Sterol carrier protein-2/sterol carrier protein-x expression differentially alters fatty acid metabolism in L cell fibroblasts

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Abstract **Sterol carrier protein-2 (SCP-2) and SCP-x are ubiquitous proteins found in all mammalian tissues. Although both proteins interact with fatty acids, their relative contributions to the uptake, oxidation, and esterification of straight-chain (palmitic) and branched-chain (phytanic) fatty acids in living cells has not been resolved. Therefore, the effects of each gene product on fatty acid metabolism was individually examined. Based on the following, SCP-2 and SCP-x did not enhance the uptake/translocation of fatty acids across the plasma membrane into the cell: *i*) a 2-fold increase in phytanic and palmitic acid uptake was observed at long incubation times in SCP-2- and SCP-x-expressing cells, but no differences were observed at initial time points; *ii*) uptake of 2-bromo-palmitate, a nonoxidizable, poorly metabolizable fatty acid analog, was unaffected by SCP-2 or SCP-x overexpression; and *iii*) SCP-2 and SCP-x expression did not increase targeting of radiolabeled phytanic and palmitic acid to the unesterified fatty acid pool. Moreover, SCP-2 and SCP-x expression enhanced fatty acid uptake by stimulating the intracellular metabolism via fatty acid oxidation and esterification. In summary, these data showed for the first time that SCP-2 and SCP-x stimulate oxidation and esterification of branched-chain as well as straight-chain fatty acids in intact cells.**—Atshaves, B. P., S. M. Storey, and F. Schroeder. **Sterol carrier protein-2/sterol carrier protein-x expression differentially alters fatty acid metabolism in L cell fibroblasts. *J. Lipid Res.* 2003.** 44: **1751–1762.**

Supplementary key words phytanic acid • palmitic acid • 2-bromopalmitate • fatty acid oxidation • fatty acid uptake

Sterol carrier protein-2 (SCP-2) gene products (SCP-2 and SCP-x) have long been identified as sterol transport proteins [reviewed in ref. (1)]. More recent studies indicate that SCP-2 also functions in the uptake and esterification of straight-chain (e.g., oleic acid) fatty acids (FAs) (2), while SCP-x participates in the oxidation of branched-chain (e.g., phytanic acid) FAs (3, 4). However, almost nothing is known about the function of SCP-x in FA uptake or esterification. Likewise, the relative roles of SCP-2 versus SCP-x in straight-chain or branched-chain FA oxidation remain unresolved. Despite the lack of conclusive evidence, several observations point to an important role for SCP-2 and SCP-x in functioning in these capacities, including FA binding affinities where SCP-2 binds straight-chain FAs (5–11) and their metabolically active fatty acyl CoA derivatives (9, 12) to a high degree, as exhibited by K_ds in the submicromolar and nM range, respectively. In addition, SCP-2 also binds branched-chain FAs such as phytanic or pristanic acid (9), as well as their respective acyl CoAs with high affinity (7, 9).

The intracellular localization of SCP-x and SCP-2 also indicate a role in FA metabolism. The highest concentration of SCP-2 and SCP-x is in peroxisomes (13), where α-oxidation of branched-chain FAs as well as the β-oxidation of some straight chain FAs occurs (14–16). Additionally, it has been shown that half or more of total SCP-2 is extraperoxisomal (13, 17), where part of the SCP-2 may arise from the partial posttranslational cleavage of the SCP-x protein (18, 19). Extraperoxisomal SCP-2 locations include: *i*) plasma membrane in caveolae, wherein several FA translocase/transporter proteins reside (20); *ii*) cytoplasm, where SCP-2 enhances diffusion of fluorescent fatty acid (21); *iii*) microsomes, where FAs are esterified (17, 22, 23); and *iv*) mitochondria, where the majority of FA β-oxidation occurs (17, 24, 25).

In terms of physiological roles of SCP-2 and SCP-x in FA metabolism, while simultaneous ablation of SCP-x and SCP-2 in mice diminished oxidation of phytanic acid (9), the liver FA binding protein (L-FABP) was upregulated 4-fold, complicating clear interpretation of results. This followed from the fact that: *i*) L-FABP enhances the uptake of both unsaturated (23, 26–28) and saturated (29–32) straight-chain FAs in intact cells; *ii*) L-FABP functions as a FA donor for both peroxisomal and mitochondrial FA oxidation in vitro (33); *iii*) L-FABP binds phytanic acid (7); and *iv*) phytanic is a PPARα ligand that, like other peroxisomal

Manuscript received 4 April 2003 and in revised form 20 May 2003.

Published, JLR Papers in Press, June 16, 2003.
DOI 10.1194/jlr.M300141-JLR200

Abbreviations: CE, cholesteryl ester; DG, diacylglyceride; FA, fatty acid; L-FABP, liver fatty acid binding protein; MG, monoacylglyceride; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; SCP, sterol carrier protein; TG, triacylglyceride.

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proliferator agents, simultaneously induces expression of L-FABP and peroxisomal FA oxidation (34–36). Thus, it is unclear whether the abnormal phytanic acid oxidation in SCP-x/SCP-2 gene-ablated mice was due to the absence of SCP-2, the absence of SCP-x, or the upregulation of L-FABP.

In summary, the above studies strongly suggest a correlation between SCP-x and/or SCP-2 and FA metabolism, including oxidation and esterification. However, this has not been definitively established in intact cells or animals. The objective of the present investigation in examining saturated FA metabolism in intact L cells overexpressing either SCP-2 or SCP-x was to answer the question of whether SCP-2 or SCP-x plays a role in peroxisomal or mitochondrial FA uptake, oxidation, and esterification in intact cells.

MATERIALS AND METHODS

Materials

Silica Gel G and Silica Gel 60 plates were obtained from Analtech (Newark, DE) and EM Industries, Inc. (Darmstadt, Germany), respectively. Lipid standards were acquired from Nu-Chek Prep, Inc. (Elysian, MN) and Avanti (Alabasta, AL). [2,3-^{3}H]phytanic acid and 2-bromo-[1-^{14}C]palmitic acid were purchased from Moravek Biochemicals, Inc. (Brea, CA), while [9,10-^{3}H]palmitic acid was from Dupont New England Nuclear (Boston, MA). All solvents and reagents were of the highest grade available as well as cell culture tested.

