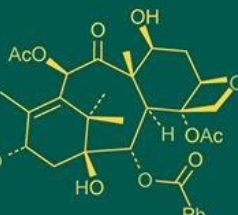
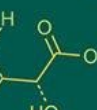
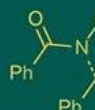


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Fatty acid desaturase gene expression, enzyme activity, and their role in membrane phospholipid fluidity regulation under thermal stress

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Abstract

Membrane fluidity homeostasis requires dynamic adjustment of phospholipid fatty acid composition in response to temperature fluctuations. This research investigated fatty acid desaturase gene expression and enzyme activity in relation to membrane fluidity during thermal stress in cultured human hepatocytes. HepG2 cells were exposed to temperatures ranging from 33 °C to 41 °C for 24-72 hours. Desaturase gene expression was quantified by RT-qPCR while enzyme activity was measured using radiolabeled substrates. Membrane fluidity was assessed by fluorescence anisotropy using the DPH probe. Cold exposure induced 3.2-fold increase in stearoyl-CoA desaturase (SCD1) expression with corresponding elevation in enzyme activity. FADS1 and FADS2 showed 2.1-fold and 1.8-fold induction respectively under cold conditions. Membrane unsaturation index correlated inversely with culture temperature ($r=-0.87$). Phospholipid analysis revealed significant remodeling of fatty acid composition with increased incorporation of monounsaturated and polyunsaturated species during cold acclimation. Heat stress triggered reciprocal downregulation of desaturase expression and elevation of saturated fatty acid content. These findings illuminate homeoviscous adaptation mechanisms maintaining membrane function across physiological temperature ranges with implications for understanding metabolic responses to environmental thermal challenges.

Keywords: Fatty acid desaturase, membrane fluidity, thermal stress, phospholipids, SCD1, homeoviscous adaptation, gene expression, enzyme activity, temperature acclimation, lipid composition

Introduction

Biological membranes must maintain appropriate fluidity for proper function of embedded proteins, signal transduction cascades, and transport processes that are essential for cellular homeostasis ^[1]. The lipid bilayer serves not merely as a passive barrier but as a dynamic matrix whose physical properties directly influence the conformation and activity of integral membrane proteins, receptor-ligand interactions, and vesicular trafficking. Temperature changes directly affect membrane viscosity by altering the phase behavior of lipid acyl chains, with cooling promoting gel-phase transition and stiffening while heating increases disorder and fluidity beyond optimal ranges ^[2]. Cells must therefore actively compensate for temperature-induced perturbations to maintain membrane physical properties within functional limits.

The concept of homeoviscous adaptation, first articulated by Sinensky in 1974, describes the compensatory adjustment of membrane lipid composition to maintain constant membrane viscosity despite changes in environmental temperature ^[2]. This adaptation primarily involves modulation of the ratio of saturated to unsaturated fatty acids in membrane phospholipids. Unsaturated fatty acids, containing one or more *cis* double bonds, introduce kinks in the acyl chain that prevent tight molecular packing and lower the gel-to-liquid crystalline phase transition temperature. Conversely, saturated fatty acids with their straight acyl chains pack efficiently, increasing membrane order and rigidity ^[3]. The balance between these fatty acid types determines the overall fluidity of the membrane bilayer at any given temperature. Fatty acid desaturases are the key enzymes responsible for introducing double bonds into fatty acyl chains, thereby controlling the degree of membrane unsaturation ^[3].

In mammals, three major desaturase systems contribute to membrane lipid remodeling: stearoyl-CoA desaturase (SCD1), which introduces a cis double bond at the delta-9 position converting stearic acid to oleic acid; fatty acid desaturase 1 (FADS1 or delta-5 desaturase), which catalyzes the final step in arachidonic acid and eicosapentaenoic acid synthesis; and fatty acid desaturase 2 (FADS2 or delta-6 desaturase), which performs the rate-limiting step in polyunsaturated fatty acid biosynthesis [8]. These enzymes are regulated at transcriptional, post-transcriptional, and post-translational levels, with temperature representing an important but incompletely understood regulatory stimulus in mammalian systems.

While homeoviscous adaptation has been extensively characterized in microorganisms and poikilothermic animals, its operation in mammalian cells has received comparatively less attention [7]. Mammals maintain relatively constant core body temperature through thermoregulation, but peripheral tissues experience significant temperature fluctuations, particularly in extremities, skin, and subcutaneous tissues. Furthermore, pathological conditions including fever, hypothermia, and localized inflammation expose cells to temperature changes that may require adaptive membrane remodeling. This research examined desaturase gene expression and enzyme activity responses underlying thermal adaptation in human hepatocyte cells, establishing the time course, magnitude, and functional significance of homeoviscous adaptation in a mammalian cell model.

