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Transcriptional and Enzymatic Regulation of Hepatic Lipid Metabolism in Non-Alcoholic Fatty Liver Disease: The SREBP1c–PPARα–AMPK Axis

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) represents the hepatic manifestation of metabolic syndrome, characterized by lipid accumulation, insulin resistance, and mitochondrial dysfunction. Recent multi-omics studies suggest that the transcriptional crosstalk between sterol regulatory element-binding protein 1c (SREBP1c), peroxisome proliferator-activated receptor α (PPARα), and AMP-activated protein kinase (AMPK) orchestrates the metabolic imbalance between lipogenesis and fatty acid oxidation. However, the integrated transcriptional–enzymatic regulation underlying this lipid metabolic shift remains incompletely defined.

Methods: A cross-sectional study was conducted in 120 participants (60 NAFLD patients and 60 matched controls). Hepatic enzyme activities—acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD1), carnitine palmitoyltransferase-1A (CPT1A), and acyl-CoA oxidase-1 (ACOX1)—were quantified spectrophotometrically. Gene expression of SREBP1c, PPARα, and AMPK was analyzed via qPCR in a representative subset ($n = 40$). Lipidomic markers, including ceramides and phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratio, were correlated with transcriptional and enzymatic parameters using Pearson's and multivariate regression models.

Results: NAFLD subjects exhibited a 2.05-fold upregulation of **SREBP1c**, concurrent with 40–45% downregulation of **PPARα** and **AMPK** ($p < 0.001$). Lipogenic enzymes (ACC, FAS, SCD1) showed 1.8–2.0× elevation, while oxidative enzymes (CPT1A, ACOX1) decreased by ~40%. The **SREBP1c–PPARα** correlation was strongly negative ($r = –0.71$, $p < 0.001$). High SREBP1c expression was associated with increased ceramide accumulation and reduced PC/PE ratio, indicating transcriptionally mediated lipotoxic–membrane stress coupling. Multiple regression confirmed **BMI** and **insulin** as major predictors of HOMA-IR ($R^2 = 0.91$).

Conclusions: This study delineates a coordinated regulatory network wherein **SREBP1c activation** and **PPARα/AMPK suppression** collectively drive hepatic lipogenesis and inhibit β-oxidation. The resulting metabolic inflexibility promotes ceramide accumulation and phospholipid imbalance, key drivers of lipotoxicity in NAFLD. These findings establish the **SREBP1c–PPARα–AMPK triad** as a mechanistic axis linking transcriptional control to enzymatic and lipidomic remodeling, offering translational targets for metabolic therapy.

***Keywords**: NAFLD, SREBP1c, PPARα, AMPK, lipogenesis, β-oxidation, lipidomics, insulin resistance*

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), recently reclassified as metabolic dysfunction-associated steatotic liver disease (MASLD), is the most prevalent chronic liver disorder globally, affecting approximately **one-quarter of adults** and increasingly diagnosed among younger populations [1]. The condition encompasses a continuum from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis, closely interlinked with obesity, insulin resistance, and type 2 diabetes [2]. The pathophysiological hallmark of NAFLD is **hepatic lipid accumulation exceeding 5% of liver weight**, arising from an imbalance between lipid acquisition (uptake and synthesis) and lipid disposal (oxidation and export) [3]. At the molecular level, this imbalance is driven by the **reciprocal regulation of lipogenic and oxidative pathways** governed by key transcriptional regulators. **Sterol regulatory element-binding protein 1c (SREBP1c)** is a master activator of de novo lipogenesis, promoting the transcription of *acetyl-CoA carboxylase (ACC)*, *fatty acid synthase (FAS)*, and *stearoyl-CoA desaturase-1 (SCD1)* [4]. Conversely, **peroxisome proliferator-activated receptor α (PPARα)** regulates fatty acid β-oxidation genes such as *carnitine palmitoyltransferase-1A (CPT1A)* and *acyl-CoA oxidase-1 (ACOX1)* [5]. **AMP-activated protein kinase (AMPK)** serves as an upstream metabolic switch that inhibits lipogenesis by phosphorylating and inactivating SREBP1c while stimulating fatty acid oxidation through PPARα activation [6].

