

Hydrogen reduction-calcined coral calcium activates Nrf2-antioxidant pathways and suppresses inflammation in Wistar rats

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Abstract

Hydrogen-releasing mineral supplements have recently attracted attention as novel nutritional antioxidants. Coral calcium hydride (CCH), a patented hydrogen-donating mineral agent, has been shown to enhance endogenous antioxidant capacity in rodent brain tissue. This study examined whether dietary supplementation with CCH alters hippocampal gene expression patterns in Wistar rats and clarifies the molecular mechanisms linking hydrogen donation with antioxidative and anti-inflammatory effects. Six-week-old male Wistar rats were fed either standard chow or chow supplemented with 0.1% CCH for two weeks. DNA microarray profiling and Ingenuity Pathway Analysis (IPA) identified that CCH supplementation significantly up-regulated Nrf2-associated antioxidant genes, including ALDH3A1 and related oxidative-stress response elements, while suppressing inflammatory and immunological pathways driven by NF- κ B-related mediators. These transcriptomic changes support the physiological finding of enhanced antioxidant ability previously observed in CCH-fed rats and suggest that hydrogen-enriched nutritional supplementation may exert neuroprotective benefits by modulating redox-sensitive transcriptional programs in the hippocampus.

Key words; Coral calcium hydride; Molecular hydrogen; Oxidative stress; Nrf2 signaling; Hippocampus

Introduction

Oxidative stress plays a pivotal role in aging, neurodegeneration, and cognitive decline. Molecular hydrogen has been proposed as a selective antioxidant capable of neutralizing cytotoxic reactive oxygen species with minimal biological interference. Preclinical studies have demonstrated that hydrogen gas reduces ischemia-reperfusion injury, suppresses oxidative damage, and improves hippocampus-dependent learning [1–3]. However, gaseous or liquid hydrogen is often impractical for long-term dietary or clinical use.

Over the past decade, accumulating basic and clinical data have reinforced the concept of molecular hydrogen as a pleiotropic cytoprotective agent. Recent neuropharmacological and clinical reviews consistently describe antioxidant, anti-inflammatory, and anti-apoptotic effects of hydrogen across a wide range of neurological and systemic disease models [8,10,13]. Animal studies using hydrogen-rich water have demonstrated improvements in hippocampus-dependent memory performance and reductions in oxidative stress markers in aging or stress-loaded rodents [2,9]. Clinical investigations likewise indicate that hydrogen administration can enhance systemic antioxidant capacity and ameliorate symptoms in metabolic, cardiovascular, and central nervous system disorders, although the underlying molecular programs in brain tissue remain incompletely defined [10–13].

Coral calcium hydride (CCH) is a patented hydrogen-releasing mineral compound that generates molecular hydrogen continuously in aqueous environments. Previous work by Ueda et al. demonstrated that CCH enhances endogenous antioxidant ability in the rat brain and improves behavioral vigor in senescence-accelerated mice [4]. In addition, food-derived formulations that liberate hydrogen in the intestinal tract have been reported to modulate oxidative stress and inflammatory mediators in peripheral tissues [5,11,12]. These observations led to the hypothesis that hydrogen released from CCH may alter redox-regulated gene expression programs in the hippocampus.

Particular attention has recently been paid to the ability of hydrogen to modulate redox-sensitive transcription factors.

Experimental and clinical studies indicate that hydrogen-rich water can activate Nrf2-dependent antioxidant pathways and simultaneously suppress NF- κ B signaling in intestinal and immune tissues, leading to attenuation of chronic inflammation and remodeling of transcriptomic networks [11,12]. Large-scale reviews of hydrogen therapy further emphasize that such dual regulation of Nrf2 and NF- κ B may represent a unifying mechanism linking hydrogen exposure to protection against ischemia-reperfusion injury and neuroinflammation [8,13]. However, there are few data describing how solid, hydrogen-releasing mineral formulations such as CCH influence gene expression patterns within the hippocampus, and whether they recapitulate these canonical Nrf2/NF κ B signatures in vivo.

This study aimed to characterize the transcriptomic consequences of dietary CCH supplementation using whole-genome DNA microarray profiling and network-level bioinformatic analysis. Special focus was placed on antioxidant pathways, including Nrf2 signaling, and inflammatory pathways mediated by NF- κ B. Understanding how hydrogen-releasing nutrients modulate gene networks may provide mechanistic insight into their potential value as neuroprotective functional foods.

