THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 271, No. 47, Issue of November 22, pp. 29854–29858, 1996
Printed in U.S.A.

Regulation of Stearoyl-CoA Desaturase 1 mRNA Stability by Polyunsaturated Fatty Acids in 3T3-L1 Adipocytes*

(Received for publication, June 28, 1996, and in revised form, September 9, 1996)

Anna M. Sessler‡, Navjot Kaur§, Jiwan P. Palta§, and James M. Ntambi‡¶‖

From the ‡Department of Biochemistry, §Department of Horticulture, and ¶Nutritional Sciences, University of Wisconsin, Madison, Wisconsin 53706

The effects of arachidonic acid (20:4, n-6) and other fatty acids on the expression of stearoyl-CoA desaturase gene 1 were investigated in fully differentiated 3T3-L1 adipocytes. Treatment of 3T3-L1 adipocytes with arachidonic acid resulted in a decrease in stearoyl-CoA desaturase (Scd) enzyme activity and *scd1* mRNA. Arachidonic acid did not alter the transcription of the *scd1* gene, whereas the half-life of the *scd1* mRNA was reduced from 25.1 to 8.5 h. Blocking the conversion of arachidonic acid to eicosanoids by pretreatment of the cells with cyclooxygenase, lipoxygenase, or cytochrome P-450 epoxygenase inhibitors did not reverse the inhibition caused by arachidonic acid, indicating that eicosanoid synthesis is not necessary for the repression of *scd1* mRNA expression. Treatment of adipocytes with linoleic (18:2, n-6) and linolenic (18:3, n-3) acids also resulted in inhibition of *scd1* mRNA accumulation. By contrast, oleic acid (18:1, n-9) and stearic acid (18:0) had no effect on *scd1* mRNA levels. Taken together, these results suggest that polyunsaturated fatty acids repress the expression of the *scd1* gene in mature adipocytes by reducing the stability of *scd1* mRNA.

The mouse embryo 3T3-L1 preadipocytes (1–4) represent a useful model system for studying the mechanisms of cellular differentiation and development. Under appropriate stimuli, these cells differentiate in culture into cells possessing the morphological and biochemical characteristics of adipocytes (5–11). Accompanying acquisition of the adipocyte phenotype, the cells become responsive to both lipogenic (insulin) and lipolytic (ACTH) hormones (10, 12) and acquire increased levels of enzymes of the glycolytic, lipogenic, and lipolytic pathways (5, 7, 10, 12) as well as other adipocyte-specific proteins such as stearoyl-CoA desaturase (13, 14), the insulin receptor (8, 15), and myelin aP2 (16), which are expressed at high levels in adipocytes.

Over the years, several differentiation-induced genes have been isolated and characterized, and their promoters have been analyzed (14, 17, 18). Two of these genes, stearoyl-CoA desaturase 1 and 2 (*scd1* and *scd2*) (14, 17), encode two isozymes of stearoyl-CoA desaturase, a key enzyme involved in the biosynthesis of unsaturated fatty acids as well as the regulation of this process. The enzyme activity increases 20–100-fold during the differentiation of 3T3-L1 preadipocytes (12). This increase is primarily due to increased transcription of the *scd* genes (14, 19). The enzyme catalyzes the Δ^9-*cis* desaturation of fatty acyl-CoAs (20); the predominant products are palmitoleoyl- and oleoyl-CoA. Palmitoleic and oleic acids are the major constituents of membrane phospholipids and triacylglycerol stores found in adipocytes (12). The ratio of stearic acid to oleic acid is one of the factors influencing cell membrane fluidity. Alteration of this ratio is implicated in aging, obesity, and various diseases such as cancer, diabetes, and heart disease (21–23).

Several studies using rat liver primary cultures and intact animals have established that genes encoding both glycolytic and lipogenic enzymes are regulated by dietary fatty acids (24–29). Polyunsaturated fatty acids (PUFAs),[1] particularly the ω-6 and ω-3 series, repress the transcription of genes such as malic enzyme, acetyl-CoA carboxylase, fatty acid synthase (*FAS*), glucose transporter 4 (*GLUT4*), *S14* protein, and *scd1* (24–27, 30–32). Saturated and monounsaturated fatty acids have no effect on the transcription of these genes.