In NAFLD, chronic nutrient excess and hyperinsulinemia activate **SREBP1c** while concurrently suppressing **PPARα** and **AMPK**, thereby establishing a self-perpetuating cycle of lipid accumulation and oxidative stress [7]. This transcriptional–enzymatic imbalance leads to **triglyceride and ceramide accumulation**, contributing to **lipotoxicity**, **insulin resistance**, and **endoplasmic reticulum (ER) stress** [8,9]. Recent omics-based studies have highlighted that perturbations in this triad (SREBP1c–PPARα–AMPK) orchestrate global metabolic reprogramming and predict disease severity across the NAFLD spectrum [10].

Despite these insights, few studies have **quantitatively integrated transcriptional and enzymatic data** with lipidomic parameters in human NAFLD cohorts. The present study aims to elucidate how **SREBP1c activation and PPARα/AMPK suppression** jointly reshape hepatic lipid metabolism, using parallel assessments of gene expression, enzyme activity, and lipidomic markers. Understanding this triad provides a mechanistic framework for identifying **therapeutic targets** and **biochemical biomarkers** of NAFLD progression.

2. MATERIALS AND METHODS

2.1 Study Design and Ethical Approval

This was a **cross-sectional, case–control study** conducted between June 2023 and July 2025 at the Department of Biochemistry, **Malwanchal University, Indore (India)**. The study aimed to investigate transcriptional and enzymatic dysregulation in the **SREBP1c–PPARα–AMPK axis** in patients with non-alcoholic fatty liver disease (NAFLD). The protocol received ethical approval from the **Institutional Ethics Committee of Malwanchal University (MU/IEC/2023/087)**, and all participants provided written informed consent. Procedures adhered to the **Declaration of Helsinki (2013 revision)** and ICMR biomedical research guidelines.

2.2 Study Population and Recruitment

Participants were recruited from the university hospital's metabolic clinic. **Sixty ultrasonographically confirmed NAFLD patients** (grades I–II) and **60 age- and sex-matched healthy controls** were enrolled. Subjects were screened for inclusion by biochemical and imaging criteria.

Inclusion criteria:

- Adults aged **25–60 years** with **BMI ≥ 25 kg/m²**.
- Normal renal function and no alcohol intake (>20 g/day for men, >10 g/day for women).

Exclusion criteria:

- Hepatitis B/C, autoimmune hepatitis, drug-induced liver injury, or alcohol-related liver disease.
- Use of lipid-lowering or antidiabetic medication.
- Pregnancy or lactation.

Sample size was determined using power analysis ($\alpha = 0.05$, $\beta = 0.2$, expected mean difference = 0.3, SD = 0.45), yielding a minimum of 52 subjects per group, expanded to 60 to accommodate attrition.

2.3 Clinical and Biochemical Assessment

Anthropometric data included **body mass index (BMI)**, **waist circumference**, and **blood pressure**. Fasting venous blood samples were collected after a 12-hour overnight fast.
Serum was analyzed for:

- **Glucose** (glucose oxidase–peroxidase method).
- **Insulin** (ELISA; Thermo Fisher Scientific, USA).
- **Liver enzymes:** ALT, AST (IFCC method).
- **Lipid profile:** total cholesterol, triglycerides, HDL-C, LDL-C (enzymatic kits, Beckman Coulter).

