

Cholesterol changes the fatty acid composition of rat enterocytes

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Abstract

The effect of free cholesterol on the fatty acid composition and growth of rat fetal enterocytes was investigated in the absence and presence of 10% (v/v) fetal calf serum. Cholesterol caused a significant reduction of cell number after 6 and 12 h in culture. The fatty acid composition of enterocytes cultured in the presence of serum was also changed by the presence of 20 μ M cholesterol. The fatty acid profile was determined by HPLC using fluorescence detection (325 nm excitation and 395 nm emission). Cholesterol (20 μ M) increased the proportion (given in percentage of the total fatty acids) of the following fatty acids in cultured cells: lauric (by 42%), oleic (by 34%), linoleic (by 44%) and γ -linolenic (by 20%) acids and reduced the proportion of palmitic (by 12%), stearic (by 20%), arachidonic (by 21%) and docosahexaenoic (by 44%) acids. In addition to modifying the content of individual fatty acids, cholesterol increased the polyunsaturated/saturated fatty acid ratio from 0.48 to 0.67 and the unsaturation index from 67.12 to 75.30. This is the first evidence that cholesterol modifies fatty acid composition possibly via *de novo* fatty acid synthesis and desaturation.

Key words

- Enterocytes
- Cholesterol
- Fatty acid composition
- Cell proliferation

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Research supported by FAPESP,
CNPq, PRONEX, and CAPES.

Received July 3, 2002
Accepted November 7, 2002

Cholesterol is a water-insoluble molecule and its intestinal absorption is very complex. In addition to diet, there are two endogenous sources of cholesterol: biliary cholesterol, which is always unesterified, and intestinal epithelial sloughing. Cholesterol absorption requires emulsification, hydrolysis of the ester bond (when esterified) by a specific pancreatic esterase, micellar solubilization, absorption in the proximal jejunum, re-esterification within the intestinal cells, and transport to the lymph in the chylomicrons (1). The intestine, however, has a limited

capacity to absorb cholesterol. The absorption of dietary cholesterol ranges from 40 to 60% independent of the amount ingested up to 600 mg/day (2). The absolute amount of cholesterol absorbed increases in parallel with intake within the normal range (3). Therefore, a significant proportion of dietary cholesterol remains in the intestinal lumen to be excreted (4).

Cholesterol and fatty acids are primary components of cell membranes. Growing cells synthesize both cholesterol and fatty acids according to their growth demands (5).

Recent evidence suggests that the synthesis of cholesterol, fatty acids and triglycerides is controlled by a common family of transcription factors designated sterol regulatory element-binding proteins (SREBP) (6). SREBP directly activate the expression of more than 30 genes dedicated to the synthesis and uptake of cholesterol, fatty acids, triglycerides, and phospholipids (7,8). When the cholesterol content of cells increases, SREBP lose their ability to bind DNA and the transcription of target genes ceases. Cholesterol acting through SREBP activates the expression of several genes of the pathways of fatty acid and triglyceride synthesis such as fatty acid synthase and glycerol-3-phosphate acyltransferase. In addition, this sterol raises the expression of long-chain fatty acyl elongase and stearoyl CoA desaturase. These observations raise the possibility that cholesterol may be able to change the fatty acid composition of the cells.

These considerations and the fact that SREBP has been reported to be present in the intestine (9) led us to investigate the effect of free cholesterol on the fatty acid composition of rat fetal enterocytes. Concomitantly, the effect of cholesterol on enterocyte growth was also evaluated. The experiments were performed with and without fetal calf serum (FCS) in the media. The latter condition mimics the luminal side of the intestinal epithelium.

HF12 medium, DMEM, streptomycin and penicillin were obtained from Invitrogen (New York, NY, USA). DMSO, collagenase type IA, hyaluronidase, and cholesterol were purchased from Sigma (St. Louis, MO, USA). FCS was from Cutilab (Campinas, SP, Brazil) and trypsin was from the Adolfo Lutz Institute (São Paulo, SP, Brazil).

