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Nuclear Receptors as Key Regulators of N-Acetyltransferase 2 Expression in Human Hepatic Cells

Abdul Karim[1], Fatima Begum[1], Nazmul Islam[1]*, Rahman Ullah[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Chittagong, Chittagong, Bangladesh.

***E-mail** ✉ nazmul.islam.pg@gmail.com

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ABSTRACT

Arylamine N-acetyltransferase 2 (NAT2) exhibits a well-characterized genetic polymorphism in humans that influences the metabolism of drugs and xenobiotics. Recent research, including genome-wide association studies, has linked NAT2 genetic variants to varying risks of dyslipidemia and cardiometabolic disorders, indicating a potential previously unrecognized role for NAT2 in metabolic disease pathophysiology. Consistent with this, our recent work demonstrated that human NAT2 expression is differentially modulated by glucose and insulin. Furthermore, in silico analyses revealed that NAT2 is co-expressed with liver-enriched nuclear receptors, such as NR1H4 (FXR) and NR1I2 (PXR), which are known to regulate hepatic glucose and lipid metabolism. Identifying the transcriptional regulators of NAT2 could provide insights into novel hepatic functions of this enzyme. Therefore, the current study was undertaken to examine whether hepatic nuclear receptors transcriptionally regulate NAT2. To investigate this, cryopreserved human hepatocytes were exposed to agonists targeting four distinct hepatic transcription factors/nuclear receptors—FXR (NR1H4), PXR (NR1I2), LXR (NR1H3), and PPARα (PPARA)—and the resulting changes in NAT2 mRNA levels were assessed. Although treatment with FXR, PXR, or LXR agonists (GW-4064, SR-12813, or GW-3965) effectively upregulated their respective target genes, these agonists did not produce a significant change in NAT2 transcript levels in human hepatocytes. Treatment with the PPARα agonist GW-7647 led to a statistically significant reduction in NAT2 mRNA, but the effect was minimal. In conclusion, the nuclear receptors assessed in this work—FXR, PXR, LXR, and PPARα—did not meaningfully influence NAT2 gene expression in cryopreserved human hepatocytes. Further investigations are necessary to determine which transcriptional factors control NAT2 expression in the liver.

Keywords: N-acetyltransferase 2, NAT2, Hepatocytes, Nuclear receptors, FXR, PPARα, PXR, LXR

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Introduction

Arylamine N-acetyltransferase 2 (NAT2) is a phase II enzyme involved in the metabolism of aromatic amines and hydrazines, as previously summarized [1]. NAT2 is characterized by distinct genetic polymorphisms, and research indicates that single nucleotide polymorphisms (SNPs) within its coding region can affect protein stability or alter its substrate specificity [2]. Depending on the combination of NAT2 variants, individuals can be classified into three acetylator types—slow, intermediate, or rapid—based on the enzyme activity produced [2, 3]. These acetylator phenotypes significantly influence how the body metabolizes certain drugs, such as isoniazid and hydralazine, and carcinogenic compounds like 4-aminobiphenyl [4].

Emerging evidence suggests that NAT2 may also impact broader aspects of human physiology. In a genome-wide association study (GWAS), the NAT2 SNPs rs1208 and rs1801280 were linked to insulin resistance, as measured by the euglycemic clamp, independent of body mass index [5]. Complementary studies in mice revealed that deletion of Nat1—the functional counterpart of human NAT2—results in metabolic disturbances, including elevated fasting glucose, insulin resistance, impaired mitochondrial function, reduced fat utilization, and increased fat deposition in tissues [6, 7]. These findings highlight a role for NAT1 in regulating insulin sensitivity and

energy balance in vivo. Although the precise mechanisms remain to be clarified, these data imply that variations in NAT2 activity may modulate energy storage and utilization.

Our recent studies indicate that NAT2 plays a significant role in regulating liver lipid and cholesterol metabolism. In liver cancer cell models, NAT2 expression increases in response to glucose and insulin, suggesting that its levels are sensitive to nutrient conditions [8]. Computational analyses further reveal that NAT2 is co-expressed with key genes involved in lipid synthesis and transport, such as APOB, ABCG8, ANGPTL3, FABP1, MOGAT2, and PLA2G12B [8]. Supporting this, multiple genome-wide association studies (GWAS) have linked NAT2 variants to differences in plasma lipid and cholesterol concentrations [9]. Interestingly, alleles associated with dyslipidemia risk, including rs1495741-A, are linked to the rapid acetylator phenotype, suggesting that individuals with higher NAT2 activity may be more prone to lipid abnormalities [9]. Evidence from rat models carrying rapid or slow Nat2 acetylator genotypes corroborates this association: rapid acetylator rats consistently exhibit elevated triglycerides and LDL alongside reduced HDL, independent of dietary intake, compared with slow acetylator counterparts [10].

