# Redox-Dependent Modulation of Lipid Synthesis Induced by Oleic Acid in the Human Intestinal Epithelial Cell Line Caco-2

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**The absorption, remodeling, and delivery of dietary lipids by intestinal cells are part of a complex multi-step process, the dynamics of which is influenced by the lipid composition of the diet and the physiological state of enterocytes. Emerging data indicate that, among the parameters known to modulate the cell functionality, the internal oxidative balance plays a pivotal role. In this study, we analyzed the effects of varying redox equilibria on the way in which the intestinal Caco-2 cell line utilize an exogenous lipid source such as oleic acid. Firstly, we manipulated the intracellular levels of soluble thiols (glutathione), and the amount of cell-associated products of lipid peroxidation, commonly regarded as two critical parameters characterizing the redox profile of the cells. Two different perturbants having opposite effects on the cell's redox profile were used: the pro-oxidizing agent $CuSO_4$ (2.5 and 10 μ*M*) and the antioxidant and thiol supplier N-acetylcysteine (NAC, 2.5 and 5 m*M*). The influence of these mild but critical manipulations on the incorporation of oleate (50 and 500 μ*M*) into cholesterol, triacylglycerol, and phospholipid was then evaluated. We found that the emerging pro-oxidant condition induced by $CuSO_4$ pre-exposure was associated with a significant up-regulation of phospholipid synthesis, while minor modifications were detected in that of triacylglycerols. Conversely, when a more reducing state was induced by NAC pre-treatment, there was a significant down-regulation of triacylglycerol synthesis, with minor modifications in that of phospholipids. In addition, the incorporation of oleic acid in the cholesteryl ester fraction appeared to be unmodified under all the redox conditions reported. On the whole, these results indicate that the pre-existing internal redox potential of the enterocytes is a critical factor that is able to differentially modulate lipid synthesis at the intestinal level. Thus, the adoption of a strategy designed to control/buffer the antioxidant capacity of the gastrointestinal tract could have important consequences for the modulation of lipid balance in the body.**

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The small intestine, the sole organ responsible for the assimilation and transport of dietary lipids, plays an important role in maintaining lipid balance within the body, acting as an interface between lipids derived from the diet and those circulating in the form of plasma lipoproteins. It is well accepted that the presence of dietary fatty acids in the proximal intestine represents the main driving force that enhances triacylglycerol and hence intestinal lipoprotein secretion (1). Moreover, it has been reported that dietary fatty acid composition affects both the biosynthesis and the secretion of lipoproteins of intestinal origin (2). Nevertheless, the active contribute of the intestinal cell on the way in which they use dietary fatty acids should be not underestimated.

During the past years, evidences have accumulated which demonstrate that the balance of the oxidative and reductive potential within the cell can have profound consequences on several cellular function (DNA synthesis, selective gene expression, different metabolic processes), through the activation/repression of a number of redox-sensitive transcription factors, and protein kinases (3, 4). On the other hand, a persistent situation of redox imbalance due to an excess of oxidants and/or a down-regulation of endogenous antioxidant defenses has been associated with a number of gastrointestinal disorders (5), often characterized by significant perturbations of plasma lipoprotein profile (6). Although informations concerning the effects of oxidized lipids or lipoproteins on several cellular functions are currently available (7–10), knowledge concerning the influence of the intracellular redox environment *per se* on different aspects of cellular lipid metabolism is still lacking. In order to evaluate the possible metabolic inter-relationships between internal redox conditions of the enterocytes and cell lipid metabolism, we planned an *in vitro* study in which the influence of different redox equilibria on the synthesis of lipids by the Caco-2 intestinal cell line was evaluated. This human cell line has been widely used in many studies

concerning the absorption of substances present in food, either as normal diet constituents or as additives, contaminants, and drugs. Thus, it represents a useful model to study many parameters of enterocyte function (11–13).

In our experimental protocol we evaluated the effects of pre-treatment with the antioxidant N-acetylcysteine or the pro-oxidizing agent $CuSO_4$ on the subsequent uptake and incorporation into intracellular lipids of oleic acid by Caco-2 cells. The results obtained indicate that changes in the cell's redox status can differentially modulate the processing of oleate by intestinal cells, suggesting that the basal redox profile of enterocytes may play a critical role for the future composition of newly synthesized intestinal lipoproteins.

## Materials and Methods

**Cells.** The human colon carcinoma cell line Caco-2 was grown in Dulbecco's modified Eagle's medium (DMEM, HyClone, Chester, UK Ltd.) supplemented with 0.2 m*M* L-glutamine, 1% non-essential amino acids (HyClone), 5 U/ml penicillin, 5 μg streptomycin, and 10% (v/v) fetal bovine serum (Northumbria Biological Ltd., Cramlington, UK), and maintained at 37°C in a atmosphere of 5% $CO_2$, 95% air. For experiments studying the evaluation of intracellular redox status, cells were seeded at a density of $9 \times 10^4$/ml in 25 $cm^2$ tissue culture flasks (Falcon). In studies where the effect of oleic acid was to be assessed, cells were seeded at the density of $4 \times 10^4$/ml in 24-well cell culture plates (Falcon). Fresh complete medium was supplied every 2 days. Cells were used 10 days after plating (three days after they reach confluence, as monitored by light microscopy), according to Field and collaborators (14).

