

***EXAMINING THE EFFECT OF STEROL CARRIER PROTEIN-2 ON  
PHYTANIC ACID TOXICITY***

A Senior Thesis

By

Cynthia Chen

1997-98 University Undergraduate Research Fellow  
Texas A&M University

Group: Biochemistry/Chemistry

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on Phytanic Acid Toxicity**

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**Cynthia Chen**

**Advisor: Dr. Friedhelm Schroeder**

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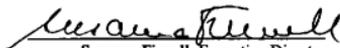
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Approved as to style and content by:



Dr. Friedhelm Schroeder (Faculty Advisor)  
Department of Physiology and Pharmacology



Susanna Finnell, Executive Director  
Honors Program and Academic Scholarships

Fellows Group: Biochemistry/Chemistry

## FOOTNOTES

### Abbreviations used:

SCP-2, sterol carrier protein-2; PDD, peroxisomal deficiency disease; PBS, phosphate-buffered saline; SCP-X, sterol carrier protein-X.

### Key words:

peroxisome, sterol carrier protein-2, phytanic acid, fatty-acid complex, flow cytometry,  $\alpha$ -oxidation,  $\beta$ -oxidation, toxicity, uptake, transfected L cells, SCP-2 over-expressors

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## ABSTRACT

Although peroxisomes are essential organelles involved in the conversion of hydrogen peroxide to water, they also contain unique metabolic pathways in lipid metabolism. One of these is the  $\alpha$ -oxidation of branched-chain fatty acids such as phytanic acid. Phytanic acid accumulates to toxic levels in patients with defective or deficient numbers of peroxisomes. Little is known about how phytanic acid is taken up into the cell and incorporated into the peroxisome. Sterol carrier protein-2 (SCP-2), a lipid-binding protein concentrated in peroxisomes, may function in phytanic acid uptake and metabolism. Fluorescence techniques have shown that SCP-2 can bind phytanic acid, but it is not known whether SCP-2 enhances uptake and/or metabolism of phytanic acid in intact cells. SCP-2 is concentrated in the peroxisomes, but significant amounts are also on the mitochondria and cytosol. To examine SCP-2 function and location of function, transfected L-cells over-expressing SCP-2 were exposed to a phytanic acid-albumin complex or a palmitic acid-albumin complex, and their relative toxicities were compared. The data show that SCP-2 enhanced the toxicity of phytanic acid, which undergoes  $\alpha$ -oxidation in the peroxisome, but not that of palmitic acid, which undergoes normal  $\beta$ -oxidation in the mitochondria. However, further study is needed to resolve the mechanism whereby SCP-2 mediates the toxicity of phytanic acid.

## INTRODUCTION

Although it is the most recently characterized organelle, much has been learned about the peroxisome and its deficiency diseases since the organelle's discovery in 1954. The peroxisome is essential to the cell, a fact underscored by the presence of peroxisomal deficiency diseases (Reddy and Mannaerts, 1994; Wanders, *et al.*, 1995).

Peroxisomal deficiency diseases (PDDs) can be classified into two groups. Zellweger syndrome and neonatal adrenoleukodystrophy patients lack peroxisomes as a result of defective peroxisome assembly. Other diseases have a single enzyme deficiency of the peroxisome; these include X-linked adrenoleukodystrophy and Refsum's disease. Clinical manifestations of the PDDs vary widely but all exhibit some degree of neurological deficit. Patients with PDDs often exhibit craniofacial dysmorphism and may die in infancy (Dodt, *et al.*, 1996). Peroxisomal functions, then, are of great interest since understanding them may aid in treatment of PDD patients.

Peroxisomes are named for their role in hydrogen peroxide metabolism, but they also contain several unique metabolic pathways, especially in lipid metabolism. This includes ether lipid biosynthesis, dolichol metabolism, bile acid synthesis, cholesterol synthesis (most enzymes), and  $\beta$ -oxidation of fatty acids (van den Busch, 1992; Reddy and Mannaerts, 1994). More importantly, peroxisomes also function in  $\alpha$ -oxidation of branched-chain fatty acids and  $\beta$ -oxidation of very long-chain fatty acids ( $>C_{22}$ ) that the mitochondria cannot metabolize. While  $\beta$ -oxidation takes place in both the peroxisome and the mitochondria,  $\alpha$ -oxidation occurs only in the peroxisome (Singh, *et al.*, 1993). Peroxisomes are clearly important in the oxidation of fatty acids, but the mechanism(s) by which these lipids are transported to and/or into the peroxisome are not known. Likewise, much remains to be learned about intraperoxisomal factors regulating  $\beta$ -oxidation and  $\alpha$ -oxidation.