Liver and adipose tissue are the two major tissues involved in lipid biosynthesis. Although the regulation of lipogenic gene expression by PUFAs in liver is currently being studied, the effects of these molecules on gene expression in mature, fully differentiated adipocytes have not been extensively investigated. In view of the potential role of polyunsaturated fatty acids in regulating total fatty acid synthesis and the role stearoyl-CoA desaturase plays in this process, we examined the effect of polyunsaturated fatty acids on the expression of the *scd1* gene in mature adipocytes. Our results suggest that PUFAs regulate the expression of the adipocyte *scd1* gene by regulating stability of mRNA transcripts.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco's modified Eagle's medium, fetal bovine serum, and actinomycin D were obtained from Life Technologies, Inc. Calf serum was purchased from Biowhittaker, and insulin was purchased from Lilly. 3-Isobutyl-1-methylxanthine was obtained from Aldrich. Nytran membranes were supplied by Schleicher & Schuell, Inc. All radiolabeled compounds were obtained through DuPont NEN unless specified. Probe-labeling kits were purchased from Promega. Silica gel plates were obtained from Analtech. All other materials were obtained from Sigma.

Cell Culture—Murine 3T3-L1 preadipocytes were cultured and differentiated into adipocytes as described previously (14). Mature 3T3-L1 adipocytes, 6–9 days after induction of differentiation, were treated with albumin-bound fatty acids. Fatty acid-albumin stocks were prepared as 100 mM fatty acid with 2 mM fatty acid-free bovine serum albumin, 0.1% butylated hydroxytoluene, and 20 μM α-tocopherol (33) to minimize oxidation of the fatty acids.

Isolation and Analysis of RNA—Total cellular RNA was isolated

* This work was supported by National Institutes of Health Grant DK42825, United States Department of Agriculture (Hatch) Grant 3784, and by multiple disciplinary Grant 5204 from the College of Agriculture and Life Sciences, University of Wisconsin, Madison, WI 53706. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‖ To whom correspondence should be addressed: Dept. of Biochemistry, 420 Henry Mall, Madison, WI 53706. Tel.: 608-265-3700; Fax: 608-265-3272.

[1] The abbreviations used are: PUFA, polyunsaturated fatty acid; AA, arachidonic acid; PIPES, 1,4-piperazinediethanesulfonic acid; SCD, stearoyl-CoA desaturase.

TABLE I
Effect of arachidonic acid (AA) on Scd enzyme activity

Day 6 3T3-L1 adipocytes were treated with the indicated concentrations (μM) of arachidonic acid (AA) for 72 h and then harvested for measurement of enzyme activity. Values are expressed as percentage conversion of [1-^{14}C]-palmitoyl-CoA to [1-^{14}C]-palmitoleoyl-CoA. Similar results were seen in two independent experiments.

AA (μM)	% Conversion
0	39.39 ± 0.27
100	30.64 ± 1.49
300	15.81 ± 0.16

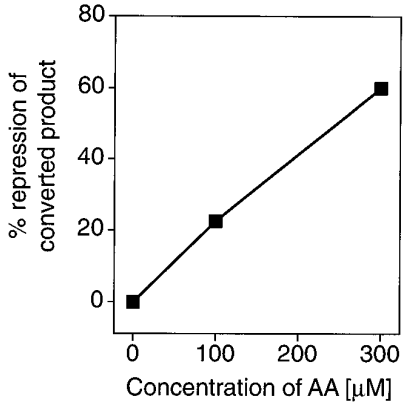

FIG. 1. **Regulation of Scd enzyme activity by arachidonic acid (AA).** Graphical representation of enzyme activity in response to AA dose. Day 6 3T3-L1 adipocytes treated with 0, 100, or 300 μM AA for 72 h were harvested for measurement of enzyme activity. Values are expressed as percentage conversion of [1-^{14}C]palmitoyl-CoA to [1-^{14}C]palmitoleoyl-CoA.

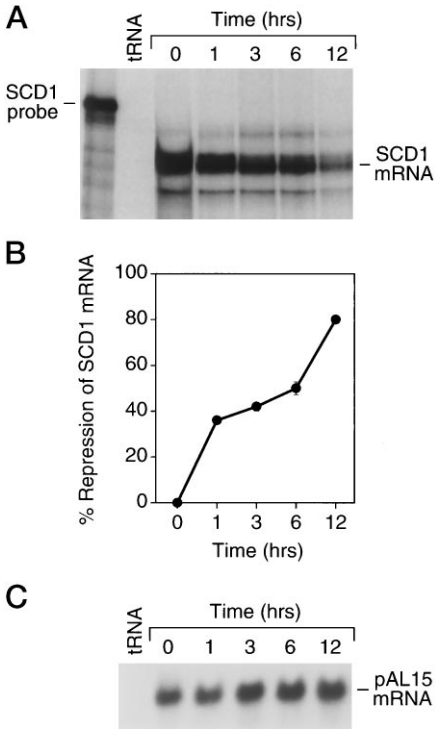

FIG. 2. **Time-dependent effect of AA on *scd1* mRNA levels.** *A*, total cellular RNA was isolated from day 6 adipocytes treated with 300 μM AA for various periods of time. Total RNA (15 μg) was subjected to RNase protection analysis using an *scd1*-specific probe. *B*, graphical analysis of results in *A*. *C*, details of this experiment are the same as in *A*, except total RNA was subjected to Northern blot analysis (20 μg/lane) and hybridized to a radiolabeled pAL15 probe. *Bars*, mean ± S.D. of three independent experiments.