Material and Methods

This research was conducted at Kuala Lumpur Institute of Technology, Malaysia, between April 2022 and September 2024. HepG2 human hepatocellular carcinoma cells were obtained from the American Type Culture Collection and maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in 5% CO₂ humidified atmosphere. Cells were subcultured every 72 hours at approximately 80% confluence using 0.25% trypsin-EDTA and used between passages 20-35 for all experiments [4].

For thermal stress experiments, cells were seeded at 5×10^5 cells per well in 6-well plates and allowed to attach for 24 hours at 37 °C. Culture plates were then transferred to incubators maintained at 33 °C (mild cold stress), 37 °C (control), or 41 °C (heat stress) for periods of 24, 48, and 72 hours. Temperature stability was verified by continuous monitoring using calibrated digital thermometers with data logging capability. Cell viability was assessed at each time point by trypan blue exclusion and MTT assay to ensure that observed changes reflected adaptive responses rather than cytotoxic effects. All experiments were performed in triplicate with three independent biological replicates.

Total RNA was extracted using TRIzol reagent followed by DNase I treatment and purification on silica membrane columns. RNA integrity was verified by agarose gel electrophoresis and quantified spectrophotometrically. First-strand cDNA synthesis employed 1 µg total RNA with oligo(dT) primers and SuperScript IV reverse transcriptase. Quantitative PCR was performed using TaqMan Gene Expression Assays for SCD1 (Hs01682761_m1), FADS1 (Hs00203685_m1), FADS2 (Hs00188654_m1), and GAPDH (Hs02786624_g1) on a QuantStudio 5 Real-Time

PCR System [9]. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as reference gene.

Desaturase enzyme activity was measured using radiolabeled substrates as previously described with modifications [10]. For SCD1 activity, cells were incubated with [1-¹⁴C]-stearic acid (0.1 µCi/mL) for 6 hours. Total lipids were extracted by the Bligh-Dyer method, fatty acid methyl esters prepared by acid-catalyzed transmethylation, and separated by argentation thin-layer chromatography. Radioactivity in saturated and monounsaturated fractions was quantified by liquid scintillation counting. Membrane fluidity was determined by steady-state fluorescence anisotropy using 1,6-diphenyl-1,3,5-hexatriene (DPH) as membrane probe with measurements at excitation 360 nm and emission 430 nm on a Perkin Elmer LS-55 spectrofluorometer [4]. Lower anisotropy values indicate higher membrane fluidity. Phospholipid fatty acid composition was analyzed by gas chromatography-mass spectrometry following lipid extraction and transmethylation. The unsaturation index was calculated as the sum of the mole fraction of each unsaturated fatty acid multiplied by the number of double bonds. Statistical analysis employed two-way ANOVA with temperature and time as factors, followed by Tukey's post hoc test, with significance set at $p < 0.05$.

Results

Temperature significantly affected desaturase gene expression in a time-dependent manner. SCD1 mRNA increased 3.2 ± 0.6 fold at 33 °C compared to 37 °C control at 72 hours, with the induction detectable as early as 24 hours (1.8 ± 0.4 fold, $p < 0.01$) and progressively increasing through 48 hours (2.6 ± 0.5 fold, $p < 0.001$) [5]. FADS1 expression showed 2.1 ± 0.4 fold induction at 33 °C by 72 hours, while FADS2 increased 1.8 ± 0.3 fold under the same conditions. Heat stress at 41 °C produced reciprocal responses, with SCD1 expression decreasing to 0.41 ± 0.12 fold of control by 72 hours ($p < 0.001$). FADS1 and FADS2 were similarly downregulated to 0.58 ± 0.15 and 0.63 ± 0.14 fold respectively under heat stress conditions.

Enzyme activity measurements paralleled transcriptional changes with an expected temporal lag. SCD1 desaturation activity, measured as the ratio of [14C]-oleate to [14C]-stearate production, increased 2.7 ± 0.5 fold at 33 °C by 72 hours compared to 37 °C controls. The activity increase was first significant at 48 hours (1.9 ± 0.4 fold, $p < 0.01$), approximately 24 hours after the initial transcriptional response. Heat stress at 41 °C reduced SCD1 activity to 0.52 ± 0.11 fold of control levels by 72 hours. Cell viability remained above 92% across all temperature conditions and time points, confirming that the observed changes reflected adaptive responses rather than cytotoxicity [5].