One protein that has come under scrutiny in peroxisomal ligand transport is sterol carrier protein-2 (SCP-2), also called non-specific lipid transfer protein. Peroxisomes contain high levels of SCP-2 gene products (Schroeder, *et al.*, 1996), some of which may also be found extraperoxisomally (Keller, *et al.*, 1989). SCP-2 is a small protein of 13.5 kD which accelerates multiple steps in cholesterol metabolism, and it binds and transfers a wide variety of ligands including fatty acids, fatty acyl CoAs, sterols, and phospholipids *in vitro* (Stolowich, *et al.*, 1997; Frolov, *et al.*, 1997a, 1996; Schroeder, *et al.*, 1998, 1996, 1990; Colles, *et al.*, 1995). The SCP-2 gene has two initiation sites encoding a 58 kD SCP-X and a 15 kD pro-SCP-2. The latter is post-translationally cleaved to form the mature 13kD SCP-2. SCP-2 has no enzyme function but binds branched-chain fatty acids such as phytanic acid (Frolov, *et al.*, 1998). Thus, it may contribute to the metabolism of branched-chain fatty acids by aiding in their uptake.

The focus of this study is on a branched-chain fatty acid known as phytanic acid (3, 7, 11, 15-tetramethylhexadecanoic acid). It is a saturated 16-carbon chain with four methyl branches. Metabolism of phytanic acid is important since it is a by-product of chlorophyll degradation by resident bacteria in the first stomach of ruminants. Thus, it accumulates in butter, cheeses, and other dairy products, which are prevalent in the average Western diet (Table 1). Phytanic acid is unable to undergo normal  $\beta$ -oxidation in the mitochondria because of a methyl group in the  $\beta$  position (Figure 1). Instead, it undergoes  $\alpha$ -oxidation in the peroxisome to remove the terminal carboxyl group which then allows normal  $\beta$ -oxidation to proceed (Figure 2). If peroxisomes are defective or deficient, phytanic acid will accumulate to toxic levels.

Genetic defects of peroxisomes not only result in decreased phytanic acid oxidative enzymes and a resulting high accumulation of phytanic acid, but concomitantly lowered levels of the phytanic acid binding protein SCP-2 as well. Elevated phytanic acid levels are characteristic of PDDs; Wanders, *et al.* found phytanic acid levels of up to 25 times the normal amount in Zellweger syndrome and other PDD patients (1995). Whether the elevated phytanic acid levels

are due only to the defective peroxisomes and/or to lowered SCP-2 levels is not known. SCP-2 is able to bind phytanic acid, as shown by fluorescence techniques (Frolov, *et al.*, 1996). Further study of the inverse correlation between SCP-2 and phytanic acid is required. Several possibilities are suggested:

- Peroxisomal and extraperoxisomal SCP-2 may increase uptake of phytanic acid into the peroxisome but do not increase metabolism.
- Extraperoxisomal SCP-2 may enhance uptake of phytanic acid into the cell but not into the peroxisome.
- Peroxisomal SCP-2 may enhance the  $\alpha$ -oxidation of phytanic acid in the peroxisome by SCP-X.

To resolve these possibilities, we used transfected L-cells (mouse fibroblast cells) transfected with cDNA encoding the 15 kD pro-SCP-2. These transfected cells express the 15 kD protein which is essentially all cleaved post-translationally to 13 kD SCP-2. Untransfected L cells have only low levels of SCP-2 (Moncecchi, *et al.*, 1996; Gossett, *et al.*, 1996). The role of SCP-2 in phytanic acid uptake and/or metabolism can be examined by looking at the toxicity of phytanic acid in cells with low levels of SCP-2 and in cells over-expressing SCP-2.

## MATERIALS AND METHODS

### *Materials.*

Phytanic acid was obtained from Matreya, Pleasant Gap, Pennsylvania; palmitic acid from Sigma, St. Louis, Missouri; and lipid-free bovine albumin from Sigma. Calcein AM and ethidium homodimer-1 were purchased from Molecular Probes, Eugene, Oregon. All reagents used in culture media were cell culture grade.

### *Preparation of Albumin-Fatty Acid Complexes for Supplementing Cells.*

Palmitic acid was complexed to lipid-free albumin using the method of Spector and Hoak (1969). The same method was adapted to addition of phytanic acid to albumin as follows.

Palmitic acid (19.2 mg) was dissolved in 13.2 mL of hexane and phytanic acid (25 mg) was dissolved in 32 mL of dimethylfluoramide (DMF). Each was added to Celite particles in a small glass beaker, using 1  $\mu$ mol fatty acid per 10 mg solid phase. The solvent was evaporated under a stream of N<sub>2</sub> gas. The fatty acid-coated particles were then transferred to a stoppered Erlenmeyer flask containing 45 mL of serum-free Higuchi media with 750 mg lipid-free albumin added. The particles were incubated with constant stirring by a magnetic bar for 30 minutes at room temperature. The mixture was decanted into plastic tubes, centrifuged for ten minutes at 1000 rpm and the Celite particles removed. Centrifugation was repeated; the supernatant was adjusted to pH 7.4 and then sterilely filtered through a Millipore filter of pore size 0.2  $\mu$ m (Corning). The palmitic acid-albumin complex and the phytanic acid-albumin complex were kept frozen at -20° C.