from 3T3-L1 cells using guanidine isothiocyanate followed by ultracentrifugation through CsCl (34). Cytoplasmic RNA was isolated from cells by modification of the procedure previously described (35) using 10% Triton X-100 and 10% SDS for cell lysis. *scd1* mRNA and *pAL15* (36) mRNA expression were measured by RNase protection or Northern blot analysis as described previously (37, 38) and quantified by laser densitometric scanning of autoradiograms.

Nuclei Isolation and Transcription Run-on Analysis—Nuclei from treated cells were isolated through a 2 M sucrose gradient after Dounce homogenization. Run-on transcription was performed as described previously (29) using a 2-kilobase cDNA probe for *scd1* designated pC3 (14).

Measurement of Scd Enzyme Activity—Desaturation of [1-^{14}C]palmitoyl-CoA was determined by modification of the procedure described previously (39, 40). Briefly, cells were washed with phosphate-buffered saline and scraped from the Petri dish. Pelleted cells were homogenized in 800 μl of 0.1 M PIPES (pH 6.0) extraction buffer containing 1% polyvinylpyrrolidone, 6000 units catalase, 0.1% bovine serum albumin, and 40 mM sodium ascorbate. The assay was conducted for 10 min at 25 °C in a total volume of 1 ml containing 1 mM dithiothreitol, 100 μg bovine serum albumin, 0.75 mM NADPH, 50 μg ferredoxin, 0.285 units Fd-NADP$^+$ oxidoreductase, 4000 units catalase, and 6 μM [1-^{14}C]palmitoyl-CoA (Amersham Corp.). The reaction was terminated with chloroform:methanol (v/v, 1:1).

Lipids from membrane samples were extracted by sequential addition of isopropyl alcohol, methanol, chloroform, and butylated hydroxytoluene. After addition of 0.8% (w/v) aqueous KCl, the chloroform phase was dried under nitrogen and converted to methyl esters with boron trifluoride and then extracted into hexane. Separation of methyl esters was done by thin layer chromatography on silica gel plates containing 15% $AgNO_3$ using a hexane:ether (9:1) solvent system. Spots were identified under UV light after spraying with 0.2% dichlorofluorescein ethanolic solution and compared with authentic standards. Plates were read on an automated thin layer chromatography analyzer (Berthold LB2842). Desaturation activity was determined by integrating the area under the peaks corresponding to 16:1 and 16:0 methyl esters and expressed as the percentage conversion of 16:0 into 16:1.

RESULTS

Arachidonic Acid Decreases Scd Enzyme Activity—The effect of arachidonic acid on Scd enzyme levels was assessed by measuring Scd enzyme activity in adipocytes treated on day 6 of differentiation. Arachidonic acid treatment caused a decrease in Scd enzyme activity. The percentage conversion of 16:0 to 16:1 by Scd enzyme activity in untreated cells was 39.39 ± 0.27% (mean ± S.D.) compared with 30 ± 1.49 and 15 ± 0.16% with 100 and 300 μM arachidonic acid, respectively (Table I). Repression of enzyme activity by AA treatment was expressed as percentage repression over control levels and was calculated from the data in Table I. As shown in Fig. 1, 100 and 300 μM AA suppressed Scd enzyme activity by 22.5 and 60%, respectively. The dose-response curve in Fig. 1 showed a linear decrease in enzyme activity in response to AA.

Arachidonic Acid Decreases scd1 mRNA Levels—To determine whether the decrease in enzyme activity was due to changes in mRNA levels, RNase protection analysis was performed on total RNA isolated from fully differentiated 3T3-L1 adipocytes treated with arachidonic acid for varying lengths of time (Fig. 2*A*). The hybridization pattern of *scd1* mRNA to its specific RNA probe was quantitated by densitometric scanning. As shown in Fig. 2*B*, the greatest repression occurred between 6 and 12 h of AA treatment. Northern blot analysis using a cDNA probe corresponding to *pAL15*, which encodes a ribosomal protein (16, 36), indicated that *pAL15* mRNA content was not significantly decreased after as much as a 48-h exposure of the cells to AA (Fig. 2*C*).

