Fatty Acid Regulation of Liver X Receptors (LXR) and Peroxisome Proliferator-activated Receptor α (PPARα) in HEK293 Cells*

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Fatty acids bind to and regulate the activity of peroxisome proliferator-activated receptor (PPAR) and liver X receptors (LXR). However, the role lipid metabolism plays in the control of intracellular fatty acid ligands is poorly understood. We have identified two strains of HEK293 cells that display differences in fatty acid regulation of nuclear receptors. Using full-length and Gal4-LBD chimeric receptors in functional assays, 20:4,6 induced PPARα activity ~2.2-fold and suppressed LXRα activity by 80% (ED50 ~25–50 μM) in HEK293-E (early passage) cells but had no effect on PPARα or LXRα receptor activity in HEK293-L (late passage) cells. LXRα was insensitive to fatty acid regulation in both HEK293 strains. Metabolic labeling studies using [14C]20:4,6 (at 100 μM) indicated that the uptake of 20:4,6 and its assimilation into triacylglycerol, diacylglycerol, and polar lipids revealed no difference between the two strains. Such treatment increased total cellular 20:4,6 (~11-fold) and its elongation product, 22:4,6 (~3.6-fold), within 6 h. Non-esterified 20:4,6 and 22:4,6 represented ~3% of the total cellular 20:4,6 and 22:4,6. In HEK293-E cells, non-esterified 20:4,6 and 22:4,6 increased 8- and 18-fold, respectively, by 6 h and was sustained at that level for 24 h. In HEK293-L cells, non-esterified 20:4,6 also increased (5-fold) at 6 h but fell by 70% within 24 h. In contrast to HEK293-E cells, non-esterified 22:4,6 did not accumulate in HEK293-L cells. Functional assays showed that 22:4,6 was ~2-fold more effective than 20:4,6 at inhibiting oxysterol-induced LXRα activity in HEK293-E cells, but had no effect on LXRα activity in HEK293-L cells. Taken together, these findings demonstrate that the rate of assimilation of exogenously added fatty acids and their metabolites into complex lipids plays an important role in regulating PPARα and LXRα activity.

Several nuclear receptors have been identified as targets for regulation by fatty acids or their metabolites. These include the peroxisome proliferator-activated receptor family (PPARα, PPARγ, PPARδ (NR1C2), PPARβ (NR1C3), liver X receptors (LXRα (NR1H3) and LXRβ (NR1H2)), RXRα, and HNF-4α (1). The best characterized are members of the PPAR family. All PPARα bind 20-carbon polyunsaturated fatty acids, e.g. 20:4,6 and 20:5,3, with an apparent Kd of ~1–4 μM (2). As class II nuclear receptors, PPARs heterodimerize with RXR and bind direct repeats (PPREs, a DR1, rat AOX PPRE: CCGAACGT-GACCTNNTGTCCT) in promoters of regulated genes. PPARs play a major role in whole body lipid metabolism, inflammatory, and immune responses (3).

Liver X receptors (LXRα and LXRβ) are also class II nuclear receptors that bind direct repeats (LXRE, a DR-4, murine Cyp7a1: TGTTGACTcaAGTTCA) as a heterodimer with RXR (4). Oxysterols, like 22(R)-hydroxycholesterol or 24,25-epoxy-cholesterol, bind and activate LXRs (5–7). LXR/RXR heterodimers bind LXREs in promoters of enzymes involved in hepatic bile acid synthesis, e.g. 7α-hydroxylase (Cyp7a1), the main route for cholesterol elimination from the body. LXRs also regulate lipogenic gene expression through two mechanisms either by controlling the expression of SREBP-1c or by direct binding to promoters of certain lipogenic genes, e.g. fatty acid synthase (5, 7). In contrast to PPARs, unsaturated fatty acids act as antagonist to oxysterol activation of LXRs in HEK293 and hepatoma cell lines (6). The hierarchy for the fatty acid effect on LXRs is 20:4,6 > 18:2,6 > 18:1,9; saturated fatty acids have no effect. The half-maximal effect for 20:4,6 antagonism of oxysterol binding to the LXR-ligand binding domain is ~1.5 μM. This level of fatty acid is comparable to the Kd for 20-carbon PUFA binding to PPARs (3).