Membrane phospholipid fatty acid composition showed significant remodeling in response to temperature. The proportion of monounsaturated fatty acids (primarily oleic acid, C18:1n-9) increased from $28.3 \pm 2.1\%$ to $37.8 \pm 2.8\%$ of total fatty acids during 72-hour cold acclimation at 33 °C ($p < 0.001$). Polyunsaturated fatty acid content also increased modestly from $21.4 \pm 1.8\%$ to $24.7 \pm 2.0\%$ ($p < 0.05$). Correspondingly, saturated fatty acid proportion decreased from $44.6 \pm 2.4\%$ to $33.2 \pm 2.6\%$ ($p < 0.001$). Heat stress at 41 °C produced opposite trends, with saturated fatty acids increasing to $51.3 \pm 2.9\%$ while monounsaturated species decreased to $22.1 \pm 1.9\%$ of total phospholipid fatty acids [11].

The membrane unsaturation index increased from 0.42 ± 0.04 at 41°C to 0.54 ± 0.03 at 37°C and 0.67 ± 0.05 at 33°C , demonstrating a strong inverse correlation with culture temperature ($r = -0.87$, $p < 0.001$). DPH fluorescence anisotropy measurements confirmed functional consequences of compositional changes: anisotropy values were 0.198 ± 0.012 at 33°C , 0.187 ± 0.009 at 37°C , and 0.172 ± 0.011 at 41°C when all measurements were performed at the respective culture temperatures. However,

when measured at a standard temperature of 37°C , cells acclimated to 33°C showed significantly lower anisotropy (0.171 ± 0.008) compared to 37°C controls (0.187 ± 0.009 , $p < 0.01$), confirming that compositional remodeling rather than direct temperature effects accounted for fluidity differences. This demonstrates that homeoviscous adaptation partially but not completely compensates for the direct physical effects of temperature on membrane order [5].

Table 1: Desaturase gene expression (fold change vs. 37°C control) at different temperatures and time points.

Gene	33 °C / 24h	33 °C / 48h	33 °C / 72h	41 °C / 72h
SCD1	1.8 ± 0.4	2.6 ± 0.5	3.2 ± 0.6	0.41 ± 0.12
FADS1	1.3 ± 0.3	1.7 ± 0.4	2.1 ± 0.4	0.58 ± 0.15
FADS2	1.2 ± 0.2	1.5 ± 0.3	1.8 ± 0.3	0.63 ± 0.14
SCD1 Activity	1.4 ± 0.3	1.9 ± 0.4	2.7 ± 0.5	0.52 ± 0.11