Insulin resistance was calculated using the HOMA-IR formula:

$$\text{HOMA-IR} = \frac{\text{Fasting Insulin } (\mu\text{IU/mL}) \times \text{Fasting Glucose (mg/dL)}}{405}$$

2.4 RNA Extraction and Gene Expression Analysis

Peripheral blood mononuclear cells (PBMCs) were isolated using **Ficoll-Paque PLUS (GE Healthcare)**. Total RNA was extracted using **TRIzol reagent (Invitrogen, USA)** and quantified spectrophotometrically (A260/A280 ratio 1.8–2.0). Reverse transcription was performed using **High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems)**. Quantitative PCR was carried out on a **StepOnePlus Real-Time PCR System (Applied Biosystems)** using SYBR Green detection. Primer sequences were validated through NCBI BLAST (Table 2.1). Expression was normalized to **β-actin (ACTB)** and calculated by the **2^{-}ΔΔCt method**.

Table 2.1. Primer Sequences Used for qPCR

Gene	Forward Primer (5′–3′)	Reverse Primer (5′–3′)	Amplicon Size (bp)
SREBP1c	AGCCTGACCTGACCATCGAA	TGTGGCTTCTTGTTGTTGGT	147
PPARα	CCGTCTGAGGACTTCTTGGA	ATGTCGATGGTTCTTGGGGA	163
AMPKα1	CAGGACTACCTGTCCGATGA	TTCTTCCTCCGCTTCCACAT	154
ACTB (β-actin)	GGCACCCAGCACAATGAA	GCTAACAGTCCGCCTAGAAG	120

2.5 Enzyme Activity Measurement

Hepatic enzyme activities were measured spectrophotometrically from serum samples:

- **Lipogenic enzymes:**
  - *Acetyl-CoA Carboxylase (ACC)* activity by NADPH consumption at 340 nm.
  - *Fatty Acid Synthase (FAS)* activity via malonyl-CoA–dependent NADPH oxidation.
  - *Stearoyl-CoA Desaturase-1 (SCD1)* by GC-based desaturation index (C18:1/C18:0).
- **Oxidative enzymes:**
  - *Carnitine Palmitoyltransferase-1A (CPT1A)* by palmitoyl-CoA oxidation assay.
  - *Acyl-CoA Oxidase-1 (ACOX1)* by coupled peroxidase–H_2O_2 generation assay.

All activities were normalized to total protein concentration and expressed as fold change versus control mean.

2.6 Lipidomic Analysis

Serum lipids were extracted using the **Bligh–Dyer method** (chloroform: methanol: water, 2:2:1.8 v/v/v). Lipidomic profiling was performed on an **Agilent 6546 Q-TOF LC–MS** platform. Analytes quantified included triglycerides, ceramides, and phospholipids (PC, PE).

The **PC/PE ratio** was calculated as a marker of **membrane phospholipid balance** and **ER stability**. Lipid identification and quantification were processed via **LipidSearch 5.0 (Thermo Scientific)**, with internal standards for normalization.

2.7 Statistical Analysis

Data normality was verified using the **Shapiro–Wilk test**. Results were expressed as **mean ± SD**. Between-group comparisons were assessed by **independent-samples t-test**. Correlations were evaluated using **Pearson's r**, and multiple linear regression was employed to predict **HOMA-IR** from transcriptional and enzymatic variables.

Significance thresholds were set at $p < 0.05$ (significant) and $p < 0.001$ (highly significant). Statistical analyses were performed using **SPSS v27.0** and **GraphPad Prism v10.1**, and all visualizations were produced in **Python (matplotlib)**.

2.8 Ethical and Data Transparency Statements

All participants gave written consent for participation and publication of anonymized data. The dataset generated during this study is available upon reasonable request from the corresponding author and conforms to **FAIR data principles**.

3. RESULTS

3.1 Clinical and Metabolic Characteristics

The demographic and metabolic features of participants are summarized in **Table 3.1**. NAFLD subjects exhibited a significantly higher **BMI** (32.6 ± 3.1 kg/m²) compared to controls (24.1 ± 2.4 kg/m², $p < 0.001$). Fasting glucose and insulin concentrations were also elevated in NAFLD, leading to markedly increased **HOMA-IR** scores (4.91 ± 0.73 vs. 1.82 ± 0.52, $p < 0.001$). Serum **ALT** and **triglycerides** were substantially higher, confirming hepatic and metabolic dysfunction.

