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# Regulation of Hepatic Stearoyl-CoA Desaturase Gene 1 by Vitamin A

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**The effect of vitamin A supplementation on stearoyl-CoA desaturase gene 1 expression in mouse liver was characterized. Normal BALB/c mice were fed 0.01% and 0.1% retinol palmitate as components of nonpurified diets. This treatment resulted in a 3-fold and a 7-fold induction of SCD1 mRNA levels, respectively, as determined by RNase protection analysis. Vitamin A-deficient animals were also fed diets containing 0.01% and 0.1% retinol palmitate, resulting in a similar pattern of SCD1 mRNA induction. Fatty acid synthase and $\beta$-actin mRNA levels did not respond consistently or significantly to retinoic acid treatment. Dietary and hormonal studies were carried out to investigate the role of the retinoid X receptor in the regulation of SCD1 by type II steroid hormones. A receptor-saturating dose of thyroid hormone, triiodothyronine, repressed vitamin A-elevated SCD1 mRNA levels in vivo. Peroxisome proliferator-elevated SCD1 mRNA levels were unaffected by administration of thyroid hormone. This suggests that the retinoic acid receptor transcriptionally regulates SCD1 through a traditional mechanism of heterodimerization with the retinoid X receptor.** 

Stearoyl-CoA desaturase (SCD) is a key enzyme in fatty acid biosynthesis. It catalyzes the $\Delta^9$-cis desaturation of fatty acyl-CoA substrates: the preferred substrates being palmitoyl-CoA and stearoyl-CoA, which are converted to palmitoleoyl- and oleoyl-CoA respectively. Oleic and palmitoleic acids are the major unsaturated fatty acids in fat depots and membrane phospholipids. The ratio of stearic to oleic acid is one of the factors influencing cell membrane fluidity and cell-cell interactions (1). Abnormal alteration of this ratio has been shown to play a role in various disease states such as aging, diabetes, neurological and vascular disease, and carcinogenesis (1).

Vitamin A (retinoic acid) belongs to the type II steroid hormone superfamily, which consists of retinoic acid receptor (RAR), thyroid hormone receptor ($T_3R$), vitamin $D_3$ receptor, and peroxisome proliferator-activated receptor (PPAR). Type II steroid hormone receptors are unique because they bind to direct repeats of AGGTCA as heterodimers with retinoid X receptor (RXR). Because they need RXR for maximum binding efficiency, the regulation of RXR and their competition for it may have important implications. This unusual mechanism through which type II steroid hormones transcriptionally regulate genes allows them to play important roles in metabolism and development.

The gene expression regulation of hepatic SCD1 and fatty acid synthase (FAS) by vitamin A was investigated to gain a better understanding of steroid hormone regulation of genes involved in fat metabolism. Administration of retinol palmitate to normal and retinoic acid-deficient mice resulted in an increase in SCD1 mRNA levels. FAS did not respond significantly to vitamin A supplementation. We have shown previously that hepatic SCD1 does not respond like lipogenic genes in response to thyroid hormone and peroxisome proliferators, ligands of type II steroid hormones. SCD1 transcription is induced by peroxisome proliferators in liver (2) and repressed by thyroid hormone (Waters et al. unpublished data), which is opposite to FAS and other lipogenic genes (3,4,5). Fatty acid remodeling enzymes and lipolytic enzymes show a similar pattern of regulation to SCD1 (6,7). We have also determined with interactive studies in mice that thyroid hormone represses vitamin A-elevated SCD1 mRNA levels in vivo. This suggests competition for RXR. Peroxisome proliferator-elevated SCD1 mRNA levels are unaffected by administration of thyroid hormone, implying a nontraditional mechanism for this steroid hormone response.

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Abbreviations used: FAS, fatty acid synthase; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; SCD, stearoyl-CoA desaturase; $T_3R$, thyroid hormone receptor.

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## MATERIALS AND METHODS

*Materials.* The fat-free, high carbohydrate diet was obtained from U.S. Biochemical Corp. General laboratory chemicals were purchased from Sigma and Fisher. The $\beta$-actin used for the RNase protection was from Ambion. The origin of the SCD1 cDNA probe was previously described (8). All plasmid DNAs were isolated by a modification of the SDS-NaOH method, or alkaline lysis method (9). Radionucleotides were from DuPont New England Nuclear. Diet 11, a diet lacking fat soluble vitamins, was provided by H. F. Deluca, University of Wisconsin-Madison. FAS cDNA probe was obtained from H. Sul, University of California.