The repression of *scd1* mRNA by arachidonic acid was also dose-dependent. Doses of AA as low as 10 μM decreased levels of *scd1* message by approximately 20 ± 1.2% from normal levels. As shown in Fig. 3, *A* and *B*, concentrations of AA greater than 50 μM exerted a significant effect on mRNA levels

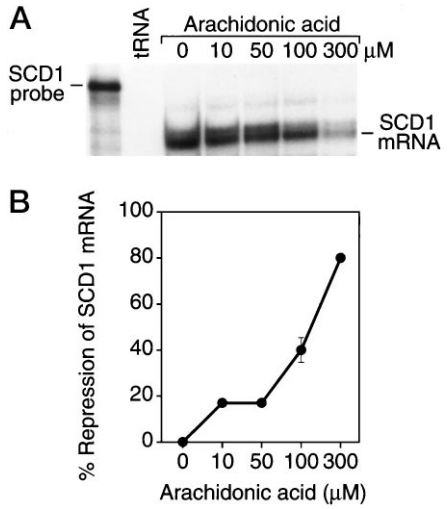

FIG. 3. **Dose-dependent effect of AA on *scd1* mRNA levels.** *A*, day 6 adipocytes were treated with different concentrations of AA for 12 h. Total RNA was isolated and analyzed for *scd1* expression by RNase protection. *B*, the autoradiogram in *A* was quantitated by laser densitometry, and the results are expressed as percentage repression over control (*0* μM lane).

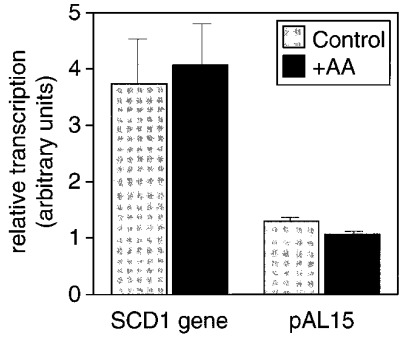

FIG. 4. **Analysis of nuclear run-on transcription of the *scd1* gene.** Isolated nuclei from cells treated with or without 300 μM AA were subject to transcription run-off assays. Transcription from the *scd1* gene was analyzed using the pC3 (2-kilobase) probe. Transcription from the gene encoded by *pAL15* was analyzed with the pAL15 probe. Results are reported as the mean ± S.D. of two independent experiments.

(ED_{50} = 160 μM). Doses as high as 300 μM resulted in the maximal repression of 80 ± 0.8%. AA had no effect on cell viability, as determined by comparison of cell numbers in control and treated cells (data not shown). These results, combined with the enzyme activity studies, show that decreased levels of enzyme activity are due, in part, to changes of *scd1* mRNA levels.

Arachidonic Acid Affects scd1 mRNA Turnover—To determine whether changes in *scd1* mRNA levels in response to AA treatment were due to alterations in gene transcription or mRNA stability, experiments were performed to examine both mechanisms. Nuclear run-on transcription assays showed no significant reduction in the transcription of the *scd1* gene to account for the 80% reduction in *scd1* mRNA accumulation (Fig. 4). As expected, transcription of *pAL15* was also unaffected by AA treatment. Therefore, studies of the stability of *scd1* mRNA upon exposure to AA were performed. Day 9 3T3-L1 adipocytes (control and 12 h after exposure to 300 μM AA) were exposed to the transcription inhibitor, actinomycin D (5 μg/ml), for increasing time periods. Levels of chased mRNA were determined by RNase protection analysis. As shown in Fig. 5*A*, treatment with arachidonic acid resulted in destabilization of *scd1* mRNA. The hybridization patterns were analyzed by laser densitometry, and the values are plotted as a