Collectively, these findings indicate that NAT2 expression is influenced by nutrient cues, such as glucose and insulin, and may also contribute to the maintenance of lipid and cholesterol balance, particularly in organs with high NAT2 expression like the liver and intestines. Although the impact of NAT2 genetic variants on xenobiotic metabolism has been widely studied, the mechanisms controlling its transcription remain poorly understood [8, 11, 12]. Our previous analyses revealed that NAT2 is co-expressed with genes encoding several hepatic nuclear receptors, including farnesoid X receptor (FXR; NR1H4) and pregnane X receptor (PXR; NR1I2), which act as ligand-dependent transcription factors [13]. While PXR is primarily recognized for activating genes involved in drug metabolism, it also influences glucose regulation and insulin sensitivity [14, 15]. Similarly, FXR, liver X receptors α/β (LXRα/β), and peroxisome proliferator-activated receptor α (PPARα) respond to intracellular lipid signals and control the expression of genes governing lipid metabolism [16, 17]. These connections prompted us to investigate whether NAT2 transcription in the liver is modulated by these nuclear receptors.

In this study, we evaluated the ability of FXR, PXR, LXR, and PPARα to regulate NAT2 expression. Cryopreserved human hepatocytes were exposed to specific agonists for each receptor, and the resulting NAT2 transcript levels were compared with those of established receptor target genes.

Materials and Methods

Cell culture

Human hepatocytes, preserved via cryopreservation, were sourced from BioIVT (http://www.bioivt.com) and maintained in liquid nitrogen until required for use. Prior to culture, cells were rapidly thawed at 37°C for 90 seconds and then suspended in InVitroGRO HT medium (BioIVT) supplemented with TORPEDO™ Antibiotic Mix (BioIVT). The hepatocytes were subsequently plated onto collagen-coated Biocoat® plates (Corning) and incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 95% air, as described previously [18].

Agonists for nuclear receptors

FXR agonist GW-4064, PXR agonist SR-12813, LXR agonist GW-3965, and PPARα agonist GW-7647 were obtained from Selleck. Stock solutions were prepared in DMSO, and the final concentrations used for treatments were 1 μM GW-4064, 1 μM SR-12813, 2 μM GW-3965, and 10 μM GW-7647. Cryopreserved human hepatocytes, plated in triplicate, were exposed to either DMSO as a vehicle control or the respective agonist for 48 hours before collection.

Gene expression analysis

Following treatment with the respective agonists, total RNA was extracted from both control and treated hepatocytes using the E.Z.N.A. Total RNA Kit 1 (Omegabiotek) according to the manufacturer's guidelines. Complementary DNA (cDNA) was then generated using the High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific). Target genes were amplified and quantified using iTaq Universal SYBR Green Supermix (Bio-Rad) on an Applied Biosystems StepOne real-time PCR system, employing the primers listed in **Table 1.** Expression levels were normalized to GAPDH, and relative changes in gene expression were calculated using the ΔΔCt ($2^{-\Delta\Delta CT}$) approach. Statistical comparisons between treated and control groups were performed using unpaired t-tests in GraphPad Prism v8.2.1 (GraphPad Software). Data are presented as mean ± SEM from three

independent experiments (n = 3), with statistical significance indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

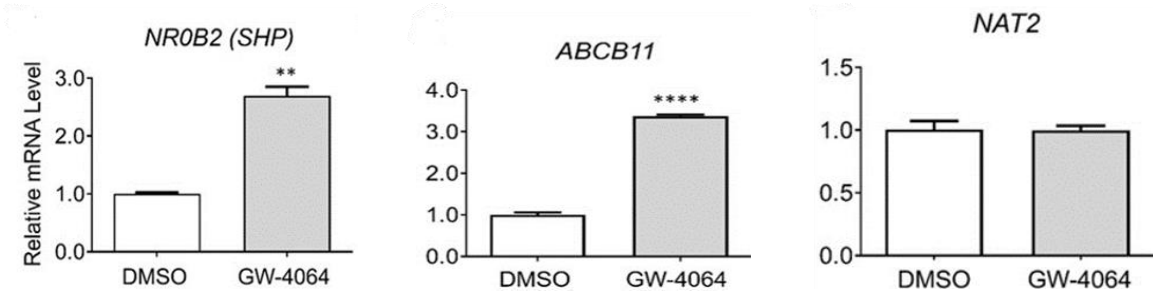
Table 1. List of PCR primer sequences used in the study.

