Arachidonic acid inhibits lipogenic gene expression in 3T3-L1 adipocytes through a prostanoid pathway

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Abstract **This report examines the effect of polyunsaturated fatty acids (PUFA) on lipogenic gene expression in cultured 3T3-L1 adipocytes. Arachidonic acid (20:4, n–6) and eicosapentaenoic acid (20:5, n–3) suppressed mRNAs encoding fatty acid synthase (FAS) and S14, but had no effect on β-actin. Using a clonal adipocyte cell line containing a stably integrated S14CAT fusion gene, oleic acid (18:1, n–9), arachidonic acid (20:4, n–6) and eicosapentaenoic acid (20:5, n–3) inhibited chloramphenicol acetyltransferase (CAT) activity with an ED_{50} of 800, 50, and 400 μm, respectively. Given the high potency of 20:4, n–6, its effect on adipocyte gene expression was characterized. Arachidonic acid suppressed basal CAT activity, but did not affect glucocorticoid-mediated induction of S14CAT expression. The effect of 20:4, n–6 on S14CAT expression was blocked by an inhibitor of cyclooxygenase implicating involvement of prostanoids. Prostaglandins (PGE_2 and $PGF_{2\alpha}$ at 10 μm) inhibited CAT activity through a pertussis toxin-sensitive G_i/G_o-coupled signalling cascade. Our results suggest that 20:4, n–6 inhibits lipogenic gene expression in 3T3-L1 adipocytes through a prostanoid pathway. This mechanism of control differs from the polyunsaturated fatty acid-mediated suppression of hepatic lipogenic gene expression.**—Mater, M. K., D. Pan, W. G. Bergen, and D. B. Jump. **Arachidonic acid inhibits lipogenic gene expression in 3T3-L1 adipocytes through a prostanoid pathway. *J. Lipid Res.* 1998.** 39: **1327–1334.**

Supplementary key words adipocytes • polyunsaturated fatty acids • arachidonic acid • transcription • PGE_2 • inositol phosphate/calcium • prostanoids

Polyunsaturated fatty acids (PUFA), particularly the highly unsaturated n–3 fatty acids, when fed to rodents inhibit hepatic de novo lipogenesis, triglyceride synthesis and secretion, and induce peroxisomal and microsomal fatty acid oxidation (1–14). PUFA effect on these metabolic pathways is controlled by the transcription of specific genes involved in these pathways. For example, PUFA rapidly inhibit the transcription of genes encoding the fatty acid synthase (FAS) and the S14 protein, while inducing expression of genes encoding acyl CoA oxidase (AOX) and cytochrome P450 4A2 (Cyp4A2), enzymes involved in peroxisomal and microsomal fatty acid oxidation, respectively (15, 16).

The molecular basis for PUFA-mediated control of hepatic gene expression involves at least two distinct pathways. One pathway requires the peroxisome proliferator activated receptor (PPAR), a member of the steroid–thyroid supergene family. In liver, PPARα is the principal PPAR subtype accounting for the fatty acid control of AOX and CYP4A2 (16). PPARα is activated by peroxisome proliferators, including fatty acids. The second pathway is independent of PPARα and is involved in the PUFA-mediated suppression of S14, FAS and l-pyruvate kinase (15–17). Specific fatty acid-regulated transcription factors have not yet been identified for the PUFA-mediated control of FAS, S14, or L-PK. While these genes are subject to complex control by insulin, T_3, and glucose (1, 2, 17), the *cis*-regulatory targets for PUFA control of S14 and L-PK do not converge with the principal targets for endocrine or carbohydrate control. Instead, the PUFA-regulatory elements converge with targets that serve an ancillary role in hormone/nutrient control of gene transcription (13, 17).

While the liver serves as a major target for fatty acid-regulated gene expression, recent studies indicate that white adipose tissue might also be a target for fatty acid control of gene expression (13, 18, 19). Fatty acids promote differentiation of preadipocytes to adipocytes, a process that involves PPARγ2. PPARγ2 is activated by thiazolidinediones, a class of insulin sensitizing drugs, as well as fatty acids and prostanoids (20–27). Prostanoids, products of arachidonic acid metabolism, have been reported to promote adipo-

Abbreviations: PUFA, polyunsaturated fatty acids; FAS, fatty acid synthase; AOX, acyl CoA oxidase; PPAR, peroxisome proliferator activated receptor; L-PK, l-pyruvate kinase; Cyp4A2, cytochrome P450 4A2; CAT, chloramphenicol acetyltransferase; DEX, dexamethasone; FBS, fetal calf serum; IBMX, isobutylmethyl xanthine; D-MEM, Dulbecco's modified Eagle's medium; MMTV, murine mammary tumor virus; NDGA, nordihydroguaiaretic acid; PMA, phorbol 12-myristate 13 acetate; PKC, protein kinase C; PKA, protein kinase A.

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cyte differentiation in culture and to affect gene expression (24, 25, 27, 28).