**Experimental Protocol.** At the start of each experiment, the maintenance medium was removed and monolayers were washed twice with phosphate-buffered saline (PBS) solution, pH 7.4. Then, in order to introduce different redox equilibrium in the cells, Caco-2 cells were pre-incubated for 24 hr respectively with the antioxidant N-acetylcysteine (NAC, Sigma Chemical Co., 2.5 or 5 m*M*) or the pro-oxidant agent $CuSO_4$ (2.5 or 10 μ*M*) or with the same volume of phosphate-buffered saline (PBS) solution, pH 7.4 (control). The medium was then removed, and the monolayers were washed twice with PBS. At this time point, for those samples destined for redox studies, the medium was removed, and 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 sec in a Soniprep 150. To address the effects of the modified redox environments on the lipid synthesis, cells incubated in 24-well plates and pre-treated with NAC or $CuSO_4$ were washed with PBS and again incubated for 4 hr with serum-free DMEM medium supplemented with two different concentrations of oleic acid (50 and 500 μ*M*) bound to 1% bovine serum albumin. At the end of experiments, cells were processed as described below.

**Evaluation of Intracellular Redox State.** To assess the redox state of the cells after NAC or $CuSO_4$ treatments and before their subsequent exposure to oleic acid, two different parameters have been considered: (A) the extent of cellular lipid peroxidation; (B) the reduced (GSH) and the oxidized (GSSG) glutathione levels.

*(A).* 4-Hydroxy-2(*E*)-nonenal (4-HNE) and malondialdehyde (MDA) are important decomposition products of peroxides derived from polyunsaturated fatty acids (PUFA) and related esters, which on the whole provide a convenient index of lipid peroxidation (15, 16). Assay for cellular 4-HNE+MDA content was carried out using the commercially available Bioxytech LPO-586 assay (Oxis International Inc., Portland, OR), following the manufacturer's instruction. Data, expressed as nanomoles of 4-HNE+MDA per mg of cell protein, were calculated on the basis of a 4-HNE calibration curve.

*(B).* All preparation steps were performed at 4°C. Aliquots of the cell lysates were diluted with 1 vol of 10% (w/v) of ice-cold 5-sulfosalicylic acid (Sigma) and centrifuged at 6,000 rpm for 10 min to remove the protein precipitate. The clear supernatants were stored at 4°C until assayed. To determine the total intracellular glutathione content, the enzymatic recycling assay with glutathione reductase (type IV, Sigma) and 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, Sigma) was used, essentially according to Anderson (17). For the measurement of GSSG, the acidified homogenates were submitted to derivatization with undiluted 2-vinylpyridine (Aldrich, Milwaukee, WI) in presence of triethanolamine (Sigma) for 1 hr at room temperature. Samples were then assayed by means of the same procedure 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. Data were expressed as nanomoles of GSH or GSSG per mg of cell protein.

**Lipid Synthesis in Caco-2 Cells.** The intracellular synthesis of lipids in enterocytic cells, pre-treated for 24 hr with NAC or $CuSO_4$ was evaluated by incorporation of [9,10(n)$^3$H]oleic acid (NEN Life Science Products Inc., Boston, MA; specific activity, 9.2 Ci/mmol) into cholesteryl ester, triacylglycerol, and phospholipids. On the day of the experiment cells were incubated in DMEM medium containing oleic acid, 2 μCi/ml [$^3$H]oleate 1% fatty acid-free albumin, for 4 hr at 37°C in a 5% $CO_2$ incubator. Two different final concentrations of 500 and 50 μ*M* of oleic acid were tested. After the incubation, cells were washed and lipids extracted with hexane/isopropyl alcohol (3:2, v/v). [1-$^{14}$C]Cholesteryl oleate (NEN; specific activity, 60 mCi/mmol, about 1,200 dpm/tube) was added to each tube as an internal standard. The lipid classes, separated in bands by TLC on silica gel (Merck, Darmstadt, Germany) run in a hexane/ether/acetic acid (70:30:1, v/v/v) and identified by comparison with cholesteryl oleate, triolein, and phospholipid mixture standard, were scraped from TLC plates and counted for $^3$H and $^{14}$C radioactivity in a LS5000 Beckman liquid scintillation counter. After extraction of cellular lipids, the cells were dissolved in 1 *M* NaOH for measurement

of cellular protein. Each point of each experiment was performed in duplicate, and data were calculated as nmol lipid/hr/mg protein.

**Evaluation of Acyl-Coenzyme A:Cholesterol Acyltransferase (ACAT) Activity.** ACAT activity was determined according to Suckling (18). Protein (0.05–0.1 mg) was incubated in 0.05 *M* phosphate buffer for 5 min at 37°C containing GSH and BSA (10 mg/ml). The reaction was started by addition of oleoylCoA substrate containing [$^{14}$C]oleoylCoA (Amersham Pharmacia Biotech Inc., Milan, Italy; specific activity, 56 mCi/mmol). The reaction was stopped with methanol and lipids extracted from the incubate by chloroform/methanol (2:1 v/v). [$^{3}$H]Cholesteryl oleate (2,000 dpm/tube) was added to estimate recovery. TLC separated lipids and the cholesteryl ester fraction was identified by comparison with a cholesteryl oleate standard and counted directly for $^{3}$H and $^{14}$C radioactivity.

**Protein Content and Cell Viability.** The protein concentration was measured by the commercially available Coomassie brilliant blue dye-binding assay (Bio-Rad, Hercules, CA), following the manufacturer's instructions. Bovine serum albumin was used as a standard. Cell viability after $CuSO_4$ and NAC treatments and before the oleate experiments was evaluated by the Trypan blue dye-exclusion test. Cell monolayers were routinely monitored for their general morphology by light microscopy observation, whether monolayer integrity, assessed as a function of the amount of cytoplasmic lactate dehydrogenase (LDH) leakage into the medium, was measured by a specific commercially available test (Sigma). Finally, the number of detached cells after drug treatment was evaluated in a cell-counting chamber by counting the freely floating cells in the medium.

