Role of cyclooxygenases COX-1 and COX-2 in modulating adipogenesis in 3T3-L1 cells

Hongyun Yan, Abdenaim Kermouni, Mohammed Abdel-Hafez, and David C. W. Lau[1]

Julia McFarlane Diabetes Research Centre, Departments of Medicine, Biochemistry and Molecular Biology, The University of Calgary, Calgary, AB, T2N 4N1, Canada

Abstract **Cyclooxygenase (COX) catalyses the rate-limiting step of prostanoid biosynthesis. Two COX isoforms have been identified, COX-1, the constitutive form, and COX-2, the inducible form. While COX-2 has been implicated in body fat regulation, the underlying cellular mechanism remains to be elucidated. The present study was undertaken to examine the potential role of COX in modulating adipogenesis and to dissect the relative contribution of the two isoenzymes in this process. COX-2 was found to be expressed in undifferentiated 3T3-L1 cells and down-regulated during differentiation, whereas the cellular level of COX-1 remained relatively constant. Abrogating the activity of either of these two isoenzymes by selective COX inhibitors accelerated cellular differentiation, suggesting that both COX isoenzymes negatively influenced differentiation. Tumor necrosis factor-α (TNFα) significantly up-regulated COX-2 expression (~2-fold) in differentiating 3T3-L1 cells, whereas similar effect was not observed with COX-1 expression. Abrogating the induced COX-2 activity reversed the TNFα-induced inhibition of differentiation by ~70%, implying a role for COX-2 in mediating TNFα signaling. Hence, both COX isoforms were involved in the negative modulation of adipocyte differentiation. COX-2 appeared to be the main isoform mediating at least part of the negative effects of TNFα.**—Yan, H., A. Kermouni, M. Abdel-Hafez, and D. C. W. Lau. **Role of cyclooxygenases COX-1 and COX-2 in modulating adipogenesis in 3T3-L1 cells.** ***J. Lipid Res.*** **2003.** 44: **424–429.**

Supplementary key words tumor necrosis factor-α • prostaglandin • prostanoid biosynthesis

Cyclooxygenase (COX) catalyzes the rate-limiting step of prostanoid biosynthesis and controls the first committed step of prostanoid formation. The availability of this enzyme directly relates to the abundance of prostaglandin (PG) production. Two COX isoforms have been identified, COX-1, the constitutive form, and COX-2, the inducible form. While the former is believed to be responsible for homeostasis, the latter is thought to exert actions mainly in pathological states (1, 2). The divergent function of these two COX isoforms may be attributable to the availability of the enzyme under particular settings or the functional coupling with different downstream terminal PG synthases (3, 4). A recent study suggested that COX-2 might be involved in body fat regulation (5). Mice heterozygous for the COX-2 gene showed increased body weight by about 30%, with fat pads enlarged 2–3-fold when compared with those from the wild-type animals. In comparison, mice lacking COX-1 gene appeared normal in phenotype and body fat content. These findings suggested that COX-2 might be involved in body fat regulation in vivo, whereas the role of COX-1 remains obscure. The underlying cellular mechanism for the COX-2 effect remains to be elucidated.

Adipogenesis is a crucial aspect in controlling body fat mass. The acquisition of the mature adipocyte phenotype is a highly regulated process in which preadipocytes undergo differentiation resulting in both increased size and number of mature adipocytes in the adipose tissue. Our previous study showed that COX pathway might be involved in regulating this process (6). The present study was undertaken to further elucidate the underlying cellular mechanisms. 3T3-L1 cells were used as the model system and a pharmacological inhibition approach using highly selective COX inhibitors was employed to dissect the relative contributions of the two COX isoenzymes. The role of COXs under stimulation of tumor necrosis factor-α (TNFα), an adipokine abundantly produced by adipocytes and a potent negative regulator of adipogenesis, was also explored.

Manuscript received 6 September 2002 and in revised form 28 October 2002.

Published, JLR Papers in Press, November 4, 2002.
DOI 10.1194/jlr.M200357-JLR200

Abbreviations: c/EBPα, aP2, adipocyte fatty acid-binding protein; CCAAT/enhancer-binding protein α; COX, cyclooxygenase; DD, day of differentiation; Dex, dexamethasone; DMEM, Dulbecco's modified Eagle's medium; GLUT4, glucose transporter-4; GPDH, glycerol-3-phosphate dehydrogenase; HRP, horseradish peroxidase; MIX, 1-methyl-3-isobutylxanthine; PG, prostaglandin; PPARγ2, peroxisome-proliferator-activated receptor γ 2; TNFα, tumor necrosis factor-α.