Because feeding rats diets containing fish oil for 5 days leads to a ~50% suppression of $mRNA_{FAS}$ and $mRNA_{S14}$ in epididymal fat (13), we were interested in determining whether the mechanism of PUFA-mediated suppression of lipogenic gene expression in adipocytes was similar to that found in liver. Accordingly, we examined the effects of specific mono- and polyunsaturated fatty acids on lipogenic gene expression in 3T3-L1 cells. L1 cells differentiate in vitro from preadipocytes (fibroblasts) to adipocytes and this differentiation is accompanied by the induction of lipogenic genes as well as receptors that bind lipogenic and lipolytic hormones (28). We examined the effect of PUFA on expression of S14 and FAS. FAS is a well-known lipogenic enzyme. S14 encodes a nuclear protein (~17 kD M_r; 4.9 pl) that is believed to participate in the regulation of transcription of other lipogenic genes like FAS, ATP citrate lyase, and malic enzyme. A recent report suggests that S14 may be co-activator affecting lipogenic gene expression (29). Our results show that while specific PUFA inhibit S14 and FAS gene expression in cultured adipocytes, the mechanism for control may involve a prostanoid pathway. This mechanism of control differs from the one previously described in liver (13).

MATERIALS AND METHODS

Cell culture

3T3-L1 cells were grown to 2 days post confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, penicillin (100 units/l), and streptomycin (0.1 mg/l). Differentiation was initiated with DMEM containing 10% fetal bovine serum (FBS), 1 mm insulin, 1 mm dexamethasone (DEX), and 250 mm isobutylmethyl xanthine (IBMX) for 48 h. After initiating differentiation, media was replaced with DMEM supplemented with 10% FBS and insulin and changed every 2–3 days.

Several 3T3-L1 cell lines containing stably transfected S14CAT fusion genes have been previously described (30). The monoclonal C11 cell line contains S14CAT124 (CAT: chloramphenicol acetyl transferase) reporter gene. S14CAT124 contains the S14 promoter to extending from −4315 to +19 bp fused upstream from CAT. L1 cells transfected with TKCAT208, MamNeoCAT, or RSVCAT are pools of G418-resistant cells. TKCAT208 contains the region extending from −1.6 to −1.4 kb upstream from the S14 transcription start site fused upstream from the thymidine kinase (TK) promoter. The −1.6/−1.4 kb region contains the glucocorticoid and adipocyte-specific elements required for S14 expression in adipocytes (30). MamNeoCAT (Clontech) contains the glucocorticoid inducible MMTV promoter while RSVCAT (from S. Conrad, Michigan State University) contains the Rous Sarcoma Virus promoter. With the exception of MamNeoCAT, these stably transfected cell lines were prepared by co-transfection with SV2Neo and colonies were selected and maintained in 0.4 mg/ml G418 (Geneticin, Life Technologies) until confluent. After initiating differentiation, cells were maintained in the absence of G418. Cells were treated with fatty acids at concentrations indicated in figures and always at a 5:1 ratio with fatty acid-free bovine serum albumin. After treatments, cells were assayed for CAT activity and protein content as previously described (13). CAT units: ^{14}C-acetylated chloramphenicol CPM/100 μg protein per hour.

RNA analysis

Total RNA was isolated from cells using RNA STAT-60 (Tel-Test B, Friendswood, TX). RNA (20 μg/lane) was electrophoretically separated in 1% agarose–formaldehyde gels (14). Northern blots were prepared and probed with radiolabeled cDNA (14) for S14 (S14ExoPEII6), FAS (Fas-1; from H.S. Sul, University of California, Berkeley) and β-actin (L. Kedes, Stanford, Palo Alto, CA).

Triglyceride and fatty acid analysis

Total triglycerides were analyzed using Sigma Triglyceride Assay Kit according to the manufacturer's protocol. Fatty acid analysis of adipocyte fatty acids involved conversion of fatty acids to methyl esters of total cell lipids by direct transesterification using boron trichloride/methanol (14% w/v, Sigma). The composition of the fatty acid methyl ester was determined by a capillary gas–liquid chromatography using a Hewlett-Packard 5890 gas chromatograph fitted with a 50 m × 0.025 mm (id) CP-Sil 88 capillary column (Chrompack, Middleburry, The Netherlands) and a flame ionization detector. A temperature gradient program from 150°C to 250°C at 1°C/min was used. Injection port and detector temperatures were 240°C.

The fatty acid methyl esters were identified by comparing their retention times versus those of authentic standards.

RESULTS

PUFA suppress S14 and FAS gene expression in L1 adipocytes

Treatment of primary hepatocytes with 20:4, n–6 or 20:5, n–3 leads to a suppression of $mRNA_{S14}$ and $mRNA_{FAS}$ with an $ED_{50} < 100$ μm (13). To determine whether PUFA inhibited the expression of these mRNAs in cultured 3T3-L1 adipocytes, cells were treated with vehicle, 18:1, n–9; 20:4, n–6; or 20:5, n–3 for 48 h (**Fig. 1**). 18:1, n–9 had no significant effect on mRNAs encoding S14, FAS or β-actin. In contrast, both 20:4, n–6 and 20:5, n–3 suppressed $mRNA_{S14}$ by 85 and 70%, respectively. $mRNA_{FAS}$ was suppressed by 70% after 20:4 treatment and ~40% after 20:5 treatment. $mRNA_{Actin}$ was unaffected by these treatments. These results indicated that the previously reported effects of dietary PUFA on adipocyte FAS and S14 gene expression may be due to direct effects of PUFA on fat cells. This effect is fat type specific and cannot be attributed to a generalized fatty acid effect as 18:1 did not affect any mRNA examined. Moreover, the lack of a PUFA effect on $mRNA_{Actin}$ suggested that the inhibition of S14 and FAS was gene specific.