**Statistical Analysis.** Data are reported as the mean ± standard deviation of at least four experiments in which each point was performed in duplicate. Statistical significance among the groups was determined using the paired two-tailed Student's *t*-test. A $P$ value <0.05 was taken to reflect a significant difference.

## Results

**Characterization of the Redox Profile of Caco-2 Cells After $CuSO_4$ or NAC Treatments.** The starting point for this study was to select the appropriate concentrations of $CuSO_4$ and NAC capable of manipulating the internal redox balance toward respectively a pro-oxidant and antioxidant status, without affecting cell viability (19, 20). Thus, an analysis performed in order to verify the internal redox condition of Caco-2 cells after NAC or $CuSO_4$ pretreatment and before the oleate incorporation was carried out. Treatment with $CuSO_4$ for 24 hr induced an increased level of cellular 4-HNE+MDA ($P < 0.01$) in a dose-dependent manner, as shown in Fig. 1a. As expected, NAC treatments induced an opposite effect, with a reduction of the enaldehyde content with respect to untreated cells. This decrease was significant at the concentration of 5 m*M* ($P <$ 0.01). Cells treated with 2.5 μ*M* $CuSO_4$ showed both a 15% drop in GSH content ($P < 0.05$, Fig. 1b) and a simultaneous 2.8-fold increase in GSSG basal levels ($P < 0.01$, Fig. 1c). Moreover, 10 μ*M* of exposure with this compound was able

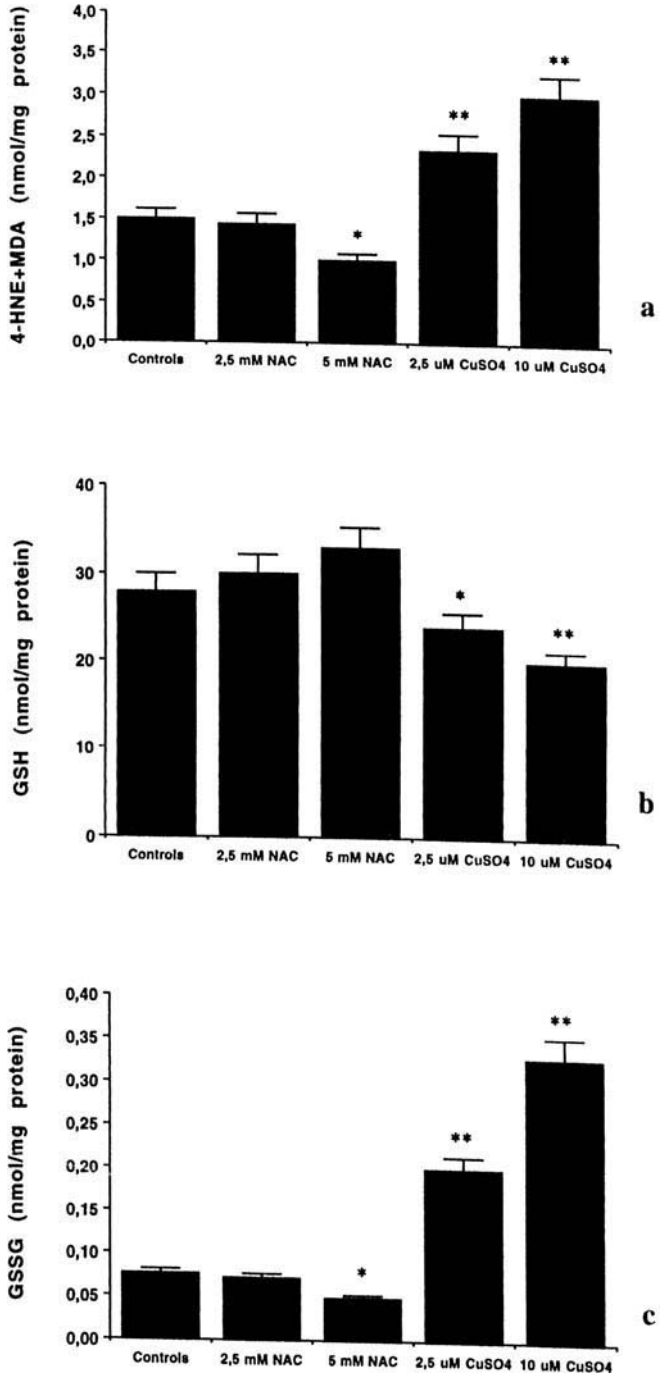



**Figure 1.** Evaluation of Caco-2 redox balance after 24 hr of NAC or $CuSO_4$ pre-treatment and before oleate exposure. Cells grown on 25 $cm^2$ flasks for 10 days were incubated for 24 hr with the antioxidant NAC (2.5 and 5 m*M*) or with the pro-oxidizing compound $CuSO_4$ (2.5 and 10 μ*M*). At the end of the incubation period, cells were washed with PBS, harvested, and sonicated as described in Materials and Methods. The following parameters have been considered: (a) cellular lipid peroxidation monitored by the formation of 4-HNE+MDA; (b) GSH levels; (c) GSSG levels. The means and standard deviations of six separate experiments are shown. *$P < 0.05$ and **$P <$ 0.01 versus untreated cells.