[1] To whom correspondence should be addressed.
e-mail: dcwlau@ucalgary.ca

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle's medium (DMEM), calf serum, and fetal bovine serum were purchased from Gibco BRL (Burlington, ON). Insulin, 1-methyl-3-isobutylxanthine (MIX), and dexamethasone (Dex) were obtained from Sigma Chemical Co. (St. Louis, MO). Rosiglitazone was purchased from SmithKline Beecham Pharma (Oakville, Canada). The selective COX-2 inhibitor celecoxib was a kind gift from Dr. Ching-Shih Chen (University of Kentucky). The other specific COX-2 inhibitor, NS-398, and COX-1 inhibitor, SC-560, were purchased from Cayman Chemicals (Ann Arbor, MI). Rabbit adipocyte fatty acid-binding protein (aP2) antiserum was obtained from Alpha Diagnostic International, Inc. (San Antonio, TX). Goat polyclonal antibodies against COX-1, COX-2, glucose transporter-4 (GLUT4), rabbit polyclonal antibodies against peroxisome-proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (c/EPBα), the blocking peptide for COX-1, and the horseradish peroxidase (HRP) conjugated secondary antibodies (anti-goat IgG and anti-rabbit IgG) were products of Santa Cruz Biotechnology (Santa Cruz, CA).

Cell culture and differentiation

3T3-L1 cells were obtained from American Type Culture Collection (Rockville, MD). Cells were maintained in DMEM containing 10% calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Differentiation induction was performed at 2 days post-confluence [day 0 of differentiation (DD0)] in DMEM supplemented with 10% fetal bovine serum. For standard induction of differentiation, cells were exposed to 1.7 μM insulin, 0.5 mM MIX, and 1 μM dexamethasone for 2 days, followed by 0.4 μM insulin and 1 μM dexamethasone for another 2 days. To avoid the potential interference of dexamethasone (Dex) on COX expression, a modified differentiation cocktail was used in most experiments, in which Dex was replaced by 2 μM of rosiglitazone in the hormonal cocktail described earlier. By using this protocol, about 60–70% of cells underwent differentiation by day 4 of differentiation. In order to accentuate the COX effect on differentiation, cells were differentiated in one set of experiments under conditions that would induce partial maturation (40% vs. ~95%): 0.17 μM insulin, 0.5 mM MIX, and 0.1 μM Dex for 2 days, followed by 0.04 μM insulin and 0.1 μM Dex for another 2 days.

Glycerol-3 phosphate dehydrogenase activity

Following different treatments as indicated, cells were washed thrice with phosphate-buffered saline (PBS), harvested in 10 mM Tris-EDTA buffer (pH 7.4), and sonicated. Following centrifugation at 100,000 *g* for 10 min at 4°C, the supernatant was collected. Protein content was determined by the Bradford method (Bio-Rad Laboratories, Inc., Mississauga, ON), and glycerol-3-phosphate dehydrogenase (GPDH) activity was quantified according to the method of Kozak and Jensen (7). One unit of specific enzyme activity corresponded to the oxidation of 1 nmol of NADH/min/mg protein.

Western blot analysis

Cells were rinsed thrice with PBS, and scraped into lysis buffer [125 mM NaCl, 2 mM EDTA, 50 mM HEPES (pH 7.4), 1% Triton X-100, 1 mM DTT] supplemented with pepstatin (5 μg/ml), leupeptin (5 μg/ml), and phenylmethylsulfonyl fluoride (1 mM). After centrifugation at 12,000 *g* for 15 min at 4°C, the soluble fraction was collected, and protein content was determined. Twenty micrograms of total protein was separated by SDS-PAGE (10–12% gel) and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad Laboratories, Inc.) Pausau S staining was performed after transfer to confirm sample loading and transfer efficiency. After blocking with 5% skimmed milk [in TBS containing 0.05% Tween 20 (TBST)] for 30–60 min at room temperature (RT), the membrane was incubated with the primary antibody at the appropriate dilution (goat anti-COX-2, 1:2,000; goat anti-COX-1 and anti-GLUT4, 1:1,000; rabbit anti-PPARγ and anti-c/EBPα, 1:1,000; rabbit anti-