PUFA regulates S14 at the transcriptional level

In an effort to establish the mechanism for control, we took advantage of L1 cells containing a stably transfected S14CAT fusion gene. This S14CAT fusion gene contains the *cis*-regulatory elements required for the adipocyte-specific glucocorticoid-mediated activation of transcription of this gene. Accordingly, basal and DEX-mediated induction of CAT activity was examined in preadipocytes and adipocytes receiving vehicle, 18:1, 20:4, and 20:5 (**Fig. 2**, **A–C**).

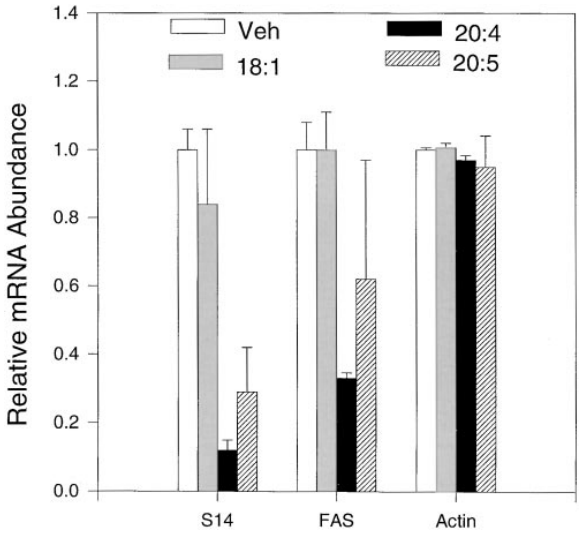

Fig. 1. Effect of fatty acids on S14 FAS and β-actin mRNAs in 3T3-L1 adipocytes. Adipocytes were treated with dexamethasone (DEX, 1 μm) for 48 h to induce S14. During this induction period, cells were also treated with 250 μm 18:1, n–9 (shaded bar), 20:4, n–6 (black bar), or 20:5, n–3 (cross-hatched bar). After treatment, cells were harvested for RNA extraction and analysis of mRNA levels by Northern analysis. The results are normalized for the vehicle-treated group. They represent the mean of duplicate samples and the error bars represent the range. These results are representative of 3 independent studies yielding similar results.

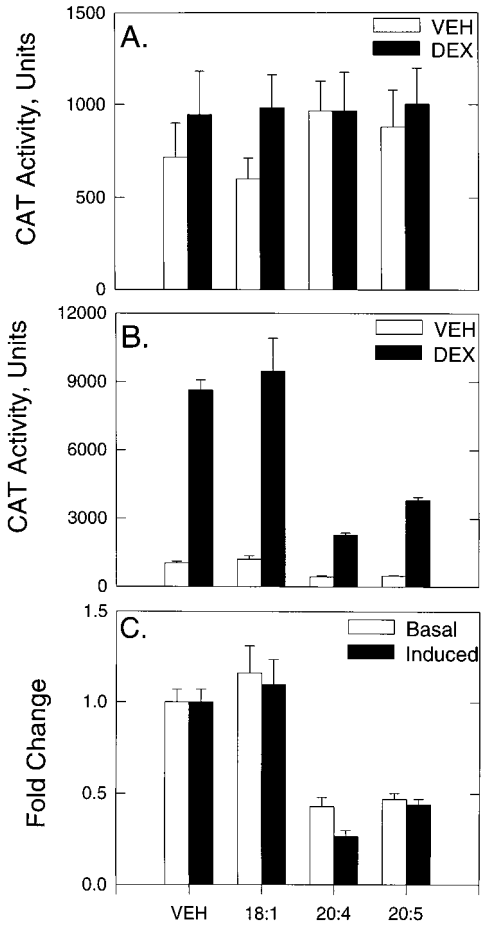

Fig. 2. PUFA regulation of S14CAT expression in L1 preadipocytes and adipocytes. 3T3-L1 preadipocytes (A) and adipocytes (B and C) were prepared as described in Materials and Methods. Cells received either vehicle or DEX for 48 h. During this time, cells were also treated with 250 μm 18:1, 20:4, and 20:5. Cells were harvested for CAT and protein analyses. Results are expressed as CAT activity units. The results are the mean ± SD of 3 samples/group. The results are representative of 2 independent studies yielding similar results. Panel C is redrawn from panel B to illustrate the Basal level of CAT activity in cells receiving no DEX and the Induced level of CAT activity in cells receiving DEX for 48 h.