L cell culture

Murine L cells were cultured to confluency at 37°C and 5% CO_2 in a chemically defined medium (37) to which 10% fetal bovine serum (Hyclone, Logan, UT) was added. Mock-transfected cells (designated as control) and cells transfected with cDNA corresponding to the pro-SCP-2 and SCP-x were obtained as described earlier (19, 38). L cell fibroblasts were used in these experiments since they have extremely low levels of endogenous SCP-2 and SCP-x, yet contain all the appropriate machinery for FA uptake, esterification, and FA oxidation (2, 3, 23, 39, 40, 41). Indeed, while fibroblasts have been used by many laboratories to study human defects of FA oxidation [reviewed in ref. (42–44)], quantitative techniques such as immunogold electron microscopy and indirect immunofluorescence performed on liver, hepatoma cells, and transfected L cells show essentially the same results, i.e., that SCP-2 and SCP-x are localized to peroxisomes, the endoplasmic reticulum, and the mitochondria to the same extent (13, 17, 40). A respective 5- and 4-fold increase in SCP-2 and SCP-x levels was observed in the overexpressing cells (**Fig. 1**). In terms of total cell protein, levels of SCP-2 and SCP-x were 0.036% and 0.01% of total protein, respectively, well within the physiological range of liver and steroidogenic tissues [reviewed in refs. (45, 46, 47)], while levels of SCP-2 and SCP-x in control and mock-transfected cells were at or below the level of detection. In addition, overexpression of SCP-2 or SCP-x in L cell fibroblasts did not affect growth curves or doubling times as compared with control cells, indicating cell viability was not affected by increased intracellular SCP-2 or SCP-x levels. While it has been shown that FAs induce lipid accumulation and toxicity in cultured cells and animals (48, 49), experiments in the present work were performed at levels well below toxic. Nevertheless, viability of the transfected cells was determined regularly based on trypan blue exclusion and morphology, where no significant differences were observed as compared with control cells grown in parallel. For all experiments, cells were cultured on 35 mm dishes (5×10^5/dish) in the above medium. After 24 h, serum was removed and cells were cultured in serum-free medium for an additional 24 h before experimentation. The absence of serum from the medium had no deleterious effect since L cells can routinely be cultured in serum-free, chemically defined Higuchi medium for months to years (50). BSA was not present in the medium or added with the FA in order to avoid confusion of the role of BSA versus L-FABP in FA uptake.

Fig. 1. Western blot analysis of sterol carrier protein-2 (SCP-2) and SCP-x expression in transfected L cells. Cell homogenates (10 μg) isolated from control (A and B, lane 1) and SCP-2 (B, lane 2) or SCP-x expressors (A, lane 2) were run on 12% Tricine gels, transferred to nitrocellulose, and probed with affinity-purified polyclonal SCP2 [as described in (19)].

Uptake of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid

In order to obtain data in the linear range of FA uptake, measurements were made at 37°C in serum-deprived cells incubated with increasing concentrations of cold FA (25 nM to 0.6 μM) supplemented with radiolabeled FA (1.5 μCi/nmol [2,3-^{3}H]phytanic acid or 1.5 μCi/nmol [9,10-^{3}H]palmitic acid) at increasing time intervals (1 min through 120 min). Based on these results, subsequent experiments with phytanic acid and palmitic acid were performed with 50 nM FA (supplemented with radiolabled probe) at time intervals from 1 min to 30 min. At indicated times, the culture medium was removed and saved while the cell monolayer was washed two times with PBS. The cell monolayer was lipid extracted with n-hexane-2-propanol (3:2; v/v) (51) and radioactivity was quantitated in liquid scintillation cocktail (Scinti Verse, Fisher Scientific, Pittsburgh, PA) on a Packard 1600TR liquid scintillation counter (Meridian, CT). The culture medium and PBS washes were combined and extracted by the method of Folch (52). Total uptake of the respective [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid was determined from the radiolabel in the cell monolayer corrected for the aqueous-soluble radiolabeled FA oxidation products (released from the cells into the medium). Cell protein content was determined on the dried sediment after lipid extraction (53). Radiolabeled FA uptake was expressed as pmol [2,3-^{3}H]phytanic acid or [9,10-^{3}H]palmitic acid taken up/mg protein.

Uptake and targeting of the nonoxidizable FA analog: 2-bromo-[1-^{14}C]palmitic acid

Conditions to measure uptake of the nonoxidizable FA analog 2-bromo-[1-^{14}C]palmitic acid were as described above. In brief, serum deprived cells were incubated at 37°C in serum-free medium supplemented with 0.2 μCi 2-bromo-[1-^{14}C]palmitic acid (53 mCi/mmol). At timed intervals from 1 min to 30 min, medium was removed and the cell monolayer was washed two times with PBS, frozen over liquid N_2, and the lipid extracted with n-hexane-2-propanol (3:2; v/v) (51). While [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid are oxidized to water-soluble products, due to the

presence of the bromo group at the 2 position in 2-bromo-[1-^{14}C] palmitic acid, oxidation cannot occur (54, 55). Therefore, total uptake of 2-bromo-[1-^{14}C]palmitic acid was determined from radiolabel taken into the cell monolayer without further correction.

Oxidation of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid

Oxidation of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid in cells expressing SCP-2 and SCP-x was measured in serum-free medium supplemented with 50 nM phytanic acid and 1.5 μCi/nmol [2,3-^{3}H]phytanic acid or (50 nM palmitic acid and 1.5 μCi/nmol [9,10-^{3}H]palmitic acid). At timed intervals from 1–30 min, [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid oxidation was measured as the release of water-soluble tritiated FA oxidation products into the culture medium (56) after extracting the medium by the method of Folch (52). Radioactivity and protein content were quantitated as described above. Radiolabeled oxidation was expressed as pmol [2,3-^{3}H]phytanic acid or [9,10-^{3}H]palmitic acid oxidized/mg cell protein.

Targeting of [2,3-^{3}H]phytanic acid, [9,10-^{3}H]palmitic acid, and 2-bromo-[1-^{14}C]palmitic acid into specific lipid classes

To determine the targeting of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid into specific lipid classes, cells were incubated with [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid and extracted as described above for the uptake and oxidation experiments. The organic phase of the lipid extract was evaporated to dryness under N_2 and redissolved in a small volume of chloroform. The lipid classes were resolved by spotting one-half of the total lipid sample on Silica gel G TLC plates developed in petroleum ether-diethyl ether-methanol-acetic acid (90:7:2:0.5; v/v/v/v). Lipid fractions [monoacylglycerides (MGs), diacylglycerides (DGs), triacylglycerides (TGs), cholesteryl esters (CEs), phospholipids (PLs), and free FA] were identified by comparison with known standards run on the same TLC plates. Each resolved lipid class was visualized by iodine vapor, scraped, eluted with chloroform, and dried under a stream of N_2, and radioactivity was quantified.

To resolve targeting of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid into individual PL classes, the other half of the total lipid sample was resolved first on the Silica gel G TLC plate as described above. The PL spot was scraped, eluted with chloroform, dried under N_2, and redissolved in a small volume of chloroform. Individual PLs were resolved on Silica Gel 60 TLC plates using the following solvent system: chloroform-methanol-water-glacial acetic acid (150:112.5:10.5:6; v/v/v/v). PL classes were visualized by iodine vapor, scraped, eluted with chloroform, and dried under a stream of N_2, and radioactivity was determined.

Although 2-bromopalmitic acid is poorly esterified to more complex lipids such as glycerides, the extent of such esterification was determined. As before, lipids were resolved into individual lipid classes on Silica gel G TLC plates developed in petroleum ether-diethyl ether-methanol-acetic acid (90:7:2:0.5; v/v/v/v), and the radioactivity in each fraction was quantified.

Determination of lipid mass and specific activity

The mass (pmol/mg cell protein) of individual lipid classes was measured as described earlier (51, 57, 58). The specific activity (dpm/pmol lipid class) of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid in each lipid class was determined by dividing the radiolabeled FA uptake data (dpm/mg protein) by lipid mass to obtain dpm/pmol lipid class.

Statistics

The values indicated in each figure or table represent the mean ± SEM with n and *P* as indicated in Results. Student's unpaired *t*-test was used for statistical comparisons (GraphPad Prism, San Diego, CA). Values with $P < 0.05$ were considered statistically significant.