Based on in vitro binding and cell culture studies both PPARs and LXR have emerged as prospective monitors of intracellular NEFA levels, and in liver these receptors would respond accordingly by altering metabolism to prevent lipid and cholesterol overload. However, there remains little information that documents changes in intracellular NEFA levels in cells or how lipid metabolism might contribute to the regulation of NEFA levels and influence nuclear receptor activity. During the course of our studies, we observed that the fatty acid regulation of PPARα and LXRα in HEK293 cells was strain-dependent. This difference in fatty acid responsiveness provided us with an opportunity to identify prospective metabolic pathways that impact intracellular NEFA levels and affect nuclear receptor activity. Our studies show that while both cell types share the same metabolic pathways for 20:4,6 assimilation onto complex lipids, there

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are clear quantitative differences. These differences contribute to the level of non-esterified PUFAs in cells that, in turn, affects LXR and PPAR activity. Using the HEK293 cell model has provided support for the notion that both LXRα and PPARα are indeed sensors of intracellular NEFA.

MATERIALS AND METHODS

Cells—Two strains of HEK293 identified as HEK293-E (early passage) and HEK293-L (late passage) were obtained from K. Olson and K. Gallo at the Department of Physiology at Michigan State University. Cells were maintained in DME/F12 (Invitrogen) with 7.5% fetal calf serum (Intergen, Purchase, NY) in plastic tissue culture vessels (Falcon, BD). Fatty acid treatment dishes or serum-free media. Cells were grown to confluence and then incubated overnight in serum-free media before fatty acid treatment.

Plasmids—CMX-hLXRα, CMX-hLXRβ, CMX-Gal4-hLXRβ, CMX-Gal4-hLXRβ, TK-LXRRE3-Luc, TK-MH100X4-Luc, and SG5-rPPARα were previously described (8, 9). pM-rPPARα-LBD was constructed using the Matchmaker kit (CLONTECH) fusing the PPAR-LBD to the Gal4–BD. The primers used for this construction are: sense, 5′-GGG ATG TCA CAC AAT GCA ACGT and antisense, 5′-GTC TCA CAT GTC TCT GTA GAT CTC. pRG-Luc was obtained from Promega and serves as an internal control for transfection efficiency. All plasmids were cesium-purified before transfection.

Transfection—Cells were grown to confluence in 6-well culture plates. Cells in each well receive 1 μg of reporter plasmid and 1 μg of receptor expression plasmid. Cells were transfected using Lipofectin (6.6 μg/ml DNA) in serum-free media. After an overnight transfection period, cells were treated with fatty acids or drugs for 24 h in serum-free media containing bovine serum albumin (BSA). The BSA was adjusted to maintain a 5:1 molar ratio of fatty acid to BSA. LXRα transfection analysis using either full-length receptors or receptor fragments using the Matchmaker kit (CLONTECH) fusing the PPAR-LBD to the Gal4–BD. The primers used for this construction are: sense, 5′-GGG ATG TCA CAC AAT GCA ACGT and antisense, 5′-GTC TCA CAT GTC TCT GTA GAT CTC. pRG-Luc was obtained from Promega and serves as an internal control for transfection efficiency. All plasmids were cesium-purified before transfection.

Analysis of PUFA—Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC) was used to analyze fatty acid composition. Cells were grown to a higher density (3.8 x 10⁶ cells/well). However, all transfections and fatty acid metabolism studies described below were carried out at full confluence in serum-free media when cell growth was minimized.