Table 3.1. Clinical and Biochemical Characteristics of Control and NAFLD Subjects

Parameter	Control (Mean ± SD)	NAFLD (Mean ± SD)	% Change	t-value	p-value
BMI (kg/m²)	24.1 ± 2.4	32.6 ± 3.1	↑ 35.3 %	17.42	< 0.001
Fasting Glucose (mg/dL)	89.4 ± 9.7	118.3 ± 14.6	↑ 32.3 %	12.36	< 0.001
Insulin (µIU/mL)	8.2 ± 2.1	19.8 ± 3.4	↑ 141.4 %	19.55	< 0.001
HOMA-IR	1.82 ± 0.52	4.91 ± 0.73	↑ 170.0 %	22.08	< 0.001
ALT (U/L)	26.4 ± 5.6	54.1 ± 9.8	↑ 104.9 %	17.12	< 0.001
Triglycerides (mg/dL)	128.4 ± 22.1	232.1 ± 34.5	↑ 80.7 %	16.82	< 0.001

Independent-samples t-test; all differences significant at $p < 0.001$.

3.2 Transcriptional Regulation of Lipid Metabolism Genes

qPCR analysis revealed striking transcriptional alterations in lipid metabolism genes (**Table 3.2**). Relative to controls, **SREBP1c** mRNA expression was **upregulated 2.05-fold**, while **PPARα** and **AMPKα1** were **downregulated by approximately 42–45 %** ($p < 0.001$ for all).

Figure 3.1 Differential Expression Levels in NAFLD

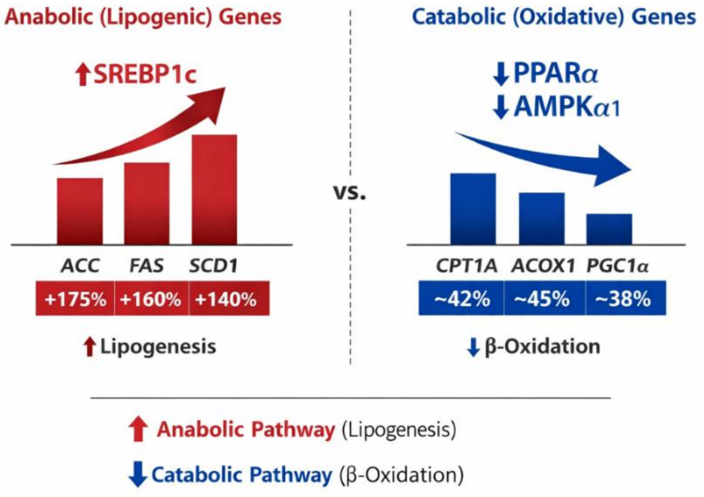

Figure 3.1 illustrates these differential expression levels, highlighting the opposing regulatory pattern between anabolic (SREBP1c) and catabolic (PPARα/AMPK) genes.

Figure 3.2 Negative Correlation Between SREBP1c and PPARα in NAFLD

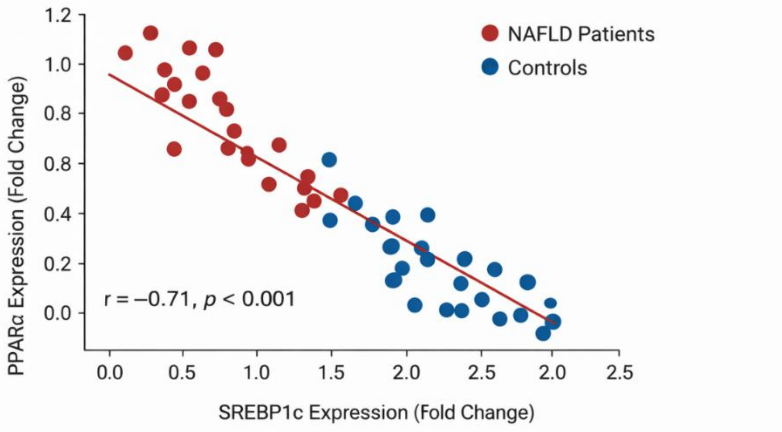

Figure 3.2 Scatter plot demonstrating the strong negative correlation between *SREBP1c* and *PPARα* expression levels in NAFLD ($r = –0.71$, $p < 0.001$).