Gene (Human)	Function / Description	Primer	Sequence
ABCA1	ATP-binding cassette transporter A1, involved in cholesterol efflux	Forward	GCTGGTGTGGACCCTTACTC
		Reverse	GCAGCTTCATATGGCAGCAC
ABCB11	ATP-binding cassette transporter B11, critical for bile acid transport	Forward	AACAGGCTCAGCTGCATGAT
		Reverse	CTGGATGGTGGACAAGCGAT
ABCG5	ATP-binding cassette transporter G5, mediates sterol transport	Forward	CTCGCAGGAACCGAATTGTG
		Reverse	GGCGTGCCACAGAAAATCAG
ACOX1	Acyl-CoA oxidase 1, key enzyme in peroxisomal fatty acid β-oxidation	Forward	GTAGCAGTCTGGCCAACCAT
		Reverse	GCTCCCCTGAAGGAAATCCC
CYP2B6	Cytochrome P450 2B6, involved in xenobiotic and drug metabolism	Forward	CCACCCTAACACCCATGACC
		Reverse	CCCAGGTGTACCGTGAAGAC
CYP3A4	Cytochrome P450 3A4, major enzyme in drug metabolism and steroid synthesis	Forward	CGGGACTATTTCCACCACCC
		Reverse	CCCACGCCAACAGTGATTA
CYP7A1	Cytochrome P450 7A1, catalyzes the rate-limiting step in bile acid synthesis	Forward	AAGCAAACACCATTCCAGCG
		Reverse	CACTGGAAAGCCTCAGCGAT
FABP1	Fatty acid-binding protein 1, involved in intracellular lipid transport	Forward	GGGAAGGGAGCCCCCTATAA
		Reverse	TGGATCACTTTGGACCCAGC
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase, housekeeping gene for normalization	Forward	GGTGAAGCAGGCGTCGGAGG
		Reverse	GAGGGCAATGCCAGCCCCAG
NAT2	N-acetyltransferase 2, important for xenobiotic metabolism	Forward	TGGACCAAATCAGGAGAGAG C
		Reverse	GCCCACCAAACAGTAAACCC
NR0B2 (SHP)	Nuclear receptor subfamily 0 group B member 2, also known as SHP, regulates bile acid and lipid metabolism	Forward	TGCTGTCTGGAGTCCTTCTG
		Reverse	CCAGGGTTCCAGGACTTCACA
SLC2A2 (GLUT2)	Glucose transporter 2, mediates glucose uptake in liver and pancreas	Forward	CCAGCTACCGACAGCCTATT
		Reverse	GGTTTGCTGATACCAGCCGT

Results and Discussion

Gene expression changes by a FXR agonist, GW-4064

To determine whether NAT2 expression is controlled at the transcriptional level by FXR, cryopreserved human hepatocytes were treated with either DMSO (vehicle control) or the FXR agonist GW-4064, and NAT2 mRNA levels were assessed. As positive controls for FXR activation, the mRNA levels of two established FXR target genes—small heterodimer partner [SHP; nuclear receptor subfamily 0 group B member 2 (NR0B2)] and ATP-binding cassette subfamily B member 11 (ABCB11)—were also measured [19]. As anticipated, treatment with GW-4064 led to a significant increase in both target genes, with NR0B2 showing a 2.7-fold upregulation ($p < 0.01$) and ABCB11 a 3.4-fold increase ($p < 0.0001$), confirming FXR activation **(Figures 1a and 1b)**. In contrast, NAT2 mRNA levels remained unchanged following FXR agonist exposure **(Figure 1c)**, indicating that FXR does not regulate NAT2 transcription in human hepatocytes.