RESULTS

Concentration and time dependence of FA uptake and oxidation

The concentration dependence of phytanic acid and palmitic acid uptake and oxidation in control L cells was established under conditions of increasing FA from 25 nM to 600 nM (**Fig. 2A**). With increasing concentration, uptake and oxidation of palmitic acid was saturated near 100 nM (Fig. 2A, inset). In contrast, while oxidation quickly saturated, phytanic acid uptake continued increasing at the highest concentration studied (600 nM). Time dependence was also determined under nonsaturating concentrations (50 nM) for timed intervals from 1–120 min (Fig. 2B). From these results, it was determined that incuba-

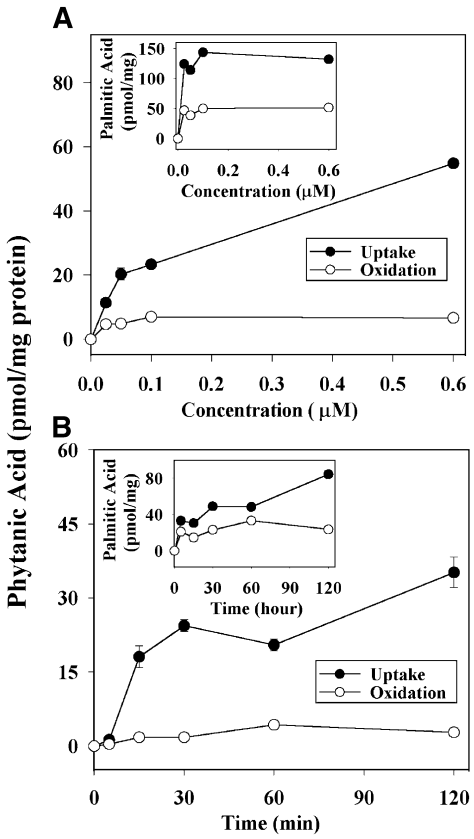

Fig. 2. Concentration and time dependence of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid uptake and oxidation. A: Total uptake (closed circles) and oxidation (open circles) of phytanic acid and palmitic acid (inset) was determined in L cells at different FA concentrations (25 nM through 1.8 μM). B: Phytanic acid and palmitic acid (inset) uptake (closed circles) and oxidation (open circles) was determined in control cells at increasing time intervals (1 min to 120 min) at 50 nM concentration of FA as described in Materials and Methods. Values represent means ± SEM (n = 3–6). * Indicates significance $P < 0.006$ as compared with control cells at the same time point.

tions at <30 min were generally sufficient to observe both phases of FA uptake.

Role of SCP-2 expression in [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid uptake in transfected L cell fibroblasts

To determine the effect of FA branching on FA uptake, incorporation of [2,3-^{3}H]phytanic acid was compared with that of [9,10-^{3}H]palmitic acid over time. While uptake of both phytanic and palmitic acid in control cells was biphasic, the initial uptake of palmitic acid (**Fig. 3**, inset) was 38-fold higher than that of phytanic acid uptake (Fig. 3; $P \leq 0.001$, n = 3–4). Incorporation of both FAs into control cells did not plateau after 1 min incubation, but continued to increase up to 30 min, at which point total incorporation of [9,10-^{3}H]palmitic acid (Fig. 3, inset) was only 2-fold faster ($P \leq 0.001$, n = 3 or 4) than that of [2,3-^{3}H]phytanic acid (Fig. 3). Thus, the presence of methyl branching in the FA significantly inhibited the transport of FA across the plasma membrane into the cell.

From studies with poorly metabolizable FAs as well as with FAs with typical lipid distributional analysis, it has long been established that cellular FA uptake is biphasic (26). The initial rapid phase during the first min of uptake has been attributed primarily to FA transport across the plasma membrane while the slower phase (2–30 min) of uptake is thought to reflect primarily effects of intracellular metabolism (esterification and/or oxidation) on driving FA uptake (23, 26, 27, 32). Comparison of FA uptake in SCP-2 overexpressing and control L cells showed that SCP-2 expression did not significantly enhance the initial uptake of phytanic acid (Fig. 3). However, after 15 min and 30 min incubations, phytanic acid incorporation was significantly increased 2-fold and 1.6-fold, respectively ($P \leq 0.003$, n = 3 or 4). Similarly, SCP-2 expression did not increase the initial uptake of palmitic acid, but increased total incorporation into the cell by 2.0- and 2.1-fold after 5 min and 30 min, respectively, as compared with control cells (Fig. 3, inset; $P \leq 0.001$, n = 3 or 4). Since SCP-2 stimulated FA uptake only at longer time points, this suggested that SCP-2 did not enhance the uptake of branched- and straight-chain FAs by increasing their transport across the plasma membrane into the cell.

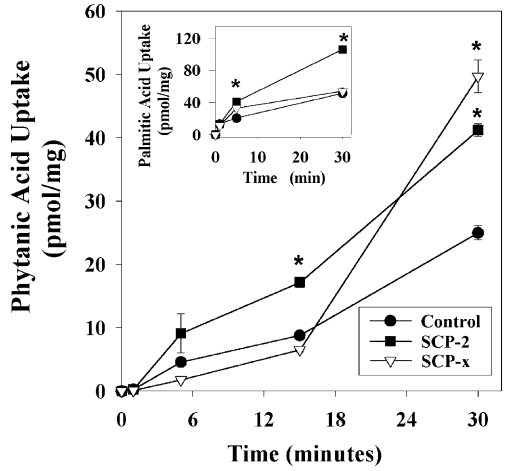

Fig. 3. Total uptake of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid in transfected L cells overexpressing SCP-2 and SCP-x. Total uptake of phytanic acid and palmitic acid (inset) was determined in control cells (closed circles), SCP-2- (closed squares), and SCP-x- (inverted triangles) expressing cells over a 30 min time interval as described in Materials and Methods. Values represent means ± SEM (n = 3–6). * Indicates significance $P < 0.006$ as compared with control cells at the same time point.

Effect of SCP-x expression on [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid uptake

Since SCP-x is a thiolase enzyme involved in peroxisomal branched-chain FA oxidation (59, 60), the protein may also have the ability to influence FA uptake, especially uptake of branched-chain FAs such as phytanic acid. Moreover, SCP-x may indirectly enhance uptake from the 13 kDa SCP-2 protein produced from posttranslationally cleavage [reviewed in ref. (19)]. However, results from uptake experiments revealed SCP-x did not enhance the initial uptake of either FA (Fig. 3, insert), but instead stimulated incorporation of [2,3-^{3}H]phytanic acid in the cell 2-fold after 30 min incubation (Fig. 3; $P \leq 0.001$, n = 3 or 4). With [9,10-^{3}H]palmitic acid, SCP-x overexpression enhanced cellular incorporation only transiently at longer time points, i.e., 1.6-fold after 5 min incubation (Fig. 3; $P \leq 0.001$, n = 3 or 4), but not after 30 min. Thus, SCP-x expression did not stimulate the translocation of branched- or straight-chain FA across the plasma membrane into the cell. It did, however, stimulate the uptake of phytanic acid > palmitic acid at later time points where intracellular metabolism may contribute. This result was in good agreement with the fact that SCP-x is a peroxisomal thiolase enzyme that exhibits high specificity for branched-chain rather than straight-chain FA oxidation (59, 60).