Male and female Wistar rats (3-4 months) obtained from the Department of Physiology and Biophysics, Institute of Biomedical Sciences, University of São Paulo, were maintained (3 females and 1 male) on a 12-h light/dark cycle for 7 days. Pregnant female rats

were housed in separate cages until the 20th day of pregnancy when they were killed by cervical dislocation, and the fetuses were collected after abdominal incision.

For the isolation and culture of enterocytes from rat fetuses, a modified protocol of the method described by Fukamachi (10) and Evans et al. (11) was used. Fetuses were washed with 70% alcohol. The small intestine was then dissected out and washed in Hank's balanced salt solution (HBSS) at 37°C. Mesenteric tissue was removed and the intestine was opened laterally under a light microscope. The intestinal mucosa was treated with collagenase type IA (458 U/ml) and hyaluronidase (320 U/ml) in HBSS, at 37°C for 60 min in a shaker bath and the preparation was centrifuged at 1,200 g for 5 min at 4°C. The pellet was resuspended in sterile HF12 medium by repeated pipetting using a Pasteur pipette. The cells were decanted in 50-ml polycarbonate tubes and centrifuged for periods of up to 10 min. Cells were then cultured in HF12 medium at 37°C in an atmosphere of 5% CO₂ and 95% air. Cells were diluted in medium without FCS or in medium containing 10% FCS to achieve a final concentration of 1.0 x 10⁵ cells/ml. Cells were plated for the periods indicated above in 24-well plates and maintained at 37°C in an atmosphere of 5% CO₂ and 95% air. Cells were removed from the plates by gentle scraping and their total number and viability index were determined with a Neubauer chamber using 0.1% Trypan blue dye solution in saline.

Before addition to the medium, cholesterol was solubilized in ethanol. For the control, the same volume of ethanol was added at a final percentage which was always less than 0.5%. Enterocyte growth curves were constructed in the presence and absence of FCS after 3, 6, 12, 24, 48 and 72 h. The effect of cholesterol on cell growth in the presence and absence of FCS was tested at 20 µM after 6 and 12 h in culture. Previous experiments using different concentrations and flow

cytometry analysis established that 20 μM cholesterol does not cause necrosis or apoptosis of enterocytes.

The effect of 20 μM cholesterol on fatty acid composition was evaluated after 24 h. The fatty acids were extracted from the enterocytes as previously described (12). After extraction and saponification (13), the fatty acids were derivatized with 4-bromomethyl-7-coumarin (14) and the analysis was performed in a Shimadzu model LC-10A liquid chromatographer. The samples were eluted using a C8 column (25 x 4.6 cm ID, 5- μm particles) with a C8 pre-column (2.5 x 4.6 cm ID, 5- μm particles), at a flow rate of 1 ml/min of acetonitrile/water (77:23, v/v) and fluorescence detector (325 nm excitation and 395 nm emission) (13). The fatty acids used as standards were obtained from Sigma: lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1 ω 6), stearic (C18:0), oleic (C18:1 ω 9), linoleic (C18:2 ω 6), linolenic (C18:3 ω 6), arachidonic (C20:4), eicosapentaenoic (C20:5 ω 3), docosahexaenoic (C22:6 ω 3), and margaric (C17:0) acids. The last fatty acid was used to calculate recovery and quantify the others. We constructed a calibration curve for each fatty acid standard, obtaining coefficients of correlation and regression. For quantification of fatty acids, the capacity factor (K'), elution sequence, linearity, recovery, precision, interference and limit of detection were determined. The minimum limit of quantification of the fatty acids ranged from 1 to 10 ng.

The unsaturation index of the fatty acids present in enterocytes was calculated as previously described (15,16). Briefly, the proportion of each fatty acid multiplied by the number of double bonds present in the molecule was summed and the values are presented in Table 1. The polyunsaturated/saturated (P/S) fatty acid ratio of the total fatty acids present in enterocytes was calculated as previously described (17). The effect of cholesterol was analyzed statistically by ANOVA followed by the Tukey-Kramer test

for comparison with control conditions (ethanol addition). The results are reported as means \pm SEM.

The experimental procedure used in this study was approved by the Ethics Committee of the Institute of Biomedical Sciences, University of São Paulo.