to deplete GSH content more seriously (–30%, $P < 0.01$), with a concomitant augmented 4.4-fold concentration in GSSG levels ($P < 0.01$). By contrast, both the NAC concentrations used maintained the intracellular content of GSH at control level (Fig. 1b), and only 5 m*M* concentration of this drug decreased significantly ($P < 0.05$) the level of its oxidized form with respect to untreated cells (Fig. 1c). The overall results can be best interpreted by evaluating the GSSG/GSH ratio, which is actually considered a more precise marker of the redox potential of the cell (21, 22). In fact, both the $CuSO_4$ concentrations used showed a severe and significant ($P < 0.01$) increase in this ratio (0.015 and 0.084, evaluated at 2.5 and 10 μ*M* $CuSO_4$, respectively), in comparison with untreated cells (ratio = 0.0024). At the same time, 5 m*M* NAC (ratio = 0.0014) treatment significantly ($P < 0.01$) reduced this ratio with respect to untreated cells, while 2.5 m*M* NAC (ratio = 0.0020) did not alter significantly this parameter from control value. A concentration of 5 m*M* NAC was therefore used in all subsequent experiments.

The viability of the cells was not significantly altered by any of the manipulations described below and ranged between 92% and 95%. In addition, no sign of cell damage was detected after drug treatments, as monitored by the relatively unchanged LDH release into the medium (8% for controls, 9% and 11% for 2.5 and 10 μ*M* $CuSO_4$, 8.5% for 5 m*M* NAC of total LDH activity, respectively). Finally, both treatments described did not induce alterations in cell morphology or an increase in propensity to detach, as detected by light microscopy observations and counting cells floating in the medium, respectively.

**Effect of Different Internal Redox Equilibrium on Lipid Synthesis by Caco-2 Cells.** A set of preliminary experiments in which the rate incorporation of labeled oleic acid into cellular lipids at different time of incubation was performed. As shown in Fig. 2a–c, a linear increase of [$^3$H]oleate over 8 hr of incubation was detected at both the oleate concentration used (50 and 500 μ*M*). Therefore an incubation time of 4 hr was chosen for all subsequent experiments. In view of these preliminary experiments, the incorporation of labeled oleic acid into cholesteryl oleate, triacylglycerols, and phospholipids was used to evaluate the synthesis of the three lipid classes in Caco-2 cells. The effects of variations of intracellular redox equilibrium on lipid synthesis in the enterocytes are shown in Figs. 3–5. The data are reported as percent of synthesis of single lipid class in control cells, which are untreated enterocytes. The synthesis of cholesteryl ester (0.26 ± 0.12 and 1.71 ± 0.5 nmol/hr/mg cell protein in control group, with 50 and 500 μ*M*, respectively) was unaffected by the changes in the cell's redox profile previously shown to be induced by cell pre-treatments (Fig. 3). The same experimental approach was used to study the synthesis of triacylglycerols (2.03 ± 0.14 and 32.6 ± 2.87 nmol/hr/mg cell protein in control group, with 50 and 500 μ*M*, respectively) and phospholipids (7.78 ± 0.67 and 29.12 ± 2.54 nmol/hr/mg cell protein in

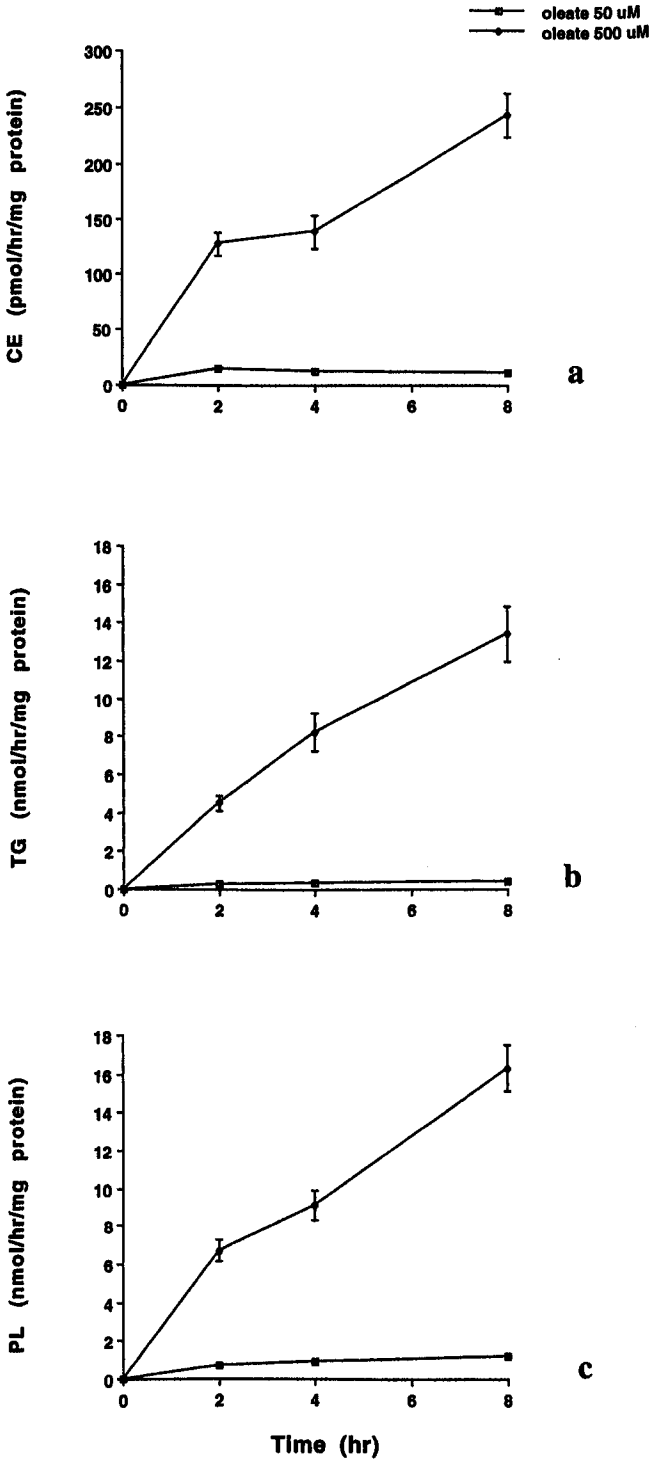
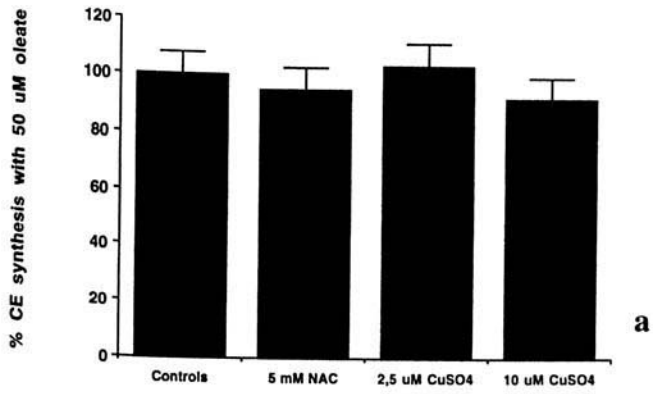