Uptake of 2-bromo-[1-^{14}C]palmitic acid in SCP-2- and SCP-x-expressing L cells

Since phytanic acid and palmitic acid are both readily metabolized, interpretations of effects of SCP-2 and SCP-x on FA uptake versus their enhancement of additional intracellular processes were potentially complicated. This issue was resolved by examining the uptake of 2-bromo-[1-^{14}C]palmitic acid, a FA analog that is taken up by cells, but is not a substrate for mitochondrial or peroxisomal oxidation, and is only poorly esterified as compared with naturally occurring FAs (55). Uptake of 2-bromo-palmitic acid was determined over the same 30 min time frame as with palmitic and phytanic acid in the preceding experiments. As expected, control L cells took up 2-bromo-palmitic acid (**Fig. 4**), did not oxidize 2-bromo-palmitic acid (not shown), and only weakly esterified 2-bromo-palmitic acid, primarily into glycerides (not shown). Moreover, overexpression of SCP-2 or SCP-x did not enhance 2-bromo-palmitic acid initial uptake or uptake over longer time points (30 min incubation). In summary, results from the nonoxidizable, weakly esterifiable 2-bromo-palmitic acid clearly established that SCP-2 and SCP-x did not enhance FA uptake over the first 30 min incubation time.

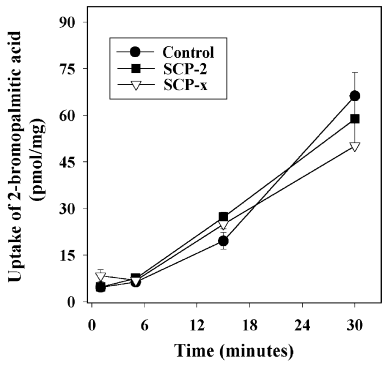

Fig. 4. Total uptake of 2-bromo-[1-^{14}C]palmitic acid in transfected L cells overexpressing SCP-2 and SCP-x. The extent of FA uptake was determined after 60 min incubation in control cells (closed circles), SCP-2- (closed squares), and SCP-x- (inverted triangles) expressing cells over a 30 min time interval, as described in Materials and Methods. Values represent means ± SEM (n = 3–6).

Effect of SCP-2 and SCP-x expression on [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid pool size of unesterified FA

One possible mechanism whereby SCP-2 and SCP-x expression may increase FA uptake is by providing additional intracellular sites for FA binding. Therefore, the effect of SCP-2 and SCP-x expression on the size of the unesterified FA fraction was determined (**Fig. 5**). After the initial uptake into the cells, both phytanic acid and palmitic acid briefly targeted the unesterified FA pool, but by 30 min had redistributed completely to other lipids fractions so that no radiolabeled FA was detected. With [2,3-^{3}H]phytanic acid, incorporation into the unesterified FA fraction of control cells after 5 min was 2.8-fold greater than that of the [9,10-^{3}H]palmitic acid, but by 15 min the [2,3-^{3}H]phytanic acid had redistributed completely from the unesterified FA pool to other lipids (Fig. 5A vs. 5B).

SCP-2 expression did not increase the quantity of [2,3-^{3}H]phytanic acid (Fig. 5A) or [9,10-^{3}H]palmitic acid (Fig. 5B) in the unesterified FA pool at any of the time points examined. On the contrary, SCP-2 expression increased the removal of [9,10-^{3}H]palmitic acid, but not [2,3-^{3}H]phytanic acid, from the unesterified FA pool by 15 min incubation. SCP-x expression also did not increase the quantity of phytanic acid (Fig. 5A) or palmitic acid (Fig. 5B) in the unesterified FA pool at any of the time points examined. Instead, SCP-x expression increased the removal of phytanic acid from the unesterified FA pool by 5 min incubation and palmitic acid by 15 min incubation. In summary, these data show that SCP-2 and SCP-x did not significantly increase the uptake of either branched- or straight-chain FAs by providing increased binding sites for these FAs within SCP-2- and SCP-x-overexpressing L cells.

Effect of SCP-2 expression on [2,3-^{3}H]phytanic acid [9,10-^{3}H]palmitic acid oxidation

Comparison of branched- versus straight-chain FA oxidation in control L cells revealed [2,3-^{3}H]phytanic acid

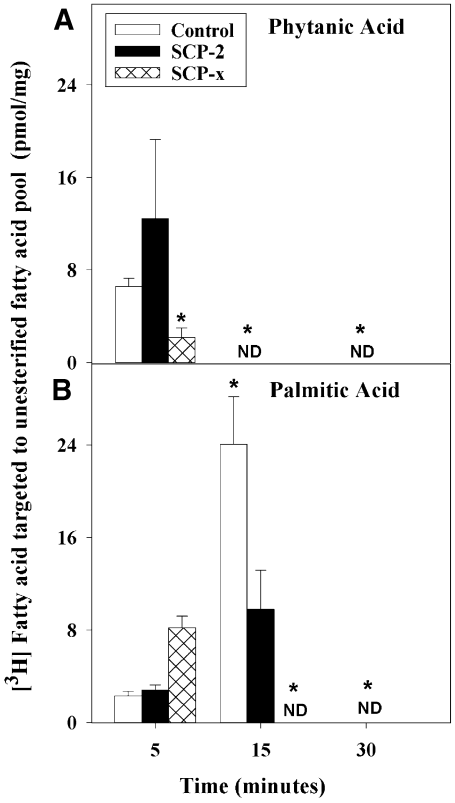

Fig. 5. Targeting of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid to the free FA pool in transfected L cells overexpressing SCP-2 and SCP-x. The extent of targeting of phytanic acid (A) and palmitic acid (B) to the free FA pool was determined in control cells (open bar), SCP-2- (filled bar), and SCP-x- (hatched bar) expressing cells over a 30 min time interval as described in Materials and Methods. Values represent means ± SEM (n = 3–6). ND was defined as values below 0.01 pmol/mg. * Indicates significance $P <$ 0.02 as compared with control cells at the same time point, labeled with the respective FA.

oxidation was 6-fold slower than that of [9,10-^{3}H]palmitic acid after 30 min incubation (data not shown). In addition, SCP-2 expression increased the oxidation of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid 3.8- and 2.2-fold, respectively, ($P < 0.002$, n = 3–6) at 5 min and 2- and 1.9-fold at 30 min ($P < 0.002$, n = 3–6).

Since the uptake of [9,10-^{3}H]palmitic acid was significantly faster than that of [2,3-^{3}H]phytanic acid, this may partially obscure the magnitude of differences in oxidation of these FAs. To resolve this potential problem, the percentage of phytanic acid taken up and oxidized was compared with that of palmitic acid in control cells (**Table 1**). Only 31.5 ± 7.2% of [2,3-^{3}H]phytanic acid taken up was oxidized by 1 min incubation, 3-fold less than that of [9,10-^{3}H]palmitic acid. After 5 min and 30 min incubation, these differences were more striking: 6.9- and 6.4-fold less, respectively (Table 1). Thus, while branched-chain FA total oxidation was up to 13-fold less than that of straight-chain FA oxidation, normalization for differences in relative uptake of these FAs revealed that the percentage of branched chain taken up that was oxidized was only 3- to 7-fold less than that of the straight-chain FA.

TABLE 1. Percent oxidation of [2,3-^{3}H]phytanic and [9,10-^{3}H]palmitic acid

Time (min)	Control	SCP-2	SCP-x
Phytanic Acid			
1	31.5 ± 7.2	63.5 ± 15.7[a]	95.1 ± 14.8[a]
5	8.8 ± 1.6	15.9 ± 5.5	28.5 ± 6.4
30	7.3 ± 0.7[b]	8.7 ± 0.7[b]	13.6 ± 1.2[a,b]
Palmitic Acid			
1	92.8 ± 4.2	90.3 ± 1.6	86.8 ± 5.2
5	60.8 ± 3.2	66.8 ± 3.5	53.2 ± 2.25
30	46.8 ± 3.6	28.9 ± 3.1[a]	34.7 ± 2.4[a]

SCP, sterol carrier protein. Percent oxidation of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic was determined as described in Materials and Methods. Values reflect the mean ± SEM.