*Animals and treatments.* The protocol to research the metabolic regulation of lipid biogenesis was approved by the University of Wisconsin Research Animal Resources Center. Male BALB/c mice and pregnant females were obtained from Harlan Sprague-Dawley. Retinoic acid-deficient mice were obtained by a standard protocol of feeding females diet 11 during pregnancy and maintaining them and their pups on the diet for 5 weeks after birth (10). Diet 11 is deficient in all fat soluble vitamins, however, vitamins D, E and K were supplemented. Pups were considered retinoic acid-deficient at 5 weeks of age when maintained on diet 11 (10). Normal mice were fed a complete Purina Formulab Diet 5008 (chow) for at least 2 days prior to the start of the experiment. Food was withheld from male mice (4-6 weeks old) for 24 hours, refed chow diet for 24 hours, starved for 24 hours (11), and then fed the test diet (chow or fat free, high carbohydrate diet with or without retinol palmitate) at intervals shown in the figures and legends. This feeding schedule insured that the mice ingested the food. Animals were given free access to tap water at all times. In each experiment one mouse was treated per sample, and mRNA was measured by RNase protection and slot blot analysis to confirm results. Experiments were repeated as indicated in the figure legends. Vitamin A was supplemented in the diets at 0.01% and 0.1% as retinol palmitate for 36 hours (12). Retinol palmitate is a vitamin A derivative commonly used as an oral vitamin A supplement (13). In designated experiments, the chow diet was supplemented with 0.5% or 1.0% clofibrate. Triiodothyronine ($T_3$) was administered by intraperitoneal injection at appropriate dosages and time courses as described in figure legends (14). Animals were killed by cervical dislocation and livers were surgically removed for further analysis.

*RNA extraction and analysis.* Total hepatic RNA was isolated according to Chirgwin et al. (15). RNase protection analysis was performed according to Melton et al. (16). Antisense RNA probes for SCD1 and $\beta$-actin were synthesized and hybridized to 15 $\mu$g RNA as previously described (17). The protected RNA hybrids were ethanol precipitated, and separated on a 7% polyacrylamide, 7M urea sequencing gel, and visualized by autoradiography. Relative levels of hybridization were quantified by laser densitometry using multiple exposures to confirm the results. Slot blots were performed using 20 $\mu$g total liver RNA per slot, denaturing with formaldehyde and formamide, and blotting on Nytran Max Strength membrane with Schleicher and Schuell Minifold II Slot Blot System. Slot blots were probed with random priming labeled cDNA probes and hybridized for 48 hours. mRNAs were quantified by calculating the average hybridization of multiple exposures from laser densitometric scanning of autoradiograms. Results were repeated as indicated in the figure legends.

## RESULTS

### *Dose-Response of SCD1 mRNA Induction by Vitamin A in Vitamin A-Deficient Animals*

To examine the effect of vitamin A on SCD1 mRNA levels in mouse liver, vitamin A-deficient mice were fed a fat-free high carbohydrate diet alone or containing 0.01% or 0.1% retinol palmitate for 36 hours. SCD1 and $\beta$-actin mRNA levels were measured by RNase protection analysis, with the hybridization signals quantitated by laser densitometry. Data are expressed graphically as a fold induction ratio of SCD1/$\beta$-actin mRNA (Fig. 1). In 36 hours, SCD1 mRNA levels were induced by 1.6-fold in mice fed 0.01% retinol palmitate, compared to 2.3-fold in animals fed 0.1%. A fat-free diet was chosen in this case because polyunsaturated fatty acids repress SCD1, and may conceal the induction by vitamin A. Refeeding a fat-free, high carbohydrate diet to fasted mice increases the rate of transcription of the SCD1 gene (17), which is partially caused by the removal of polyunsaturated fatty acids from the diet. Therefore, the fold induction over the diet alone is low, but still clearly present.

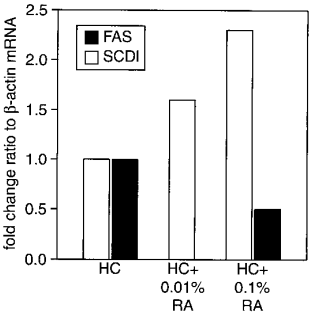


**FIG. 1.** Dose-response of SCD1 mRNA induction by vitamin A in vitamin A-deficient mice. Vitamin A-deficient mice were fasted and then refed a fat-free high carbohydrate diet (HC) alone or supplemented with 0.01% or 0.1% retinol palmitate (RA) for 36 hours. Total RNA was isolated and analyzed by RNase protection analysis using an SCD1-specific, FAS-specific, or $\beta$-actin-specific complementary riboprobe. Autoradiograms were subjected to laser densitometric scanning. Data are expressed graphically as a fold induction ratio of SCD1 or FAS/$\beta$-actin mRNA.