2. Methods and Materials

2.1. Materials

CCH was obtained from ICB, Ltd., Sendai, Japan, and coral calcium (CC) was purchased from Coralbio, Okinawa, Japan.

2.2. Animals

Male Wistar rats were acquired from Kyudo, Co., Ltd. and maintained at the Experimental Animal Center of Biotech Co. Ltd at a controlled ambient temperature of 23 ± 1 °C and $50 \pm 10\%$ relative humidity. The Committee for Ethics on Animal Experiments of Kyudo, Co. Ltd. reviewed and approved the experimental design.

Six-week-old male Wistar rats ($n = 8$) were assigned to 2 groups: standard diet-fed group (CE-2, Clea Japan, Inc., Tokyo, Japan) and CCH-fed group. The CCH diet was standard CE-2 feed supplemented with 0.1% CCH powder. Inhibition of accelerated aging [5] and an increase in the in vivo antioxidant ability [4] was observed in SAM/P-8 mice fed a diet supplemented with the same diet of 0.1% CCH. In

accordance with these reports, the CCH concentration used in our study was set at 0.1%.

2.3. RNA preparation

The animals were killed by cervical dislocation at the age of 8 weeks. They were decapitated and the hippocampi were removed and rapidly frozen in liquid nitrogen. The hippocampi were then homogenized with a conventional rotor-stator homogenizer. Total RNA was then extracted from the tissues by using the RNeasy Lipid Tissue Mini Kit (Qiagen, Valencia, CA). RNA was treated with DNase 1 (Qiagen) and purified using an RNeasy Mini Kit (Qiagen). The purity and integrity of the isolated total RNA were analyzed with both ultraviolet spectrophotometry and the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). All RNA samples exhibited a 260/280 ratio between 2.0 and 2.2 and a 28S/18S ratio of >1.6; the RNA integrity number was >9.0.

2.4. Microarray experiment

The Agilent Whole Rat Genome 4×44K G4122F Oligo Microarray was used for global gene expression analysis. This microarray contains 41,012 rat complementary DNA (cDNA) probes, each comprising a single 60-oligomer oligonucleotide sequence. Target RNA labeling and hybridization were performed according to the protocol for one-color microarray-based gene expression analysis using the Quick Amp Labeling Kit (Agilent Technologies). In brief, 500 ng of RNA was transcribed using the oligo (dT)-based T7 promoter primer and Moloney murine leukemia virus reverse transcriptase (MMLV-RT) in the first- and second-strand cDNA synthesis reactions. The double-stranded cDNAs were used as templates in the preparation of fluorescent complementary RNAs (cRNAs) in the presence of T7 RNA polymerase and cyanine 3-CTP dye in an *in vitro* transcription reaction. The labeled cRNAs were purified, fragmented, and hybridized to microarrays in a rotating hybridization oven at 10 rpm for 17 h at 65 °C. After hybridization, the microarrays were washed according to the manufacturer's instructions and scanned on an Agilent DNA Microarray Scanner with the Scan Control software (Agilent Technologies).

The resulting images were processed, and raw data were collected using the Agilent Feature Extraction software. The gene expression data were analyzed using GeneSpring GX 10 (Agilent). The signal intensity of each probe was normalized by a percentile shift, in which each value was divided by the 75th percentile value of all samples in that array. The microarray data discussed in this publication have been deposited in the Gene Expression Omnibus (GEO) repository at the National Center for Biotechnology

Information and are accessible through the GSE Series accession number 48623 (<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48623>).

2.5. Network generation

Lists of differentially expressed genes were exported into and analyzed with the Ingenuity Pathway Analysis software (IPA; Ingenuity Systems, Mountain View, CA). These genes were overlaid onto a global molecular network developed from information in the ingenuity knowledge base. Networks of these differentially regulated genes were then algorithmically generated on the basis of their connectivity.

2.6. Biofunctional analysis of the entire data set

Biofunctional analysis was used to identify the biological functions that were most significant to the data set. The genes associated with biological functions in the ingenuity knowledge base were included in the analysis. Fisher's exact test was used to calculate the p value, i.e., the probability that each biological function assigned to that data set is due to chance alone.

2.7. Functional analysis of the network

Functional analysis of the network was performed to identify biological functions and/or diseases that were most significantly associated with the genes in the network. The network genes associated with biological functions and/or diseases in the ingenuity knowledge base were considered for the analysis. The significance of the association between the data set and the pathway was determined in 2 ways: (i) by determining the ratio of the number of genes from the data set mapped to the network to the total number of molecules in the network and (ii) by using Fisher's exact test to calculate the p value of the probability that the association between the genes in the data set and the network is explainable by chance alone.