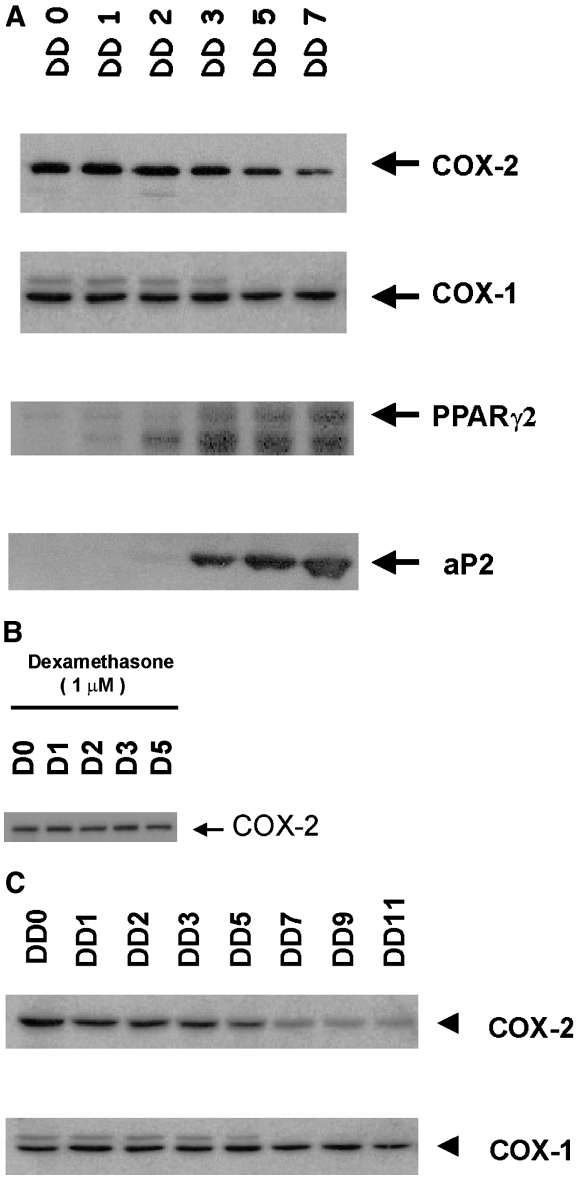

Fig. 1. Cyclooxygenase (COX)-2 and COX-1 expression in 3T3-L1 cells. 3T3-L1 cells at 2 days post confluence [day 0 of differentiation (DD0)] were induced to differentiate by a standard hormonal cocktail consisting of 1.7 μM insulin, 0.5 mM 1-methyl-3-isobutylxanthine (MIX), and 1 μM dexamethasone (Dex) for the initial 2 days, followed by 0.4 μM insulin and 1 μM Dex for another 2 days (A); or by a Dex-free modified hormonal cocktail (Dex-free) in which Dex was replaced by 2 μM rosiglitazone in the above cocktail (C); or the cells were exposed to Dex (1 μM) alone up to 5 days (B). Cells were harvested at the indicated times from day 0 (DD0) to day 7 (DD7) of differentiation in A, and to day 11 (DD11) in C. Twenty micrograms of total protein was separated by SDS-PAGE and immunoblotted with specific antibodies for COX-1, COX-2, and/or aP2, and PPARγ. Two bands were detected in the COX-1 blots; the lower band was confirmed to be the specific band by peptide blocking.