[a] Indicates significance $P < 0.05$, n = 3–6 as compared with control cells with the same fatty acid (FA).

[b] Indicates significance $P < 0.005$, n = 3–6 as compared with the respective cell line labeled with [9,10-^{3}H]palmitic acid at the same time point.

SCP-2 expression increased the percentage of [2,3-^{3}H] phytanic acid that was oxidized by 2-fold after 1 min and 5 min incubation (Table 1). However, once oxidation plateaued (i.e., saturated after 30 min incubation) SCP-2 did not significantly alter the percentage of phytanic acid oxidized. In contrast, since [9,10-^{3}H]palmitic acid oxidation was already saturated by 1 min incubation, SCP-2 expression did not significantly increase the percentage of [9,10-^{3}H]palmitic acid oxidized regardless of time point examined.

In summary, whether the oxidation was expressed as oxidation of [2,3-^{3}H]phytanic acid or the percentage of [2,3-^{3}H]phytanic acid oxidized, the oxidation of phytanic acid was significantly less than that of palmitic acid at all time points regardless of the presence of SCP-2 overexpression. Because palmitic acid oxidation already appeared saturated by 1 min incubation, SCP-2 expression did not stimulate the percentage of palmitic acid taken up and oxidized. In contrast, since phytanic acid oxidation was not saturated until extended incubation times, SCP-2 expression increased the percentage [2,3-^{3}H]phytanic acid oxidized about 2-fold at early time points. The latter suggested for the first time that, once phytanic acid was taken up, SCP-2 expression also enhanced the peroxisomal oxidation of phytanic acid.

Role of SCP-x in [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid oxidation

Although SCP-x is the only known peroxisomal thiolase involved in oxidation of branched chain FAs in vitro (59, 61), the relative role of SCP-x in straight- (palmitic acid) versus branched- (phytanic acid) chain FA oxidation in intact cells is not known. Therefore, oxidation of the straight-chain [9,10-^{3}H]palmitic acid and the branched-chain [2,3-^{3}H]phytanic acid was determined in control and SCP-x-expressing cells.

SCP-x expression enhanced phytanic acid oxidation only at longer incubation times: 2.4- and 3.7-fold at 15 min and 30 min incubation, respectively (data not shown). In contrast, at the same time points, SCP-x expression enhanced oxidation of palmitic acid significantly less: 1.4- and 1.9-fold at 5 min and 30 min incubation, respectively ($P < 0.002$, n = 4–5). When the effect of SCP-x on palmitic acid and phytanic acid oxidation was normalized for differences in their respective uptake, SCP-x expression increased the percentage of [2,3-^{3}H]phytanic acid oxidized at all time points by as much as 3-fold at 1 min and 5 min incubations and 1.9-fold at 30 min (Table 1). However, SCP-x expression did not increase the percentage of [9,10-^{3}H]palmitic acid taken up and oxidized at any time point examined.

In summary, SCP-x expression enhanced the oxidation of the branched-chain phytanic acid more than that of straight-chain palmitic acid in intact cells. However, while SCP-x expression increased by several-fold, the percentage of [2,3-^{3}H]phytanic acid oxidized at all time points examined, and SCP-x did not enhance the percentage of [9,10-^{3}H]palmitic acid taken up that was oxidized. These data show for the first time that SCP-x expression in intact cells significantly and preferentially stimulates the peroxisomal oxidation of branched chain FAs.

Effect of SCP-2 and SCP-x expression on intracellular esterification of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid

SCP-2 and SCP-x may also stimulate FA uptake during the second, longer time phase of FA uptake by enhancing FA esterification into complex lipids. In control cells, [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid targeting to total esterified lipid was greatest after 30 min of incubation (**Fig. 6A**, Table 1). The [2,3-^{3}H]phytanic acid was incorporated into total esterified lipids 2.4-fold more than [9,10-^{3}H]palmitic acid (Fig. 6A). Expression of SCP-2 and SCP-x increased the esterification of phytanic acid by 1.6- and 1.8-fold, respectively ($P \leq 0.0008$, n = 3 or 4). In contrast, while the expression of SCP-2 stimulated the esterification of [9,10-^{3}H]palmitic acid by 1.8-fold (Fig. 6A; $P \leq 0.002$, n = 3 or 4), SCP-x expression did not significantly increase esterification of [9,10-^{3}H]palmitic acid (Fig. 6A).

In summary, SCP-2 and SCP-x enhanced the esterification of phytanic acid in transfected L cells. In contrast, only SCP-2 stimulated the esterification of palmitic acid. Thus, enhancement of FA esterification accounted at least in part for the increased FA uptake noted during the longer phase of uptake for the branched-chain phytanic acid in SCP-2- and SCP-x-overexpressing cells, as well as for the straight-chain palmitic acid in SCP-2-overexpressing cells.

Effect of methyl branching on FA distribution into PLs and esterified neutral lipids

To determine whether branched-chain FA was selectively esterified to the polar PLs versus esterified neutral lipids, these two groups of esterified lipids were resolved as described in Materials and Methods. In control cells, the branched-chain [2,3-^{3}H]phytanic acid was evenly distributed between PLs and neutral lipids (Fig. 6B, C). Meanwhile, the specific activity of [2,3-^{3}H]phytanic acid in PLs increased nearly 150-fold from 1 min to 30 min incubation time (**Fig. 7A**). In contrast, the straight-chain palmitic acid was preferentially (i.e., 5.1-fold) esterified into PLs as com-

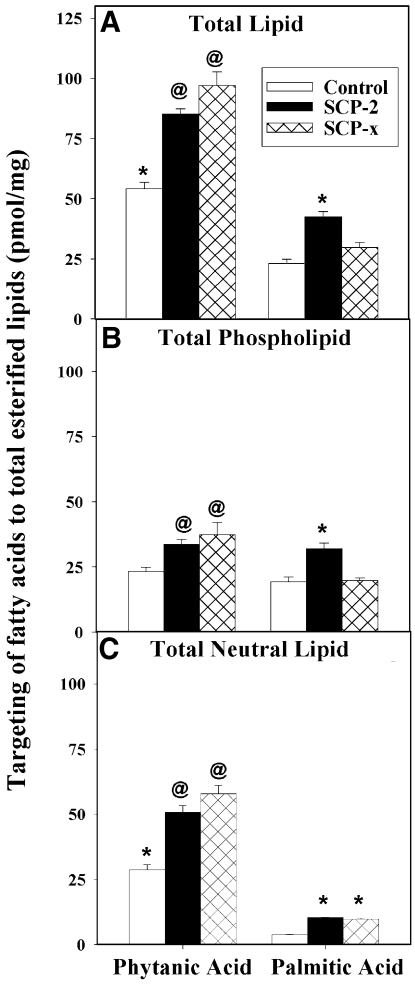

Fig. 6. Targeting of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid to esterified lipids in transfected L cells overexpressing SCP-2 and SCP-x. The extent of targeting of phytanic acid and palmitic acid to total esterified lipids (A), total phospholipids (PLs) (B), and total neutral lipids (C) were determined in control cells (open bars), SCP-2- (closed bars), and SCP-x- (hatched bars) expressing cells over a 30 min time interval as described in Materials and Methods. Values represent means ± SEM (n = 3–6). * Indicates significance $P < 0.002$ as compared with cells incubated with palmitic acid. @ Indicates significance $P < 0.05$ as compared with control cells incubated with the respective FA.