**Figure 2.** Rate of incorporation of labeled oleic acid into cholesteryl ester (CE), triglyceride (TG), and phospholipid (PL) fractions, evaluated at different times of incubation in Caco-2 cells. Cells grown on 24-well plates for 10 days were washed with PBS and then incubated for 8 hr with serum-free DMEM containing 50 or 500 μ*M* oleate and 2 μCi/ml [$^3$H]oleate bound to 1% fatty acid-free albumin. A linear increase of [$^3$H]oleate incorporation over 8 hr of incubation was detected at both oleate concentrations used. The means and standard deviations of four separate experiments are reported.

control group, with 50 and 500 μ*M*, respectively). Preexposure to 5 m*M* NAC significantly ($P < 0.05$) reduced Caco-2 triacylglycerol synthesis by 24% when the cells



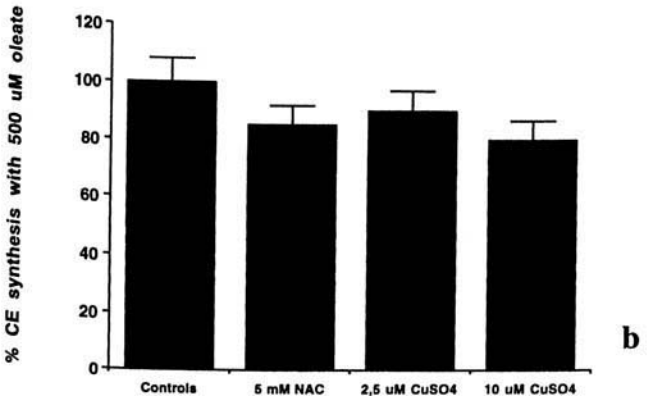
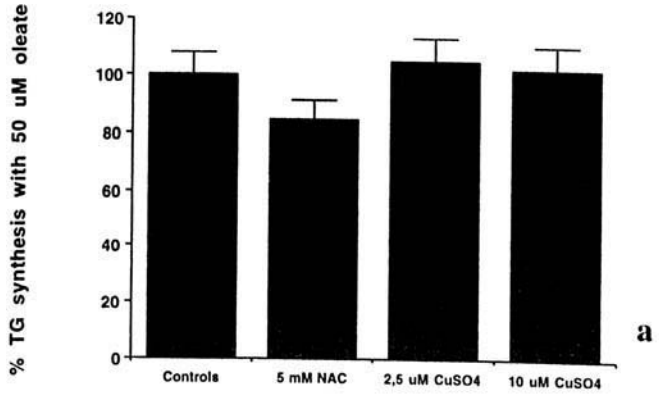




**Figure 3.** Effect of NAC or $CuSO_4$ pre-treatment on the cholesteryl ester synthesis induced by 50 $\mu M$ (a) and 500 $\mu M$ (b) oleic acid in Caco-2 cells. Cells were grown on 24-well plates for 10 days and were exposed to 5 m$M$ NAC or 10 $\mu M$ $CuSO_4$ for 24 hr. The medium was then removed, and the cells were washed with PBS and, finally, incubated for 4 hr at 37°C with serum-free DMEM containing 50 or 500 $\mu M$ oleate and 2 μCi/ml [$^3$H]oleate bound to 1% fatty acid-free albumin. Cholesterol ester synthesis was determined as described in materials and methods. Data are expressed as percentage of controls, which was considered as 100%. Control values were respectively 0.26 ± 0.12 nmol/hr/mg protein with 50 $\mu M$ oleate and 1.71 ± 0.5 nmol/hr/mg protein with 500 $\mu M$ oleate. The means and standard deviations of six separate experiments are reported.