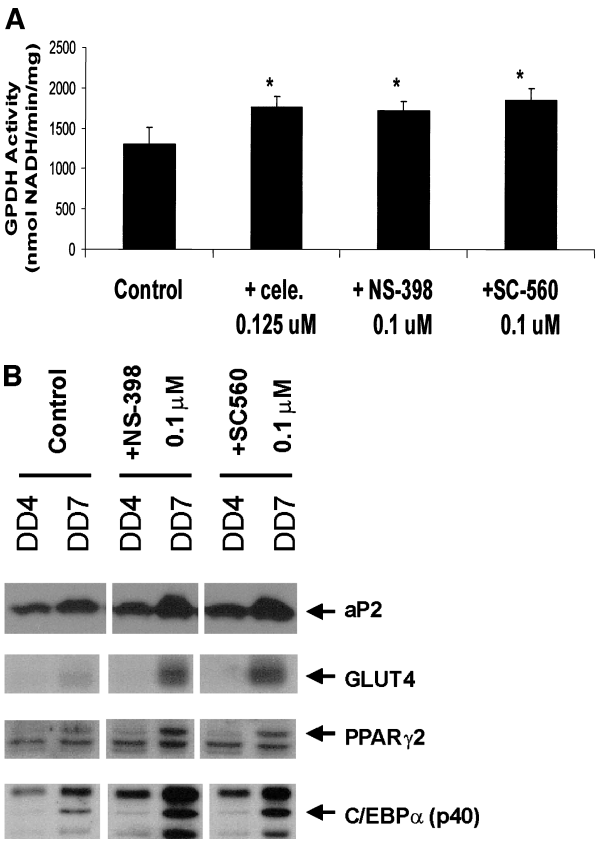

Fig. 2. Abrogating COX-2 and COX-1 activity enhanced differentiation in 3T3-L1 cells. A: 3T3-L1 cells (DD0) were induced to undergo differentiation by the modified hormonal cocktail (Dex free) in the presence or absence of COX-2 inhibitors (celecoxib and NS-398) or COX-1 inhibitor (SC-560) at the indicated concentrations. Cells were harvested on differentiation day 5 and glycerol-3-phosphate dehydrogenase (GPDH) activity was assayed. Results represent the mean ± SD of three experiments. An asterisk denotes $P < 0.05$ versus control [hormonal cocktail (Dex free) + vehicle]. B: 3T3-L1 cells were induced to undergo partial differentiation by using a hormonal cocktail consisting of 0.17 μM insulin, 0.5 mM MIX, and 0.1 μM Dex for the initial 2 days, followed by 0.04 μM insulin and 0.1 μM Dex for another 2 days. COX-2 inhibitor (NS-398) or COX-1 inhibitor (SC-560) was added to the differentiation medium at the indicated concentrations. Cells were harvested on day 4 (DD4) and 7 (DD7) of differentiation. Twenty micrograms of total protein was separated by SDS-PAGE and immunoblotted with specific antibodies for aP2, glucose transporter-4 (GLUT4), PPARγ, and CCAAT/enhancer binding protein α (c/EBPα).

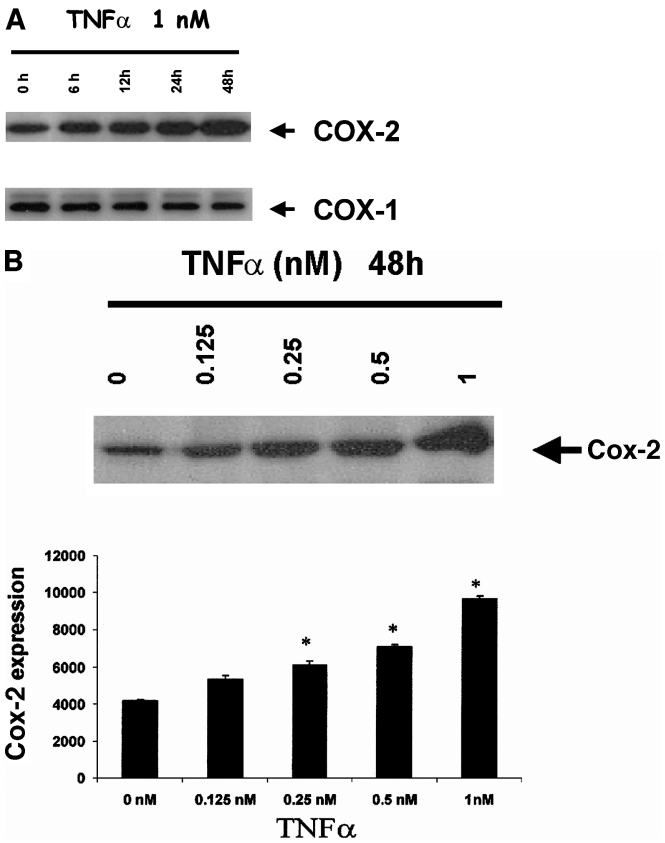

Fig. 3. Tumor necrosis factor-α (TNFα) upregulated COX-2 protein expression in differentiating 3T3-L1 cells. A: Time course: 3T3-L1 cells were exposed to 1 nM TNFα in the presence of the modified hormonal cocktail (Dex free) and cells were collected at the indicated times. B: Dose-response: 3T3-L1 cells were exposed to increasing doses of TNFα in the presence of the modified hormonal cocktail (Dex free), and cells were harvested at 48 h after treatment. COX-2 and COX-1 expression was determined by immunoblotting. Quantitative data represent the mean ± SD of three experiments. Asterisk denotes $P < 0.05$ versus control (Dex free cocktail alone).