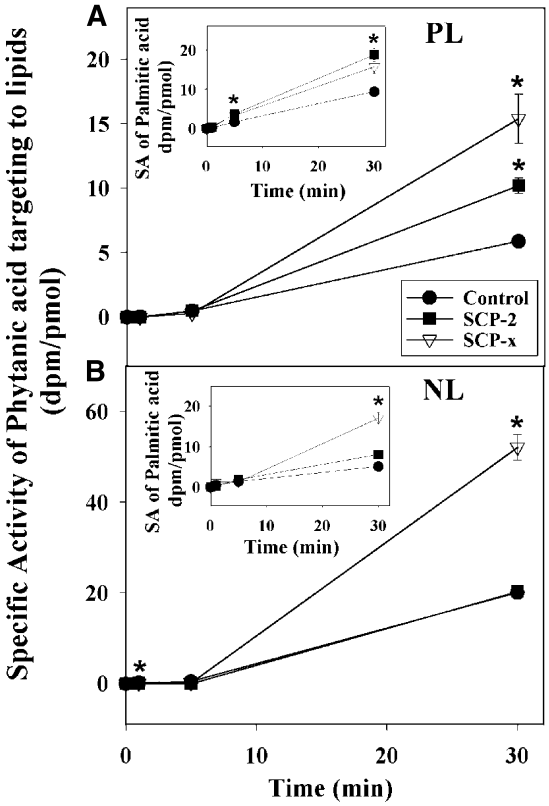

Fig. 7. [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid-specific activity in PL and neutral lipid fractions in transfected L cells overexpressing SCP-2 and SCP-x. The extent of targeting of phytanic acid and palmitic acid (inset) to PLs (A) and neutral lipids (B) was determined as specific activity (dpm/pmol) in control cells (closed circles), SCP-2- (closed squares), and SCP-x- (inverted triangles) expressing cells as described in Materials and Methods. NL indicates neutral lipids. Values represent means ± SEM (n = 3–6). * Indicates significance $P < 0.05$ as compared with control cells incubated with the respective FA.

pared with neutral lipids (Fig. 6B, 6C). The specific activity of [9,10-^{3}H]palmitic acid in PLs increased 47-fold from 1 min to 30 min incubation time. Furthermore, the specific activity of [9,10-^{3}H]palmitic acid in the PL fraction was higher than that of [2,3-^{3}H]phytanic acid at all time points of incubation: 5-, 3.4-, and 1.6-fold higher at 1 min, 5 min, and 30 min, respectively ($P < 0.04$, n = 3–6).

Effect of SCP-2 and SCP-x expression on FA distribution into PLs and individual PL species

Since cellular PLs represent 50% to 80% of total esterified lipids (Fig. 6B vs. 6A), the possibility that SCP-2 and SCP-x expression differentially stimulated incorporation of branched-chain versus straight-chain FAs into PLs was examined. With [2,3-^{3}H]phytanic acid, SCP-2 expression increased the targeting into PLs 1.5-fold (Fig. 6B; $P \leq 0.01$, n = 4–6) and increased its specific activity 1.7-fold at 30 min (Fig. 7A). SCP-x expression similarly increased the targeting of phytanic acid into PLs 1.6-fold (Fig. 6B; $P \leq 0.05$, n = 4–6), and increased its specific activity therein 2.6-fold at 30 min (Fig. 7A). Similarly, SCP-2 expression increased the targeting of palmitic acid into PLs by 1.7-fold after 30 min incubation (Fig. 6B), and likewise significantly increased the specific activity of [9,10-^{3}H]palmitic acid about 2-fold at all time points examined (Fig. 7A). Although SCP-x did not appear to significantly alter the esterification of [9,10-^{3}H]palmitic acid into total PLs (Fig. 6B); nevertheless SCP-x increased the specific activity of [9,10-^{3}H]palmitic acid about 2-fold at all time points examined (Fig. 7A).

In summary, SCP-2 and SCP-x expression increased the incorporation of the branched-chain phytanic acid into PLs, while only SCP-2 enhanced incorporation of the straight-chain palmitic acid into PLs. Thus, only SCP-x expression differentially targeted [2,3-^{3}H]phytanic acid, but not [9,10-^{3}H]palmitic acid, away from PLs, consistent with its ability to preferentially enhance the peroxisomal oxidation of phytanic acid (Table 1).

Individual PL classes were also resolved in order to determine whether phytanic acid and palmitic acid were differentially targeted. SCP-2 expression increased targeting of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid into some PL species [5- to 6-fold for phosphatidylinositol (PI) and 3- to 6-fold for phosphatidylserine (PS); **Fig. 8**; $P \leq 0.05$, n = 4–6]. In contrast, SCP-x expression significantly decreased targeting to several PL species [46–84% for phosphatidic acid and 90–8% for PS; Fig. 8; $P \leq 0.05$, n = 4–6]. In summary, SCP-2 and SCP-x differentially modulated targeting of both phytanic acid and palmitic acid into specific PL classes, especially phosphatidic acid, PE, and PS.

Effect of methyl branching on FA distribution into total neutral lipids and individual neutral lipid species

Since both phytanic acid and palmitic acid were differentially incorporated into PLs, it was important to determine if either FA was differentially esterified into neutral lipids. Comparison of control cells incubated with [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid for 30 min showed a 7.6-fold ($P < 0.0001$, n = 4–6) preferential targeting of [2,3-^{3}H]phytanic acid targeting toward neutral lipids (Fig. 6C). This was in agreement with the 4-fold increase in specific activity in [2,3-^{3}H]phytanic acid targeted to neutral lipids (Fig. 7B).

The neutral lipid fraction was further resolved into specific neutral lipid classes, which included DGs, MGs, TGs, and CEs. The distribution of the individual neutral lipids after 30 min incubation showed that a small percentage of both [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid was targeted to CE and TG, with the majority targeted to DG (data not shown). Furthermore, while distribution to the TG fraction was 7-fold ($P < 0.0001$, n = 3–6) greater with the phytanic acid label as compared with palmitic acid in control cells, the percent distribution of both [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid in the DG fraction was the highest, accounting for as much as half of the total. In summary, phytanic acid preferentially accumulated in neutral lipids more so than palmitic acid in control cells. However, within the neutral lipids, both phytanic acid and palmitic acid distributed to DG > TG > CE.