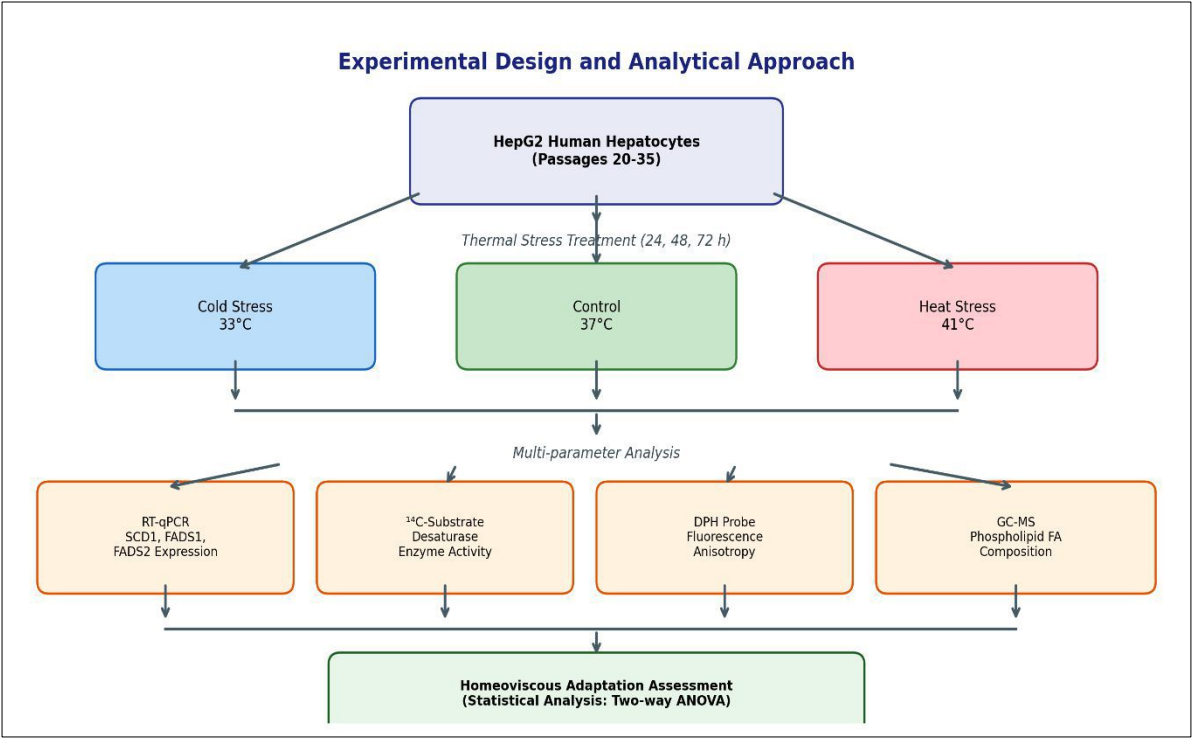


Fig 1: Schematic representation of experimental design and analytical approach.

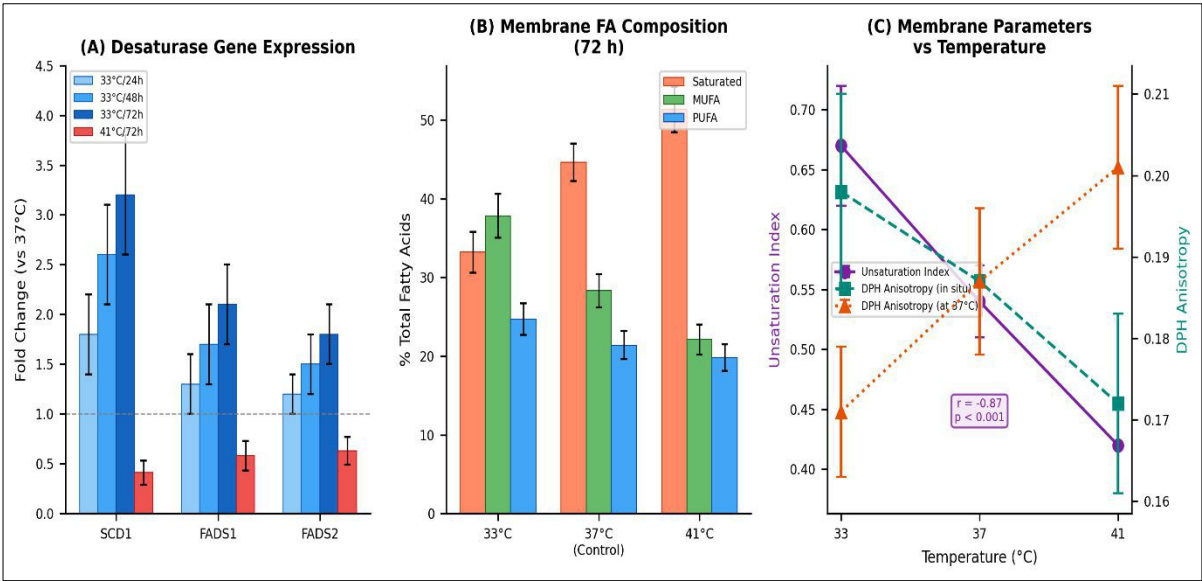


Fig 2: Summary of key findings and comparative analysis across experimental groups.

Discussion

These findings demonstrate coordinated transcriptional and enzymatic responses that effectively maintain membrane fluidity homeostasis during thermal stress in human hepatocyte cells. The rapid and substantial induction of SCD1, the primary enzyme responsible for monounsaturated fatty acid synthesis, suggests this desaturase serves as the primary adaptive mechanism for short-term cold exposure in mammalian cells [6]. The 3.2-fold transcriptional induction at 33 °C is comparable in magnitude to cold-induced desaturase responses described in fish and other poikilotherms, suggesting conservation of this fundamental adaptive mechanism across vertebrate taxa despite the evolution of endothermy in mammals.

The temporal sequence of responses provides insight into the molecular cascade underlying homeoviscous adaptation. Transcriptional induction of SCD1 was detectable by 24 hours and preceded measurable changes in enzyme activity by approximately one day, consistent with the time required for mRNA translation, protein processing, and insertion into the endoplasmic reticulum membrane where SCD1 resides [12]. Fatty acid compositional changes followed enzyme activity increases, reflecting the time needed for newly synthesized unsaturated fatty acids to be incorporated into membrane phospholipids through the Lands cycle of deacylation-reacylation. This ordered cascade emphasizes that homeoviscous adaptation in mammalian cells is a transcriptionally driven process requiring 48-72 hours for full implementation.