serum against aP2, 1:2,000) at 4°C overnight or at RT for 1 h. After washing thrice with TBST, the membrane was probed with HRP conjugated secondary antibody (anti-goat or anti-rabbit IgG at 1:2,000 for COX-2 and aP2, and 1:1,000 for COX-1, PPARγ, c/EBPα, and GLUT4) at RT for 1 h. The membrane was then washed thrice with TBST, and signal was visualized by enhanced chemiluminescence (Amersham, Buckinghamshire). After exposure to Kodak X-OMAT AR film, the immunoblot exposures were scanned, and bands were quantified using NIH Image 1.55.

Statistical analysis

Quantitative data were expressed as means ± SD from at least three independent experiments, and analyzed by the two-tailed Student's *t*-test.

RESULTS

Expression pattern of COX-1 and COX-2 during 3T3-L1 differentiation

The expression of COX-1 and COX-2 during differentiation was determined by immunoblotting. COX-2 was found to be expressed in undifferentiated 3T3-L1 cells, and the expression was down-regulated during differentiation. COX-1 expression, in comparison, remained relatively stable over the 7-day period (**Fig. 1A**). As Dex, a key ingredient in the standard differentiation cocktail, has been shown to down-regulate COX-2 expression by affecting its mRNA stability (8), we examined the direct effect of Dex on COX-2 expression in 3T3-L1 cells. COX-2 protein expression was unaltered up to 5 days following Dex treatment (Fig. 1B). To further ascertain the notion that decreased COX-2 was a differentiation-dependent event, a modified Dex-free hormonal cocktail containing rosiglitazone was used to induce differentiation. Similar expression patterns of COXs were observed under this condition (Fig. 1C).

COX inhibitors enhanced differentiation in 3T3-L1 cells

Both COX-1 and COX-2 inhibitors were found to enhance differentiation, as suggested by the augmented GPDH-specific activity (**Fig. 2A**). A more profound effect was observed when cells were induced to undergo partial differentiation, as indicated by the enhanced expression of PPARγ2, c/EBPα, aP2, and GLUT4 (Fig. 2B), as well as increased GPDH-specific activity (data not shown).

TNFα upregulated COX-2 expression and COX-2 inhibitors partly reversed TNFα-induced inhibition of differentiation

Adipogenesis is subject to both positive and negative regulation in vivo (9–11). While COX-1 and COX-2 both exerted an inhibitory effect on differentiation in the presence of positive adipogenic stimuli, the next question was whether they functioned in concert in response to negative signals. To this end, we examined the role of these two COX isoenzymes in the presence of TNFα, a potent negative regulator of adipogenesis. TNFα was found to up-regulate COX-2 expression in a dose- and time-dependent fashion, with the earliest induction seen at 6 h and a maximum induction of up to 2-fold (**Fig. 3**). No induction was observed with COX-1 expression (Fig. 3A). To further examine whether the induced COX-2 activity mediated the TNFα signaling, specific COX-2 inhibitors were employed. Abrogating the induced COX-2 activity by specific COX-2 inhibitors reversed the TNFα-induced differentiation inhibition by ~70%, as assessed by GPDH-specific activity (**Fig. 4A**) as well as aP2 and GLUT4 expression (Fig. 4B).

DISCUSSION

Prostaglandins are a class of lipid mediators comprised of PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$, and their metabolites. COX catalyzes the first committed step of PG biosynthesis and plays a central role in PG production (12). Two COX isoforms have been identified, COX-1, the constitutive form, and COX-2, the inducible form (1, 2). The two isoforms are encoded by different genes located at different chromosomes and appear to exert different actions in the organism (13). Our earlier work and that of others (6, 14, 15) have demonstrated that the COX pathway is involved in the regulation of adipogenesis. However, the relative contributions of the two COX isoenzymes to the differentiation program as well as the underlying mechanisms remain to be elucidated. In the present study we mapped the COX protein expression profile during differentiation in 3T3-L1 cells. Both COX-1 and COX-2 proteins were detectable in undifferentiated 3T3-L1 cells. COX-1 protein level remained unchanged, whereas COX-2 expression was down-regulated during differentiation. The decrement in COX-2 expression appeared to be a differentiation-dependent event based on two separate observations. First, cells differentiated by different induction cocktails, in the absence or presence of Dex, showed similar expression patterns. Second, Dex did not exert direct influence on COX-2 protein expression in 3T3-L1 cells. Thus, COX-2 appeared to the COX isoform regulated during differentiation. It is known that COX-2 expression is highly regulated at transcription level, mainly via the NF-κB pathway (2). Recent studies showed that the NF-κB pathway is suppressed by PPARγ (16), the key regulator of adipocyte dif-