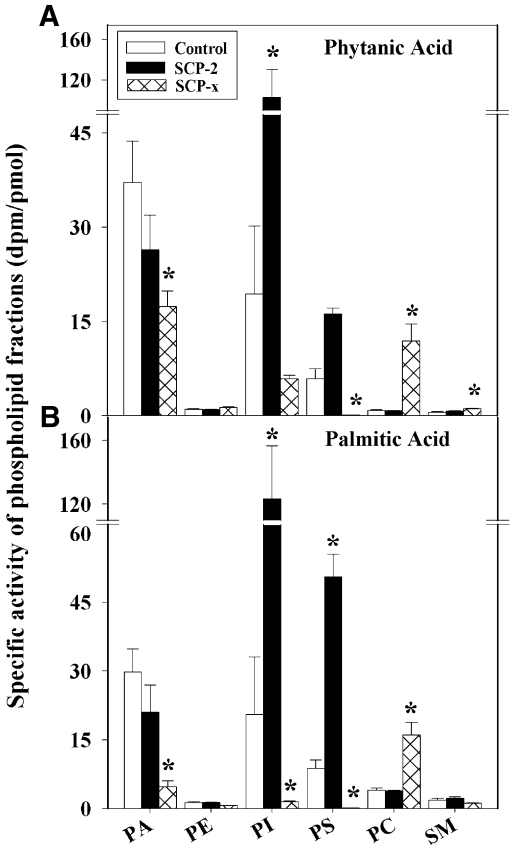

Fig. 8. Targeting of [2,3-^{3}H]phytanic acid and [9,10-^{3}H]palmitic acid to individual PLs in transfected L cells overexpressing SCP-2 and SCP-x. The extent of targeting of phytanic acid (A) and palmitic acid (B) to individual PLs was determined as specific activity (dpm/pmol) after 30 min incubation in control cells (open bars), SCP-2- (closed bars), and SCP-x- (hatched bars) expressing cells as described in Materials and Methods. PLs were defined as phosphatidic acid, PA; ethanolamine glyceroPL, PE; phosphatidylinositol, PI; phosphatidylserine, PS; choline glycerophospholipid, PC; and sphingomyelin, SM. Values represent means ± SEM (n = 3–6). * Indicates significance $P < 0.05$ as compared with control cells incubated with the respective FA.

Effect of SCP-2 and SCP-x expression on FA distribution into total neutral lipids and individual neutral lipid species

SCP-2 and SCP-x expression increased targeting of [2,3-^{3}H]phytanic acid to neutral lipids up to 2-fold (Fig. 6C), but only SCP-x expression increased the specific activity of phytanic acid in neutral lipids at the 30 min time point (Fig. 7B). With palmitic acid, both SCP-2 and SCP-x expression increased the incorporation of [9,10-^{3}H]palmitic acid targeted to total neutral lipids 2- to 3-fold at 30 min (Fig. 6C; $P < 0.002$, n = 4–6), in good agreement with the specific activity in [9,10-^{3}H]palmitic acid targeted to neutral lipids (Fig. 7B). The extent of targeting into specific neutral lipid classes was also determined (data not shown). SCP-2 and SCP-x expression increased targeting of phytanic acid to DG/MG by 2.1- and 2.3-fold, respectively ($P < 0.0001$, n = 3–6). Furthermore, SCP-2 and SCP-x increased the distribution of phytanic acid to the TG fraction 1.3- and 1.6-fold, respectively ($P < 0.04$, n = 3–5). In contrast, levels of CE were decreased 8-fold ($P < 0.004$, n = 3–5) in SCP-2-expressing cells. Similar to phytanic acid, a respective 3.7- and 3.4-fold increase in the distribution of palmitic acid to the DG/MG fraction was observed in SCP-2- and SCP-x-expressing cells ($P < 0.0001$, n = 3–6). However, no significant difference was observed with the TG or CE fractions.

DISCUSSION

Although the SCP-2 gene is now known to code for two gene products, SCP-2 and SCP-x [reviewed in ref. (46)], the relative roles of these proteins in FA uptake, oxidation, and esterification are not completely resolved. While it is generally agreed that the 58 kDa SCP-x protein is exclusively peroxisomal, results from immunogold electron

microscopy and indirect immunofluorescence has revealed more than half of the total 13.2 kDa SCP-2 protein is present extraperoxisomally (13, 17). In addition, subcellular fractionation data showed that while the 58 kDa SCP-x and 46 kDa thiolase copurified with the peroxisomal matrix marker catalase, the 13.2 kDa SCP-2 did not (13, 18, 62). The idea of exclusive peroxisomal localization was suggested based on two factors: *i*) anti-SCP-2 immunoreactivity is present at highest concentration in peroxisomes (13, 17), and *ii*) data from cDNA-derived amino acid sequence where a SKL peroxisomal targeting signal (PTS1) at the C terminus was found (46). However, it must be considered that peroxisomes generally constitute <1% of cell volume (13, 17), and intracellular targeting is influenced by the presence of additional amino acid sequence at the N-terminus (40). For example, based on the presence of the SKL targeting sequence in the three proteins of the SCP-2 family (SCP-2, pro-SCP-2, and SCP-x), it was assumed that all three proteins were strictly peroxisomal. However, recent work with the pro-SCP-2 has shown that the SKL sequence is heavily influenced by the presence of the 20-amino acid presequence located in pro-SCP-2 (40). Once cleaved, a subtle conformational change in the protein occurs, leading to poor exposure of the SKL site and little peroxisomal targeting. Since the 20-amino acid presequence is missing from the mature form of SCP-2, this clearly supports immunogold EM and indirect immunofluorescence results showing SCP-2 localized extraperoxisomally (40, 46).

Some studies suggest that SCP-2 and SCP-x may exhibit distinctly different roles in FA metabolism. With regards to SCP-2, recent in vitro studies show that SCP-2 binds FAs (5) and fatty acyl CoAs (12) with high affinity. However, while these discoveries suggest that SCP-2 may be involved in FA oxidation and/or esterification [reviewed in ref. (63)], this has not previously been demonstrated in intact cells or animals. Likewise, with regards to SCP-x, in vitro studies indicate that this protein is the only known enzyme with 2/3-oxoacyl-CoA thiolase activity essential for the oxidation of branched-chain FAs (61). While SCP-x protein appears almost exclusively localized in peroxisomes, the fact that it is partially posttranslationally cleaved to SCP-2 complicates interpretations of exclusive actions for SCP-x (18, 19). Likewise, studies with SCP-2/SCP-x gene-ablated mice do not resolve the relative contributions of either protein (9, 64) and are complicated by concomitant up-regulation of L-FABP (9, 65, 66). To more clearly resolve the potential roles of SCP-2 and SCP-x in branched- and straight-chain FA uptake, oxidation, and esterification, the effects of these SCP-2 gene products on phytanic (branched-chain) and palmitic (straight-chain) acid uptake and metabolism were examined in transfected L cell fibroblasts. The data presented herein demonstrate for the first time differential role(s) of SCP-2/SCP-x gene products in the metabolism of branched-chain (phytanic acid) and straight-chain (palmitic acid) FAs in intact cells.

The presence of four methyl branches in phytanic acid versus palmitic acid dramatically reduced both the uptake and oxidation. Furthermore, the uptake appeared linear over the first 15 to 30 min. Phytanic acid was also much more incorporated into esterified lipids, especially in PL species. With palmitic acid, like that of other straight-chain FAs (21, 26, 27, 32, 67–70), the uptake appeared biphasic (rapid during first 1 min, followed by slower phase from 1–30 min). Similarly, the oxidation of palmitic acid was biphasic. These data indicate that the patterns of phytanic and palmitic acid uptake were due, at least in part, to substantial differences in intracellular metabolism. Indeed, although phytanic acid and palmitic acid are both 16 carbons long, the presence of methyl branches in phytanic acid precludes straightforward oxidation in mitochondria. Instead, phytanic acid is sequentially oxidized first in peroxisomes (α- and β-oxidation) and then in mitochondria (β-oxidation), while β-oxidation of the unbranched palmitic acid occurs primarily in mitochondria (71).