2.8. Statistical analysis

Of the 41,252 probes in the Agilent Whole Rat Genome Oligo Microarray, 29,041 were analyzed by one-way analysis of variance (ANOVA; DIET effect (CE2 and CCH) with multiple testing corrections using the Benjamini-Hochberg false discovery rate (the overall p value was <0.05). To analyze the effects of accelerated senescence and CCH feeding independently, we used t-test multiple testing corrections using the Benjamini-Hochberg false discovery rate (overall p value: <0.05).

Results

3. Results

3.1. Definition of the gene expression signatures in CCH feeding

To identify differentially expressed genes between CE-2-fed rats and CCH-fed rats, probes were selected for statistical analysis if they had detected flags in all samples in at least 1 out of 2 conditions. Out of 41,090 probes on the array, 30,667 were selected in this manner. A ttest with a significance level set at $p < 0.05$ was performed, which left 4,689 out of 30,667 probes. The remaining probes were further selected using the criterion of at least a 1.5-fold change. We identified 791 out of 4,689 probes using this protocol. These 791 probes were subdivided into up- and down-regulated (322 up-regulated and 469 down-regulated) probes and analyzed with the IPA software, which were scatter plot (Figure 1). Up and down expression of genes were indicated in Table 1 of canonical pathway list. The numbers of successfully mapped up- and down-regulated probes were 174 (assigned to 162 genes after removal of duplicated entities) and 335 (319 genes), respectively.

3.2. Gene network analysis and biofunctional imputation

To identify the functional categories of the genes, pathway analysis was performed using IPA. In the IPA, each probe was mapped to a gene in the ingenuity knowledge base and used for network generation. Each generated network was scored using the probability of its generation. Nine networks from up-regulated genes (Figure 2 and

Supplemental table S1) and 182 genes and 17 networks from down-regulated genes (Figure 3 and Supplemental table S2) had a generated network score ($= -\log P$) larger than 10.

4. Discussion

In the present study, we demonstrated that dietary administration of coral calcium hydride (CCH), a sustained hydrogen-releasing mineral formulation, markedly reshaped the hippocampal transcriptomic landscape in Wistar rats. By integrating microarray profiling with IPA-based network and canonical pathway analyses, we identified two convergent molecular signatures: (1) activation of antioxidant and metabolic defense pathways mediated primarily by the Nrf2-ARE transcriptional axis, and (2) suppression of NF- κ B-associated inflammatory signaling, including cytokine production, chemokine activity, and immune-metabolic amplification loops. These findings support the idea that hydrogen donors exert biological effects not only through direct radical scavenging, as originally shown by Ohsawa et al. [1], but also through transcriptional reprogramming of redox-sensitive gene networks.

4.1. Integration of the present findings with hydrogen biology

Hydrogen has been widely investigated as a selective antioxidant since its discovery as a neutralizer of hydroxyl radicals and cytotoxic ROS [1]. Subsequent studies demonstrated that hydrogen influences electrophile response systems, mitochondrial redox processes, and inflammatory pathways [8,10]. Recent reviews by Hu et al. (2024) and Wang et al. (2025) highlighted hydrogen's effects on transcriptional hubs such as Nrf2, NF- κ B, STAT3, and metabolic checkpoints [14,15]. Our dataset aligns with these findings and expands them by showing that CCH, via gradual systemic hydrogen release, activates a coordinated neuroprotective gene-expression program within the hippocampus—a region particularly vulnerable to oxidative stress because of its high metabolic activity and lipid composition.

4.2. Nrf2-driven antioxidant response and metabolic resilience

A prominent feature of our results is the upregulation of Nrf2-regulated cytoprotective genes, including Hmox1, Gsta1, Gclc, and Aldh3a1. Nrf2 activation enhances glutathione synthesis, increases detoxification capacity, and stabilizes mitochondrial redox balance [6,7]. These findings parallel earlier reports showing that

hydrogen improves mitochondrial membrane potential, suppresses mitochondrial permeability transition pore opening, and limits ROS propagation [9]. Upregulation of ALDH3A1 is particularly notable, as aldehyde dehydrogenases detoxify reactive aldehydes formed during lipid peroxidation. This suggests that CCH may enhance metabolic resilience by accelerating clearance of oxidative metabolites. Overall, these results support the hypothesis that CCH extends hydrogen's biological actions beyond radical scavenging into broader genomic control mechanisms [4].