In preadipocytes, CAT activity is expressed at low levels and is not induced by DEX or affected by any fatty acid treatment (Fig. 2A). In contrast, DEX induced S14CAT ~18-fold in adipocytes (Fig. 2B). While 18:1 treatment had no effect, 20:4, n–6 and 20:5, n–3 both inhibited CAT activity by >70%. The effect of the fatty acid treatment on both basal (no DEX treatment) and induced (DEX treatment) was comparable (Fig. 2C) indicating that 20:4, n–6 and 20:5,n–3 acted on the basal expression and not on DEX-mediated transactivation.

To reinforce the notion of specificity of PUFA action and that PUFA did not have generalized effects on glucocorticoid activation of gene transcription, the effect of PUFA on the expression of the glucocorticoid inducible MMTV promoter was examined. DEX induced CAT activity ~8-fold and PUFA did not affect either the basal or induced level of CAT activity (not shown). Similar results were obtained in cells containing stably integrated TKCAT208 (not shown). This plasmid contains the S14 glucocorticoid response region fused upstream from the thymidine kinase basal promoter (TKCAT208, Methods and Materials). These results argue against the S14 glucocorticoid response region (between −1.4 and −1.6 kb) as the *cis*-regulatory target for PUFA-mediated suppression of S14 gene expression in adipocytes. Based on our understanding of the functional elements controlling S14 gene expression in liver and preadipocytes (13, 30), these findings implicate the S14 proximal promoter (i.e., −290 to −1 bp) as a likely target for PUFA action. However, additional studies will be required to localize the *cis*-regulatory target for PUFA action.

Dose response analysis

In primary hepatocytes, both 20:4, n–6 and 20:5, n–3 are equipotent inhibitors of S14CAT activity (13). To determine whether adipocytes respond to PUFA like hepatocytes, L1 adipocytes containing the stably integrated S14CAT gene were treated with fatty acids ranging from 50 to 1000 μm (**Fig. 3**). While treatment of cells with 18:1, n–9 up to 500 μm had no significant effect on CAT activity; 1 mm 18:1, n–9 reduced CAT activity by ~60%. Both 20:4, n–6 and 20:5, n–3 inhibited S14CAT activity. However, ED_{50} for 20:4, n–6 was 6-fold lower than 20:5, n–3. In contrast to liver (13), 20:4, n–6 was a more potent inhibitor of S14 gene expression than 20:5, n–3 in adipocytes.

Effect of PUFA on adipocyte differentiation

Saturated and polyunsaturated fatty acids have been reported to stimulate adipocyte differentiation in OB1771

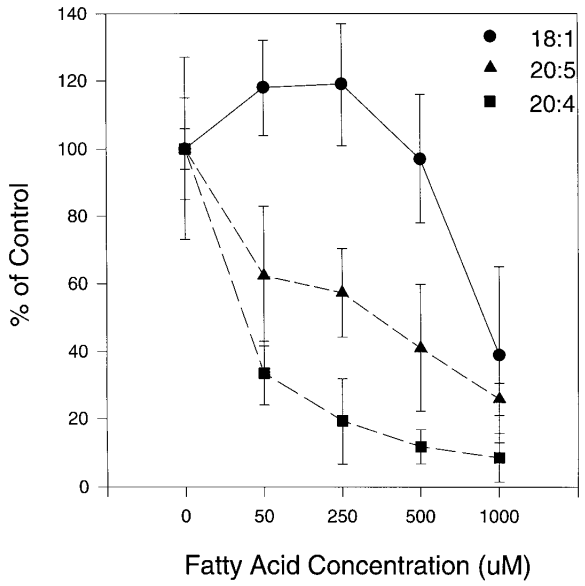

Fig. 3. Dose response of PUFA on S14CAT expression in adipocytes. L1 Adipocytes containing stably integrated S14CAT were treated with DEX for 48 h and during that time received varying concentrations of fatty acids (from 50 to 1000 μm) in serum-free medium. After the 48-h treatment, cells were harvested for CAT and protein assays. The results are represented as mean ± SD of 3 samples and are representative of 2 independent studies.

cells (18, 19, 28). To determine whether PUFA affected adipocyte differentiation, specific fatty acids were added to the medium after removal of the differentiation medium and maintained in the medium for 10 days at 50 μm. Continuous treatment of cells with 50 μm fatty acid had no obvious effect on differentiation as assessed by triglyceride accumulation (**Table 1**) or oil red staining (not shown). Fatty acid analysis of cells treated with the specific fatty acids showed an expected accumulation of the specific fatty acid in the triglyceride/phospholipid pool (Table 1). For example, 20% of the fatty acids within the phospholipid/triglyceride pool was 20:4, n–6 in cells receiving continuous 20:4, n–6 treatment. In contrast, cells treated with either no fat or 18:1, n–9 showed less than 3% 20:4, n–6.