### *Experimental Controls.*

Transfected L-cells over-expressing SCP-2 were compared with mock-transfected L-cells, used as the control. The use of mock-transfectants was to ensure that differences in cell toxicity were not due to genetic manipulation. L cells were transfected in this lab to carry a mock vector or the vector for SCP-2 over-expression. Northern blot was done on the 2A cells to test for SCP-2 RNA and Western blot done to ensure presence of the protein. Scanning densitometry of Western blots showed that the level of SCP-2 in the control mock-transfected cells was at the limit of detectability. In contrast, SCP-2 over-expressing cells had SCP-2 levels equal to  $0.038 \pm 0.01\%$  of the total protein (Moncecchi, *et al.*, 1996).

Palmitic acid (hexadecanoic acid) was chosen as the control fatty acid since it is a saturated 16-carbon chain fatty acid lacking methyl branches. Phytanic acid is also a saturated 16-carbon chain, differentiated by the presence of four methyl groups (Figure 3).

### *Cell Culture and Growth Conditions.*

A strain of mouse fibroblast cells, L cells, was grown in monolayer on 10 cm plastic plates (Corning). Higuchi medium (described in Higuchi, 1970) was used with the addition of 250 units each of penicillin and streptomycin (GibCo BRL) and 10% fetal bovine serum (Sigma). Cells were grown at 37° C and 5% CO<sub>2</sub>. Confluent monolayers were trypsinized and diluted in new media every two days.

### *Flow Cytometry.*

Toxicity of the fatty acids was quantified using flow cytometry to measure live/dead cell ratios. Flow cytometry, simply, examines cell properties as cells in suspension flow single-file through a laser-illuminated chamber. The cells, to which probes have been added, scatter light and fluoresce at different wavelengths. Appropriate probes are chosen that will distinguish one cell property from another.

Since determination of cell viability was the object of this experiment, a dye solution of calcein AM and ethidium homodimer-1 was chosen. Calcein AM, which is colorless, is taken up by live cells and hydrolyzed by cytosolic esterases to calcein, which fluoresces green. Ethidium homodimer-1 is not taken up by live cells but instead passes through the permeable membranes of dead cells. It stains the nucleus which then fluoresces red.

In the flow cytometer, the fluorescence emissions and scattered light are detected by a phototube, amplified, and digitized. The digitized values are stored in a data file on a personal computer. This study used a Becton Dickinson FACSCalibur cytometer in conjunction with the Macintosh software program CellQuest.

#### *Analysis of Live/Dead Ratios*

Two cell lines were tested: mock-transfected L-cells used as the control and L-cells transfected to over-express SCP-2. Thus, over-expressors can be compared to mock-transfectants to look at toxicity of phytanic acid in the presence of high levels of SCP-2 and at very low levels of SCP-2.

Plates of each cell line were grown to confluency. The serum-containing media was then aspirated off, and replaced with serum-free media under sterile conditions. Cells were washed once with three mL phosphate-buffered saline (PBS) before addition of the new media. Each cell culture dish was then inoculated with lipid-free albumin, the palmitate-albumin complex, or the phytanate-albumin complex for a final concentration of 25  $\mu$ M albumin. Plates were incubated for 24, 48, 72, or 96 hours.

After the appropriate incubation time, the cells were prepared for analysis. All culture media and washes were saved and centrifuged to prevent loss of floating cells. First, the culture media were removed from the plate. Cells were washed once with three mL of PBS. Cells were detached using three mL trypsin and the reaction stopped after five minutes with three mL serum-containing media. A small aliquot of the cells, now in suspension, was used to determine the cell

number with a hemacytometer. The rest of the culture medium was centrifuged along with the original media and the PBS wash for 6 minutes at 1000 rpm. The supernatant was aspirated off and the pelleted cells resuspended in 10 mL Puck's medium to obtain a cell density of approximately  $3.5 \times 10^5$  cells/mL. Aliquots of cells in suspension were incubated with the dye solution of calcein AM and ethidium homodimer-1 in Falcon tubes. Each sample was then run through the flow cytometer at low speed. At this speed and with this concentration of cells, the flow cytometer counted cells at a rate of approximately 670 cells/second.

## RESULTS

Toxicity of the fatty acids were measured and compared by looking at the percentages of live and dead cells after the proper incubation time. Data output by CellQuest included the total number of counted cells, the number and percentage of those cells which were counted as "live," and the number and percentage of cells which were counted as "dead" (Figure 4).