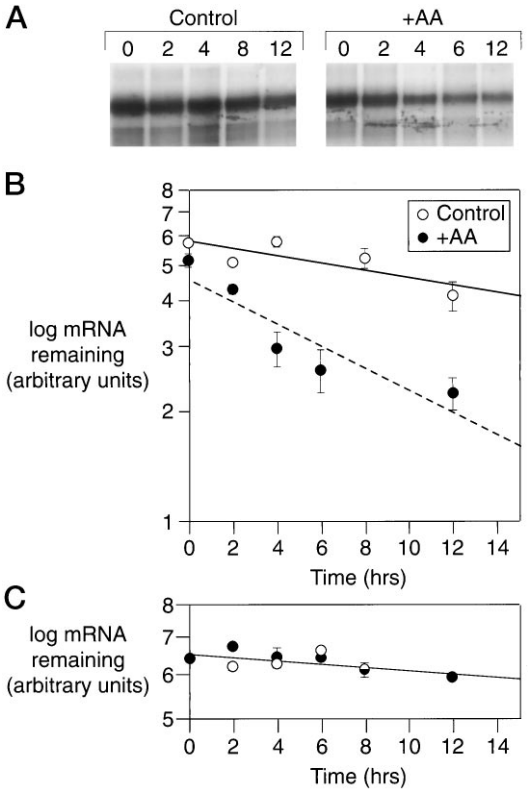

FIG. 5. **Effects of AA on the stability of *scd1* mRNA.** *A*, cytoplasmic RNA from control cells and cells pretreated for 12 h with 300 μM AA was isolated after the addition of actinomycin D (5 μg/ml) for the indicated times. RNA was subjected to RNase protection using an *scd1*-specific probe. Similar results were obtained from three independent experiments. *B*, graphical representation of the effect of AA on the stability of *scd1* mRNA. Densitometric data were plotted as mRNA remaining in 3T3-L1 adipocytes exposed to either actinomycin D alone (control, ○) or both actinomycin D and AA (+AA, ●). The mRNA half-lives were calculated from the curve-fits of a linear plot. *Bars*, mean value ± S.D. *C*, graphical representation of the half-lives of *pAL15* mRNA in response to actinomycin D alone or actinomycin D and AA treatment. Experimental conditions are the same as in *A*, except Northern blot analysis was performed with a pAL15 radiolabeled probe, and the autoradiogram was subjected to laser densitometry.

function of time (Fig. 5*B*). *scd1* mRNA levels decreased more rapidly with time in cells treated with AA. The $t_{1/2}$ of *scd1* declined from 25.1 ± 4.1 to 8.5 ± 0.6 h as calculated by linear regression analysis. In contrast, the $t_{1/2}$ of *pAL15* mRNA did not decrease in response to AA treatment (Fig. 5*C*). The stability of actin mRNA was also not significantly affected by AA (data not shown). These results show that enhanced *scd1* mRNA turnover is primarily responsible for AA-mediated repression of *scd1* gene expression in 3T3-L1 adipocytes.

Eicosanoid Synthesis Inhibitors Have no Effect on AA Suppression of scd1 mRNA Levels—To determine whether the AA-induced regulation of *scd1* mRNA was an arachidonic acid-specific effect or required oxidative metabolism, we investigated the effects of eicosanoid biosynthesis inhibitors on the levels of *scd1* mRNA. Cyclooxygenase, lipoxygenase, and cytochrome P-450 epoxygenase inhibitors were employed to block metabolism of exogenously added AA to active eicosanoids. As shown in Fig. 6, cells pretreated for 30 min with ibuprofen (50 μM), nordihydroguariatic acid (10 μM), or caffeic acid (10 μM) followed by a 12-h treatment with AA still showed repression of *scd1* mRNA over control levels. When quantitated by densitometric scanning, the repression by AA in the presence of ibuprofen (78 ± 1.4%) is similar to that observed with AA alone. The repression in the presence of nordihydroguariatic acid and caffeic acid was 78.5 ± 3.5 and 77.4 ± 10%,

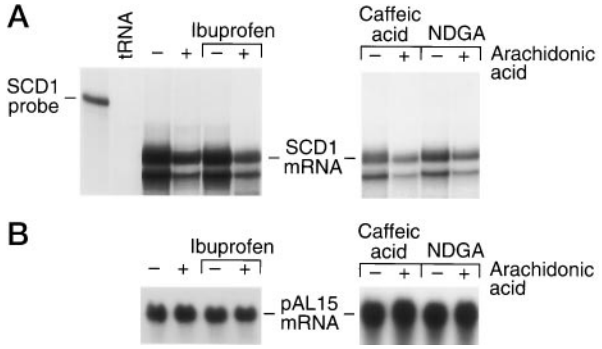

FIG. 6. **Effects of eicosanoid biosynthesis inhibitors on AA suppression of *scd1* mRNA levels.** *A*, day 6 3T3-L1 adipocytes were pretreated with ibuprofen (50 μM), caffeic acid (10 μM), or nordihydroguariatic acid (*NDGA*) (10 μM) for 30 min. After pretreatment, cells were exposed to 300 μM AA for 12 h in the presence of inhibitor. RNA was then isolated and subjected to RNase protection analysis. *B*, Northern blot analysis of RNA in *A* using a pAL15 radiolabeled cDNA probe.