TABLE 1. Fatty acid composition in adipocytes treated with different fatty acids

	Treatment						
	FCS	Alb	18:1	18:2	18:3	20:4	20:5
	mg/plate						
Triglyceride content		34 ± 5	37 ± 1	33 ± 1	40 ± 4	35 ± 8	39 ± 7
Fatty acid profile	*percent of total fatty acid*						
16:0	59.8	55.3	46.7	44.4	46.3	45.3	46.2
18:0	10.4	9.7	8.6	8.8	8.3	10.5	8.0
18:1, n–9	20.9	24.5	32.9	19.1	17.9	18.2	19.7
18:2, n–6	1.9	2.0	2.1	16.0	4.1	1.8	1.8
18:3, n–6	0.7	1.0	0.5	0.9	11.0	0.6	0.4
18:3, n–3	0.4	0.8	1.5	1.6	4.0	0.4	0.5
20:4, n–6	3.3	3.5	3.0	3.8	4.2	20.0	2.7
20:5, n–3	0.7	1.1	2.9	3.4	2.8	1.2	19.0
22:6, n–3	1.8	2.2	1.7	2.0	1.4	2.0	1.6

Triglycerides and fatty acid analysis was described in Material and Methods. Fatty acid results are shown as a percentage of total fatty acids. FCS: fetal calf serum, fetal calf serum was used at 10% in the medium; Alb: albumin.

To assess the effect of these treatments on S14CAT expression at the end of the 10-day treatment, cells were treated with vehicle or DEX for 48 h (**Fig. 4**). While 18:1 had no effect on DEX-induced S14CAT expression, 18:2, n–6, 18:3, n–6, and 20:5, n–3 suppressed CAT activity by ~30%. In contrast, cells treated with 20:4, n–6 showed a >90% suppression of both basal and DEX-induced S14CAT activity. A corresponding decline in $mRNA_{S14}$ and $mRNA_{FAS}$ was also found after this treatment (not shown). The decline in FAS and S14 gene expression would suggest that de novo lipogenesis was inhibited in these cells. However, the triglyceride levels and the level of 16:0 (Table 1) were unaffected by these treatments. Presumably, these fatty acids accumulated from the fetal calf serum supplement in the culture medium. Thus, in the presence of a continuous supply of exogenous PUFA, an inhibition of de novo lipogenesis may have little impact on triglyceride accumulation in adipocytes.

Arachidonic acid inhibits S14CAT expression through a prostanoid pathway

Our goal has been to determine whether the mechanism of PUFA regulation of adipocyte gene expression was similar to that found in liver. The dose response studies already indicate that, in contrast to liver, 20:4, n–6 is a 6-fold more potent inhibitor of lipogenic gene expression than 20:5, n–3. When compared to 20:4, n–6, 20:5, n–3 is a poor substrate for the synthesis of prostaglandins by cyclooxygenase 1 and 2 (31). Thus, the differential potency

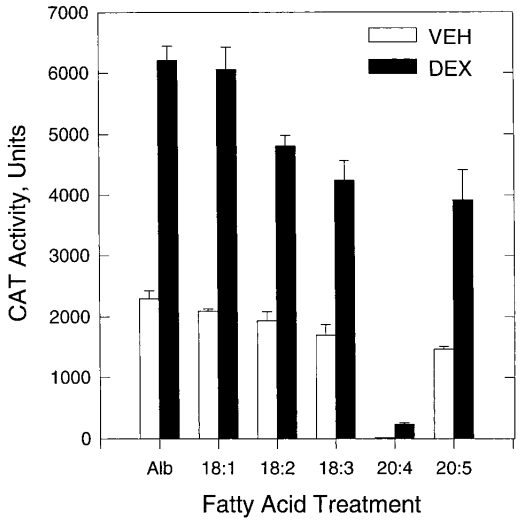

Fig. 4. Effect of long term fatty acid treatment on S14CAT expression. L1 adipocytes containing the S14CAT fusion gene were treated with various fatty acids at 50 μm for 10 days. Treatment started immediately after initiating differentiation and continued for 10 days, a time when >80% of the cells were adipocytes as judged by the accumulation of triglycerides. During the last 48 h of treatment, cells received DEX to induce S14CAT activity. Results are represented as mean ± SD with an n = 3. This study is representative of 3 separate studies.

of 20:4, n–6 and 20:5, n–3 suggests that 20:4, n–6 may be converted to prostanoids which, in turn, induce changes in adipocyte lipogenic gene expression. This is in keeping with others who have established that adipocytes convert 20:4, n–6 to the prostaglandins, PGE_2, $PGF_{2\alpha}$, and PGI (32–35).

To determine whether 20:4, n–6 requires metabolism to prostanoids, we used the cyclooxygenase inhibitor flurbiprofen. Cells were also treated with the nordihydroguaiaretic acid (NDGA) and clotrimazole, inhibitors of lipoxygenase and monooxygenase activity, respectively. While flurbiprofen blocked the 20:4, n–6 inhibitory effect on CAT activity (**Fig. 5A**) and $mRNA_{S14}$ (not shown), NDGA and clotrimazole (not shown) had no effect. Thus, 20:4, n–6-mediated inhibition of S14 gene expression requires cyclooxygenase and implicates a role for prostanoids in regulating adipocyte lipogenic gene expression. To further examine this possibility, the effect of specific prostanoids on adipocyte lipogenic gene expression was evaluated by treating L1 adipocytes with PGE_2 or $PGF_{2\alpha}$ at 10 μm (Fig. 5B). Both PGE_2 and $PGF_{2\alpha}$ inhibited CAT activity. Dose response analyses show that PGE_2 inhibits S14CAT expression with an ED_{50} of ~5 μm, a concentration well below the ED_{50} of ~50 μm for 20:4, n–6. Analysis of $mRNA_{S14}$ and $mRNA_{FAS}$ after PGE_2 treatment showed a similar decline (not shown). Based on these results, we conclude that 20:4, n–6 is converted to prostaglandin in adipocytes and that PGE_2 and $PGF_{2\alpha}$ inhibit adipocyte lipogenic gene expression.