### *Optimization of Dyes*

One of the major artifacts of flow cytometry is the accumulation of fluorescent dyes at high levels. This can be sufficient to self-quench, thereby leading to an underestimation of live and dead cells. Therefore, the proper concentration of calcein AM and ethidium homodimer-1 probes has to be determined for each cell system. The goal was to use the smallest dye concentration necessary to dye the cells so that background fluorescence, self-quenching artifacts, and/or toxic effects of the dyes to the cells would be minimized.

While a solution of 1  $\mu\text{M}$  calcein AM and 8  $\mu\text{M}$  ethidium homodimer-1 is optimal for many cell types, this was too high for L-cells. Likewise, a solution of 0.5  $\mu\text{M}$  calcein AM and 4  $\mu\text{M}$  ethidium homodimer-1 was still too high. It was found that a solution of 0.1  $\mu\text{M}$  calcein AM and 0.8  $\mu\text{M}$  ethidium homodimer-1 was appropriate for L cells (Figure 5).

### *Cell Death in Mock Transfectants.*

Figure 6 demonstrates the effects of the fatty acid-albumin complexes added to mock transfected cells. Lipid-free albumin added at a concentration of 25  $\mu\text{M}$  did not significantly affect the cells. In contrast, treatment of the cells with palmitic acid-albumin or the phytanic acid-albumin complexes resulted in toxicity, although not until after 72 hours of incubation with the fatty acid complexes. At this point, the addition of palmitic acid caused a 5% cell death and the phytanic acid resulted in 14% cell death. Toxicity increased to 8% cell death after 96 hours of

incubation in mock transfectants exposed to palmitic acid. The addition of phytanic acid resulted in cell death of 25% after 96 hours. Figure 7 compares the live and dead cell populations obtained for each treatment after 96 hours of incubation. The plot of the mock-transfectants exposed to lipid-free albumin shows that a small number of dead cells, while the plots of the mock transfectants treated with palmitic acid or phytanic acid show a larger population of dead cells.

Statistical analysis of the means using Student's t-test showed that cell death caused by the phytanic acid was significantly higher than that of the palmitic acid at 72 and at 96 hours ( $p < 0.01$ ). The addition of lipid-free albumin did not cause significant changes in live cell number over the period.

#### *Cell Death in SCP-2 Over-expressors.*

Exposure of transfected cells over-expressing SCP-2 to lipid-free albumin did not have toxic effects on the cells. At 72 hours, cells treated with the palmitic acid showed 12% cell death while those exposed to phytanic acid had 17% cell death (Figure 8). Cell death due to palmitic acid was greatest after 96 hours of incubation, at 10%. The addition of phytanic acid resulted in 40% cell death after 96 hours of incubation. Figure 9 shows the live and dead cell populations obtained from each treatment. The plot of the SCP-2 over-expressors exposed to phytanic acid shows a large distinct dead cell population as opposed to those treated with lipid-free albumin.

Again, statistical analysis of the means showed that addition of lipid-free albumin did not cause significant cell death. The greater percentages of dead cells seen in phytanic acid were statistically higher than those obtained from palmitic acid at both 72 and at 96 hours ( $p < 0.01$ ).

*Comparison of the Effect of Palmitic acid on Cells.*

Figure 10 compares the cells which had palmitic acid added. The mock transfectants and the over-expressors both exhibited about 10% death after 96 hours of incubation. Plots of the cells over the 96 hour incubation period were similar, and statistical analysis of the means showed no significant difference in the two cell lines (Table 2).

*Comparison of the Effect of Phytanic Acid on Cells.*

Figure 11 shows the cell growth for cells inoculated with phytanic acid. Unlike palmitic acid, the addition of phytanic acid was more toxic to the cells over-expressing SCP-2. Cell death in the two cell lines paralleled each other over the first 48 hours. At 72 hours, though, the over-expressors exhibited 18% cell death while the mock transfectants had 14% cell death. Statistical analysis of these means found that they were significantly different ( $p < 0.01$ ). At 96 hours, the difference in toxicity was even greater. The over-expressors experienced 40% cell death while the mock transfectants demonstrated a 25% cell death. Again, the means were found to be significantly different ( $p < 0.01$ ).

## DISCUSSION

### *Effects of Lipid-free Albumin on Cells.*

The addition of lipid-free albumin did not affect the growth of either the mock transfected cells or the SCP-2 over-expressors. The small percentages of cell death seen over the 96 hour incubation period (2-3%) may have been due to the albumin itself. However, since cell death did not fluctuate or increase over the period, it is more likely that the cells died from mechanical manipulations when suspending the cells in medium, toxic effects of the dyes used, or other minor causes.

Since the albumin alone is not responsible for cell death, the method of adding fatty acids complexed to albumin seems to be effective in isolating the toxic effects of the fatty acids.