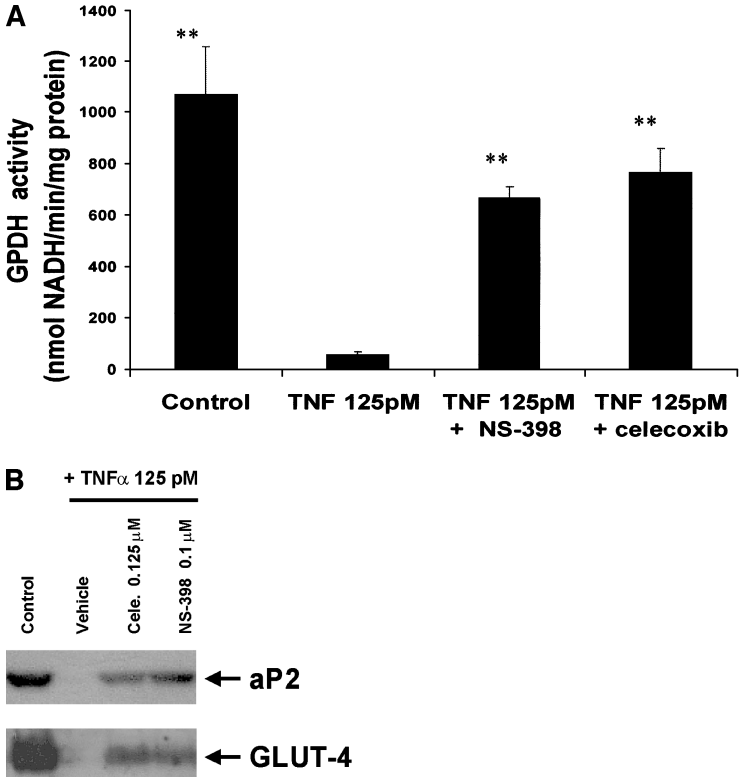

Fig. 4. COX-2 inhibitors partly reversed TNFα-induced differentiation inhibition. 3T3-L1 cells were induced to undergo differentiation by the modified hormonal cocktail (Dex free). TNFα (125 pM) was concomitantly added to the differentiation medium in the presence or absence of the specific COX-2 inhibitors (0.125 μM celecoxib or 0.1 μM NS-398). Cells were harvested at differentiation day 8. GPDH-specific activity was quantified (A) and protein expression of aP2 and GLUT4 was determined by immunoblotting (B). Quantitative data represent the mean ± SD of three experiments. Double asterisks denote $P < 0.01$ versus TNFα treated cells.

ferentiation. It is thus plausible that the constitutive activation of PPARγ during differentiation, possibly via suppression of the NF-κB pathway, down-regulates COX-2 expression. This cellular mechanism may explain how COX-2 expression is down-regulated during differentiation and warrants further investigation.

To our knowledge, this is the first report that has examined COX protein expression, coupled with functional studies, during differentiation. A previous study has reported COX transcription profile in OB177A cells, but functional studies were not carried out (17). The detection of COX-2 in 3T3-L1 cells in the basal state was somewhat unexpected, as COX-2 is generally considered to be the inducible form of COX, present mainly in the stimulated state. However, recent studies suggest that this isoform may indeed be present under unstimulated conditions in such tissues as brain (18), kidney (19), and trachea (20). As COX-1 and COX-2 showed distinct expression patterns during differentiation (Fig. 1A, C), one would expect that they might exert different effects on adipose cell differentiation. However, blocking the activity of either of the two COX isoforms with specific inhibitors revealed similar effects. Differentiation was augmented in both cases, suggesting that both COX-1 and COX-2 were involved in the negative modulation of differentiation. Moreover, the enhancement on differentiation was comparable under both conditions (Fig. 2A, B), suggesting that both COX-1 and COX-2 contributed to a similar extent toward preadipocyte differentiation. As both isoforms negatively influenced the signals for differentiation, it would appear unlikely that any downstream products catalyzed by the COX pathway would exert a major positive effect on adipose cell differentiation. Hence, our findings raised the question of the physiological relevance of PGJ_2, a downstream prostanoid of the COX pathway purported to be an endogenous ligand of PPARγ and a potent inducer of differentiation (21). The fact that no appreciable quantity of PGJ_2 has been detected in vivo lends further support for the potential role of COX-1 and COX-2 as negative modulators of adipose cell differentiation.