Rat fetal enterocytes presented a consistently higher number of cells when cultured in the absence of FCS for all periods studied (Figure 1A). For the period between 24 and 72 h in culture, the number of cells was about 42% higher in the absence than in the presence of serum. These findings are compatible with the fact that intestinal epithelial cells actively proliferate in the intestinal lumen, which occurs in the absence of serum.

Cholesterol has been shown to reduce the proliferation of various cell types such as lymphocytes (18), fibroblasts (6), intestinal epithelial cells (10) and tumor cells (5). Therefore, the reduction in the number of

Table 1. Effect of cholesterol on the fatty acid composition of rat fetal enterocytes after 24 h in culture.

Fatty acids	Without 10% FCS and ethanol	Without 10% FCS and 20 μM cholesterol	With 10% FCS and ethanol	With 10% FCS and 20 μM cholesterol
12:0 Lauric acid	4.81 \pm 0.71	5.33 \pm 0.17	5.50 \pm 0.39	7.81 \pm 0.51*
14:0 Myristic acid	5.17 \pm 0.28	4.86 \pm 0.30	4.74 \pm 0.25	4.26 \pm 0.20
16:0 Palmitic acid	26.42 \pm 1.30	26.03 \pm 1.72	27.07 \pm 0.42	23.83 \pm 0.39*
16:1 Palmitoleic acid	3.24 \pm 0.60	3.65 \pm 0.51	3.36 \pm 0.63	3.02 \pm 1.00
18:0 Stearic acid	30.44 \pm 2.30	30.54 \pm 1.66	29.98 \pm 1.72	23.93 \pm 1.43*
18:1 Oleic acid	11.93 \pm 1.18	11.17 \pm 0.46	11.14 \pm 0.80	14.93 \pm 0.30*
18:2 Linoleic acid	12.09 \pm 1.33	10.69 \pm 1.69	11.31 \pm 0.69	16.29 \pm 0.85*
18:3 γ -Linolenic acid	1.30 \pm 0.21	1.10 \pm 0.20	1.17 \pm 0.04	1.40 \pm 0.07*
20:4 Arachidonic acid	2.38 \pm 0.49	4.48 \pm 0.52*	3.53 \pm 0.59	2.79 \pm 0.93*
20:5 EPA	1.15 \pm 0.17	0.85 \pm 0.07	0.98 \pm 0.07	1.06 \pm 0.06
22:6 DHA	1.07 \pm 0.15	1.30 \pm 0.13	1.22 \pm 0.09	0.68 \pm 0.04*
P/S ratio	0.49	0.48	0.48	0.67
Unsaturation index	64.45	69.60	67.12	75.30

The cells (10^6) were cultured in HF12 medium in the presence and absence of 10% fetal calf serum (FCS). The values are reported as means \pm SEM of eight samples from three experiments. P/S, polyunsaturated/saturated ratio; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

* $P < 0.05$ compared to no cholesterol addition (ANOVA followed by Tukey-Kramer test).

cells (Figure 1) may result from a low proliferative activity of the enterocytes as reported for the other cells. In the absence of serum, cholesterol reduced the number of cells by 41% after 6 and 12 h in culture. This effect of cholesterol was less pronounced in the presence of serum for both 6 (26%) and 12 h (29%). Thus, free cholesterol in the intestinal lumen may play a role in the control of epithelial cell proliferation.

The fatty acid composition of enterocytes cultivated in the absence of serum was only slightly changed by cholesterol (Table

1). This sterol raised the proportion of arachidonic acid by 88%, whereas the unsaturation index was increased from 64.45 to 69.60.

