0.1 mg of cell protein were assayed for ALP activity by the addition of *p*-nitrophenyl phosphate substrate. Results, evaluated on the basis of OD values obtained at 405 nm, were expressed as U/mg cell protein.

2.4. Assessment of cell monolayer integrity

The transepithelial electrical resistance (TEER), as the index of confluence and integrity of monolayers [12,16], was measured at 37°C over the period of culture, using a Millicell-ERS apparatus (Millipore, Bedford, MA, USA). Results, expressed as Ω/cm^2, were corrected by subtraction of the blank values (filters without cells). Then, the values obtained were multiplied by the surface area of the monolayer.

Cell damage was evaluated by the amount of cytosolic lactate dehydrogenase (LDH) leakage into the basolateral medium, measured by a specific commercially available test (Sigma Chemical Co, MO, USA). Total cellular LDH was measured after solubilizing the monolayer with 0.1% Triton X-100 in PBS followed by centrifugation and assaying of the supernatant. Results were expressed as mU/ml.

2.5. Micelle preparation

Micelles were prepared essentially as described by Field et al. [17]. Stock solutions of cholesterol, monoolein, and phosphatidylcholine were prepared in chloroform, while a stock solution of sodium taurocholate was made in 95% ethanol. The lipids and bile salt were mixed and the solvents were evaporated under a stream of nitrogen. Serum-free DMEM was then added so that the final concentrations of the micelle were: 150 μM cholesterol, 0.3 mM monoolein, 50 μM phosphatidylcholine and 5 mM taurocholate. In the experiments performed to evaluate the cholesterol uptake by the cells, [1-^{14}C]-cholesterol (NEN Life Science Products Inc., Boston, MA, USA; specific activity 14666 dpm/μmol) was added to the micelle preparations. The micellar solution was vortexed and stirred overnight at 37°C before use.

2.6. Experimental protocol

At the start of each experiment, the maintenance medium was removed and monolayers were washed twice with PBS, pH 7.4. Then, in order to introduce different redox equilibrium in the cells, Caco-2 cells were pre-incubated for 24 h with DMEM containing the antioxidant *N*-acetylcysteine (NAC, Sigma, 5 mM) or the pro-oxidant agent $CuSO_4$ (10 μM) or with the same volume of DMEM alone (control). The concentrations used were chosen on the basis of a published report, in which we demonstrated that they were efficacious as redox modulators without causing toxicity and/or cell alterations [7]. Then, the medium was removed and, for the samples destined for redox studies, the cells were harvested, centrifuged for 5 min at 900 rpm, washed twice with PBS, resuspended in the same buffer, and lysed by sonication for 30 s (two times) in a Soniprep 150. To address the effects of the modified redox environments on the micellar cholesterol incorporation, monolayers were washed with PBS, then the apical chamber was rinsed with serum-free DMEM containing [1-^{14}C]-cholesterol-micelles, and the basolateral chamber was rinsed with serum-free DMEM medium alone. After 24 h of incubation at 37°C, cells were harvested and processed as described below.

2.7. Evaluation of the redox profile of the cells

To assess the redox state of the cells after NAC or $CuSO_4$ treatments, before their subsequent exposure to micelles, GSH and GSSG levels were considered. To determine the total intracellular glutathione content, the enzymatic recycling assay with glutathione reductase (type IV, Sigma) and 5,5′-dithiobis-2-nitrobenzoic acid (Sigma) was used, essentially according to Anderson [18]. For the measurement of GSSG, the acidified homogenates were submitted to derivatization with undiluted 2-vinylpyridine (Aldrich, Milwaukee, WI, USA) in the presence of triethanolamine (Sigma) for 1 h at room temperature. Samples were then assayed by means of the same procedure as described above for total glutathione measurement. The amount of GSH present in the samples was calculated as the difference between total glutathione and GSSG levels. As an additional redox parameter, protein sulfhydryl groups (SH) were measured as described by Di Monte et al. [19].

2.8. Cholesterol handling by intestinal cells

At the end of incubation period with labelled micelles, both the basolateral and the apical medium were removed. The cells were washed with PBS, lipids were extracted with hexane/isopropyl alcohol (3:2 v:v), and separated by thin layer chromatography (TLC), as previously described [7]. The radioactivity associated with free cholesterol (FC) and CE was measured in LS 5000 Beckman. The basolateral medium was processed according to Folch et al. [20], to detect the relative amount of both the cholesterol forms released by the cells.