The distinctly different expression patterns of COX-1 and COX-2 during differentiation suggested that these isoforms might play different roles in adipose cell biology. While both COX isoenzymes appeared to function similarly in the presence of positive adipogenic stimuli, the possibility existed that they might act differently in response to negative signals. We used TNFα, a negative regulator of adipogenesis, in our next series of experiments to address this question. TNFα is a cytokine produced by adipocytes and is present in abundance in the obese states (22). It is known to inhibit adipocyte conversion (23) and has also been shown to be a potent inducer for COX-2 but not for the constitutive COX-1 (24). It is thus feasible that COX-2, but not COX-1, might function as a mediator in the presence of negative signals such as TNFα. Indeed, our experiments demonstrated that COX-2 was up-regulated by TNFα in differentiating 3T3-L1 cells, whereas a similar effect was not observed with COX-1 expression. Abrogating the induced COX-2 activity by specific COX-2 inhibitors reversed the TNFα-induced inhibition of differentiation by about 70%, suggesting that COX-2 was the principal isoenzyme mediating TNFα action. A similar modulating effect of COX-2 has also been reported in adiponectin signaling (25). Our present findings are in keeping with the observations reported in knockout mice deficient in the COX isoforms. Mice heterozygous for the COX-2 gene were found to be obese (5), with 30% more body fat than the wild-type animals. The phenotype for COX-1 knockout mice was identical to the wild-type, with no change in body fat content. Hence, the differential responses of the two COX isoenzymes to negative stimuli may explain the different phenotypic changes in adiposity between the COX-2 and COX-1 knockout animals.

In conclusion, both COX-1 and COX-2 negatively influenced adipose cell differentiation. COX-2 was the major isoform involved in mediating some of the TNFα negative effects on adipogenesis. It could be reasoned that COX-2 may play a more important role in body fat regulation in vivo. Further dissection of the complex action of this isoenzyme and the underlying mechanisms regulating COX-2 may provide new insights into the control of regional and total body fat and potential new targets for the treatment of obesity.

This study was supported by a grant-in-aid from the Heart and Stroke Foundation of Alberta, Canada. M.A-H. was supported by the University of Calgary Graduate Fellowship Program and by a studentship from the Julia McFarlane Diabetes Research Centre. The authors thank Dr. Marilyn J. Mooibroek for her critical review and constructive comments on this manuscript. The superb technical assistance of Lucia Ma and Baoying Gu is gratefully acknowledged.

REFERENCES

1. Smith, W. L., and D. L. Dewitt. 1996. Prostaglandin endoperoxide H synthases-1 and -2. *Adv. Immunol.* **62:** 167–215.
2. Smith, W. L., D. L. DeWitt, and R. M. Garavito. 2000. Cyclooxygenases: structural, cellular, and molecular biology. *Annu. Rev. Biochem.* **69:** 145–182.
3. Smith, W. L., and R. Langenbach. 2001. Why there are two cyclooxygenase isozymes. *J. Clin. Invest.* **107:** 1491–1495.
4. Ueno, N., M. Murakami, T. Tanioka, K. Fujimori, Y. Urade, and I. Kudo. 2001. Coupling between cyclooxygenases, terminal prostanoid synthases and phospholipase A{sub2}s. *J. Biol. Chem.* **276:** 34918–34927.
5. Fain, J. N., L. R. Ballou, and S. W. Bahouth. 2001. Obesity is induced in mice heterozygous for cyclooxygenase-2. *Prostaglandins Other Lipid Mediat.* **65:** 199–209.
6. Shillabeer, G., V. Kumar, E. Tibbo, and D. C. Lau. 1998. Arachidonic acid metabolites of the lipoxygenase as well as the cyclooxygenase pathway may be involved in regulating preadipocyte differentiation. *Metabolism.* **47:** 461–466.
7. Kozak, L. P., and J. T. Jensen. 1974. Genetic and developmental control of multiple forms of L-glycerol 3-phosphate dehydrogenase. *J. Biol. Chem.* **249:** 7775–7781.
8. Lasa, M., M. Brook, J. Saklatvala, and A. R. Clark. 2001. Dexamethasone destabilizes cyclooxygenase 2 mRNA by inhibiting mitogen-activated protein kinase p38. *Mol. Cell. Biol.* **21:** 771–780.
9. MacDougald, O. A., and M. D. Lane. 1995. Transcriptional regulation of gene expression during adipocyte differentiation. *Annu. Rev. Biochem.* **64:** 345–373.