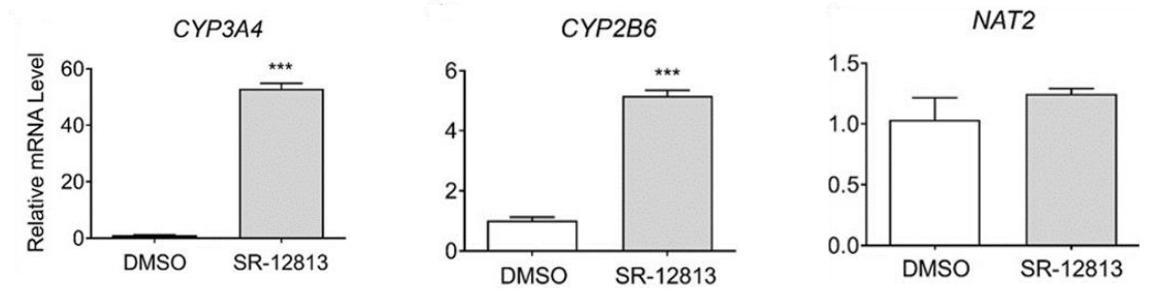


a) c) d)

Figure 1. Effects of FXR activation on gene expression. Cryopreserved human hepatocytes were exposed to the FXR agonist GW-4064 for 48 hours. Transcript levels of the indicated genes were quantified by RT-qPCR and normalized to GAPDH. Expression in the treated cells is shown relative to the DMSO control. Panels A and B depict the FXR target genes NR0B2 and ABCB11, respectively, which were significantly upregulated. In contrast, NAT2 mRNA levels remained unchanged following GW-4064 treatment (c). Data are presented as mean ± SEM. Statistical significance is indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Gene expression changes by a PXR agonist, SR-12813

To examine whether PXR influences NAT2 transcription, cryopreserved human hepatocytes were treated with either the vehicle DMSO or the PXR agonist SR-12813, and NAT2 mRNA levels were analyzed. In parallel, two well-characterized PXR-responsive genes, CYP3A4 and CYP2B6, were measured as positive controls [20]. Exposure to SR-12813 strongly induced both control genes, with CYP3A4 showing a 52.9-fold increase and CYP2B6 a 5.2-fold increase ($p < 0.001$ for both), confirming successful PXR activation **(Figures 2a and 2b)**. Notably, NAT2 expression remained unchanged after PXR agonist treatment **(Figure 2c)**, suggesting that NAT2 transcription is not directly regulated by PXR in these human hepatocytes.



a) c) d)

Figure 2. Assessment of PXR-mediated gene regulation in human hepatocytes. Human hepatocytes, previously cryopreserved, were treated for 48 hours with the PXR agonist SR-12813. Transcript levels were quantified by RT-qPCR and normalized to GAPDH. Data are shown as fold changes relative to DMSO-treated controls. Panels A and B illustrate robust induction of the canonical PXR targets CYP3A4 and CYP2B6 following agonist exposure. In contrast, NAT2 expression remained unchanged, as shown in Panel C. Results are presented as mean ± SEM. Statistical significance is indicated as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Gene expression changes by a LXR agonist, GW-3965

To examine whether LXR regulates NAT2 transcription, cryopreserved human hepatocytes were exposed to either DMSO or the LXR agonist GW-3965, and NAT2 mRNA levels were measured. As positive controls, we monitored the expression of two well-characterized LXR target genes, ABCA1 and ABCG5 [21]. Treatment with GW-3965 significantly increased both control genes, with ABCA1 showing an 18.2-fold induction ($p < 0.0001$) and ABCG5 a more modest 1.3-fold increase ($p < 0.01$) **(Figures 3a and 3b)**. In contrast, NAT2 expression remained unchanged following LXR agonist treatment **(Figure 3c)**, indicating that NAT2 is not transcriptionally regulated by LXR in human hepatocytes.