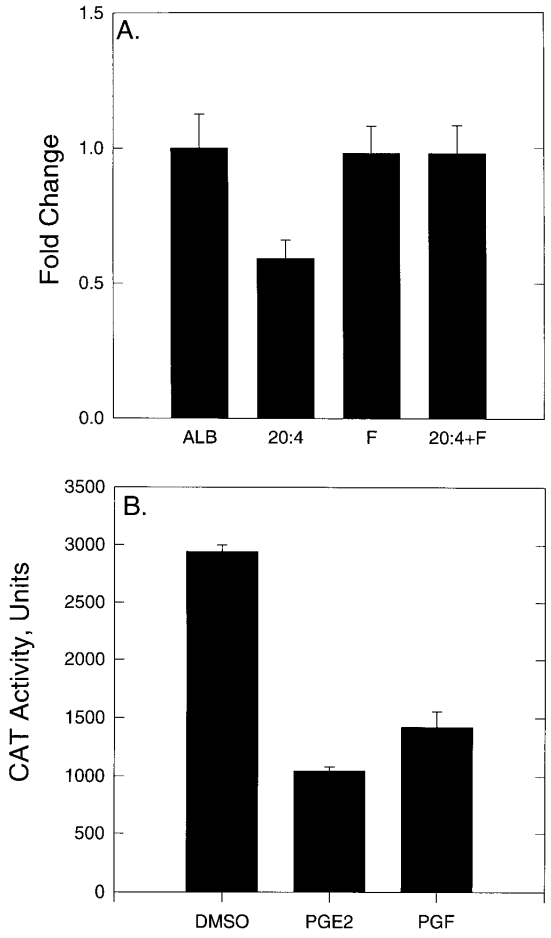

Fig. 5. Arachidonic acid inhibition of S14CAT expression is mediated through a prostanoid pathway. A: L1 adipocytes expressing S14CAT were treated with DEX as described above. Cells were also treated with 20:4 (250 μm), flurbiprofen (100 μm), or a combination of both 20:4 and flurbiprofen. After a 48-h treatment period, cells were harvested for CAT and protein assays. Results are the mean and standard deviation of triplicate samples and are representative of 2 separate studies. B: L1 adipocytes expressing S14CAT were treated as described above with PGE_2 or $PGF_{2\alpha}$ at 10 μm. After a 48-h treatment period, cells were harvested for CAT and protein assays. Results are the mean and standard deviation of triplicate samples and are representative of 3 separate studies.

Signal transduction pathway for PGE_2 control of S14 gene transcription

PGE_2 and $PGF_{2\alpha}$ regulate cell function through G-protein-linked plasma membrane receptors (36–41). Depending on the G-protein linkage, PGE_2 can increase or decrease cellular cAMP levels or elevate IP_3 and Ca^{+2} levels. In addition, some studies suggest that the peroxisome proliferator activated receptor γ2, an adipocyte-specific nuclear receptor, might be activated by prostaglandins (25, 26).

To determine whether changes in intracellular Ca^{+2}, cAMP or activation of PPARγ2 is involved in prostaglandin-mediated suppression of S14 gene expression, L1 adipocytes containing the stably integrated S14CAT fusion gene were treated with A23187 (a calcium ionophore), 8-CTP-cAMP, and isobutylmethyl xanthine (IBMX) or pioglitazone to elevate intracellular calcium or cAMP or to activate PPARγ2, respectively (**Fig. 6**). Treatment of cells with 8-CTP-cAMP plus IBMX or the A23187 inhibited CAT activity by ≥50% or $mRNA_{S14}$ (not shown). Treatment of cells with pioglitazone had no consistent effect on CAT activity. These studies demonstrate that alterations in intracellular cAMP or Ca^{+2} markedly suppress S14 gene transcription in fully differentiated adipocytes. In contrast, activation of PPARγ2 by pioglitazone had no effect on S14 gene expression.