4.3. Suppression of NF- κ B-associated inflammatory signaling
Down-regulated genes clustered around inflammatory pathways, including Tnf, Il1b, and Ccl family chemokines. Canonical pathways enriched among down-regulated genes included NF- κ B activation, Toll-like receptor signaling, and cytokine-receptor cascades. These results echo hydrogen's documented anti-inflammatory effects [11–13]. CCH appears to attenuate transcriptional programs responsible for cytokine production and immune amplification, suggesting a role in immunometabolic normalization. Given that hippocampal inflammation contributes to neuronal dysfunction, stress vulnerability, and degenerative processes, suppression of NF- κ B-driven pathways may represent a key mechanism underlying CCH's neuroprotective potential.

4.4. Systems-level convergence of antioxidant and anti-inflammatory pathways
A notable feature of the dataset is the convergence of Nrf2-driven antioxidant activity and suppression of NF- κ B-associated inflammatory circuits. Network coherence and upstream regulator analysis support the involvement of Nrf2 as an activated upstream regulator and NF- κ B-related molecules as suppressed regulators. These interactions align with current conceptual models of hydrogen biology, which emphasize modulation of transcriptional nodes controlling redox and metabolic homeostasis rather than isolated radical scavenging [8,13]. CCH thus appears to reorganize molecular networks in a direction consistent with cellular resilience and diminished oxidative-inflammatory burden.

4.5. Relation to existing HRCC/CCH studies
Our findings extend the growing body of work on hydrogen-releasing coral calcium formulations. Recent multi-omics research in a murine ulcerative colitis model demonstrated that hydrogen-rich coral calcium (HRCC) restored inflammatory balance

and modulated microbiota–host interactions through integrated metabolic and immunological pathways [16]. Previous studies from your research group have shown that CCH and related hydrogen-releasing coral calcium compounds improve antioxidant capacity and modify inflammatory cytokine profiles in rodents and humans [4,5]. The present hippocampal dataset adds a neural dimension to these findings and supports the notion that CCH confers systemic and central redox-immune benefits.

4.6. Possible implications for brain aging and neuroprotection
Because oxidative stress and immune dysregulation drive hippocampal vulnerability, the transcriptional impacts of CCH may have implications for aging, mild cognitive impairment, and neurodegeneration. Activation of Nrf2 and suppression of NF- κ B are hallmark protective responses against age-associated hippocampal dysfunction [6–9]. Although behavioral or biochemical validation is still required, our findings raise the possibility that long-term CCH supplementation may promote neuroprotective adaptation.

4.7. Study limitations and future directions
This study has several limitations. First, transcriptomic endpoints were not accompanied by protein-level assays, metabolic indices, or behavioral outcomes. Second, in vivo hydrogen release kinetics of CCH were not quantified. Third, cell-type-specific contributions (e.g., neurons vs. microglia vs. astrocytes) were not examined. Future research should integrate proteomics, metabolomics, microbiome profiling, single-cell transcriptomics, and longitudinal CCH exposure models. Given the emerging links between hydrogen biology and gut–brain interaction, multi-omics integration may provide broader insight into the systemic actions of CCH [10,11].

4.8. Overall conclusion
Together, these findings indicate that CCH acts as a biologically active hydrogen-delivery system capable of reshaping hippocampal gene expression toward enhanced antioxidant capacity, greater metabolic resilience, and suppressed inflammatory burden. These transcriptional changes align with modern paradigms of hydrogen biology and further establish coral-derived hydrogen-releasing minerals as promising candidates for redox-immune modulation and neuroprotective applications.

Conclusion

CCH supplementation induces robust transcriptomic alterations in the rat hippocampus characterized by Nrf2 activation and suppression of inflammatory gene networks. These findings provide mechanistic insight into the antioxidant and neuroprotective effects associated with hydrogen-releasing nutrients and support their development as functional food compounds.

6. Acknowledgments

CCH was kindly given from TAANE, Co. Ltd., and partially supported by TAANE, Co. Ltd.

7. Conflicts of Interest

The authors declare no conflict of interest.