10. Smas, C. M., and H. S. Sul. 1995. Control of adipocyte differentiation. *Biochem. J.* **309:** 697–710.
11. Gregoire, F. M., C. M. Smas, and H. S. Sul. 1998. Understanding adipocyte differentiation. *Physiol. Rev.* **78:** 783–809.
12. Smith, W. L., and L. J. Marnett. 1991. Prostaglandin endoperoxide synthase: structure and catalysis. *Biochim. Biophys. Acta.* **1083:** 1–17.
13. Langenbach, R., C. Loftin, C. Lee, and H. Tiano. 1999. Cyclooxygenase knockout mice: models for elucidating isoform-specific functions. *Biochem. Pharmacol.* **58:** 1237–1246.
14. Mater, M. K., D. Pan, W. G. Bergen, and D. B. Jump. 1998. Arachidonic acid inhibits lipogenic gene expression in 3T3–L1 adipocytes through a prostanoid pathway. *J. Lipid Res.* **39:** 1327–1334.
15. Casimir, D. A., C. W. Miller, and J. M. Ntambi. 1996. Preadipocyte differentiation blocked by prostaglandin stimulation of prostanoid FP2 receptor in murine 3T3–L1 cells. *Differentiation.* **60:** 203–210.
16. Setoguchi, K., Y. Misaki, Y. Terauchi, T. Yamauchi, K. Kawahata, T. Kadowaki, and K. Yamamoto. 2001. Peroxisome proliferator-activated receptor-gamma haploinsufficiency enhances B cell proliferative responses and exacerbates experimentally induced arthritis. *J. Clin. Invest.* **108:** 1667–1675.
17. Borglum, J. D., B. Richelsen, C. Darimont, S. B. Pedersen, and R. Negrel. 1997. Expression of the two isoforms of prostaglandin endoperoxide synthase (PGHS-1 and PGHS-2) during adipose cell differentiation. *Mol. Cell. Endocrinol.* **131:** 67–77.
18. Yamagata, K., K. I. Andreasson, W. E. Kaufmann, C. A. Barnes, and P. F. Worley. 1993. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron.* **11:** 371–386.
19. Harris, R. C., J. A. McKanna, Y. Akai, H. R. Jacobson, R. N. Dubois, and M. D. Breyer. 1994. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J. Clin. Invest.* **94:** 2504–2510.
20. Walenga, R. W., M. Kester, E. Coroneos, S. Butcher, R. Dwivedi, and C. Statt. 1996. Constitutive expression of prostaglandin endoperoxide G/H synthetase (PGHS)-2 but not PGHS-1 in hum an tracheal epithelial cells in vitro. *Prostaglandins.* **52:** 341–359.
21. Kliewer, S. A., J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann. 1995. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell.* **83:** 813–819.
22. Hotamisligil, G. S., N. S. Shargill, and B. M. Spiegelman. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* **259:** 87–91.
23. Sethi, J. K., and G. S. Hotamisligil. 1999. The role of TNF alpha in adipocyte metabolism. *Semin. Cell Dev. Biol.* **10:** 19–29.
24. Chen, C. C., Y. T. Sun, J. J. Chen, and K. T. Chiu. 2000. TNF-alpha-induced cyclooxygenase-2 expression in human lung epithelial cells: involvement of the phospholipase C-gamma 2, protein kinase C-alpha, tyrosine kinase, NF-kappa B-inducing kinase, and I-kappa B kinase 1/2 pathway. *J. Immunol.* **165:** 2719–2728.
25. Yokota, T., C. S. Meka, K. L. Medina, H. Igarashi, P. C. Comp, M. Takahashi, M. Nishida, K. Oritani, J. Miyagawa, T. Funahashi, Y. Tomiyama, Y. Matsuzawa, and P. W. Kincade. 2002. Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins. *J. Clin. Invest.* **109:** 1303–1310.