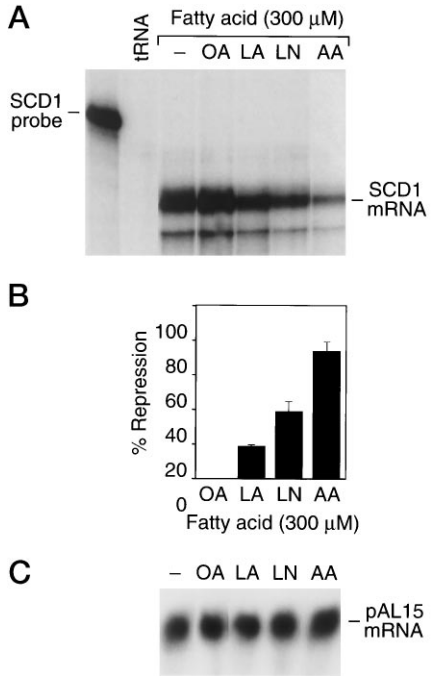

FIG. 7. **Effect of fatty acids on *scd1* mRNA levels.** *A*, day 6 adipocytes were exposed to 300 μM oleic acid (*OA*), linoleic acid (*LA*), linolenic acid (*LN*), and arachidonic acid (*AA*) for a 12-h period. Total cellular RNA was subjected to RNase protection analysis using an *scd1*-specific probe. The results are representative of several independent experiments. *B*, the autoradiogram in *A* was quantified by laser densitometry and the results reported as percentage repression relative to the maximum level of expression. *C*. RNA, as in *A*, was subjected to Northern blot analysis and probed with a radiolabeled pAL15 probe.

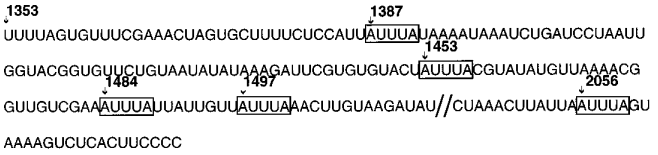

FIG. 8. **Partial sequence of the 3′ untranslated region of mouse *scd1* mRNA.** Nucleotides from 1353–2079 are shown with AU-rich elements indicated by *boxed* sequences. *Arrows* show the location by base numbers. The // symbol represents a portion of the sequence not shown.

respectively. In addition, the acetylenic analog of AA (eicosatetraynoic acid) that cannot be metabolized to eicosanoids was able to repress *scd1* mRNA accumulation (data not shown). Levels of *pAL15* mRNA remained unaffected by either AA or AA + ibuprofen, nordihydroguariatic acid, or caffeic acid treatments (Fig. 6*B*). These results suggest that AA acts independently of eicosanoid metabolism to affect *scd1*-specific gene expression.

scd1 mRNA Levels in Mature Adipocytes Are Decreased by Other Polyunsaturated Fatty Acids—To determine if the observed repression was a general response to PUFAs or specific to AA only, additional fatty acids were tested for their effects on *scd1* mRNA expression. As shown in Fig. 7*A*, 300 μM linoleic (*LA*, 18:2) and linolenic (*LN*, 18:3) acids, in addition to AA, repressed the level of *scd1* mRNA within 12 h of treatment. In contrast, oleic acid and stearic acid (data not shown) did not decrease the amount of *scd1* mRNA (Fig. 7). Eicosapentaeinoic acid (20:5) reduced *scd1* mRNA as did arachidonic acid (data not shown). The level of *pAL15* mRNA, analyzed by Northern blot, did not change significantly in response to any fatty acid (Fig. 7*C*). These results demonstrate that PUFAs have an apparent, specific effect on the levels of *scd1* mRNA in mature adipocytes.

DISCUSSION

In the present study, we have demonstrated that exposure of 3T3-L1 adipocytes to 300 μM arachidonic acid results in a decrease in Scd enzyme activity as well as *scd1* mRNA levels (Figs. 1 and 2). As much as a 60% decrease in enzyme activity was observed, whereas mRNA levels were repressed by 80% of the original level. Treatment of 3T3-L1 adipocytes with AA also caused a 3-fold decrease in the half-life of *scd1* mRNA (Fig. 5) and no apparent decrease in *scd1* gene transcription. The pretranslational regulation of *scd1* gene expression by PUFAs seems, then, to result primarily from the decrease of mRNA stability. Furthermore, the repression was independent of AA metabolism to eicosanoids because cyclooxygenase, lipoxygenase, and epoxygenase inhibitors did not abolish the effect (Fig. 6). Other polyunsaturated fatty acids, such as linoleic, linolenic, and eicosapentaeinoic acids, also decreased the *scd1* mRNA levels when added exogenously to mature adipocytes. By contrast, oleic acid (Fig. 7) and stearic acid did not decrease *scd1* mRNA levels; therefore, this response is unique to polyunsaturated fatty acids.

Stearoyl-CoA desaturase gene expression has previously been shown to be repressed by polyunsaturated fatty acids in liver tissue principally at the level of gene transcription (29–30). Until now, the effect of polyunsaturated fatty acids on *scd1* gene expression in adipose tissue had not been studied. The rate of transcription from the *scd1* gene was not dramatically affected in this adipocyte system. Although transcriptional regulation can not be completely ruled out by these experiments, changes in transcription that are below detectable levels suggest that transcriptional regulation does not play a significant role in PUFA suppression of adipocyte *scd1* gene expression. Our results also suggest that posttranslational regulation is not a major factor in AA-mediated *scd1* repression. The observed reduction in enzyme activity (60%) could be completely accounted for by decreases in *scd1* mRNA levels (80%). Thus, there seems to be no additional down-regulation occurring posttranslationally. As opposed to hepatocytes, changes in mRNA stability are the major determinant of *scd1* mRNA abundance in adipocytes.