### *Effects of Palmitic Acid on Cells.*

It was found that the addition of palmitic acid to culture media resulted in moderate toxicity in the cells. No significant difference between the mock transfectants and the over-expressors was seen. Palmitic acid is a fatty acid that is metabolized through normal  $\beta$ -oxidation in the mitochondria (van den Busch, 1992). SCP-2 is present in the mitochondria, although in much smaller amounts than are found in the peroxisome (Keller, *et al.* 1989). The similar response of the two cell lines to palmitic acid shows that mitochondrial  $\beta$ -oxidation is normal in SCP-2 over-expressors. Thus, mitochondrial SCP-2 does not alter the toxicity of palmitic acid by affecting its uptake or metabolism.

*Implications of the Effects of Phytanic Acid on SCP-2 Over-expressing Cells.*

The addition of phytanic acid to the cells over-expressing SCP-2 resulted in significantly greater cell death than in the mock transfectants, which contain very low levels of SCP-2. This increased toxicity in the presence of high levels of SCP-2 raises several possibilities:

1. Peroxisomal SCP-2 is involved in the uptake of phytanic acid into the peroxisome but does not enhance the enzymes involved in  $\alpha$ -oxidation of phytanic acid. Thus the increased levels of phytanic acid are not metabolized quickly enough and build up to toxic levels.
2. Peroxisomal SCP-2 is involved in both the uptake and the metabolism of phytanic acid. However, since the cells only over-expressed SCP-2, there were not sufficient levels of the enzymes involved in  $\alpha$ -oxidation to metabolize the additional phytanic acid.
3. Extraperoxisomal SCP-2 enhances uptake of phytanic acid into the cell and peroxisomal SCP-2 enhances uptake into the peroxisome. However, enzymes involved in  $\alpha$ -oxidation are not present in sufficient amounts to metabolize all of the phytanic acid.
4. Extraperoxisomal SCP-2 enhances the uptake of phytanic acid into the cell, but peroxisomal SCP-2 is not involved in transfer of the fatty acid into the peroxisome. The cell cannot transfer enough of the phytanic acid into the peroxisome for metabolism, so phytanic acid accumulates to toxic levels.

Assessment of each of these possibilities shows that the fourth explanation is the least likely. SCP-2 is primarily found in the peroxisome. Significant amounts are also present in the mitochondria, with some associated with the cytosol and endoplasmic reticulum (Keller, *et al.*, 1989). It is unlikely that the small amount of extraperoxisomal SCP-2 would enhance uptake of

phytanic acid into the cell without the large concentration of SCP-2 in the peroxisome enhancing phytanic acid uptake into the peroxisome for metabolism.

The third possibility, in which both extraperoxisomal and peroxisomal SCP-2 enhance uptake of the phytanic acid, is more feasible, but isolation of the two would be necessary for confirmation. Alternatively, the experiment could be repeated for transfected L cells over-expressing a protein known as liver fatty acid binding protein (L-FABP). It is known that L-FABP is present in the cytosol and participates in fatty acid binding and transport. L-FABP also binds phytanic acid (Frolov, 1997b). Thus, a toxicity curve in L-FABP over-expressors paralleling the toxicity shown in SCP-2 over-expressors would provide evidence for extraperoxisomal SCP-2's involvement in phytanic acid uptake.

Conversely, a toxicity curve with L-FABP over-expressing cells differing from that of SCP-2 over-expressors would provide evidence for the first two possibilities in which only peroxisomal SCP-2 is involved in phytanic acid transport. However, it would not be known if SCP-2 stimulates enzymes in  $\alpha$ -oxidation of phytanic acid (#2) or not (#1) since only normal levels of these enzymes are present in the SCP-2 over-expressors. To distinguish between the two, the experiment could be repeated for transfected L cells over-expressing both SCP-2 and SCP-X. Some evidence supports the hypothesis that some 13 kD SCP-2 is cleaved from the C-terminus of 58 kD SCP-X. The 58 kD SCP-X is a thiolase protein with enzymatic activity at its N-terminus and the SCP-2 portion at the C-terminus. SCP-X has been shown to react with phytanic acid derivatives and is an enzyme that selectively  $\alpha$ -oxidizes phytanic acid *in vitro* (Wanders, *et al.*, 1997). SCP-2 has no enzyme function but binds branched-chain fatty acids such as phytanic acid (Frolov, *et al.*, 1998). Thus, it may contribute to the reactivity of SCP-X by:

- (i) aiding in the uptake of branched-chain fatty acids;
- (ii) stimulating simultaneous conversion of phytanic acid to phytanoyl CoA and translocation into the peroxisome;

(iii) stimulating intraperoxisomal delivery of phytanic acid intermediate substrates to SCP-X.

Decreased toxicity of phytanic acid in these cells would support the explanation that toxicity in the SCP-2 over-expressors was due to enhanced uptake by peroxisomal SCP-2 without a concomitant increase in  $\alpha$ -oxidation enzymes.