2.9. Acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity

ACAT activity was determined according to Suckling [21]. Cell homogenates (0.1–0.2 mg) were incubated in phosphate buffer 0.05 M for 5 min at 37°C containing GSH (10 mg/ml) and bovine serum albumin (10 mg/ml). The reaction was started by addition of oleoylCoA substrate (10 nmol/tube) containing [^{14}C]oleoylCoA (Amersham Pharmacia Biotech Inc., Milan, Italy; 15000 dpm/nmol). The reaction was stopped with methanol and lipids extracted from the incubate by chloroform/methanol (2:1 v/v). [^{3}H]cholesteryl oleate (700 dpm/tube) was added as an internal standard to estimate recovery. Lipids were separated by TLC and the CE fraction was identified by comparison with a cholesteryl oleate standard. The radioactivity associated with the lipid bands, previously scraped off from the plate, was measured in a LS 5000 Beckman liquid scintillation counting.

2.10. Cholesteryl ester hydrolase (CEH) activity

CEH activity was determined on Caco-2 homogenates by measuring the release of [^{14}C]oleic acid from the cholesteryl[1-^{14}C]oleate substrate. Assay of CEH activity was carried according to Martinez and Botham [22]. The substrate was prepared as a mixed micelle with CE, phosphatidylcholine and sodium taurocholate in a molar ratio of 1:4:2. Cholesteryl[1-^{14}C]oleate (NEN, 6000 dpm/nmol) phosphatidylcholine (57.6 μmol) and cholesteryl oleate (14.4 μmol) were resuspended in 50 mM Tris–HCl buffer, pH 7.4, containing 28.8 μmol sodium taurocholate, and then sonicated at 46°C for 1 h (5 min sonication, 5 min rest). 0.1–0.2 mg protein of homogenized cell in Tris–HCl buffer, pH 7.4, was incubated for 30 min at 37°C with 50 μl substrate solution. The reaction was stopped by the addition of 1.5 ml of chloroform/methanol/toluene (2:2.4:1 v/v/v) containing

0.29 mM oleic acid as a carrier and centrifuged to separate the organic layer and the aqueous phase. The amount of [1-^{14}C]oleate released into the upper phase was counted in a LS 5000 Beckman.

2.11. Protein content

Protein concentration was measured by the commercially available Coomassie brilliant blue dye binding assay (Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions. Bovine serum albumin was used as a standard.

2.12. Statistical analysis

All results are expressed as means ± S.E.M. determined from at least four different experiments, each performed in duplicate. For TEER analysis, eight different filters for each condition were considered. Statistical analysis was performed by InStat-3 statistical software (GraphPad Software Inc, San Diego, CA, USA) using one-way ANOVA test with Tukey–Kramer's correction for multiple comparison. The significance was taken at 5% level.

3. Results

3.1. Characterization of Caco-2 cell monolayer

The endpoint morphology of a typical monolayer of Caco-2 cells cultured on polycarbonate filters was evaluated by SEM (Fig. 1). As can be observed, all the surface of the monolayer was covered with a well-developed brush border microvilli.

Biochemical differentiation of Caco-2 cells was monitored by measuring the specific ALP activity, a brush border marker enzyme, at different stages of monolayer maturation. ALP activity increased linearly from 24.28 ± 2.88 U/mg protein detected two days post-seeding the cells to 368 ± 15.77 U/mg protein evaluated after 21 days of culture, with a general trend consistent with that reported previously [12,14].

The assessment of a complete structural and functional monolayer was also evaluated by TEER measurement. Electrical resistance across tissue culture plate inserts without cells was 188 ± 3.4 Ω/cm^2. The determination of TEER values of Caco-2 cell monolayers during the entire period of maturation showed that this parameter increased from 255 ± 4.38 Ω/cm^2 at day 2 to 930 ± 6.64 Ω/cm^2 at day 21, with a general trend consistent with that previously reported [12].

Thus, in view of the results obtained, all the experiments were carried out 21–23 days post-seeding the cells.