Destabilization of mRNA encoding the predominantly expressed form of stearoyl-CoA desaturase in adipocytes may be regulated through mRNA sequences in the 3′ untranslated region. Both the mouse and rat *scd1* cDNAs contain an unusually long 3′ untranslated region (14, 41). The role of such a long 3′-noncoding stretch is currently unknown, though it contains several structural motifs (AUUUA) characteristic of mRNA

destabilization sequences (42–43). Four of these sequences are clustered close to the 3′ end of the coding region (Fig. 8). Because these AU-rich elements play active roles in the selective degradation of several mRNAs in response to various factors, these sequences could be possible targets of PUFA effects on *scd1* mRNA (44–47). For example, Pekala and Long (42) have suggested that such a motif in the *GLUT4* gene expressed in 3T3-L1 adipocytes may confer destabilization of mRNA in response to tumor necrosis factor α treatment. With such generalized effects of AU-rich elements, it is possible to speculate that this motif plays a role in the adipocyte regulation of desaturase gene expression by regulating mRNA stability in response to PUFAs. Additional mapping studies would be necessary to identify whether the AU-rich elements in the *scd1* 3′ untranslated region are involved in this destabilization.

The nature of the PUFA metabolite that mediates the observed mRNA destabilization is currently unknown. As demonstrated in the present study, inhibiting eicosanoid synthesis (Fig. 6) did not prevent the PUFA suppression of *scd1* gene expression. Consistent with other studies on PUFA-regulated genes (25, 27, 31, 48), our investigations of AA-oxidative metabolism suggest that the products of eicosanoid synthesis are not involved in the AA-mediated decrease of *scd1* mRNA stability in 3T3-L1 adipocytes. Furthermore, the desaturase gene is regulated by a range of polyunsaturated acids and not by mono- or unsaturated fatty acids (Fig. 7), suggesting that repression is PUFA-specific.

The data presented here suggest that PUFAs regulate *scd1* gene expression through different mechanisms in different tissue types, the reasons for which are not yet understood. However, *scd1* provides a good model to study the effects of PUFAs on mRNA stability. The ongoing search, in our lab and others, for possible protein mediators that destabilize *scd1* mRNA may provide further definition to the molecular basis of PUFA regulation of lipogenic gene expression.

Acknowledgments—We thank Tsestka Takova for technical assistance, Deborah Loo and Erin Dickerson for helpful discussion and comments, and the members of the Department of Biochemistry Media Lab, University of Wisconsin-Madison.