The effect of vitamin A on FAS mRNA levels in retinoic acid-deficient mouse liver was examined to determine if all lipogenic genes respond similarly to SCD1. Mice were once again fed a high carbohydrate diet containing 0.1% retinol palmitate or vehicle alone for 36 hours. FAS and $\beta$-actin mRNA levels were measured by slot blot analysis, with the hybridization signals quantitated by laser densitometry. In 36 hours, FAS mRNA levels were slightly repressed in response to vitamin A treatment (Fig. 1).

### *Dose-Response of SCD1 mRNA Induction by Vitamin A in Normal Animals*

Retinol palmitate was also fed to normal mice to determine if vitamin A deficiency was required for SCD1 mRNA induction. Mice were administered a chow diet alone or containing 0.01% or 0.1% retinol palmitate for 36 hours. SCD1 and $\beta$-actin mRNA levels were measured by RNase protection analysis.

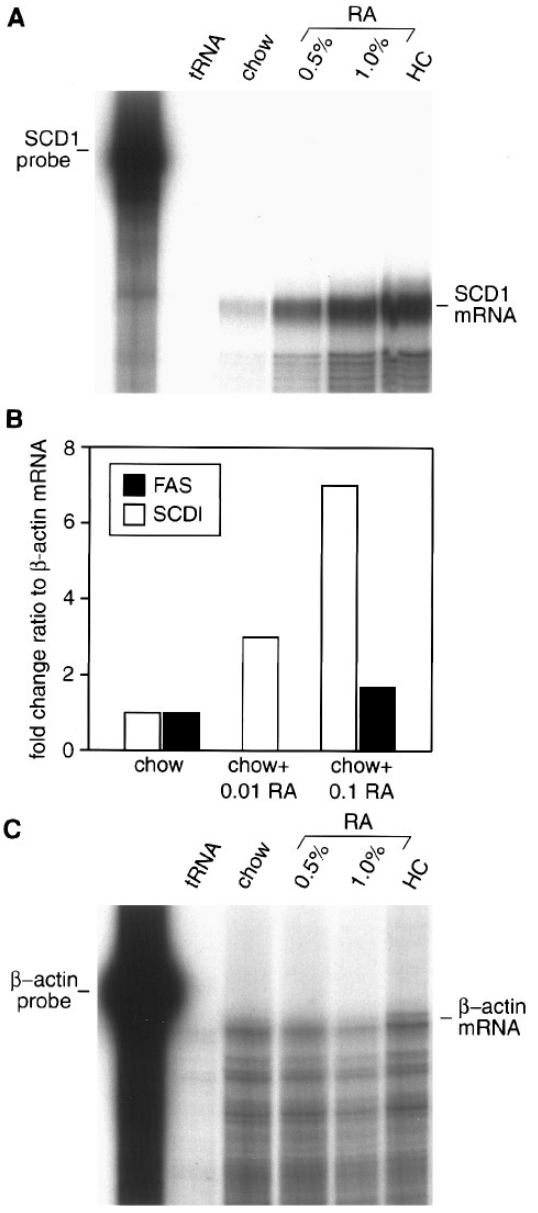


**FIG. 2.** Dose-response of SCD1 mRNA induction by vitamin A in normal mice. Normal mice were fasted and then refed a Purina chow diet alone or supplemented with 0.01% or 0.1% retinol palmitate (RA) for 36 hours. Total RNA was isolated and analyzed by RNase protection assay using an (A) SCD1-specific complementary riboprobe or a (C) $\beta$-actin-specific complementary probe, (B) or by slot blot analysis using an FAS-specific probe. Data are expressed graphically as a fold induction ratio of SCD1 or FAS/$\beta$-actin mRNA. $\beta$-actin was used as a control to show that $\beta$-actin mRNA levels do not change significantly in response to retinoic acid treatment. Autoradiograms were subjected to laser densitometric scanning. Data are expressed graphically as a fold induction ratio of SCD1 or FAS/$\beta$-actin mRNA. These data are representative of several experiments with essentially identical results.