3.2. Evaluation of the internal thiol profile of Caco-2 cells before their following exposure to micellar cholesterol

The effects of incubation of Caco-2 cells with 10 μM $CuSO_4$ or 5 mM NAC for 24 h on their oxidative state was evaluated by measuring the intracellular levels of GSH and GSSG and protein SH groups. The results obtained, showed in Table 1(a), indicate that the GSH concentration was reduced after exposure to $CuSO_4$, while the GSSG level was significantly raised, leading to a net reduction in the GSH/GSSG ratio of about 65%. Conversely, NAC treatment induced a significant increase in GSH content which was associated with a consistent parallel decrease in GSSG content, leading to a net augmentation in the GSH/GSSG ratio of about 30%. An analysis of protein-bound SH groups showed a clear reduction of this value after $CuSO_4$ treatment, while a slight but net increment after NAC treatment was appreciable. On the whole, these results indicate that the $CuSO_4$-treated cells have been shifted into a pro-oxidant state compared to the untreated cells and, conversely, cells treated with the antioxidant and thiol supplier NAC were in a more reduced state. Both the induced redox perturbations were not accompanied by a simultaneous loss of cell viability, as indicated by the unchanged amount of LDH leakage into the basolateral medium. Interestingly, TEER showed a slight but consistent increase in NAC-treated cells (958 ± 6.14 Ω/cm^2), compared with those detected in controls (922 ± 7.66 Ω/cm^2, $P < 0.01$ vs. NAC) and in $CuSO_4$-treated cells (907 ± 5.42 Ω/cm^2, $P < 0.001$ vs. NAC).

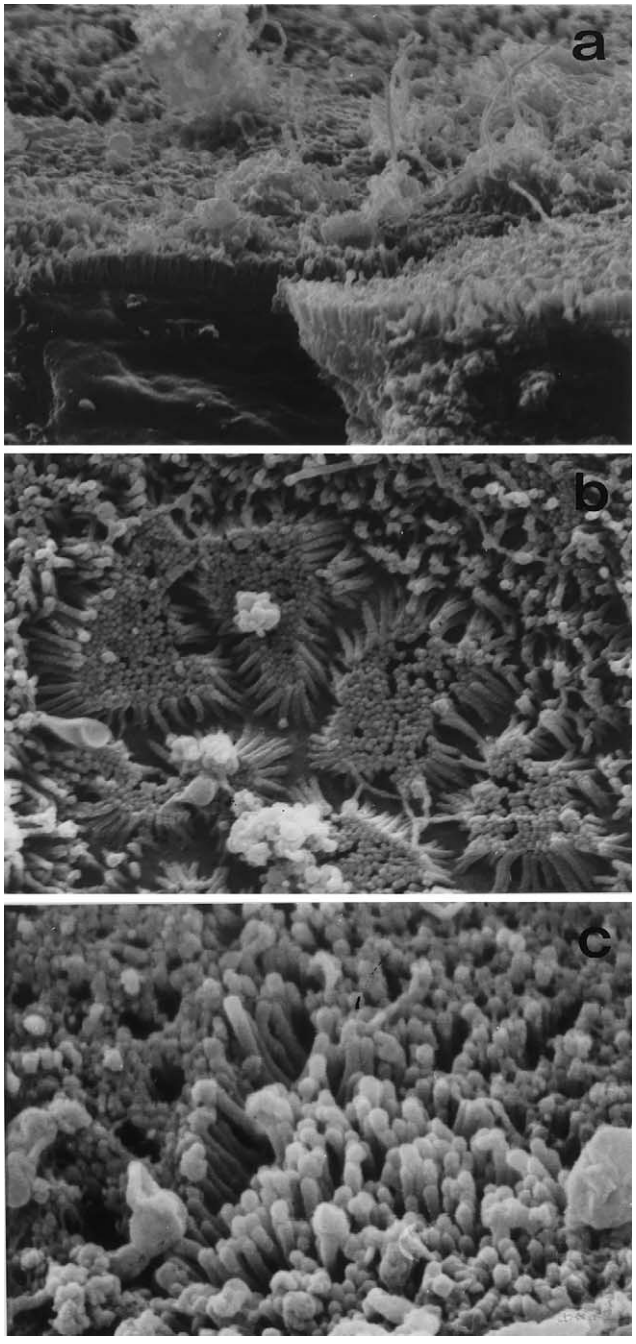

Fig. 1. Scanning electron micrographs of the Caco-2 intestinal cell monolayer after 21 days of culture. As can be seen, cells show morphological characteristics resembling those of small intestinal enterocytes. In fact, a well-polarized columnar body (a) with typical brush border microvilli projecting perpendicular to the cell surface was evident (b,c). Magnifications: (a) 5400×, (b) 10 000×, and (c) 16 000×.