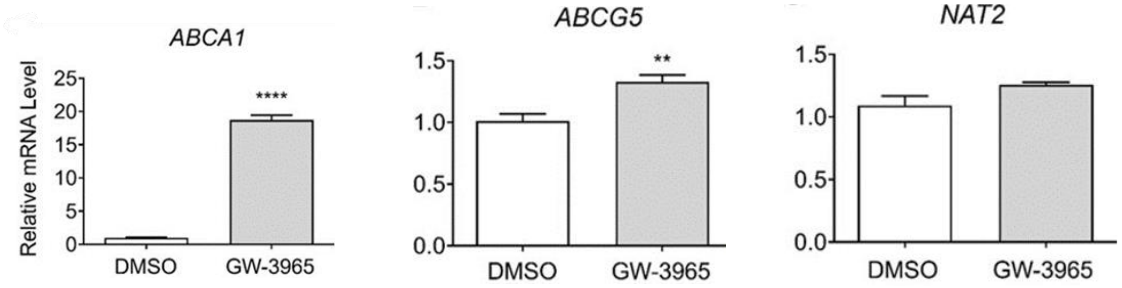


Figure 3. Effects of LXR activation on gene expression in human hepatocytes. Cryopreserved human hepatocytes were exposed to the LXR agonist GW-3965 for 48 hours, and transcript levels were quantified by RT-qPCR with GAPDH as the normalization control. Gene expression in treated cells is presented relative to DMSO-treated controls. Panels A and B show the LXR-responsive genes ABCA1 and ABCG5, both of which were significantly upregulated following agonist treatment. In contrast, NAT2 expression remained unchanged (Panel C). Data are presented as mean ± SEM. Statistical significance is indicated as: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Gene expression changes by a PPARα agonist, GW-7647

To explore whether NAT2 is under transcriptional control of PPARα, cryopreserved human hepatocytes were exposed to the PPARα agonist GW-7647 or DMSO as a vehicle control. Expression of two well-established PPARα target genes, ACOX1 and FABP1, was measured as positive controls [22]. As expected, GW-7647 treatment robustly elevated the transcripts of ACOX1 (1.8-fold, $p < 0.001$) and FABP1 (4.0-fold, $p < 0.001$) **(Figures 4a and 4b)**. NAT2 mRNA, however, exhibited a modest reduction of 0.8-fold ($p < 0.05$) **(Figure 4c)**. To further validate these findings, two additional genes known to be suppressed by PPARα—CYP7A1 and GLUT2 (SLC2A2)—were examined [23]. While CYP7A1 levels remained stable **(Figure 4d)**, GLUT2 transcript abundance decreased significantly by 0.8-fold ($p < 0.05$) **(Figure 4e)**. Overall, although NAT2 showed a statistically significant downregulation, the small magnitude of change suggests that PPARα-mediated suppression of NAT2 may have limited physiological relevance.