Depending on the G-protein linkage, PGE_2 can activate protein kinase A, protein kinase C, or calcium-regulated mechanisms. To determine which pathway affects S14 gene expression, PGE_2-treated adipocytes were treated with H7 and staurosporin, inhibitors of A and C-kinases, or pertussis toxin as inhibitor of G_i/G_o-linked processes. G_i/G_o-linked processes promote a decrease in intracellular cAMP or an activation of phospholipase C_β and elevation in intracellular Ca^{+2} through release of inositol 1,4,5-phosphate [IP_3] (40, 41). A rise in cAMP is associated with inhibition of S14CAT expression, therefore, pertussis toxin will provide a means to evaluate Ca^{+2}-regulated processes. While PGE_2 treatment of cells inhibits CAT by ~60%, co-treatment with H7 or staurosporin at doses sufficient to inhibit both A and C-kinases did not block the PGE_2 effect (**Fig. 7**). Treatment of cells with the calcium channel blocker, verapamil, also failed to block the PGE_2 effect (not shown). Only pertussis toxin blocked the PGE_2 effect (Fig. 7). Treatment of cells with pertussis

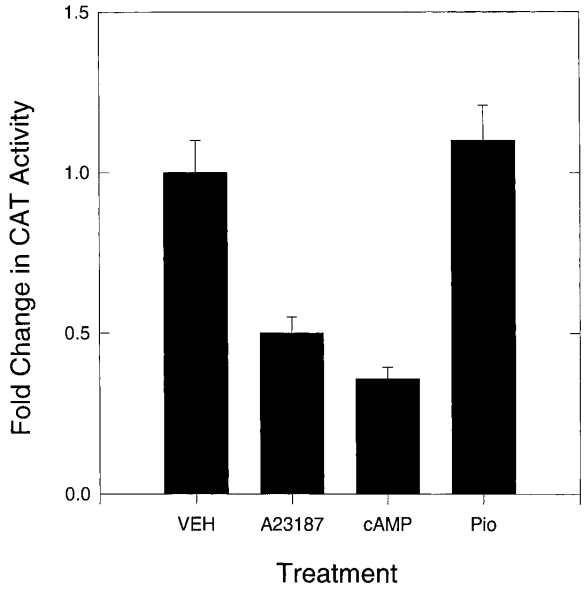

Fig. 6. Regulation of S14CAT expression by cAMP, calcium, and pioglitazone. L1 adipocytes expressing S14CAT were treated with DEX for 48 h as described above. At the initiation of DEX treatment, cells were also treated with either 50 μm 8-CTP-cAMP + 100 μm IBMX, 1 μm A23187, or 5 μm pioglitazone for 48 h. After treatment, cells were harvested for CAT and protein analyses. Results are the mean and standard deviation of triplicate samples and are representative of 2 separate studies.

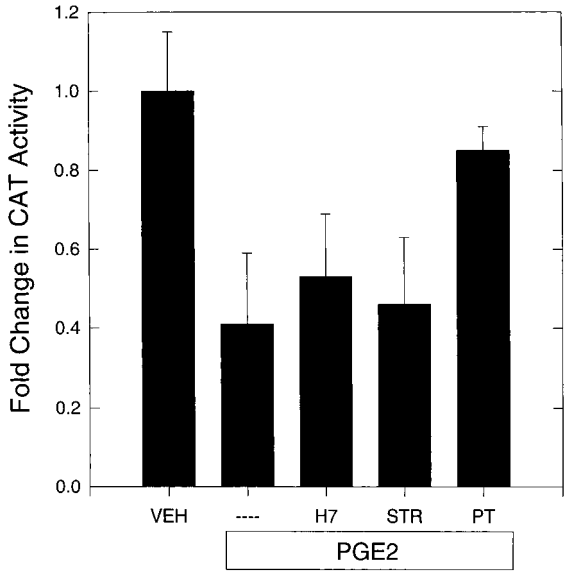

Fig. 7. Reversal of PGE_2 suppression of S14CAT expression by H7, staurosporin, and pertussis toxin. L1 cells were treated with VEH and PGE_2 for 48 h as described above. Where indicated in the figure, cells also received H7 (10 mm), staurosporin (5 nm, STR) or pertussis toxin (25 ng/ml, PT). After treatment, cells were harvested for CAT and protein assays as described. Results are the mean and standard deviation of triplicate samples and are representative of 3 separate studies.

toxin in the absence of PGE_2 had no effect on S14CAT expression (not shown). Taken together, these studies suggest that PGE_2 operates through a G_i/G_o-linked signaling system which may involve changes in intracellular calcium.

DISCUSSION

Treatment of adipocytes with fatty acids have profound effects on differentiation and gene expression (18, 19, 22, 27, 28, 43, 44). The principal mechanisms for the effects of fatty acids on adipocyte gene expression involve either prostanoid or PPAR-mediated pathways. Our studies show that the dominant negative suppression of lipogenic gene expression by PUFA in the 3T3-L1 cell line is mediated by a prostanoid pathway and not through a PPAR-mediated pathway. PUFA suppress $mRNA_{S14}$ and $mRNA_{FAS}$ levels and S14CAT expression in cultured 3T3-L1 adipocytes and this effect occurs only in fully differentiated adipocytes. The suppression of S14 gene transcription is 6-fold more sensitive to 20:4, n–6 than 20:5, n–3. 20:5, n–3 is a poor substrate for both cyclooxygenase 1 and 2 (36, 42). This observation, coupled with the finding that flurbiprofen, a cyclooxygenase inhibitor, blocks the 20:4, n–6 effect on S14 gene expression (Fig. 5) suggests that 20:4, n–6 effects on 3T3-L1 lipogenic gene expression may be mediated through prostanoids. Both PGE_2 and $PGF_{2\alpha}$ inhibit lipogenic gene expression and this effect is blocked with pertussis toxin implicating an G_i/G_o-coupled signaling pathway.