3.3. Evaluation of cholesterol uptake by intestinal cells

To evaluate the micellar cholesterol uptake under the redox

condition described above, Caco-2 cells previously exposed to NAC or $CuSO_4$ were incubated with micellar solution for 24 h at 37°C. Then, the radioactivity associated with FC and CE fractions was evaluated. As can be observed in Table 1(b), a significant reduction in FC incorporation was detected in NAC-treated cells with respect to controls, and $CuSO_4$ pre-treatment did not significantly affect this value, even if a slight trend towards an enhanced incorporation appeared evident. A different trend regarding the esterification of micellar cholesterol was also detected. In fact, an increase of CE-associated radioactivity in NAC-treated cells was evident, and a concomitant decrease in this value in cells treated with $CuSO_4$ was found. On the whole, these results affect the basal FC/CE ratio, with an obvious increment in $CuSO_4$-treated cells (+129%) and a concomitant reduction in NAC-treated cells (−46%) with respect to basal values.

3.4. Effect of different internal thiol profiles on the Caco-2 cholesteryl esterl cycle

The results obtained on cholesterol ester cycle are summarized in Table 1(c). A significant increment of ACAT activity was detected in cells pre-treated with NAC, while a decrement in the activity of this enzyme was evident following a pre-treatment with $CuSO_4$. A similar trend was detected for CEH, for which an augmented activity was monitored after a pre-treatment with NAC, while a loss in the activity of this enzyme was evident when the cells were pre-treated with $CuSO_4$. As a consequence, oxidized cells showed a lower ACAT/CEH activity ratio, in comparison with both untreated and NAC-treated cells.

3.5. Effect of different internal thiol profiles on cholesterol extrusion by Caco-2 cells

The results on cholesterol output by Caco-2 cells into the basolateral medium are summarized in Table 1(d). Caco-2 cells secreted more FC than CE under basal conditions. When the cells were pre-treated with the antioxidant NAC, a significant increase of FC with a concomitant loss in CE output was evident. These events resulted in a more than two-fold increase of the FC/CE ratio. Conversely, when the cells were pre-treated with the oxidizing agent $CuSO_4$, a strong reduction in both FC and CE outputs after micellar exposure was evident, leading to a 19% decrease of the FC/CE ratio.

3.6. Endpoint evaluation of monolayer integrity

At the end of the experiments and before the cell harvest, an evaluation of the integrity of the monolayer was carried out. Interestingly, both the TEER and LDH values showed slight but consistent alterations in $CuSO_4$-treated cells, with an increment of LDH released into the basolateral compartment (225 ± 6.5 mU/ml, 15% of total LDH activity) coupled with a drop in TEER value (746 ± 5.55 Ω/cm^2). Conversely, nor controls and NAC-treated cells showed significant perturbations in both the parameters, with LDH values, respectively, of 66 ± 2.4 and 80 ± 3.2 mU/ml (4% and 6% of total LDH activity, $P < 0.001$ vs. controls), and TEER values of 928 ± 9.2 Ω/cm^2 for controls ($P < 0.001$ vs. $CuSO_4$) and 944 ± 7.92 Ω/cm^2 for NAC ($P < 0.001$ vs. $CuSO_4$).

4. Discussion

Cells have evolved regulated pathways to maintain an appropriate cholesterol content, as cholesterol in excess can be detrimental to normal cell function [1]. The oxidative stress-mediated cholesterol accumulation has been documented for several cell types, including macrophages, vascular smooth muscle cells and nerve cells [23–27], but this association in enterocytes has been poorly investigated.

Because of its anatomical position, the gut is continuously exposed to external and internal stimuli able to depress the oxidative state of the enterocytes. At the same time, the gastrointestinal tract remains the most popular route of administration for drugs having antioxidants properties, a useful approach utilized in the treatment of inflammatory bowel diseases [28]. With this in mind and in view of the reported observation on the influence of thiol balance on dietary fatty acids processing by Caco-2 cells [7], we have designed an experimental protocol organized in two steps. Firstly, the oxidative status of polarized Caco-2 intestinal cells was manipu-

Table 1
(a) Evaluation of thiol redox balance on polarized Caco-2 cells treated for 24 h with NAC or $CuSO_4$, before their following exposure to micellar cholesterol; (b) effect of NAC or $CuSO_4$ pre-treatment on the micellar cholesterol uptake by polarized Caco-2 cells; (c) evaluation of ACAT and CEH activities in polarized Caco-2 cells pre-treated with NAC or $CuSO_4$ and then exposed to micellar cholesterol; (d) evaluation of FC and CE released in the basolateral chamber by polarized Caco-2 cells pre-treated with NAC or $CuSO_4$ and then exposed to micellar cholesterol