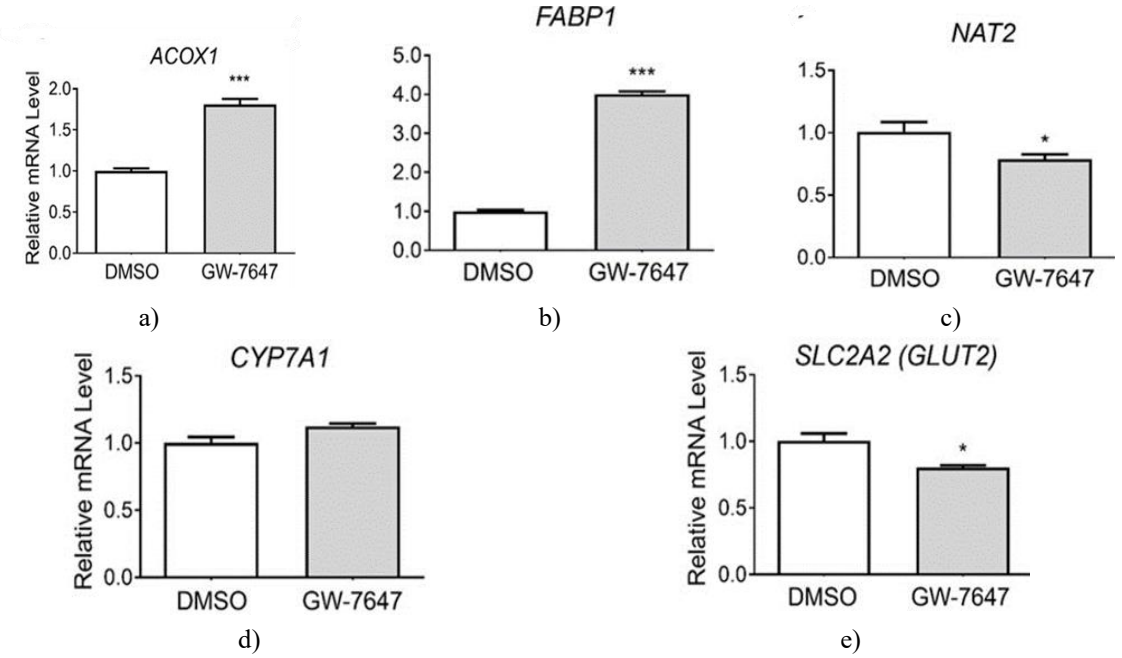


Figure 4. Effects of PPARα activation on gene expression in human hepatocytes. Cryopreserved human hepatocytes were treated with the PPARα agonist GW-7647 for 48 hours. Gene expression was measured by RT-qPCR and normalized to GAPDH. Values are expressed relative to DMSO-treated controls. Panels A and B show PPARα-responsive genes previously reported to be upregulated, ACOX1 and FABP1, while Panels D and E show genes known to be downregulated, CYP7A1 and GLUT2, respectively. Treatment with GW-

7647 resulted in a modest but statistically significant reduction in NAT2 and GLUT2 transcripts (Panels C and E). Data are presented as mean ± SEM, with significance indicated as: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

In cryopreserved human hepatocytes, activation of FXR, PXR, or LXR with their respective agonists did not produce meaningful changes in NAT2 mRNA levels, indicating that these nuclear receptors are unlikely to serve as primary transcriptional regulators of NAT2 under the conditions tested. By contrast, treatment with the PPARα agonist GW-7647 caused a slight but statistically significant reduction in NAT2 transcripts, though the functional relevance of this modest decrease remains uncertain.

PPARα is known to be activated by endogenous fatty acids and related metabolites [24, 25] and orchestrates the transcription of genes involved in fatty acid uptake, β-oxidation, and lipid biosynthesis, including SREBP-1c (SREBF1), fatty acid synthase (FASN), and acetyl-CoA carboxylase 1 (ACC1; ACACA) [24]. In this study, ACOX1 and FABP1 were examined as prototypical PPARα-upregulated genes [22], while CYP7A1 and GLUT2 (SLC2A2) were chosen as examples of PPARα-repressed targets **(Figure 4)**. CYP7A1 encodes cholesterol 7-α-monooxygenase, the rate-limiting enzyme in the conversion of cholesterol to bile acids [26], and prior studies have reported its suppression by the PPARα agonist Wy14643 in HepG2 and primary hepatocytes [23, 27]. Similarly, Wy14643 has been shown to downregulate glucose transporter genes such as GLUT2 and villin-1 (VIL1) in both human and mouse hepatocytes [23]. In the present experiments, GW-7647 did not reduce CYP7A1 levels but did significantly lower GLUT2 expression. The absence of CYP7A1 suppression may reflect indirect regulation, as previous work suggested that Wy14643 affects CYP7A1 via modulation of HNF4α rather than direct PPARα binding [27]. Other factors could also contribute to these differences, including the use of different agonists, variations in hepatocyte batches, and differences in HNF4α expression between experimental systems. Collectively, these results indicate that PPARα can modestly repress NAT2 transcription, although the biological significance of this effect is likely limited.

Activation of FXR, PXR, and LXR using their respective agonists did not noticeably alter NAT2 transcript levels in cryopreserved human hepatocytes, suggesting that these receptors are unlikely to serve as primary regulators of NAT2 expression in this context. Interestingly, treatment with the PPARα agonist GW-7647 led to a slight but statistically significant reduction in NAT2 mRNA. However, the biological relevance of this modest change remains unclear.

PPARα is naturally activated by fatty acids and related metabolites, where it modulates gene networks involved in fatty acid uptake, β-oxidation, and lipid biosynthesis [24, 25]. In addition to promoting genes such as ACOX1 and FABP1 [22], PPARα activation can indirectly suppress other genes through the proteasomal degradation of HNF4α. This mechanism reduces the expression of enzymes involved in amino acid catabolism, including HAL and SDS [23, 28, 29]. Transcriptomic studies in mouse and human hepatocytes treated with Wy14643, another PPARα agonist, have identified “alpha-amino acid catabolic process” (GO:1901606) and “cellular amino acid catabolic process” (GO:0009063) as significantly downregulated biological processes [23]. These observations raise the possibility that the modest NAT2 downregulation observed with GW-7647 is an indirect consequence of reduced HNF4α activity rather than a direct PPARα effect. Future experiments could test whether HNF4α directly regulates NAT2 transcription.