The data described in this paper show that SCP-2 does indeed play a role in phytanic acid toxicity. However, further study is needed to confirm the location of SCP-2 function and its involvement in phytanic acid metabolism. It is suggested that this experiment be repeated for L-FABP over-expressors to clarify the location of SCP-2 function and for SCP-X/SCP-2 over-expressors to examine the role of SCP-2 in the metabolism of phytanic acid.

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**TABLE 1.** Phytanic acid content of various foods.

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<u>Food</u>	<u>mg per 100 g wet weight</u>
Butter	50-500
Margarine	6-130
Cheeses	5-50
Palm oil	11
Beef liver	2.6
Lard	1.8
Veal	0.75
Milk	0.26
Egg yolk	0.22

The typical Western diet provides 50-100 mg of phytanic acid per day.

The variations in phytanic acid content of dairy products reflect, in part, differences in cattle feed and total fat content. For margarine, content reflects sources of fat used in production.

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Source: Adapted from Steinberg, 1993; Singh, et al., 1993.

**TABLE 2.** Percentages of dead cells for each cell line after 72 and 96 hours  
of incubation.

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mock transfected cells

albumin	1.73 $\pm$ 0.2	1.74 $\pm$ 0.3
palmitate-albumin	5.11 $\pm$ 0.3	6.94 $\pm$ 0.3
phytanate-albumin	13.54 $\pm$ 0.8	23.04 $\pm$ 0.7

SCP-2 over-expressing cells

albumin	3.44 $\pm$ 0.2	2.46 $\pm$ 0.5
palmitate-albumin	11.43 $\pm$ 0.8	8.29 $\pm$ 0.4
phytanate-albumin	18.08 $\pm$ 0.5	39.33 $\pm$ 0.5

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## FIGURE CAPTIONS

Figure 1. Structure of phytanic acid (3, 7, 11, 15-tetramethylhexadecanoic acid).

Figure 2. Pathway for phytanic acid degradation.

Figure 3. Comparison of structures of palmitic acid and phytanic acid.

Figure 4. Samples of data output from CellQuest:

- A. L cells are indicated by the dots in the polygon-demarcated region of the plot of forward vs. side scatter. The L-cell region is used as a gate to analyze fluorescence emission from cells only and not from cellular debris.
- B. Plot showing fluorescence of live and dead cells. Calcein AM and ethidium homodimer-1 are used to obtain two distinct cell populations.

Figure 5. Data output for L cells using different dye concentrations:

- A. Fluorescence of L cells in a solution of 1  $\mu\text{M}$  calcein AM and 8  $\mu\text{M}$  ethidium homodimer-1.
- B. Fluorescence of L cells in a solution of 0.1  $\mu\text{M}$  calcein AM and 0.8  $\mu\text{M}$  ethidium homodimer-1.

Figure 6. Percentage of live cells – mock transfectants.

Figure 7. Data plots comparing populations of live and dead cells in mock transfectants.

- A. Cells treated with lipid-free albumin.
- B. Cells treated with palmitic acid.
- C. Cells treated with phytanic acid.

Figure 8. Percentage of live cells – SCP-2 over-expressors.

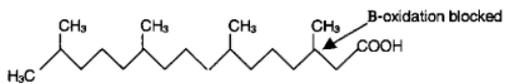
Figure 9. Data plots comparing populations of live and dead cells in SCP-2 over-expressors.

- A. Cells treated with lipid-free albumin.
- B. Cells treated with palmitic acid.
- C. Cells treated with phytanic acid.

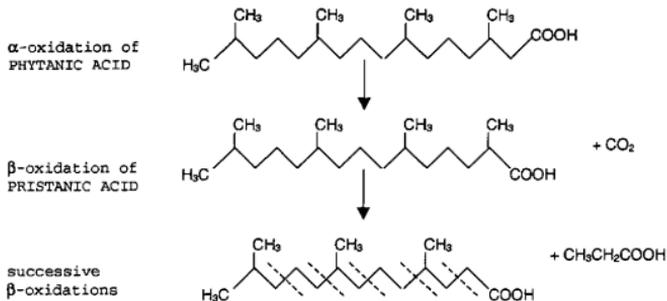
Figure 10. Comparison of percentages of live cells incubated in palmitic acid.

Figure 11. Comparison of percentages of live cells incubated in phytanic acid.

**FIGURE 1.** Structure of phytanic acid (3,7,11,15-tetramethylhexadecanoic acid).



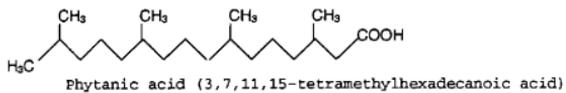
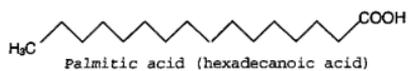
**FIGURE 2.** Pathway for phytanic acid degradation.



Source: Adapted from Singh, et al., 1993; Steinberg, 1993.