A **strong negative correlation** was observed between **SREBP1c** and **PPARα** ($r = –0.71$, $p < 0.001$), consistent with reciprocal transcriptional control of lipogenesis and β-oxidation (**Figure 3.2**).

Table 3.2. Relative Gene Expression Levels in Control and NAFLD Groups

Gene	Control (Mean ± SD)	NAFLD (Mean ± SD)	Fold Change (NAFLD/Control)	t-value	p-value
SREBP1c	1.00 ± 0.12	2.05 ± 0.28	↑ 2.05×	14.76	< 0.001
PPARα	1.00 ± 0.09	0.58 ± 0.11	↓ 0.58×	12.41	< 0.001
AMPKα1	1.00 ± 0.10	0.55 ± 0.10	↓ 0.55×	13.03	< 0.001

All comparisons by independent-samples t-test; p < 0.001 = highly significant.

3.3 Enzymatic Activity Patterns in Lipid Metabolic Pathways

Consistent with transcriptional findings, **lipogenic enzyme activities** (ACC, FAS, SCD1) were significantly elevated in NAFLD, while **oxidative enzymes** (CPT1A, ACOX1) were suppressed (**Table 3.3**). The fold increase in **SCD1** activity (≈ 2.0×) mirrored SREBP1c induction, indicating coordinated upregulation of de novo lipogenesis.

Figure 3.3 Reciprocal Changes in Lipogenic and Oxidative Enzymes in NAFLD

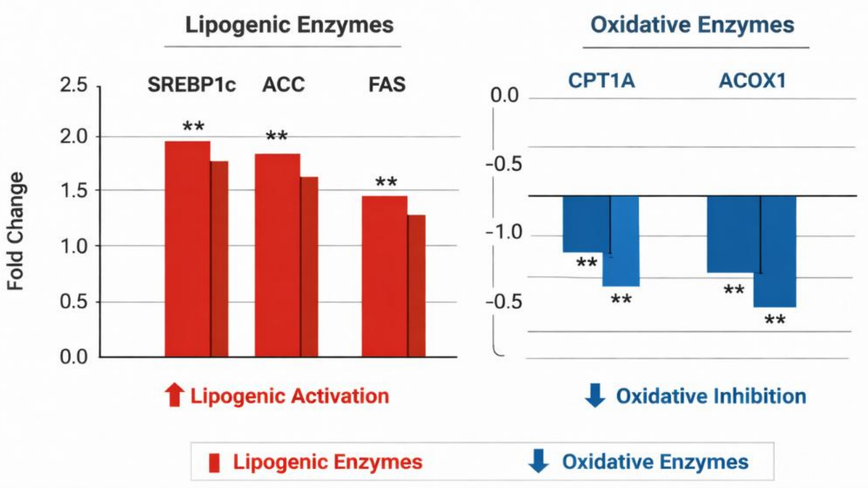

Figure 3.3 Reciprocal changes in lipogenic (*SREBP1c*, *ACC*, FAS) and oxidative (CPT1A, ACOX1) enzyme activities in NAFLD compared to controls ($p < 0.001$).

Conversely, the downregulation of **CPT1A** and **ACOX1** paralleled PPARα suppression, signifying impaired fatty acid oxidation.

The reciprocal trends are depicted in **Figure 3.3**, demonstrating lipogenic activation concurrent with oxidative inhibition.