REFERENCES

1. Green, H., and Kehinde, O. (1974) *Cell* **1,** 113–116
2. Green, H., and Kehinde, O. (1975) *Cell* **5,** 19–27
3. Green, H., and Kehinde, O. (1976) *Cell* **7,** 105–113
4. Green, H., and Meuth, M. (1974) *Cell* **3,** 127–133
5. Mackall, J. C., Student, A. K., Polakis, S. E., and Lane, M. D. (1976) *J. Biol. Chem.* **251,** 6462–6464
6. Coleman, R. A., Reed, B. C., Mackall, J. C., Student, A. K., Lane, M. D., and Bell, R. M. (1978) *J. Biol. Chem.* **253,** 7256–7261
7. Student, A. K., Hsu, R. Y., and Lane, M. D. (1980) *J. Biol. Chem.* **255,** 4745–4750
8. Reed, B. C., and Lane, M. D. (1980) *Proc. Natl. Acad. Sci. U. S. A.* **77,** 285–289
9. Rosen, O. M., Smith, C. J., Hirsch, A., Lai, E., and Rubin, C. S. (1979) *Recent Prog. Horm. Res.* **35,** 477–499
10. Cornelius, P., MacDougald, O. A., and Lane, M. D. (1994) *Annu. Rev. Nutr.* **14,** 99–129
11. Green, H., and Kehinde, O. (1979) *J. Cell. Physiol.* **101,** 169–171
12. Kasturi, R., and Joshi, V. C. (1982) *J. Biol. Chem.* **257,** 12224–12230
13. Weiner, F. R., Smith, P. J., Wertheimer, S., and Rubin, C. S. (1991) *J. Biol. Chem.* **266,** 23525–23528
14. Ntambi, J. M., Buhrow, S. A., Kaestner, K. H., Christy, R. J., Sibley, E., Kelly, T. J., Jr., and Lane, M. D. (1988) *J. Biol. Chem.* **263,** 17291–17300
15. Reed, B. C., Ronnett, G. V., Clements, P. R., and Lane, M. D. (1981) *J. Biol. Chem.* **256,** 3917–3925
16. Bernlohr, D. A., Angus, C. W., Lane, M. D., Bolanowski, M. A., and Kelly, T. J., Jr. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 5468–5472
17. Kaestner, K. H., Ntambi, J. M., Kelly, T. J., Jr., and Lane, D. M. (1989) *J. Biol. Chem.* **264,** 14755–14761
18. Christy, R. J., Yang, V. W., Ntambi, J. M. Geiman, D. E., Landschulz, W. H., Friedman, A. D., Nakabeppu, Y., Kelly, T. J., and Lane, M. D. (1989) *Genes Dev.* **3,** 1323–1335
19. Bernlohr, D. A., Bolanowski, M. A., Kelly, T. J., Jr., and Lane, M. D. (1985) *J. Biol. Chem.* **260,** 5563–5567
20. Enoch, H. G., Catala, A., and Strittmatter, P. (1976) *J. Biol. Chem.* **251,** 5095–5103
21. Jeffcoat, R. (1979) *Essays Biochem.* **15,** 1–36
22. Clandinin, M. T., Cheema, S., Field, C. J., Garg, M. L., Benkatraman, J., and Clandinin, T. R. (1991) *FASEB J.* **5,** 2761–2769
23. Spector, A. A., and Yorek, M. A. (1985) *J. Lipid Res.* **26,** 1015–1035
24. Tebbey, P. W., McGowan, K. M., Stephens, J. M., Buttke, T. M., and Pekala, P. H. (1994) *J. Biol. Chem.* **269,** 639–644
25. Clarke, S. D., Armstrong, M. K., and Jump, D. B. (1990) *J. Nutr.* **120,** 225–231
26. Jump, D. B., Clarke, S. D., Thelen, A., and Liimatta, M. (1994) *J. Lipid Res.* **35,** 1076–1084
27. Clarke, S. D., and Jump, D. B. (1994) *Annu. Rev. Nutr.* **14,** 83–98
28. Liimatta, M., Towle, H. C., Clarke, S. D., and Jump, D. B. (1994) *Mol. Endocrinol.* **8,** 1147–1153
29. Ntambi, J. M. (1992) *J. Biol. Chem.* **267,** 10925–10930
30. Landschulz, K. T., Jump, D. B., MacDougald, O. A., and Lane, M. D. (1994) *Biochem. Biophys. Res. Commun.* **200,** 763–768
31. Clarke, S. D., and Jump, D. B. (1993) *Prog. Lipid Res.* **32,** 139–149
32. Salati, L. M., and Clarke, S. D. (1986) *Arch. Biochem. Biophys.* **246,** 82–89
33. Jump, D. B., Clarke, S. D., MacDougald, O., Thelen, A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 8454–8458
34. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299
35. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
36. Nagae, Y., Fujii, H., Yoneyama, Y., Goto, Y., and Okazaki, T. (1988) *Nucleic Acids Res.* **16(21),** 10363
37. Melton, D. A., Krieg, P. A., Rebaghiati, M. R., Maniatis, T., Zinn, K., and Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056
38. Semenkovich, C. F., Wims, M., Noe, L., Etienne, J., and Chan, L. (1989) *J. Biol. Chem.* **264,** 9030–9038
39. Montgomery, M. R., and Cinti, D. L. (1977) *Mol. Pharmacol.* **13,** 60–69
40. Palta, J. P., Whitaker, B. D., and Weiss, L. S. (1993) *Plant Physiol.* **103,** 793–803
41. Theide, M. A., Ozols, J., and Strittmatter, P. (1986) *J. Biol. Chem.* **261,** 13230–13235
42. Long, S. D., and Pekala, P. H. (1996) *Biochem. Biophys. Res. Commun.* **220,** 949–953
43. Marzulff, W. F., (1992) *Genes Exp.* **2,** 93–97
44. Alberta, J. A., Rundell, K., and Stiles, C. D. (1994) *J. Biol. Chem.* **269,** 4532–4538
45. Shyu, A., Belasco, J. G., and Greenberg, M. E. (1991) *Genes Dev.* **5,** 221–231
46. Wolford J. K., and Signs, S. A. (1995) *Biochem. Biophys. Res. Commun.* **211,** 819–825
47. Shaw, G., and Kamen R. (1986) *Cell* **46,** 659–667
48. Schwartz, R. S., and Abraham, S. (1982) *Biochim. Biophys. Acta* **711,** 316–326