Figures Legend

Figure 1

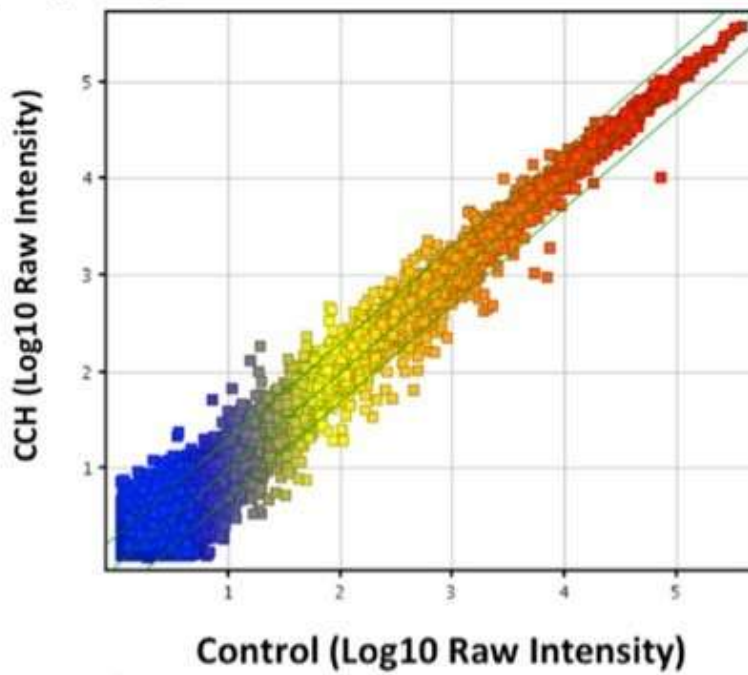


Figure 1. Scatter plot representing gene expression changes in the hippocampus of Wistar rats fed with coral calcium hydride (CCH).

Figure 2.