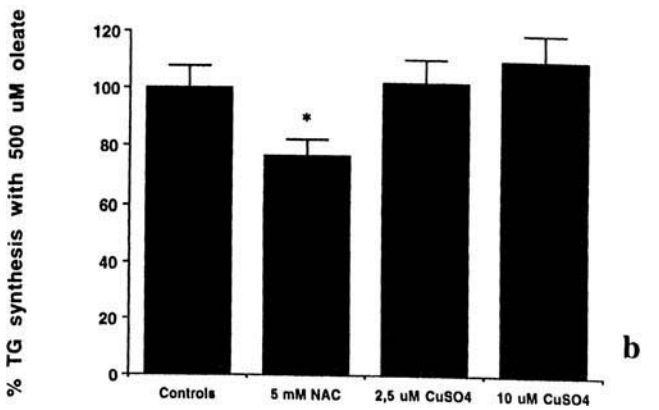




**Figure 4.** Effect of NAC or $CuSO_4$ pre-treatment on the triglyceride synthesis induced by 50 $\mu M$ (a) and 500 $\mu M$ (b) oleic acid in Caco-2 cells. Cells grown on 24-well plates for 10 days were exposed to 5 m$M$ NAC or 10 $\mu M$ $CuSO_4$ for 24 hr. The medium was then removed, and the cells were washed with PBS and, finally, incubated for 4 hr at 37°C with serum-free DMEM containing 50 or 500 $\mu M$ oleate and 2 μCi/ml [$^3$H]oleate bound to 1% fatty acid-free albumin. Triacylglycerol synthesis was determined as described in Materials and Methods. Data are expressed as percentage of controls, which was considered as 100%. Control values were respectively 2.03 ± 0.14 nmol/hr/mg protein with 50 $\mu M$ oleate, and 32.6 ± 2.87 nmol/hr/mg protein with 500 $\mu M$ oleate. The means and standard deviations of six separate experiments are reported. *$P < 0.05$ versus controls (cells incubated with oleate but not previously treated with NAC or $CuSO_4$).

were incubated with 500 $\mu M$ of oleate (Fig. 3b), although this effect appeared to be less important with 50 $\mu M$ oleate (Fig. 4a). The pro-oxidant conditions induced by $CuSO_4$ pre-treatment did not significantly affect triacylglycerol synthesis by the cells (Fig. 4), although a trend to enhanced triacylglycerol synthesis was apparent with the increase of pro-oxidant state of the cells in presence of 500 $\mu M$ oleate (Fig. 4b). Figure 5 shows that 5 m$M$ NAC pre-treatment did not affect phospholipid synthesis at either 50 $\mu M$ (Fig. 5a) and 500 $\mu M$ (Fig. 5b) oleate concentration in comparison with untreated cells. The two concentrations of $CuSO_4$ exerted different effects. At the lower concentration of $CuSO_4$ in the presence of both 50 and 500 $\mu M$ oleate concentration, the phospholipid synthesized did not differ in control and NAC-treated cells. The effects observed with the higher concentration of $CuSO_4$ varied profoundly at the different oleate concentrations. With 50 $\mu M$ we had a significant increase ($P < 0.01$) in phospholipid synthesis, while at the higher concentration of oleate there was a 20% decrease in the same parameter, although the difference was not statistically significant.

Table I shows the molar ratio values between cholesteryl ester, triacylglycerols, and phospholipids synthesized at 500 $\mu M$ with respect to those synthesized at 50 $\mu M$ of oleate. The 10-fold increase in the concentration of oleate augmented the synthesis of esterified cholesterol in all experimental conditions tested from 6.6- to 8.1-fold. The increase of triacylglycerols ranged from 15.3- to 19-fold,