The hybridization signals were quantitated by laser densitometry. Retinol palmitate (0.01% and 0.1%) significantly induced SCD1 mRNA (Fig. 2A), without changing levels of $\beta$-actin (Fig. 2C), showing that the retinol palmitate-mediated increase in SCD1 mRNA is specific. Data are expressed graphically as a fold induction ratio of SCD1/$\beta$-actin mRNA (Fig. 2B). In 36 hours, SCD1 mRNA levels in chow fed animals were induced by 3-fold with 0.01% retinol palmitate, compared to 7-fold with 0.1%. The induction pattern of SCD1 mRNA in animals fed the fat-free diet was similar to that seen in the vitamin A-deficient animals. High carbohydrate, fat-free diet alone was administered as a control, to reaffirm induction of SCD1 mRNA under these conditions (17). FAS hepatic mRNA levels were measured by slot blot analysis and were not significantly changed in response to 0.1% retinol palmitate treatment. Data are shown as a fold induction ratio of FAS/ $\beta$-actin mRNA (Fig. 2B). These data indicate that the lipogenic genes SCD1 and FAS may respond differently to vitamin A treatment.

### *Interaction Studies between Retinoic Acid, Thyroid Hormone, and Peroxisome Proliferators*

To investigate the physiological relevance of the induction of SCD1 by vitamin A, we treated normal mice with a combination of triiodothyronine ($T_3$) and dietary retinol palmitate. Thyroid hormone ($T_3$) has been shown to repress hepatic SCD1 mRNA expression (Waters et al., unpublished data). We hypothesized that $T_3$ would repress vitamin A elevated SCD1 levels by competition of its receptor with RAR for their heterodimerization partner RXR. For this study, normal mice were fasted and refed a diet supplemented with 0.01% and 0.1% retinol palmitate as indicated in Figure 3. Where indicated, mice were given receptor-saturating doses of 10 and 100$\mu$g $T_3$/ 100g BW upon feeding and again 24 hours later (14). Mice were sacrificed 36 hours after the initial feeding/treatment, with total RNA analyzed by RNase protection (Fig. 3A). Data are also represented graphically as fold change in SCD1 mRNA (Fig. 3B). Retinol palmitate-induced elevation of SCD1 mRNA levels was repressed 70% by treatment with 100$\mu$g/100g body weight $T_3$, whereas lower doses of $T_3$ resulted in less of a competition. $\beta$-actin mRNA levels did not change significantly in response to any feedings or treatments (data not shown). FAS appears to be unregulated by retinol palmitate in normal animals, and therefore, was not tested. These data suggest that $T_3$R and RAR compete in vivo for RXR, resulting in antagonistic responses.

A similar experiment was performed to study the interaction of peroxisome proliferators and $T_3$ in vivo. We hypothesized that $T_3$ would repress the induction of SCD by peroxisome proliferators if both needed the retinoid X receptor for maximum binding. Normal mice were fasted and refed a diet supplemented with 1.0% clofibrate, a peroxisome proliferator. Mice were given 2 and 20$\mu$g $T_3$ (10 and 100$\mu$g/100g BW) upon