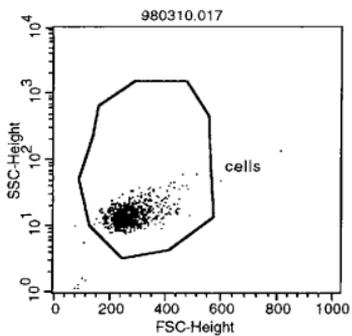
**FIGURE 3.** Comparison of structures of palmitic acid and phytanic acid.

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**FIGURE 4.** Samples of data output from CellQuest.

A.



B.

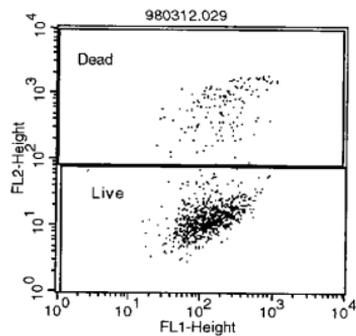
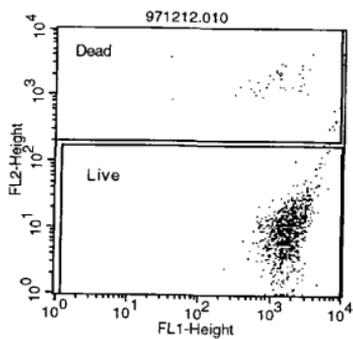
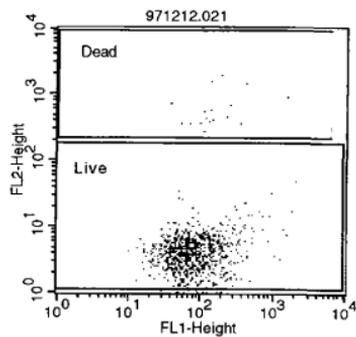


FIGURE 5. Data output for L cells using different dye concentrations.

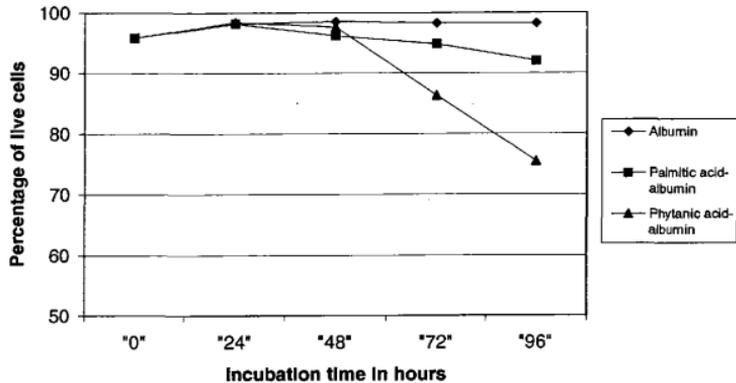
A.



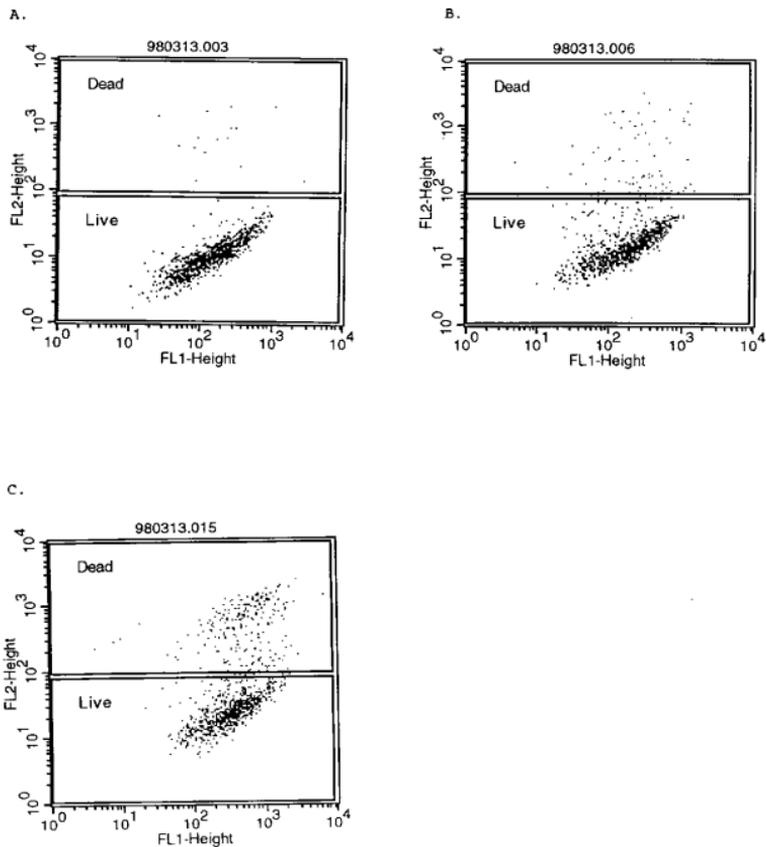
B.