Our previous findings also indicated that NAT2 expression in HepG2 cells is sensitive to extracellular glucose levels [8]. Given this, the slight decrease in GLUT2 (SLC2A2) following PPARα activation might limit glucose uptake and indirectly affect NAT2 expression. Yet, the reduction in GLUT2 was minimal, calling into question whether this mechanism has meaningful physiological impact.

Although NAT2 activity is clinically important, influencing drug metabolism and lipid homeostasis, its transcriptional regulation has been largely unexplored. One study demonstrated that NAT2 expression can be upregulated by the vitamin D receptor (VDR), with $1,25(OH)_2D_3$ treatment increasing NAT2 levels and VDR binding to its promoter in colorectal cancer cells [12]. Whether a similar VDR-mediated regulatory pathway exists in hepatocytes remains to be determined.

Conclusion

In summary, our data indicate that FXR, PXR, LXR, and PPARα activation exerts minimal effects on NAT2 transcription in cryopreserved human hepatocytes. Investigating other nuclear receptors, particularly HNF4α and VDR, may provide insight into mechanisms controlling hepatic NAT2 expression.

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Conflict of Interest: None

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Ethics Statement: Ethical approval was not required for the studies on humans in accordance with the local legislation and institutional requirements because only commercially available cryopreserved human hepatocyte samples were used.

References

1. McDonagh EM, Boukouvala S, Aklillu E, Hein DW, Altman RB, Klein TE. PharmGKB summary: very important pharmacogene information for N-acetyltransferase 2. Pharmacogenet Genomics. 2014;24(8):409–25. doi:10.1097/FPC.0000000000000062
2. Hein DW. Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. Mutat Res. 2002;506–507:65–77. doi:10.1016/S0027-5107(02)00153-7
3. Sim E, Abuhammad A, Ryan A. Arylamine N-acetyltransferases: from drug metabolism and pharmacogenetics to drug discovery. Br J Pharmacol. 2014;171(11):2705–25. doi:10.1111/bph.12598
4. Hein DW, Millner LM. Arylamine N-acetyltransferase acetylation polymorphisms: paradigm for pharmacogenomic-guided therapy—a focused review. Expert Opin Drug Metab Toxicol. 2021;17(1):9–21. doi:10.1080/17425255.2021.1840551
5. Knowles JW, Xie W, Zhang Z, Chennemsetty I, Assimes TL, Paananen J, et al. Identification and validation of N-acetyltransferase 2 as an insulin sensitivity gene. J Clin Invest. 2015;125(4):1739–51. doi:10.1172/JCI74692
6. Chennamsetty I, Coronado M, Contrepois K, Keller MP, Carcamo-Orive I, Sandin J, et al. Nat1 deficiency is associated with mitochondrial dysfunction and exercise intolerance in mice. Cell Rep. 2016;17(2):527–40. doi:10.1016/j.celrep.2016.09.005
7. Camporez JP, Wang Y, Faarkrog K, Chukijrungroat N, Petersen KF, Shulman GI. Mechanism by which arylamine N-acetyltransferase 1 ablation causes insulin resistance in mice. Proc Natl Acad Sci U S A. 2017;114(52):E11285–E11292. doi:10.1073/pnas.1716990115
8. Hong KU, Salazar-González RA, Walls KM, Hein DW. Transcriptional regulation of human arylamine N-acetyltransferase 2 gene by glucose and insulin in liver cancer cell lines. Toxicol Sci. 2022;190(1):158–72. doi:10.1093/toxsci/kfac103
9. Hong KU, Walls KM, Hein DW. Non-coding and intergenic genetic variants of human arylamine N-acetyltransferase 2 (NAT2) gene are associated with differential plasma lipid and cholesterol levels and cardiometabolic disorders. Front Pharmacol. 2023;14:1091976. doi:10.3389/fphar.2023.1091976
10. Hong KU, Doll MA, Lykoudi A, Salazar-González RA, Habil MR, Walls KM, et al. Acetylator genotype-dependent dyslipidemia in rats congenic for N-acetyltransferase 2. Toxicol Rep. 2020;7:1319–30. doi:10.1016/j.toxrep.2020.09.011
11. Zou C, Mifflin L, Hu Z, Zhang T, Shan B, Wang H, et al. Reduction of mNAT1/hNAT2 contributes to cerebral endothelial necroptosis and Aβ accumulation in Alzheimer's disease. Cell Rep. 2020;33(8):108447. doi:10.1016/j.celrep.2020.108447
12. Zhu C, Wang Z, Cai J, Pan C, Lin S, Zhang Y, et al. VDR signaling via the enzyme NAT2 inhibits colorectal cancer progression. Front Pharmacol. 2021;12:727704. doi:10.3389/fphar.2021.727704