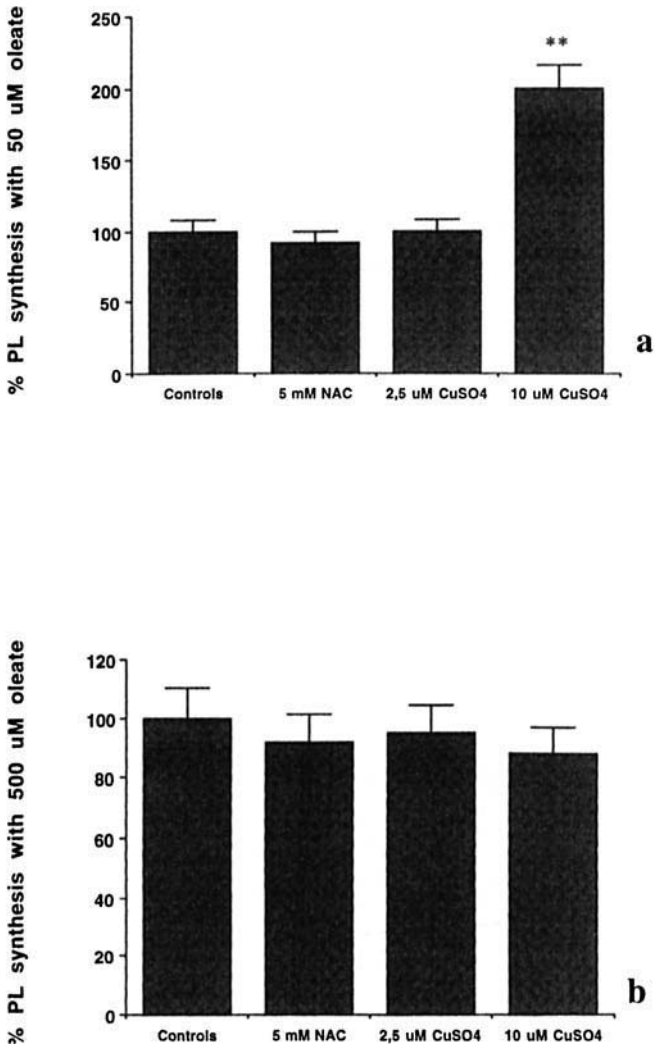



**Figure 5.** Effect of NAC or $CuSO_4$ pre-treatment on the phospholipid synthesis induced by 50 $\mu M$ (a) and 500 $\mu M$ (b) oleic acid in Caco-2 cells. Cells grown on 24-well plates for 10 days were exposed to 5 m$M$ NAC or 10 $\mu M$ $CuSO_4$ for 24 hr. The medium was then removed, and the cells were washed with PBS and, finally, incubated for 4 hr at 37°C with serum-free DMEM containing 50 or 500 $\mu M$ oleate and 2 μCi/ml [$^3$H]oleate bound to 1% fatty acid-free albumin. Phospholipid synthesis was determined as described in Materials and Methods. Data are expressed as percentage of controls, which was considered as 100%. Control values were respectively 7.78 ± 0.56 nmol/hr/mg protein with 50 $\mu M$ oleate, and 29.12 ± 2.57 nmol/hr/mg protein with 500 $\mu M$ oleate. The means and standard deviations of six separate experiments are reported. $^{**}P < 0.01$ versus controls (cells incubated with oleate but not previously treated with NAC or $CuSO_4$).

without significant differences among different intracellular redox state, while the phospholipid synthesis was raised about 5–6-fold in all groups except in cells treated with 10 $\mu M$ $CuSO_4$. In these cells, we observed that an increase in oleate concentration enhanced phospholipid synthesis when the lower concentration of pro-oxidant was used, while cells treated with the higher concentration of $CuSO_4$ did not respond to the increased availability of oleate with an increase in its incorporation into phospholipids.

**Effect of Different Internal Redox Equilibrium on ACAT Activity of Caco-2 Cells.** Caco-2 cells were pre-treated with NAC or $CuSO_4$, in the absence or presence of exogenous 500 $\mu M$ oleate for 24 hr. After this treatment the cells were harvested as described in Materials and Methods, and kept at −80°C, before the enzymatic activities were determined. The results obtained (Table II) showed that different internal redox conditions did not affect ACAT activity in all the experimental conditions reported.

## Discussion

The present study was undertaken to assess the possibility that *in vitro* redox modulation may alter the lipid synthesis induced by oleic acid in the Caco-2 intestinal cell line. Our results suggest that the pre-existing internal redox potential of the cells is a modulating factor able to affect lipid synthesis.

The major lipid classes synthesized in control cells incubated with 50 $\mu M$ oleate, in absence of any treatment to introduce redox perturbation, were phospholipids, followed by triglycerides and cholesteryl ester. The intracellular synthesis of the three lipid classes here examined appeared to be dependent on the oleate concentration used. This when 500 $\mu M$ oleate was used, a significant increase in the incorporation of labeled oleate in all the lipid classes was detected. In our experimental conditions, we observed that the 10-fold increase in the concentration of the fatty acid in the incubation medium induced a 16-fold rise in triacylglycerol synthesis. Field and colleagues (14) found, in similar experimental conditions, that the incorporation of labeled glycerol into cellular triacylglycerols increases in step-wise fashion up to 1 m$M$ of oleic acid, reporting a 3-fold increase in triacylglycerol synthesis in the range of oleate concentration used in our experiments. Notwithstanding the apparent discrepancy, we believe that these data are generally in agreement, considering that for the synthesis of triacylglycerol three fatty acids are used for each molecule of glycerol. We also observed an augmented incorporation of labeled oleic acid into phospholipid (6.8-fold) and cholesteryl ester (5.3-fold) when the cells were incubated with 500 m$M$ as compared to 50 $\mu M$ oleate. These data suggest that in presence of low concentrations of lipid in the lumen of the gastrointestinal tract the prevalent biosynthetic enterocytic pathway is that of phospholipid, while the presence of higher concentration of fatty acids, compatible with a post-prandial condition, causes to an increase in triacylglycerol synthesis, which is accompanied by a weaker enhancement of the synthesis of other lipid classes, necessary for enterocytic lipoprotein secretion.

Once we had evaluated the oleate incorporation into lipids in control cells, we studied the intracellular redox influence on such a function by using two different redox perturbants having opposite mechanisms of action: the pro-oxidant agent $CuSO_4$ and the antioxidant NAC. The intention of the authors was to induce consistent and significant alterations of cell redox potential without affecting cell viability, structure, or function. In this sense the redox modifications reported may be considered "mild". As would be

**Table I.** Molar Ratio Variation Between [$^3$H]Lipid Synthesized (Expressed as nmol/hr/mg Cell Protein) in Presence of 500 μM Oleate With Respect to Those Synthesized at 50 μM Oleate, in Enterocytes Having a Different Intracellular Redox Equilibrium

| | Controls | NAC (5 mM) | $CuSO_4$ (2.5 μM) | $CuSO_4$ (10 μM) |
|---|---|---|---|---|
| Cholesteryl ester | 6.8 ± 2.1 | 6.6 ± 3.1 | 8.1 ± 3.5 | 7.8 ± 2.3 |
| Triacylglycerols | 16.8 ± 6.5 | 15.7 ± 4.1 | 15.3 ± 5 | 19.0 ± 5.1 |
| Phospholipids | 3.85 ± 1.1 | 6.2 ± 2.4 | 5.3 ± 2.3 | 0.8 ± 0.4[a,b] |