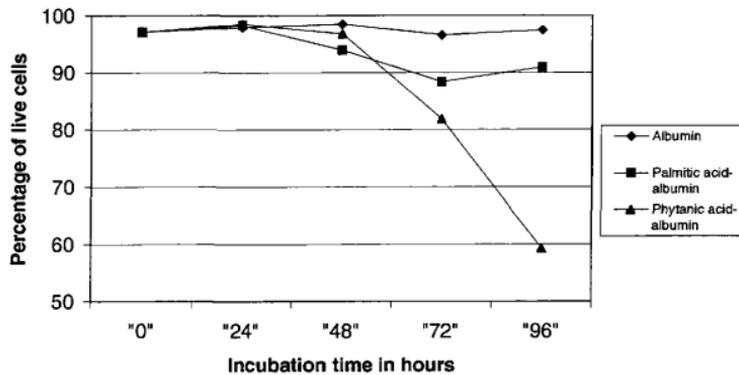
**FIGURE 6.** Percentage of live cells - Mock-transfected cells.



**FIGURE 7.** Data plots comparing populations of live and dead cells in mock transfectants.



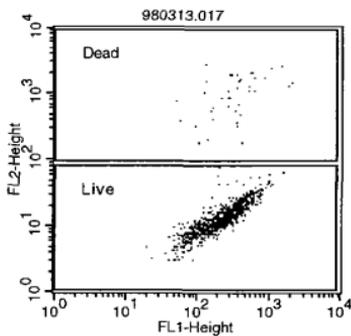
**FIGURE 8.** Percentage of live cells - SCP-2 Over-expressors.



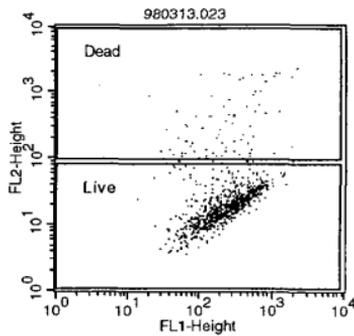
**FIGURE 9.** Data plots comparing populations of live and dead cells  
in SCP-2 over-expressors.

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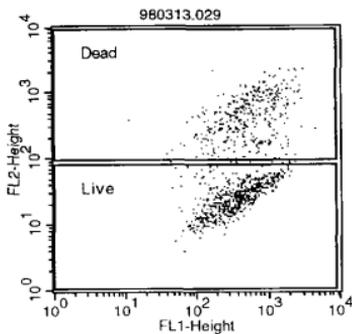
A.



B.



C.



**FIGURE 10.** Comparison of live cells incubated in palmitic acid.

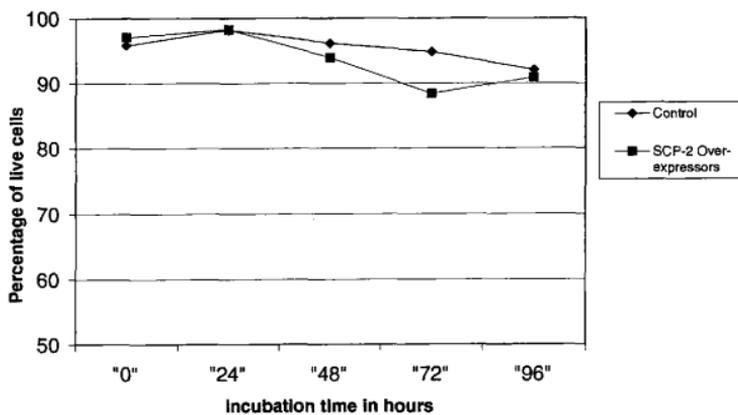
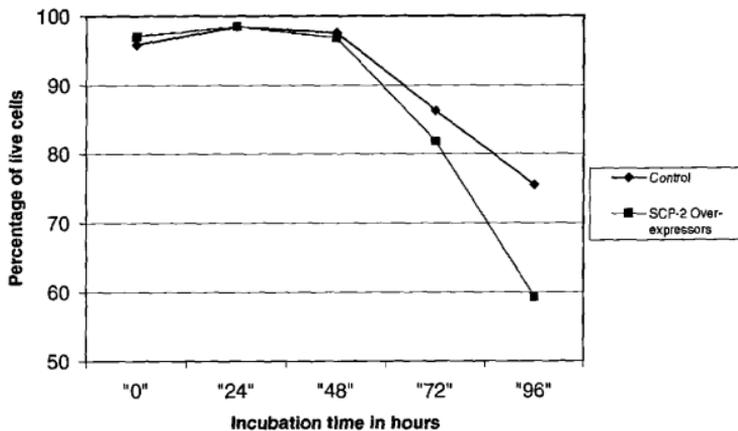


FIGURE 11. Comparison of live cells incubated in phytanic acid.



## ACKNOWLEDGMENTS

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