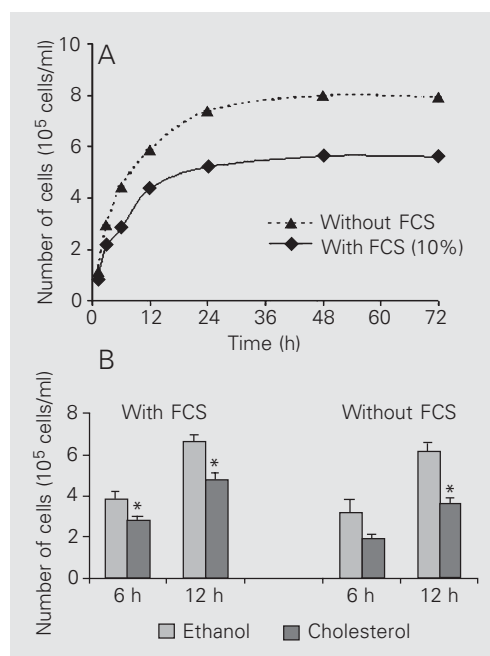
On the other hand, there was a marked effect of cholesterol on the fatty acid composition of cells cultivated in the presence of serum. Cholesterol (20 μ M) increased the proportion of lauric (by 42%), oleic (by 34%), linoleic (by 44%) and γ -linolenic (by 20%) acids and reduced that of palmitic (by 12%), stearic (by 20%), arachidonic (by 21%) and docosahexaenoic (by 44%) acids. In addition to the changes in individual fatty acids, cholesterol increased the P/S ratio (from 0.48 to 0.67) and the unsaturation index (from 67.12 to 75.30). Therefore, cholesterol treatment in the presence of serum (that mimics the conditions on the basolateral side of the intestinal epithelium) causes significant changes in fatty acid composition.

The present report is the first evidence that cholesterol modulates fatty acid composition of membranes possibly via *de novo* fatty acid synthesis and desaturation. These effects may be important for the function of the intestinal epithelium.

Acknowledgments

The authors are indebted to J.R. Mendonça and G. de Souza for technical assistance.

Figure 1. A, Effect of 10% fetal calf serum (FCS) on the growth curve of rat fetal enterocytes cultivated in HF12 medium. B, Effect of cholesterol (20 μ M) and FCS on the total number of rat fetal enterocytes after 6 and 12 h in culture. Data are reported as means \pm SEM of nine determinations from three cell preparations. * $P < 0.05$ compared to no cholesterol addition (ANOVA followed by Tukey-Kramer test).

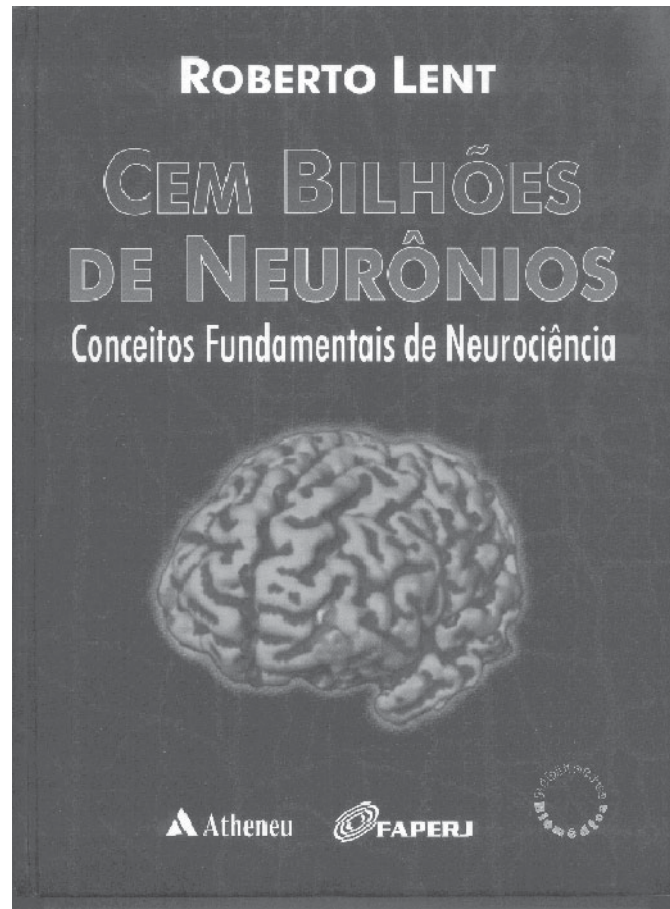


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