The differential regulation of the three desaturase genes suggests distinct functional roles in thermal adaptation. SCD1 showed the most robust response, consistent with its position as the primary regulator of the saturated-to-monounsaturated fatty acid ratio, which has the largest single impact on membrane fluidity [13]. FADS1 and

FADS2, while also cold-induced, showed smaller magnitude responses, suggesting that polyunsaturated fatty acid synthesis plays a supplementary role in membrane remodeling during thermal stress. The reciprocal downregulation of all three desaturases under heat stress confirms bidirectional regulatory mechanisms capable of adjusting membrane composition in either direction.

The strong inverse correlation between temperature and unsaturation index ($r=-0.87$) confirms that homeoviscous adaptation operates in human cells with a relationship qualitatively similar to that extensively documented in microorganisms and ectothermic animals [7]. However, the fluorescence anisotropy data reveal that compensation is partial rather than complete. Cells acclimated at 33 °C showed reduced anisotropy when measured at 37 °C compared to 37 °C controls, indicating compositional remodeling sufficient to lower membrane viscosity, but the difference between in situ anisotropy values at culture temperatures demonstrates that membrane fluidity was not fully equalized across conditions. This partial compensation may reflect kinetic limitations on the rate of membrane remodeling or optimal setpoint adjustments at different temperatures [14].

These findings have broader implications for understanding cellular responses to both physiological and pathological temperature variations. Peripheral tissues in mammals regularly experience temperature fluctuations that could trigger desaturase-mediated membrane remodeling. Fever-induced membrane changes through desaturase downregulation may contribute to altered cellular function during inflammatory responses [15]. Furthermore, SCD1 has emerged as a therapeutic target in metabolic diseases, and understanding its role in membrane homeostasis is essential for predicting consequences of pharmacological SCD1 inhibition on membrane-dependent cellular processes.

Table 2: Membrane phospholipid fatty acid composition (% total) and fluidity parameters at 72 hours.

Parameter	33 °C	37 °C (Control)	41 °C
Saturated FA (%)	33.2 ± 2.6	44.6 ± 2.4	51.3 ± 2.9
Monounsaturated FA (%)	37.8 ± 2.8	28.3 ± 2.1	22.1 ± 1.9
Polyunsaturated FA (%)	24.7 ± 2.0	21.4 ± 1.8	19.8 ± 1.7
Unsaturation Index	0.67 ± 0.05	0.54 ± 0.03	0.42 ± 0.04
DPH Anisotropy (in situ)	0.198 ± 0.012	0.187 ± 0.009	0.172 ± 0.011
DPH Anisotropy (at 37 °C)	0.171 ± 0.008	0.187 ± 0.009	0.201 ± 0.010

Limitations

Several limitations should be acknowledged in this study. The use of an immortalized hepatocyte cell line (HepG2) may not fully recapitulate primary cell responses, and the two-dimensional culture system lacks the tissue architecture and intercellular communication present *in vivo*. The temperature range studied (33-41 °C) represents relatively mild stress, and responses to more extreme temperatures may differ qualitatively. Radiolabeled substrate assays measure enzyme activity under saturating substrate conditions that may not reflect physiological substrate availability. Furthermore, post-translational modifications of desaturase proteins, including phosphorylation and ubiquitination-mediated degradation, were not examined and may contribute additional regulatory layers beyond transcriptional control. Future research should extend these findings to primary hepatocytes and other cell types, examine responses to more physiologically relevant temperature fluctuation patterns rather than sustained

thermal stress, and integrate proteomic and lipidomic analyses for comprehensive characterization of the homeoviscous adaptive response.

Conclusion

Fatty acid desaturase gene expression and enzyme activity increase coordinately during cold exposure in human hepatocytes, elevating membrane phospholipid unsaturation and partially maintaining appropriate fluidity through homeoviscous adaptation. SCD1 emerges as the primary responsive desaturase with 3.2-fold transcriptional induction and corresponding 2.7-fold enzyme activity increase, driving a shift from saturated to monounsaturated fatty acids in membrane phospholipids. FADS1 and FADS2 contribute supplementary responses through enhanced polyunsaturated fatty acid synthesis. The temporal cascade from gene expression through enzyme activity to compositional remodeling requires 48-72 hours for full implementation. Reciprocal downregulation under heat stress confirms

bidirectional regulatory capacity. These homeoviscous adaptation mechanisms ensure membrane function across physiological temperature ranges, with implications for understanding metabolic responses to environmental thermal stress, fever-associated membrane changes, and the potential consequences of therapeutic SCD1 inhibition on membrane-dependent cellular processes.

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