While both SCP-2 and SCP-x stimulated the uptake of phytanic and palmitic acid, it was only at later time points. In contrast, neither SCP-2 nor SCP-x enhanced the uptake of the nonoxidizable, poorly metabolizable FA analog, 2-bromo-palmitic acid. Since the 2-bromo-palmitate can be activated to the CoA form but binds irreversibly to carnitine palmitoyl transferase (CPT I) to prevent oxidation and is only weakly esterified (55), these data suggested that SCP-2 and SCP-x did not enhance the uptake of phytanic or palmitic acid by stimulating the translocation of these FAs across the plasma membrane into the cell. Instead, results indicated that SCP-2 and SCP-x elicited enhanced FA uptake by stimulating intracellular process(es).

The possibility that SCP-2 and SCP-x expression increased the longer time phase of FA uptake by providing additional intracellular sites for FA binding was also considered. This was based on the following: *i*) SCP-2 binds phytanic acid and palmitic acid to high degree (7, 9), and *ii*) SCP-x shares these binding sites since SCP-2 includes the entire C terminus of SCP-x [reviewed in ref. (46)]. Nevertheless, SCP-2 and SCP-x expression did not increase the phytanic acid and palmitic acid present in the unesterified FA pool. Thus, neither protein enhanced the longer phase of phytanic or palmitic acid uptake by providing an increased number of intracellular binding sites for these unesterified FAs.

While both SCP-2 and SCP-x increased FA oxidation, it was only at longer time points of incubation in the order SCP-x > SCP-2. Since the expression of SCP-x in transfected L cells enhanced the oxidation of branched-chain phytanic acid 2-fold more than that of palmitic acid, the data presented herein clearly established for the first time the physiological significance of SCP-x in preferential utilization of branched-chain versus straight-chain FAs in a living cell, consistent with in vitro studies that showed that SCP-x exhibited 2- to 3-fold substrate preference for the branched-chain phytanic acid versus straight-chain palmitic acid. It was also shown for the first time that SCP-2 expression stimulated the oxidation of both branched- and straight-chain FAs, but less so than did SCP-x. Since branched-chain oxidation is peroxisomal, this finding suggests that, although SCP-2 is not an enzyme, it may en-

hance phytanic acid peroxisomal oxidation by transporting this FA to the peroxisome and/or by facilitating its oxidation within peroxisomes. Similarly, the fact that SCP-2 stimulated palmitic acid oxidation suggested an important role of extraperoxisomal SCP-2 (13) in FA oxidation in mitochondria, the primary site for palmitic acid oxidation (3, 25, 40). In support of the possibility that both effects of SCP-2 may be due to enhancement of FA transport through the cytoplasm, SCP-2 expression enhanced the cytoplasmic diffusion of a fluorescent FA nearly 2-fold (21, 23). Finally, since SCP-2 also binds the acyl CoA derivatives of straight-chain FAs with nanomolar K_ds (5, 12), SCP-2 may act to facilitate straight-chain fatty acyl CoA utilization by carnitine palmitoyl acyltransferase I, an enzyme involved in the rate-limiting step in mitochondrial FA oxidation. Also, the observation that both SCP-2 and SCP-x enhanced the oxidation of phytanic acid in transfected cells may be physiologically significant, especially in explaining results from the SCP-2/SCP-x gene-ablated mice where a dramatic reduction in phytanic acid oxidation was observed (9). The present data with transfected cells for the first time suggest that this effect in SCP-2/SCP-x gene-ablated mice may be due not only to the absence of SCP-x, but also to the loss of SCP-2 (9).

SCP-2 and SCP-x expression also differentially stimulated the esterification of phytanic acid and palmitic acid. While both proteins similarly enhanced incorporation of phytanic acid into PLs, only SCP-2 increased targeting of palmitic acid to PLs. These observations may be explained by the presence of significant levels of SCP-2 (partially derived from SCP-x in SCP-x-expressing cells) in the endoplasmic reticulum (17), the intracellular organelle wherein fatty acyl CoAs are transesterified to PLs. Furthermore, SCP-2 is known to bind both branched-chain and straight-chain fatty acyl CoAs with high affinity (5, 12) and to stimulate their transacylation to PLs (17, 72). Interestingly, SCP-2 stimulated the incorporation of phytanic acid and palmitic acid 5.3- and 6-fold, respectively, into PI, a precursor of polyphosphoinositide signaling molecules in the cell. It was recently shown that SCP-2 may participate in cellular polyphosphoinositide signaling as indicated by: *i*) SCP-2 enhanced PI transfer between membranes in vitro; *ii*) SCP-2 stimulated phospholipase C mediated IP3 release in vitro; *iii*) SCP-2 colocalized with caveolae and caveolar vesicles in intact cells; and *iv*) SCP-2 expression significantly redistributed PI from intracellular sites to the plasma membrane (20).

SCP-2 and SCP-x expression similarly enhanced incorporation of phytanic acid and palmitic acid into total neutral esterified lipids in the order of DG >> TG > CE. Indeed, since both proteins stimulated incorporation into TGs, consistent with studies performed with oleic acid on intact cells (22), this result was in good agreement with the SCP-2/SCP-x gene-ablated mice exhibiting 2-fold reduced levels of TGs (9). However, the saturated FAs (phytanic acid and palmitic acid) were poorly targeted to CEs. This was opposite to the effect of SCP-2 and SCP-x on incorporation of unsaturated FA (oleic acid) into CEs in vitro (1, 45, 73, 74) and in intact cells (2, 19). Thus, while incorporation into TGs was stimulated, SCP-2 and SCP-x did not appear to enhance incorporation of saturated FAs (regardless of whether they were branched or nonbranched) into CEs.

In summary, the data presented herein for the first time directly examined the individual effects of SCP-2 and SCP-x expression on the uptake, oxidation, and esterification of branched-chain (phytanic acid) as well as straight-chain (palmitic acid) saturated FAs in intact cells. Both SCP-2 and SCP-x expression stimulated the cellular incorporation of phytanic and palmitic acid at longer incubation times. This effect was not due to enhanced uptake/translocation across the plasma membrane or to the presence of an increased number of intracellular FA binding sites. Instead, both proteins enhanced the incorporation of phytanic acid and palmitic acid by stimulating their metabolism by oxidation and esterification. Interestingly, when expressed as the percent palmitic acid taken up that was oxidized, neither protein appeared to stimulate palmitic acid oxidation. This was consistent with the uptake of palmitic acid being so fast that oxidation was almost completely saturated within the first min of uptake. This rapid uptake and oxidation obscured any effects of SCP-2 or SCP-x on palmitic acid oxidation when expressed as the percentage palmitic acid taken up that was oxidized. In contrast, since the uptake of phytanic acid was slow, SCP-2 and SCP-x significantly enhanced the oxidation of this branched-chain FA, regardless of how its oxidation was expressed. Finally, both proteins also enhanced the intracellular esterification of phytanic acid and palmitic acid. Thus, for the first time SCP-2 and SCP-x were shown to individually affect the oxidation and esterification of branched- and straight-chain FAs in transfected cells. These observations were consistent with SCP-2 and SCP-x stimulating FA oxidation both in peroxisomes (the primary site of branched chain FA oxidation and where the concentration of SCP-x and SCP-2 is the highest) and in mitochondria (the primary site for straight-chain FA oxidization and which also contains some SCP-2), along with stimulating esterification in the endoplasmic reticulum (which also contains some SCP-2) [reviewed in refs. (17, 46)].

This work was supported in part by a Grant from the United States Public Health Service, National Institutes of Health (DK-41402).

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