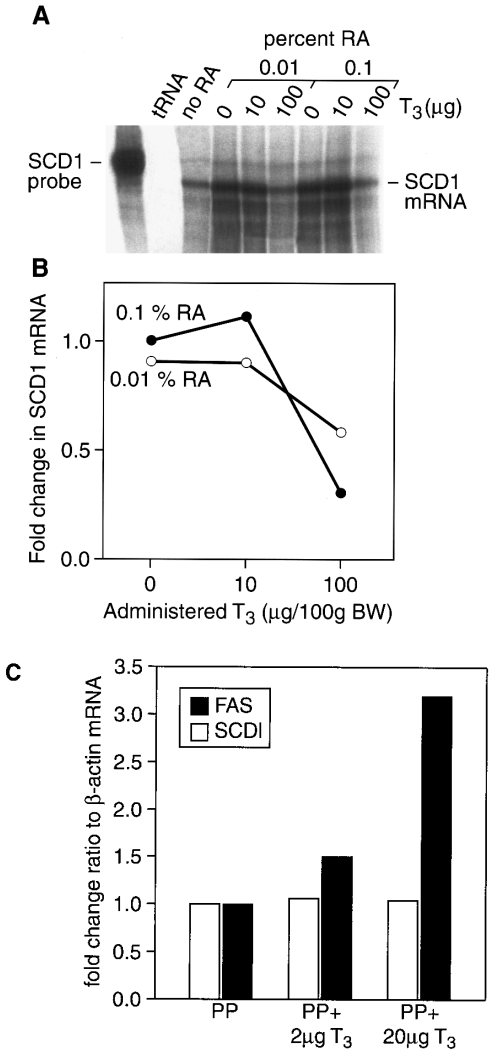


**FIG. 3.** Interaction studies between retinol palmitate, thyroid hormone and peroxisome proliferators. Normal mice were administered IP injections of either 10$\mu$g or 100$\mu$g $T_3$/100g BW upon feeding and again 24 hours later. Simultaneously, mice were fasted and refed a fat-free high carbohydrate diet containing (A and B) 0.01% or 0.1% retinol palmitate (RA) or (C) 1.0% clofibrate (PP). Total RNA was isolated after 36 hours and analyzed by RNase protection/slot blot assay using an SCD1-specific, FAS-specific, or $\beta$-actin-specific complementary probe. mRNAs were quantitated by laser densitometric scanning of autoradiograms, with data represented as -fold change in SCD1 or FAS/$\beta$-actin mRNA. These data are representative of several experiments with essentially identical results.

feeding and 24 hours later. Mice were sacrificed 36 hours after initial feeding/treatment, total SCD1, FAS and $\beta$-actin mRNA were analyzed, and data are expressed graphically as a fold ratio of SCD1 or FAS/$\beta$-actin mRNA (Fig. 3C). Peroxisome proliferator-elevated SCD1 mRNA levels were unaffected by administration of thyroid hormone. However, FAS mRNA repression by peroxisome proliferators was reversed by $T_3$ administration. These data indicate that transcriptional regulation of SCD1 mRNA by vitamin A and $T_3$ occurs through the traditional type II steroid hormone mechanism, but that peroxisome proliferators may not.

## DISCUSSION

Stearoyl-CoA desaturase has been used as a model for studying dietary and hormonal manipulations of lipogenic genes. The regulation of SCD1 in response to insulin and polyunsaturated fatty acids has been characterized, and is similar to that of other lipogenic genes (17,18,19). Here we show that SCD1 gene expression is induced by vitamin A in liver. FAS mRNA levels do not respond significantly to retinol palmitate treatment in normal animals. This is the first study with conclusive results involving vitamin A regulation of hepatic lipogenic gene expression. Vitamin A regulation of lipogenic gene expression has been demonstrated with S14 and glycerophosphate dehydrogenase in 3T3 adipocytes (20). We have previously shown that SCD1 does not respond like a lipogenic gene in response to other type II steroid hormones, such as thyroid hormone and peroxisome proliferators. SCD1 transcription is repressed by thyroid hormone in liver (Waters et al. unpublished data), and induced by peroxisome proliferators (2). Fatty acid remodeling enzymes and lipolytic enzymes show the same pattern of regulation as SCD1 to steroid hormones (6,7). Therefore, the unique pattern of SCD1 regulation by retinoic acid is consistent with that of other type II steroid hormones.

We have also determined with interactive studies in mice that thyroid hormone represses retinol palmitate-elevated SCD1 mRNA levels in vivo. Peroxisome proliferator-elevated SCD1 mRNA levels are unaffected by administration of thyroid hormone. These in vivo studies indicate that vitamin A and thyroid hormone transcriptional regulation of SCD1 is mediated through a heterodimerization with the retinoid X receptor. This method of regulation is common with type II steroid hormones, and competition for this receptor may be important for regulation. These in vivo studies indicate that peroxisome proliferator transcriptional regulation of SCD may be mediated through a slightly different mechanism.

Interaction studies also determined that thyroid hormone induced peroxisome proliferator repressed FAS mRNA levels in vivo. Hepatic FAS regulation by thyroid hormone has not been previously studied in mice. However, thyroid hormone has been shown to positively regulate adipose and chick embryo fatty acid synthase (3,5). These data indicate that thyroid hormone and peroxisome proliferators transcriptionally regulate FAS through the traditional type II steroid hormone mechanism.

Our transcriptional studies have lead to the interesting observation that hepatic SCD is regulated by insulin and polyunsaturated fatty acids similarly to lipo-

genic genes but differently in response to thyroid hormone, peroxisome proliferators, and vitamin A. This presents SCD as a unique model system because of two SCD isoforms (SCD1 and SCD2), which are tissue-specifically regulated (8), and because it does not follow the same pattern of regulation in liver as other lipogenic enzymes. More studies involving type II steroid hormone regulation of SCD and lipogenic genes must be completed before it can be determined why the pattern of SCD regulation is unique.

## ACKNOWLEDGMENT

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