	Parameter	Control	5 mM NAC	10 μM $CuSO_4$
a	GSH (nmol/mg protein)	35.24 ± 4.11	45.12 ± 6.18*	26.58 ± 3.08°°
	GSSG (nmol/mg protein)	0.58 ± 0.022	0.41 ± 0.053**	1.16 ± 0.081***
	GSH/GSSG ratio	60.75	77.8	23
	Protein SH groups	148 ± 18	166 ± 21	108 ± 11* °°
b	FC (nmol/h/mg protein)	3.97 ± 0.13	3.23 ± 0.10**	4.18 ± 0.16°°
	CE (nmol/h/mg protein)	0.0120 ± 0.002	0.021 ± 0.0038	0.0092 ± 0.0016°°
	FC/CE ratio	330	213	426
c	ACAT (nmol/h/mg protein)	3.33 ± 0.28	4.87 ± 0.44*	2.18 ± 0.2°°°
	CEH (nmol/h/mg protein)	4.09 ± 0.28	5.3 ± 0.35	3.28 ± 0.37°°
	ACAT/CEH ratio	0.81	0.91	0.66
d	FC (pmol/mg protein)	511.7 ± 51.2	777 ± 73.6*	248.3 ± 50.4* °°°
	CE (pmol/mg protein)	10.87 ± 0.87	8.3 ± 0.52*	6.58 ± 0.36**
	FC/CE ratio	47.07	93.61	38.14

The means ± S.E.M. of four sepearate experiments, each performed in duplicate, are hown. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. untreated cells; °°$P < 0.01$ and °°°$P < 0.001$ vs. NAC-treated cells.

lated into a physiological range by two compounds having an opposite effect on GSH. Then, cells having different redox profiles were exposed to micellar cholesterol to evaluate how intestinal cells process dietary cholesterol.

An interesting change in the proportion of FC and CE absorbed in oxidized cells with respect to untreated cells was clearly appreciable. On the whole, the increased FC/CE ratio coupled with both the reduced rate of cholesterol esterification (evidenced by the lower ACAT/CEH ratio) and the drop in the FC extrusion into the basolateral medium are suggestive for FC accumulation within oxidized cells, a condition largely considered dangerous for cell viability and function [2]. In fact, a post-treatment evaluation of the parameters known to give information on the integrity of the monolayer, such as TEER and LDH release, has confirmed the occurrence of significant monostrate perturbations. The whole situation appeared completely reversed if cells were pre-treated with NAC. Indeed, the reduced FC uptake by the cells observed was coupled with both a higher cholesterol esterification activity within the cells and a very strong FC output into basolateral chamber. This suggests that a higher GSH/GSSG ratio is associated with an improvement of those mechanisms that are the basis for an efficient removal of FC excess from intestinal cells.

Beside the above-mentioned effects on cholesterol homeostasis, other thiol-dependent mechanisms should be considered to explain, at least partially, the concomitant emerging alterations in the oxidized monolayer. For instance, the thiol modulation of numerous cytoskeleton-dependent cell activities (i.e. receptor expression, adhesion properties, vesicular trafficking) has been documented [29]. The loss of protein SH, considered to be a downstream event of GSH depletion and here detected in $CuSO_4$-treated intestinal cells, is an index of redox-dependent alterations in the components of cytoskeleton involved in both cell–cell and cell–substrate contacts [30–34]. Conversely, it has been reported how NAC administration to cells results in a general improvement of a number of cell properties, including cell–cell and cell–substrate adhesion processes, which depend on a more favorable redox-dependent conformation of the cytoskeletal network [30,31,35,36]. Therefore, the increased TEER value reported after NAC treatments should be interpreted as the result of a general reinforcement of all junctional complexes of the monolayer.

Finally, we cannot exclude redox influences on other mechanisms involved in both cholesterol absorption and efflux throughout the cells, such as the ATP binding cassette transporters system, and a recent investigation seems to support this possibility [37], suggesting a new way to address the cholesterol metabolism at intestinal level by antioxidants.

In summary, we provide evidence for the first time that the pre-existing redox profile of intestinal cells affects the way in which cells absorb and utilize dietary cholesterol delivered to them by mixed micelles. This fact may have a remarkable relevance at the subcellular level, influencing several mechanisms involved in the lipid transport throughout the intestinal barrier. We suggest that, as reported by others [38,39], the adoption of a strategy designed to control/buffer the antioxidant capacity of the gastrointestinal tract could have important consequences in the modulation of lipid balance in the body.

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