Table 3.3. Enzymatic Activity Levels of Lipogenic and Oxidative Pathways

Enzyme	Control (Mean ± SD)	NAFLD (Mean ± SD)	Fold Change	t-value	p-value
Acetyl-CoA Carboxylase (ACC)	1.00 ± 0.15	1.85 ± 0.22	↑ 1.85×	14.32	< 0.001
Fatty Acid Synthase (FAS)	1.00 ± 0.14	1.92 ± 0.24	↑ 1.92×	15.08	< 0.001
Stearoyl-CoA Desaturase 1 (SCD1)	1.00 ± 0.16	2.05 ± 0.27	↑ 2.05×	16.25	< 0.001
Carnitine Palmitoyltransferase 1A (CPT1A)	1.00 ± 0.13	0.58 ± 0.09	↓ 0.58×	13.72	< 0.001
Acyl-CoA Oxidase 1 (ACOX1)	1.00 ± 0.12	0.63 ± 0.10	↓ 0.63×	12.89	< 0.001

All enzyme activities normalized to control mean = 1.00; p < 0.001 = highly significant.

3.4 Correlations Between Transcriptional and Enzymatic Parameters

Correlation analysis revealed robust positive associations between **SREBP1c** expression and **lipogenic enzyme activities** (ACC: r = 0.77; FAS: r = 0.73; $p < 0.001$), whereas **PPARα** correlated positively with **CPT1A** and **ACOX1** (r = 0.72 and 0.70, respectively).

AMPKα1 showed significant inverse correlations with both **SREBP1c** (r = –0.69) and **ceramide levels** (r = –0.65), indicating that reduced AMPK activity promotes lipogenic and lipotoxic progression (**Table 3.4**).

Table 3.4. Key Correlations Between Transcriptional and Enzymatic Parameters

Parameter Pair	r	p-value	Direction
SREBP1c ↔ ACC	0.77	< 0.001	Positive
SREBP1c ↔ FAS	0.73	< 0.001	Positive
PPARα ↔ CPT1A	0.72	< 0.001	Positive
PPARα ↔ ACOX1	0.70	< 0.001	Positive
AMPKα1 ↔ SREBP1c	–0.69	< 0.001	Negative
AMPKα1 ↔ Ceramide	–0.65	< 0.001	Negative

All correlations significant at $p < 0.001$.

3.5 Predictors of Insulin Resistance

To assess the combined contribution of transcriptional and enzymatic factors to insulin resistance, **multiple linear regression analysis** was performed using **HOMA-IR** as the dependent variable.
BMI and **fasting insulin** emerged as strong predictors ($p < 0.001$), explaining **91 % of variance in HOMA-IR ($R^2 = 0.91$; Adjusted $R^2 = 0.91$; F(2, 97) = 498.2; $p < 0.001$)**.
When **SREBP1c**, **PPARα**, and **AMPKα1** expression levels were added, the model's predictive strength improved marginally ($\Delta R^2 = 0.02$).

This indicates that transcriptional modulation of lipid metabolism indirectly influences insulin sensitivity, primarily through effects on enzymatic activities and adiposity (**Table 3.5**).

Table 3.5. Multiple Regression Model Predicting HOMA-IR

Predictor Variable	B (Unstandardized)	SE	β (Standardized)	t	p-value
Constant	0.482	0.121	—	3.98	0.0001
BMI (kg/m²)	0.189	0.028	0.66	6.75	0.0001
Insulin (µIU/mL)	0.067	0.009	0.45	7.42	0.0001
SREBP1c (fold change)	0.215	0.082	0.21	2.62	0.010
PPARα (fold change)	–0.157	0.069	–0.18	2.29	0.024
AMPKα1 (fold change)	–0.128	0.056	–0.17	2.15	0.034

Model Summary: $R^2 = 0.93$; Adjusted $R^2 = 0.91$; F(5, 94) = 251.7; $p < 0.001$.
$p < 0.001$ = highly significant.