The values are reported as mean ± SD of at least four different experiments.
[a] $P < 0.005$ versus untreated cells.
[b] $P < 0.01$ versus 5 mM NAC- and 2.5 μM $CuSO_4$-treated cells.

expected, pre-treating the cells with $CuSO_4$, an evident switch to an emerging internal pro-oxidant condition due to GSH loss and an increased level of cell lipid peroxides was detected. Our subsequent studies of the incorporation of labeled oleic acid into cellular lipids demonstrated that the induced oxidative stress is capable to significantly affecting lipid synthesis by intestinal cells, essentially through a trend toward up-regulation of the synthesis of phospholipids. By contrast, on using the antioxidant and thiol supplier NAC, for which an opposite effect characterized by a more reducing state has been found, we observed a significant down-regulation of triacylglycerol synthesis with a minor modification in that of phospholipids. This observation appears particularly interesting considering that lowered triacylglycerol synthesis has recently been associated with a presumptive pro-oxidant stimulus due to exposure of intestinal cells to oxidized fatty acid (23). Interestingly, in all the experimental conditions reported here, the incorporation of labeled oleic acid into the cholesteryl ester fraction appeared to be unmodified. This observation, suggesting that esterification of cholesterol is a relatively redox-independent process, appears to be reinforced by a relatively unchanged cell activity of the enzyme responsible for cholesteryl ester synthesis, ACAT.

The intestinal epithelium is exposed to oxidants derived from the diet, bacterial metabolic products, and endogenously formed cellular metabolites. Impaired mucosal antioxidative capability increases gut susceptibility to oxidative stress injury. In fact, as reported by Privett and Cortesi (24), the capability of fatty acids to promote an evident oxidation attack to intestinal epithelium is strongly related to the basal content of dietary (vitamin E, carotenoids, selenium, etc.) and endogenous (in particular the GSH/GSSG system) antioxidants, rather than dietary oxidants *per se*. This condition is relevant for the development and progression of mucosal alteration associated with several gastroenterologic disorders (25, 26). In this paper we point out that nontoxic modifications of the intracellular oxidative balance could play a role in addressing numerous physiological functions, including absorption, processing, and, probably, output of lipid sources. The experimental conditions reported above do not resemble aberrant conditions of the gut, which are often characterized by an evident presence of diffuse cytopathology. Rather, with this protocol, we have considered those alterations of the redox balance that fall in a physiological or subtoxic range. These conditions occur frequently *in vivo*, as the gut is continuously exposed to external (food, contaminants, drugs, microorganisms) and internal (cytokines, inflammatory mediators such as prostaglandins) stimuli that are potentially able to alter the oxidative state of the enterocytes. In addition, the entire antioxidant defense system of the intestinal mucosa (many epithelia characterized by continuous renewal of the cells) is not as structurally or functionally important as, for example, those of the liver and the lung, which are provided with high concentrations of GSH and GSH-dependent enzymes, SOD, and catalase (27, 28).

**Table II.** Effect of NAC or $CuSO_4$ Pre-treatment on ACAT Activity in Caco-2 Cells Exposed to 500 μM Oleic Acid

| ACAT (nmolCE/hr/mg protein) | Without oleate | 500 μM oleate |
|---|---|---|
| Controls | 5.2 ± 2.4 | 6.84 ± 1.96 |
| 5 mM NAC | 6.53 ± 3.06 | 6.92 ± 3.33 |
| 2.5 μM $CuSO_4$ | 6.11 ± 2.17 | 6.22 ± 2.9 |
| 10 μM $CuSO_4$ | 6.87 ± 3.7 | 6.07 ± 2.17 |

The values are reported as mean ± SD of at least four different experiments.

The gastrointestinal tract remains the most popular and acceptable route of administration for drugs. Actually, there is great interest in the use of antioxidants to control and/or counteract the deleterious effects of bowel inflammation. In particular, besides drugs extensively used in inflammatory bowel diseases such as 5-aminosalicylic acid (29), oral supplementation of cysteine or NAC displays beneficial effects for some of the above mentioned diseases, due to their radical-scavenging activity and their action in helping to replenish intracellular GSH stores (25). In general, glutathione is essential for functional and structural integrity of the gut (30). At the subcellular level, soluble thiols concentration may modulate numerous activities, including some that are cytoskeleton-dependent (i.e., receptor expression, adhesion properties, vesicular trafficking) (31), and recent evidence suggests the possibility of thiol-dependent modulation of lipoprotein assembly and secretion (32). Moreover, as reported by others (33), also slight but consistent alterations of the redox state of the cells (particularly of the thiol balance) are capable to affect/modulate several subcellular and metabolic functions. Thus, the redox potential of the

cell could be interpreted *per se* as a factor able to influence/drive cell signaling.

In our study, the use of an *in vitro* model has demonstrated that by manipulations on the GSSG/GSH balance with two drugs having opposite effects we can introduce significant modifications in the way in which intestinal cells process lipids. This situation could represent only the first step of downstream events leading to a remodulation of lipoprotein synthesis and secretion by enterocytes (this aspect is currently under investigation by our group), influencing plasma lipoprotein profile. Present data suggest that 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, supporting the hypothesis (34–36) that the use of antioxidants drugs and/or the intake of nutritional antioxidants represents a possible way to control, at least partially, both the level and profile of circulating lipids.

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