Fatty acid regulation of nuclear receptors was evaluated by transfection analysis using either full-length receptors or Gal4-LBD chimeric receptors. Accordingly, cells were transfected with the LXRE-TK-Luc reporter plasmid and expression vectors containing full-length LXRs or LXRβ (Fig. 1A). The LXRE ligand, 22(R)-hydroxycholesterol induced a 6- and 2-fold increase in LUC activity in HEK293-E cells transfected with full-length LXRs and LXRβ, respectively. 22(R)-hydroxycholesterol had no effect on LUC activity when LXR expression vectors were omitted from the transfection mixture.

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Further characterization of the fatty acid effects on LXR used Gal4-chimeric receptors containing the LXR LBD fused to the Gal4 DNA binding domain (Fig. 1B). Transfection of HEK293-E cells with Gal4-LXRα-LBD led to a comparable level of induction of LUC activity (∼4-fold) following 22(R)-hydroxycholesterol treatment as seen with the full-length receptor. Moreover, the effect of fatty acids on the oxysterol regulation of LXR-LBD was essentially the same as that seen with the full-length receptor. Again, LXRα, but not LXRβ, was responsive to unsaturated fatty acid regulation. Substituting T0-091317 (at 1 μM), a potent LXR agonist (9), for 22(R)-hydroxycholesterol yielded essentially the same results for both the LXRα and LXRβ (not shown) as seen in Figs. 1, A and B. Finally, n3-PUFAs were tested for their effects on Gal4-LXRα-LBD activation (Fig. 1C). 22:6, n3 was as effective...
as 20:4,n6 at suppressing oxysterol-activated LXRα, while 18:3,n3 was the least effective at antagonizing oxysterol-regulation of LXRα.

These results confirm previous reports on the antagonistic effect of unsaturated fatty acids on the oxysterol regulation of LXRα (6). We have extended these observations to include 20- and 22-carbon n3 PUFAas as effective inhibitors of oxysterol activation of LXRα. However, neither n3 nor n6 PUFA affected oxysterol activation of LXRβ.

LXRαs and PPARαs Are Not Responsive to Fatty Acid Regulation in HEK293-L Cells—A similar analysis in the HEK293-L cells (Fig. 2) displayed a very different response pattern to fatty acids. Oxysterol 22(R)-hydroxysterol induced LUC activity by 8- and 2-fold in cells transfected with the LXRα and LXRβ expression vectors. This response is comparable to that seen in the HEK293-E cells (Fig. 1A). In contrast to the HEK293-E cells, oxysterol activation of LXRα and LXRβ in HEK293-L cells was insensitive to fatty acid regulation. In addition, both the LXRα and β chimeric Gal4-LBD receptors were insensitive to fatty acid regulation in HEK293-L cells. Even higher levels of fatty acids (300 μM) had no effect on LUC activity (Fig. 8).

Because fatty acid regulation of LXRα was cell-dependent, we examined the fatty acid regulation of a second fatty acid-regulated nuclear receptor, i.e. PPARα, in the two HEK293 strains. As noted above, the fatty acid regulation of PPARα is well established (2, 3). The two HEK293 cell strains were transfected with a reporter vector containing the acyl CoA oxidase PPRE fused upstream from the TK promoter and the CAT reporter gene (AOX-PPRE-TK-CAT). Cells were cotransfected with the SG5-PPARα expression vector. Cells were treated with 100 μM 20:4,n6 or WY14,643 (a strong PPARα agonist) for 24 h. In HEK293-E transfected with SG5-PPARα, CAT activity was induced −2.2-fold by 20:4,n6 or WY14,643 (Fig. 3). While WY14,643 induced CAT activity 2.7-fold in HEK293-L cells, 20:4,n6 had no effect on CAT activity. In the absence of SG5-PPARα transfection, CAT activity was not affected by either 20:4,n6 or WY14,643. Thus, like LXRα, PPARα is not sensitive to fatty acid regulation in HEK293-L cells.