Cyclooxygenases convert 20:4, n–6 to prostanoids and prostanoids elicit a wide range of biological effects through plasma membrane receptors coupled to G-proteins. Prostaglandins are produced locally and act in a paracrine fashion (36). PGE_2, PGI_2, and PGF_2 are the most predominant prostaglandins produced in 3T3-L1 cells, although more prostaglandins are produced in preadipocytes than adipocytes (32, 43–46). Long and Pekala (43) showed that GLUT4 expression was depressed by 20:4, n–6 and PGE_2 and further showed that cAMP was produced in adipocytes upon treatment with PGE_2. While activation of protein kinase A by cAMP had a strong suppressive effect on S14CAT expression in L1 adipocytes (Fig. 6), treatment of adipocytes with the phorbol ester, PMA, an activator of PKC (not shown), had no effect on CAT activity. Treatment of cells with inhibitors of both protein kinase A and C, i.e., H7 and staurosporin, failed to block the PGE_2-mediated inhibition of CAT activity. Therefore, it is unlikely that the PGE_2-mediated inhibition of CAT activity is due to an increase in cAMP and subsequent activation of protein kinase A or C. In contrast, treatment of cells with pertussis toxin, which blocks G_i/G_o-linked pathways, was the only agent found to block the PGE_2 inhibition on S14CAT activity (Fig. 7). G_i/G_o-linked signaling pathways inhibit G_s-linked activation of adenylate cyclase and promote an elevation in inositol 1,4,5-phosphate (IP_3) and intracellular Ca^{+2} (45). As a decrease in cAMP would be expected to induce S14CAT expression, the involvement of the G_i/G_o-linked pathway implicates activation of phospholipase C_β, release of IP_3, and

elevation of intracellular free Ca^{+2}. Ca^{+2}-activated mechanisms suppress S14CAT expression (Fig. 6). The Ca^{+2}-mediated control of S14 gene transcription will be defined in future studies.

Some studies suggest that PGE_2 action on adipocytes is anti-lipolytic and acts via blocking an increase in cAMP (44, 46, 47). Others suggest that PGE_2 acts through protein kinase C via a receptor coupled to phospholipase C in Swiss 3T3 fibroblasts (41). $PGF_{2\alpha}$ treatment of 3T3-L1 preadipocytes leads to an increase in intracellular calcium, activation of calcium/calmodulin dependent protein kinase (CaM kinase) and inhibition of differentiation (48). Ntambi and Takova (49) reported that A23187 inhibited differentiation of 3T3-L1 cells but had no effect when used 2 days after differentiation. These confusing reports make it difficult to determine how prostaglandins act on only differentiated adipocytes. In contrast to the Long and Pekala (43) studies, our results suggest that the inhibition of S14CAT expression is not due to an increase in cAMP but through a separate IP_3/Ca^{+2} pathway.

Another possibility is that prostaglandins work through a nuclear receptor pathway. Prostaglandins are reported to bind PPARγ2, a nuclear receptor (23–26). While PPARγ2 plays an important role in adipogenesis, which includes the induction of lipogenic enzymes, our results show that pioglitazone, a PPARγ2 activator, does not affect S14CAT activity in L1 adipocytes (Fig. 6). This is interesting because the S14 promoter contains a binding site for PPARγ2 (not shown).

Finally, our results would appear to conflict with reports by others documenting that rats fed vegetable oils do not show significant changes in adipose tissue lipogenesis (50, 51). One explanation is that the dose of 20:4, n–6 used in culture is higher than in vivo levels of 20:4, n–6. Our results do agree with these in vivo studies by showing that linoleic (18:2, n–6), α linolenic (18:3, n–3) and γ linolenic (18:3, n–6) acids, fatty acids found in vegetable oils, were ineffective suppressers of S14CAT activity (Fig. 4). Interestingly, epididymal fat mRNAs encoding S14 and FAS were suppressed 50% after 5 days on a diet containing 10% fish oil (13). 20:5, n–3, a component of fish oil, effectively suppressed S14 and FAS mRNAs (Fig. 1) and S14CAT activity (Fig. 2) in cultured adipocytes. When compared to 18-carbon PUFA, the highly unsaturated fatty acids found in fish oil were more potent suppressers of lipogenic gene expression (1, 2, 14). Thus, any conflict with earlier reports can be explained by the use of fish oil vs vegetable oil for in vivo studies and the dose of 20:4, n–6 or 20:5, n–3 used in the culture studies.

In summary, our results show that lipogenic gene expression is suppressed by PUFA in cultured 3T3-L1 adipocytes. The results presented here are consistent with a requirement for 20:4, n–6 conversion to prostanoids. The prostanoids may activate a G_i/G_o-linked signaling cascade that leads to the inhibition of S14 gene transcription. This mechanism for control is different from the mechanism for PUFA-mediated suppression of lipogenic gene expression in liver.

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