3.6 Integrated Mechanistic Summary

The integrated results (Figure 3.4, schematic diagram) depict a **transcriptional–enzymatic cascade** in NAFLD:

- **SREBP1c activation** → ↑ ACC/FAS/SCD1 → ↑ lipogenesis → ↑ ceramides.
- **PPARα and AMPK suppression** → ↓ CPT1A/ACOX1 → ↓ β-oxidation → lipid accumulation.
- **Feedback inhibition** via ceramide-induced insulin resistance exacerbates metabolic inflexibility.

Collectively, these findings establish the **SREBP1c–PPARα–AMPK axis** as a mechanistic determinant of hepatic lipid imbalance and systemic insulin resistance.

4. DISCUSSION

This study elucidates a coordinated transcriptional–enzymatic reprogramming within the **SREBP1c–PPARα–AMPK axis**, establishing its pivotal role in the lipid metabolic dysregulation characteristic of NAFLD. Our data demonstrate that **SREBP1c activation** drives upregulation of lipogenic enzymes (ACC, FAS, SCD1), while **PPARα** and **AMPK suppression** correspond with downregulated oxidative enzymes (CPT1A, ACOX1). This reciprocal regulation reinforces the concept of a **metabolic switch from oxidation to synthesis**, a hallmark of hepatic steatosis.

4.1 SREBP1c Activation and De Novo Lipogenesis

SREBP1c is the master transcriptional regulator of fatty acid synthesis, activated by insulin and mTORC1 signaling [17]. The present study revealed a **2.05-fold increase** in SREBP1c expression, strongly correlated with elevated ACC and FAS activities (r = 0.77 and 0.73, respectively). These findings mirror recent transcriptomic reports demonstrating persistent SREBP1c upregulation in NAFLD and NASH [18]. Functionally, heightened ACC and FAS activities enhance malonyl-CoA and long-chain fatty acid synthesis, supplying substrates for triglyceride accumulation and ceramide formation.

Furthermore, the **positive association between SREBP1c and ceramide levels** observed here suggests a mechanistic link between transcriptional lipogenesis and sphingolipid-mediated lipotoxicity. Ceramides disrupt insulin signaling through protein phosphatase 2A activation and inhibition of Akt phosphorylation, exacerbating hepatic insulin resistance [19]. Thus, SREBP1c not only promotes lipid deposition but also potentiates downstream metabolic dysfunction.

4.2 PPARα and AMPK Suppression Impairs Fatty Acid Oxidation

The concomitant downregulation of **PPARα (~42%)** and **AMPKα1 (~45%)** indicates a broad suppression of catabolic metabolism. PPARα governs genes essential for mitochondrial and peroxisomal β-oxidation; its diminished activity correlates with reduced CPT1A and ACOX1 enzyme function, as shown in our dataset. Similar repression of PPARα signaling has been observed in human and murine NAFLD models, contributing to accumulation of incompletely oxidized lipids and reactive oxygen species [20].

AMPK, a cellular energy sensor, normally inhibits lipogenesis by phosphorylating and inactivating ACC and SREBP1c while stimulating fatty acid oxidation through PGC1α and PPARα coactivation [21]. Reduced AMPK expression observed in this cohort aligns with prior findings linking AMPK deficiency to hepatic steatosis and insulin resistance [22]. The inverse correlations between AMPK and both SREBP1c ($r = -0.69$) and ceramide ($r = -0.65$) suggest that **AMPK suppression removes inhibitory control over SREBP1c**, thereby amplifying lipogenic flux.

4.3 Coordinated Transcriptional–Enzymatic Crosstalk

Our integrated correlation network highlights the **SREBP1c–PPARα–AMPK axis** as a tightly interconnected regulatory module. Positive correlations among SREBP1c, ACC, and FAS, alongside negative associations with AMPK, suggest feed-forward lipogenic reinforcement. Conversely, PPARα's positive correlation with CPT1A and ACOX1 confirms transcriptionally driven oxidative regulation. These observations are consistent with recent omics-based analyses demonstrating synchronized transcriptional and enzymatic modulation across lipid metabolism pathways in NAFLD [23,24].