PPARαs and LXRαs Display Equal Sensitivity to Exogenous Fatty Acid—We evaluated the fatty acid sensitivity of LXRα and PPARα in HEK293-E cells by carrying out a dose-response analysis (Fig. 4, A and B). In this study, the chimeric receptor Gal4-LRXα-LBD was used to assess the sensitivity of LXRα to fatty acid regulation. The maximum effect of 20:4,n6 on 22(R)-hydroxysterol-induced LUC activity was an 80% inhibition at 200 μM 20:4,n6. Fifty percent inhibition (IC50) of oxysterol-mediated induction of LXRα by 20:4,n6 was at ~60 μM. Assessment of fatty acid activation of PPARα used both the full-length SG5-PPARα expression vector and the Gal4-PPARα-LBD to activate the CAT or LUC reporter genes, respectively. At 200 μM, 20:4,n6 induced reporter gene activity 2.2-fold. This level was comparable to the effect of WY14,643 (100 μM) on PPARα-regulated reporter gene activity. The ED50 for the inductive effect of 20:4,n6 was ~50 μM. These results indicate that in HEK293-E cells, both LXRα and PPARα display a similar sensitivity to exogenously added 20:4,n6.

Fatty Acid Metabolism Studies—The fatty acid regulation of LXRα and PPARα is clearly different in the two HEK293 strains. As noted above, there are several factors that can account for this difference such as cell growth and density. However, the use of cells at confluence and fatty acid...
representative of two separate studies.

HEK293 cells were treated with [14C]20:4,n6 at 100 µM under the same conditions as used for the transfection studies. Fig. 5A illustrates the assimilation of [14C]20:4,n6 into the chloroform/methanol-soluble fraction of cells and the fractional distribution of [14C]20:4,n6 into various lipid fractions. No significant difference in fatty acid uptake or its assimilation into the chloroform-methanol-soluble fraction was observed. Thin-layer chromatographic separation of lipids allowed for the analysis of the fractional distribution of [14C]20:4,n6 between TAG, DAG, and polar lipids (lyso phosphatic acid, phosphatidic acid, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, sphingomyelin) (Fig. 5B). At all time points examined, there was no significant differences in the fractional distribution of [14C]20:4,n6 between TAG, DAG, or polar lipids between the two cells. However the fraction labeled “Other” is clearly different between the two cell types at all time points examined. This fraction is composed of cholesterol esters, neutral plasmalogens and NEFA (Fig. 5C). There are clear differences in the proportional distribution of 14C-labeled fatty acids in these three components. First, the non-esterified 14C-labeled fatty acid represents ~3% of the total 14C-labeled fatty acid incorporated into the chloroform/methanol extract of these cells. HEK293-E cells assimilate a greater fraction of the 14C-labeled fatty acid into cholesterol ester, while HEK293-L have a greater proportion of exogenous fatty acid assimilated into a fraction having chromatographic properties characteristic of neutral plasmalogens. In fact the level of 20:4,n6 assimilation into neutral plasmalogens far exceeds the level of fatty acid assimilation into CE. Together, these studies reveal differences in the level of 14C-fatty acid retained in the NEFA pool and its assimilation into two neutral lipid fractions, i.e. cholesterol ester and neutral plasmagen.