13. Frigo DE, Bondesson M, Williams C. Nuclear receptors: from molecular mechanisms to therapeutics. Essays Biochem. 2021;65(6):847–56. doi:10.1042/EBC20210020
14. Kliewer SA, Goodwin B, Willson TM. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. Endocr Rev. 2002;23(5):687–702. doi:10.1210/er.2001-0038
15. Spruiell K, Richardson RM, Cullen JM, Awumey EM, Gonzalez FJ, Gyamfi MA. Role of pregnane X receptor in obesity and glucose homeostasis in male mice. J Biol Chem. 2014;289(6):3244–61. doi:10.1074/jbc.M113.494575
16. Calkin AC, Tontonoz P. Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. Nat Rev Mol Cell Biol. 2012;13(4):213-24. doi:10.1038/nrm3312
17. Hong C, Tontonoz P. Liver X receptors in lipid metabolism: opportunities for drug discovery. Nat Rev Drug Discov. 2014;13(6):433-44. doi:10.1038/nrd4280
18. Walls KM, Hong KU, Hein DW. Heterocyclic amines reduce insulin-induced AKT phosphorylation and induce gluconeogenic gene expression in human hepatocytes. Arch Toxicol. 2023;97(6):1613-26. doi:10.1007/s00204-023-03488-2
19. Rausch M, Samodelov SL, Visentin M, Kullak-Ublick GA. The farnesoid X receptor as a master regulator of hepatotoxicity. Int J Mol Sci. 2022;23(22):13967. doi:10.3390/ijms232213967
20. Hariparsad N, Chu X, Yabut J, Labhart P, Hartley DP, Dai X, et al. Identification of pregnane-X receptor target genes and coactivator and corepressor binding to promoter elements in human hepatocytes. Nucleic Acids Res. 2009;37(4):1160-73. doi:10.1093/nar/gkn1047
21. Fessler MB. The challenges and promise of targeting the liver X receptors for treatment of inflammatory disease. Pharmacol Ther. 2018;181:1-12. doi:10.1016/j.pharmthera.2017.07.010
22. Rakhshandehroo M, Knoch B, Müller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. PPAR Res. 2010;2010:612089. doi:10.1155/2010/612089
23. Rakhshandehroo M, Hooiveld G, Müller M, Kersten S. Comparative analysis of gene regulation by the transcription factor PPARalpha between mouse and human. PLoS One. 2009;4:e6796. doi:10.1371/journal.pone.0006796
24. Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RRV, Xu HE, Turk J, et al. Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. Cell. 2009;138:476-88. doi:10.1016/j.cell.2009.05.036
25. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. J Hepatol. 2015;62:720-33. doi:10.1016/j.jhep.2014.10.039
26. Noshiro M, Okuda K. Molecular cloning and sequence analysis of cDNA encoding human cholesterol 7 alpha-hydroxylase. FEBS Lett. 1990;268:137-40. doi:10.1016/0014-5793(90)80992-R
27. Marrapodi M, Chiang JY. Peroxisome proliferator-activated receptor α (PPARα) and agonist inhibit cholesterol 7α-hydroxylase gene (CYP7A1) transcription. J Lipid Res. 2000;41:514-20. doi:10.1016/S0022-2275(20)32398-1
28. Alemán G, Ortiz V, Contreras AV, Quiroz G, Ordaz-Nava G, Langley E, et al. Hepatic amino acid-degrading enzyme expression is downregulated by natural and synthetic ligands of PPARα in rats. J Nutr. 2013;143:1211-8. doi:10.3945/jn.113.176354
29. Tobón-Cornejo S, Vargas-Castillo A, Leyva-Martínez A, Ortíz V, Noriega LG, Velázquez-Villegas LA, et al. PPARα/RXRα downregulates amino acid catabolism in the liver via interaction with HNF4α promoting its proteasomal degradation. Metabolism. 2021;116:154705. doi:10.1016/j.metabol.2021.154705