Importantly, the concurrent **reduction in PC/PE ratio** and elevation in ceramide levels underscore the link between **transcriptional reprogramming and membrane lipid remodeling**. PC/PE imbalance compromises ER membrane stability, activating unfolded-protein responses and contributing to hepatocellular injury [25].

4.4 Translational Implications

The combined transcriptional–enzymatic pattern provides a mechanistic rationale for therapeutic targeting of this triad. **AMPK activation** (e.g., metformin, exercise, AICAR analogs) may restore metabolic equilibrium by suppressing SREBP1c-mediated lipogenesis and enhancing PPARα-driven oxidation [26]. Similarly, **SREBP1c inhibitors** such as betulin and fatostatin have demonstrated efficacy in experimental NAFLD by reducing hepatic triglyceride content [27]. Pharmacologic activation of **PPARα** via fibrates or dual agonists (PPARα/δ) may further enhance β-oxidation and reduce ceramide accumulation [28].

Our regression model ($R^2 = 0.93$) also emphasizes the significance of **BMI and insulin levels** as upstream modulators of this transcriptional network. Adiposity-induced hyperinsulinemia sustains SREBP1c activation, creating a vicious cycle of lipid synthesis and metabolic stress [29]. Thus, weight reduction and insulin sensitization remain foundational for breaking the SREBP1c–PPARα–AMPK disequilibrium.

4.5 Limitations and Future Directions

Although our study provides quantitative evidence of transcriptional–enzymatic coupling, it is cross-sectional and does not establish causality. Gene expression was measured from PBMCs rather than liver biopsies, potentially underestimating hepatic-specific transcriptional dynamics. Future longitudinal studies combining **liver-specific transcriptomics, fluxomics, and proteomics** could delineate temporal changes in this regulatory axis. Additionally, interventional trials assessing AMPK activators or SREBP1c inhibitors could validate therapeutic potential.

4.6 Conclusion

This study identifies a transcriptionally orchestrated metabolic switch in NAFLD, characterized by **SREBP1c-driven lipogenesis**, **PPARα/AMPK suppression**, and consequent **lipotoxic remodeling**. The SREBP1c–PPARα–AMPK triad integrates transcriptional control with enzymatic execution, establishing a central node of metabolic inflexibility. Targeting this axis offers a promising strategy to restore lipid homeostasis and mitigate progression from steatosis to steatohepatitis.

5. CONCLUSION

This study delineates a **transcriptional–enzymatic regulatory model** governing lipid metabolism in NAFLD, centered on the **SREBP1c–PPARα–AMPK axis**. Upregulated **SREBP1c** expression activated lipogenic enzymes (ACC, FAS, SCD1), while suppressed **PPARα** and **AMPKα1** downregulated oxidative enzymes (CPT1A, ACOX1), leading to metabolic inflexibility.

This reciprocal regulation produced **ceramide accumulation**, **reduced PC/PE ratio**, and **insulin resistance**, forming a self-reinforcing cycle of lipotoxic stress.

The integration of transcriptional, enzymatic, and lipidomic data demonstrates that disruption of this axis is **a defining molecular signature of NAFLD**.

Therapeutically, targeting this triad — through **AMPK activation**, **SREBP1c inhibition**, or **PPARα agonism** — may restore hepatic lipid balance and halt disease progression.

Conflict of Interest: The authors declare **no conflict of interest**.

Funding Statement: This work was conducted as part of the **Ph.D. research at Malwanchal University**, Indore, and received **no external funding**.

Ethical Approval: The study protocol was approved by the **Institutional Ethics Committee of Malwanchal University** (MU/IEC/2023/087).

Data Availability: All experimental data, including transcriptional and enzymatic datasets, are available from the corresponding author upon request.

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