The use of 14C-labeled fatty acids provides important clues as to how exogenous fatty acids are distributed to various lipid fractions. However, to determine how addition of exogenous fatty acids affect levels of endogenous fatty acids as well as the generation of fatty acid metabolites, we examine the fatty acid composition in the total chloroform/methanol extract as well as the fractionated NEFA pool. Accordingly, cells were treated with non-radioactive 20:4,n6 at 100 µM (as described above) and total lipid was extracted from cells at 0, 1.5, 6, and 24 h after initiating treatment. Total lipids in the chloroform-methanol extract were saponified and fractionated by RP-HPLC. Although this analysis is restricted to unsaturated fatty acids, all fatty acid profiles were confirmed using gas chromatography-mass spectrometry (see “Methods and Materials”). 18:1n9 is a major unsaturated fatty acid (~1200 nmol/mg protein) in both cell types (Fig. 6A). The gas chromatography-mass spectrometry analysis indicated that both 16:0 and 18:0 are prominent lipids in HEK293 cells (not shown). Linoleic (18:2n6), arachidonic (20:4n6), and adrenic (22:4n6) are minor PUFA at < 100 nmol/mg protein. Treatment of cells with 100 µM 20:4,n6 leads to a 6.3, 11.8, and 8.7-fold increase in total 20:4,n6 in HEK293-E cells and a 8.1, 12, and 11.8-fold increase in HEK293-L cells after 1.5, 6, and 24 h, respectively. Adrenic acid (22:4n6) increased in each cell type after 20:4,n6 addition, reflecting the enzymatic conversion of 20:4,n6 to 22:4,n6 by an elongase. The fold changes in 22:4,n6 were 3.5, 6.3, and 9.2-fold for HEK293-E cells and 5.5, 8.8, and 12.5-fold for HEK293-L cells. Clearly, the addition of 20:4,n6 leads to major changes in cellular 20:4,n6 and 22:4,n6 levels in both cell types. Addition of 20:4,n6 to cells did not induce major changes in cellular levels of 18:1n9, 18:2n6, or 22:6n3 during the time course of this study.
To determine the NEFA fraction of the total lipid extract, the chloroform-methanol extract was fractionated on amino-propyl columns and analyzed by RP-HPLC (Fig. 6B). The results of this analysis are represented as a percent of total fatty acid extracted from the cell (Fig. 7, A and B). The relative abundance of 18:1,n9, 18:2,n6, 20:4,n6, and 22:4,n6 in the NEFA pool parallels the distribution of these fatty acids in the total cell extract. In untreated HEK293-E cells, these fatty acids were at 17.9, 1.5, 1.1 and 0.15 nmol/mg protein and in untreated HEK293-L cells the fatty acids were 26.2, 1.8, 1.2, and 0.3 nmol/mg protein. Each of these non-esterified fatty acids represents $\frac{1}{1153}$% of total saponified 18:1,n9, 18:2,n6, 20:4,n6, or 22:6,n3 in the cell. After initiating treatment, 20:4,n6 remains between 1.7 and 2.8% of the total cellular 20:4,n6 over the 24-h treatment period in HEK293-E cells (Fig. 7A). In contrast, non-esterified 20:4,n6 in HEK293-L cells falls from 2.5% in untreated cells to 0.5% after 24 h of treatment. This difference reflects more rapid assimilation of 20:4,n6 into complex lipids in HEK293-L cells than HEK293-E cells.

A similar analysis of 22:4,n6 indicates that this fatty acid increases from 0.5% at the start of the experiment to 3.1% of the total 22:4,n6 within 6 h in HEK293-E cells, but remains...
between 0.3 and 0.5% in HEK293-L cells. As with 20:4,n6, 22:4,n6 is more rapidly removed from the NEFA pool and assimilated into complex lipids in HEK293-L cells than in HEK293-E cells. Other unsaturated fatty acids in the NEFA pool, i.e. 18:1,n9, 18:2,n6, 22:6,n3, remain unchanged during the 24-h treatment period.

Adrenic Acid Is More Potent than 20:4,n6 at Inhibiting LXRα—Finding that adrenic acid (22:4,n6) is formed in cells following 20:4,n6 treatment indicates active elongase activity in both cell types. To determine the effect of adrenic acid on oxysterol-regulated LXRα activity, a dose-response analysis was carried out. Adrenic acid (22:4,n6; ED$_{50}$ ~ 20 μM) was ~2-fold more potent than 20:4,n6 (ED$_{50}$ ~ 40 μM) at inhibiting LXRα activity in HEK293-E cells. Neither fatty acid inhibited LXRα activity in HEK293-L cells, even up to 300 μM. Based on these metabolism studies, we conclude that non-esterified 20:4,n6 and its metabolite, 22:4,n6, are more rapidly assimilated into complex lipids in HEK293-L cells than in HEK293-E cells. This difference in clearance of exogenous fatty acid and its metabolite from the NEFA pool may account for the differences in fatty acid regulation of LXRα and PPARα in these two cell lines.