20

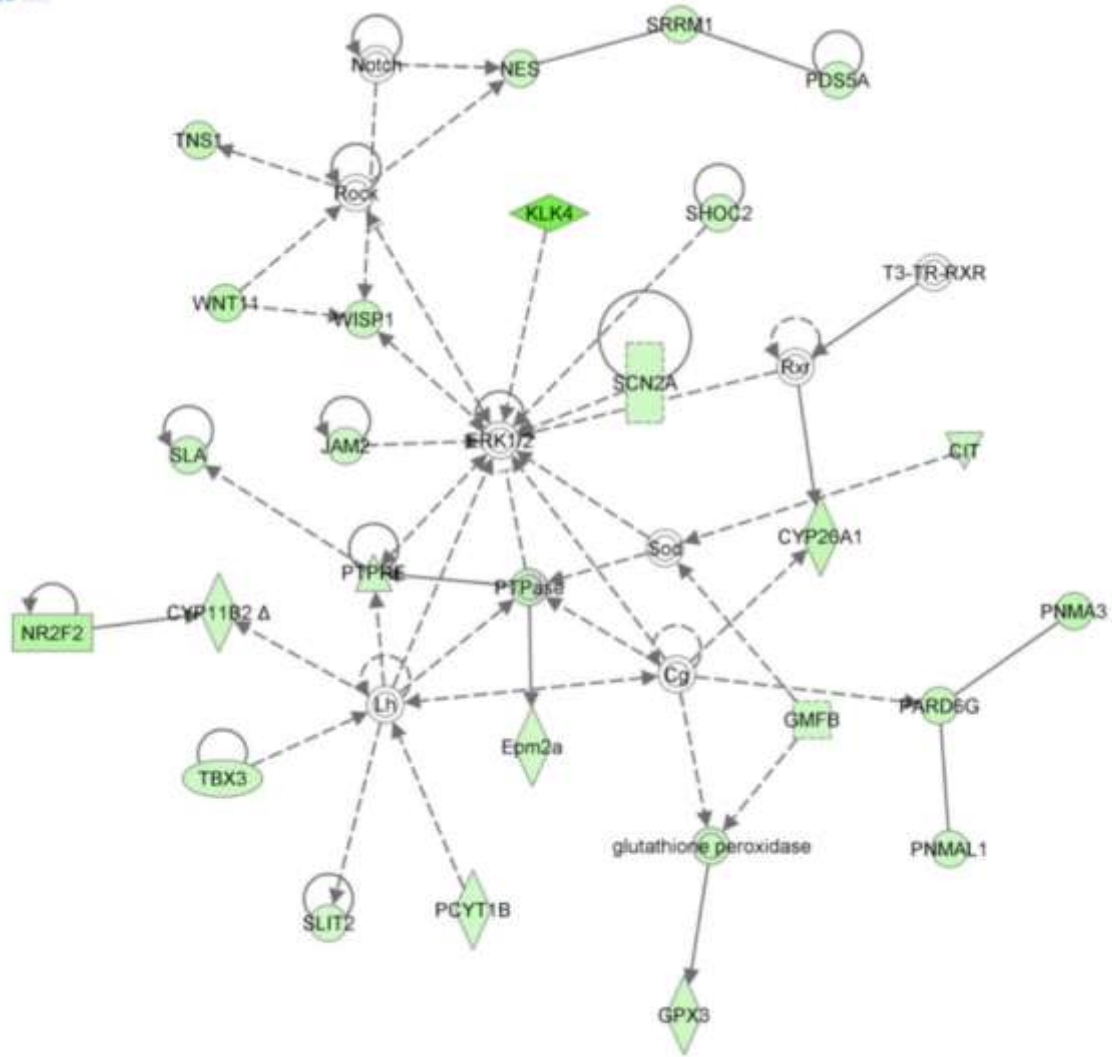


Figure 2. Representative gene interaction network constructed from up-regulated transcripts in the hippocampus of Wistar rats fed coral calcium hydride (CCH). Nodes represent gene products identified by microarray analysis and Ingenuity Pathway Analysis (IPA); green nodes indicate genes significantly up-regulated by CCH feeding ($p < 0.05$, fold change ≥ 2.0), whereas white nodes denote non-significant neighboring molecules present in the canonical network. Solid lines denote direct interactions, and dashed lines denote indirect relationships as curated in the IPA knowledge base.

Figure 3.

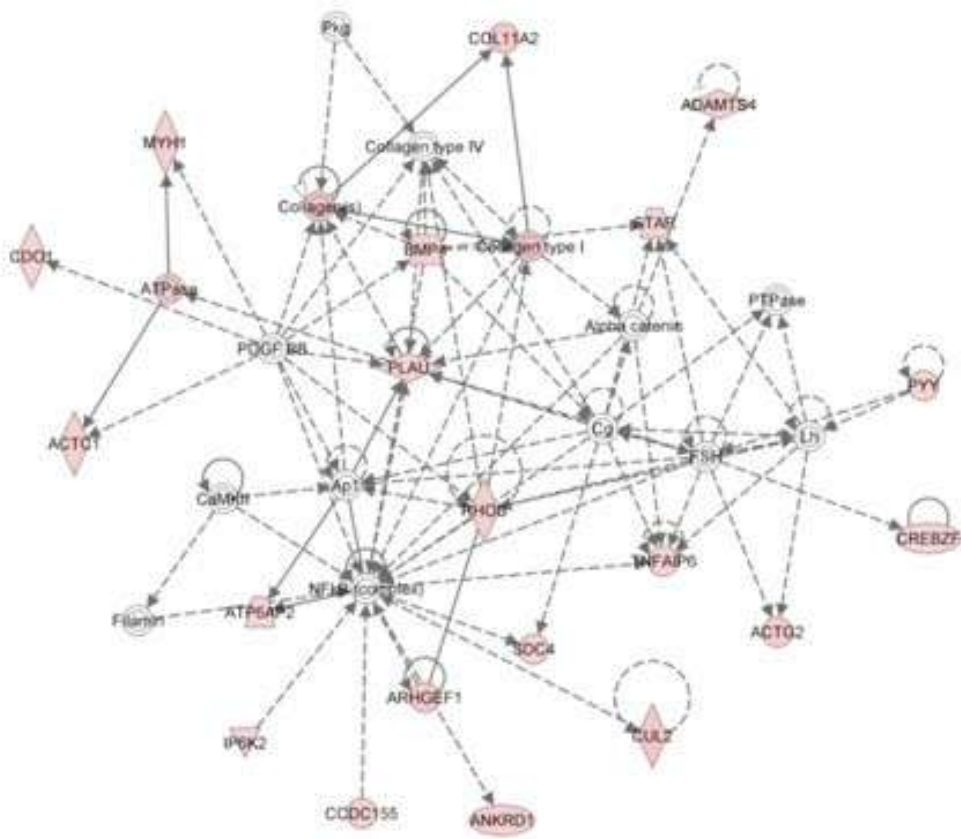


Figure 3. Representative gene interaction network constructed from down-regulated transcripts in the hippocampus of Wistar rats fed coral calcium hydride (CCH). Nodes represent gene products identified by microarray analysis and Ingenuity Pathway Analysis (IPA); red nodes indicate genes significantly down-regulated by CCH feeding ($p < 0.05$, fold change ≤ 0.5), whereas white nodes denote non-significant neighboring molecules present in the canonical network. Solid lines denote direct interactions, and dashed lines denote indirect relationships as curated in the IPA knowledge base.