**DISCUSSION**

This study was initially undertaken to evaluate the fatty acid regulation of LXRα. After beginning these studies we quickly discovered that cell specific-factors significantly affected the outcome of these studies. Because PPARα and LXRα bind NEFA with an apparent $K_d$ of 1–4 μM (3, 6), both receptors should respond to a rise in intracellular NEFA following administration of fatty acids to cells. However, our studies with two strains of HEK293 cells reveal very different responses to exogenous fatty acid challenge. The notion that cell-specific lipid metabolism contributes to nuclear receptor regulation has been described in the context of eicosanoid regulation of PPARs (12, 13) or the differential effects on mono- and polyunsaturated fatty acids on lipogenic gene expression (1, 14, 15). However, we are unaware of any report that has examined the rate of change in intracellular NEFA or their metabolites following administration of fatty acids to cells and related these changes to nuclear receptor control.

Our studies confirm an earlier report (6) by showing that oxysterol regulation of LXRα is suppressed by unsaturated fatty acids. We have extended this observation to include the 20- and 22-carbon n3-PUFA as regulators of LXRα at 100 μM 20:4,n6 = 22:6,n3 > 20:5,n3 > 18:1,n9 = 18:3,n3 > 16:0. While 20:4,n6 binds the LXRα LBD with an apparent $K_d$ of 1–5 μM (6), the IC$_{50}$ for the inhibitory effect on LXRα activity is ~40–60 μM (6) (Figs. 4 and 8). Both the $K_d$ for fatty acid binding and the ED$_{50}$ for the activation of PPARα is in this same range (3) (Fig. 4). Thus, at a given intracellular level of non-esterified 20- or 22-carbon n6 or n3 PUFA, both PPARα and LXRα regulatory networks will be affected. Our studies with primary hepatocytes indicate that this concept applies to some PPARα and LXR-regulated genes.

Although LXRβ is known to be less sensitive than LXRα to oxysterol activation (9), we were surprised to find that, in contrast to LXRα, LXRβ was insensitive to fatty acid regulation (Fig. 1). The amino acid sequence of the LBD of the two receptors differs at several locations. Presumably these differences...
account for the levels of oxysterol, fatty acid, and co-activator binding. This observation also points out that in liver, where both receptors are expressed, only LXRα will be sensitive to fatty acid control. Our studies with primary hepatocytes have revealed some LXR-regulated genes are sensitive to PUFA regulation, while others are not. Whether these two receptors regulate different sets of genes may be resolved using LXRβ and LXRβ knockout mice (16).

The differences we described in nuclear receptor regulation by fatty acids in the two HEK293 strains prompted the analysis of how these cells handle exogenous 20:4,n6. The use of both metabolic labeling and mass analysis of 20:4,n6 and its elongation product, 22:4,n6, by RP-HPLC provides insight into fatty acid metabolism and rates of clearance of exogenously added fatty acids from the intracellular NEFA pool. Both methods provide clear evidence for a more rapid assimilation of exogenous fatty acid as well as its metabolite, 22:4,n6, in the HEK293-L cells than in HEK293-E cells. This evidence is based on the rate of reduction of [14C]20:4,n6 (Fig. 5C) as well as the mass of 20:4,n6 and 22:4,n6 (Fig. 7, A and B) in the NEFA pool. We found no evidence of enhanced oxidation of [14C]20:4,6 in HEK293-L cells. This was based on the finding that nearly equal levels of fatty acid were assimilated into cells at the various time points (Fig. 5B) and that the mass of 20:4,n6/mg of protein was actually higher in HEK293-L cells (Fig. 6A). In addition, analysis of culture media for rates of depletion of [14C]-20:4,n6 or the generation of lipid oxidation products failed to reveal major differences between these two cell lines. Instead, the difference between these two cell lines is in the assimilation of exogenously added fatty acid into complex lipids. This difference may reflect levels of expression of acyl CoA synthetase. At least five acyl CoA isozymes have been identified, each capable of forming fatty acyl CoA thioesters using saturated and unsaturated fatty acids of chain lengths ranging from 12–20 carbons (17). However, ACS4 is most active on 20- and 22-carbon PUFA (17). Whether the difference in fatty acid sensitivity of the HEK293-E and HEK293-L cells is due to levels of expression of a specific acyl CoA synthetase remains to be determined.

Other differences in fatty acid metabolism are seen in the assimilation of 13C-labeled fatty acid into cholesterol esters and a fraction consistent with the chromatographic properties of neutral plasmalogens (Fig. 5). These differences likely reflect levels of expression of key enzymes involved in the biosynthesis of these complex neutral lipids; i.e. microsomal acyl-CoA:cholesterol acyltransferase 1 or 2 for cholesterol ester synthesis or peroxisomal alkaldihydroxyacetone phosphate synthase and microsomal phosphatidate phosphatase for neutral plasmalogen synthesis (18, 19). The higher capacity of the HEK293-L cells to assimilate exogenously added fatty acid into neutral lipids provides a convenient explanation for the disappearance of non-esterified 20:4,n6 and 22:4,n6 from the NEFA pool of cells. The more rapid depletion of 20- and 22-carbon n6 PUFA from the NEFA pool apparently reduces these NEFA to a level insufficient to affect LXRα or PPARα activity as measured by reporter assays.

A third mechanism that might account for the difference in nuclear receptor responsiveness between the two cell types is the assimilation of 22:4,n6 into complex lipids (Figs. 6 and 7). Both cell types equally elongate exogenously added 20:4,n6 to 22:4,n6. However, when compared with HEK293-L cells, HEK293-E cells are slow to assimilate 22:4,n6 into complex lipids. A dose response shows that 22:4,n6 is 2-fold more potent than 20:4,n6. Thus, the generation of a more potent ligand coupled with its slow assimilation in to complex lipids can account for the increased sensitivity of HEK293-E cells to fatty acid regulation of nuclear receptor.

Finally, the question remains as to whether the mechanisms reported here have any bearing on PPARα or LXRα regulation in vivo, e.g. in liver or primary hepatocytes. Many of the same studies on lipid metabolism reported here have been carried out in primary hepatocytes, and the results with primary hepatocytes over HEK cells is that we can carry out detailed kinetic analyses of key genes targeted by PPARα and LXRα rather than rely on receptor activation assays. It is clear from these kinetic analyses that intracellular NEFA levels are elevated in response to extracellular fatty acid load and fall as a result of metabolism. Kinetic analysis of PUFA metabolism in primary hepatocytes is very dynamic. Analysis of key target genes for PPARα and LXRα reveals dynamic changes in expression.

In summary, we have identified two strains of HEK293 cells that display differences in nuclear receptor signaling. This difference can be ascribed to different rates of assimilation of exogenous fatty acid (20:4,n6) and its elongation product (22:4,n6) into complex lipids. Both PPARα and LXRα, but not LXRβ, display comparable sensitivity to fatty acid regulation. This finding suggests that as PPARα is activated by elevated intracellular NEFA, LXRα will be inhibited. If this same mechanism prevails in tissues like the liver that encounter major changes in plasma lipid levels (as triacylglycerols and NEFA), then elevated intracellular NEFA will likely affect both pathways. In the liver, it is well established that both n6 and n3 PUFA are feed forward activators PPARα and stimulate pathways leading to enhanced fatty acyl CoA formation and the flow of fatty acids into various oxidation pathways (1, 2). Elevated NEFA serve as feed back regulators of LXRα and may lead to the suppression of transcription of SREBP-1c, a key transcription factor controlling de novo lipogenesis. PUFA also regulate SREBP-1c mRNA levels by enhanced mRNA turnover (20–22). Thus, PPARα and LXRXα should be viewed as monitors of intracellular NEFA levels and respond accordingly to prevent lipid overload (23).

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