Figure 4.

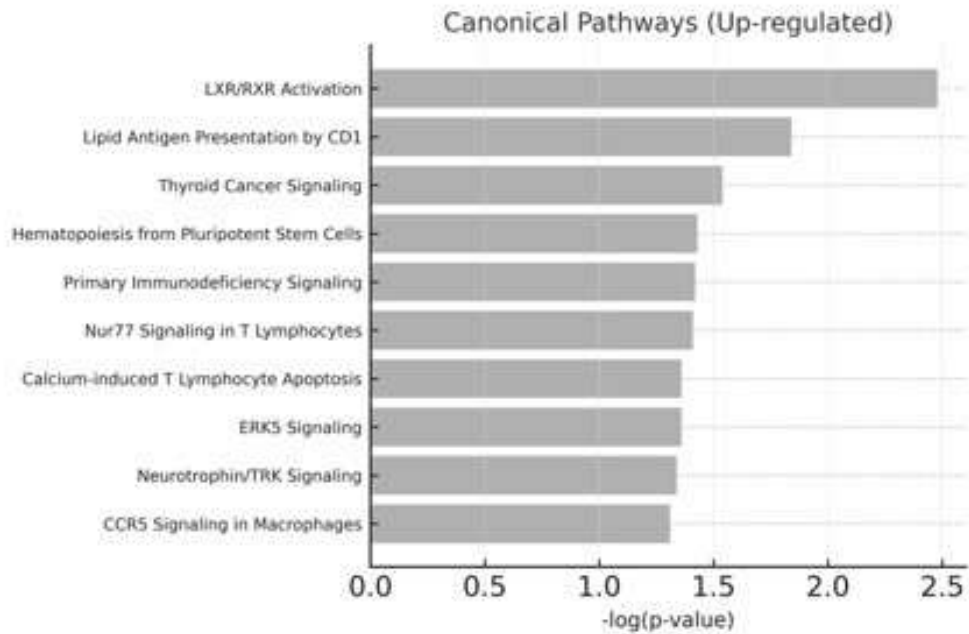


Figure 4. Canonical pathways enriched among up-regulated genes in the hippocampus of CCH-fed rats. Bars represent the magnitude of pathway enrichment based on $-\log(p\text{-value})$. Pathways reflect activation of antioxidant and cytoprotective responses, including Nrf2-associated detoxification.

Figure 5.

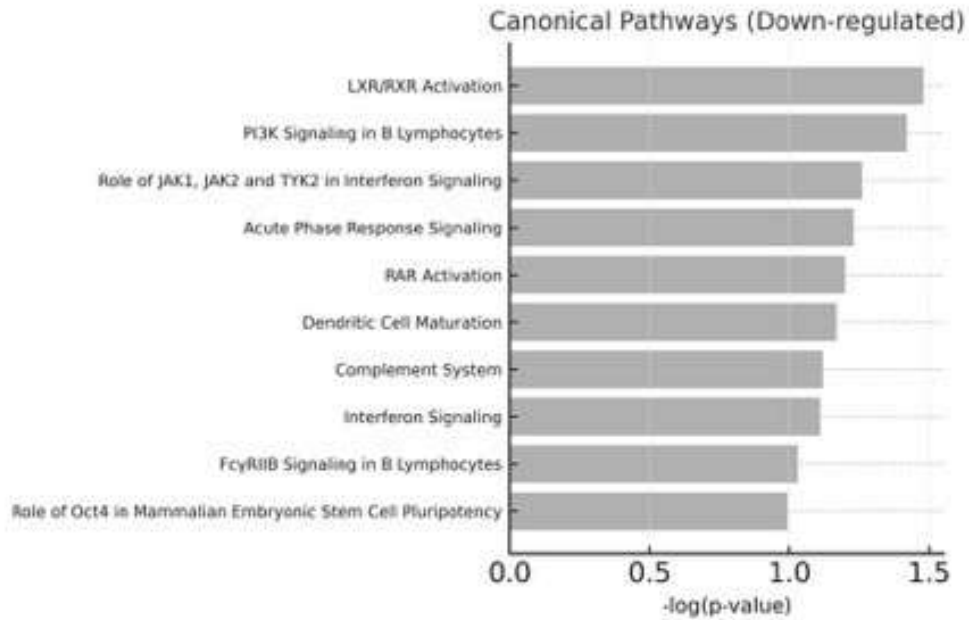


Figure 5. Canonical pathways enriched among down-regulated genes in the hippocampus of CCH-fed rats. Bars show $-\log(p\text{-value})$ for the top 10 pathways. Suppressed pathways include inflammation-associated and metabolic regulatory signals, consistent with NF- κ B suppression.

Table 1. Canonical pathway list.

Table 1. Up and down regulation by CCH fed on gene expression

ID	Up regulated Molecules in Network	Score	Focus Molecules	Top Functions
1	Cg,CIT,CYP11B2,CYP26A1,Epm2a,ERK1/2,glutathione peroxidase,GMFB,GPX3,JAM2,KLK4,Lh,NES,Notch,NR2F2,PARD6G,PCYT1B,PDS5A,PNMA3,PNMAL1,PTPase,PTPRE,Rock,Rxr,SCN2A,SHOC2,SLA,SLIT2,Sod,SRRM1,T3-TRRXR,TBX3,TNS1,WISP1,WNT11 45 25 Organ Morphology, Renal and Urological System Development and Function, Cardiovascular System Development and Function	45	25	Organ Morphology, Renal and Urological System Development and Function, Cardiovascular System Development and Function
2	ADD3,ANXA4,BATF,BCR (complex),C3,CALCA,CARD11,CPNE4,DAPP1,DBP,EDA2R,F,CGR2B,Fibrinogen,FUCA1,GADD45B,GNL3L,Iga,Ige,IgG1,Igg3,IgG,IgG2a,Igm,Immunoglobulin,LCA5,LDB2,LSP1 (includes EG:16985),MAN2A1,NFkB (complex),NR1D1,RC3H1,RELT,RRM2,TSHB,ZC3H13	42	24	Humoral Immune Response, Protein Synthesis, Hematological System Development and Function
3	Alp,ASH1L,BMPR2,BRCC3,CD3,Creb,Cyclin A,DDX6,DNMT3A,EID1,estrogen receptor,GLI1,Hdac,HIST1H1B,HISTONE,Histone h3,Histone h4,HNRNPR,HNRNPUL1,Hsp90,Integrin,ITGA8,ITGAD,KCNA1,PDE3A,PHF15,PHF17,PI3K (complex),Plas,PNLIPRP2,PPIL4,RAB3B,TOPORS,TP73,Usp7	36	22	Cell Death and Survival, Tumor Morphology, Cancer
ID	Down regulated Molecules in Network	Score	Focus Molecules	Top Functions
1	Cg,CIT,CYP11B2,CYP26A1,Epm2a,ERK1/2,glutathione peroxidase,GMFB,GPX3,JAM2,KLK4,Lh,NES,Notch,NR2F2,PARD6G,PCYT1B,PDS5A,PNMA3,PNMAL1,PTPase,PTPRE,Rock,Rxr,SCN2A,SHOC2,SLA,SLIT2,Sod,SRRM1,T3-TRRXR,TBX3,TNS1,WISP1,WNT11	45	25	Organ Morphology, Renal and Urological System Development and Function, Cardiovascular System Development and Function
2	ADD3,ANXA4,BATF,BCR (complex),C3,CALCA,CARD11,CPNE4,DAPP1,DBP,EDA2R,F,CGR2B,Fibrinogen,FUCA1,GADD45B,GNL3L,Iga,Ige,IgG1,Igg3,IgG,IgG2a,Igm,Immunoglobulin,LCA5,LDB2,LSP1 (includes EG:16985),MAN2A1,NFkB (complex),NR1D1,RC3H1,RELT,RRM2,TSHB,ZC3H13	42	24	Humoral Immune Response
3	Alp,ASH1L,BMPR2,BRCC3,CD3,Creb,Cyclin A,DDX6,DNMT3A,EID1,estrogen receptor,GLI1,Hdac,HIST1H1B,HISTONE,Histone h3,Histone h4,HNRNPR,HNRNPUL1,Hsp90,Integrin,ITGA8,ITGAD,KCNA1,PDE3A,PHF15,PHF17,PI3K (complex),Plas,PNLIPRP2,PPIL4,RAB3B,TOPORS,TP73,Usp7	36	22	Cell Death and Survival

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