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Caffeine-Driven Modulation of Hepatic Lipid Metabolism: A Network-Based Gene Expression Analysis in Mice

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ABSTRACT

Coffee is one of the most widely consumed beverages worldwide, and numerous studies have reported its health-related benefits. In this study, the impact of caffeine, a major bioactive constituent of coffee, on mouse liver function was investigated using network-based analysis and gene ontology enrichment approaches. Gene expression data from the GSE53131 dataset were retrieved from the Gene Expression Omnibus (GEO). Differentially expressed genes (DEGs) between mice fed caffeinated coffee and those receiving decaffeinated coffee were identified and further analyzed through protein–protein interaction (PPI) network construction and gene ontology enrichment. Data analysis was performed using the STRING database and Cytoscape software. Caffeine-associated effects on liver function were reflected in distinct hepatic gene expression patterns between mice consuming caffeinated versus decaffeinated coffee. Key discriminative genes—including Acly, Acss2, Acat2, Akr1d1, Elovl2, Ehhadh, Fdps, Fasn, Hmgcr, Gsta3, Ldlr, Lss, Mmab, Mvk, Mvd, Nsdhl, Prodh, Rdh11, and Thrsp—were primarily involved in lipid metabolism and cholesterol biosynthetic pathways. Overall, transcriptomic analysis of mouse liver revealed that consumption of caffeinated coffee markedly influences hepatic lipid metabolism compared with decaffeinated coffee intake. Based on these findings, caffeine may play a potential protective role in the prevention of hepatic metabolic disorders.

Keywords: Coffee, Lipid metabolism, Liver, Decaffeinated

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Introduction

Coffee is consumed globally and is regarded as one of the most popular beverages. Numerous epidemiological studies have associated coffee intake with beneficial health outcomes, including lower overall mortality and decreased incidence of chronic disorders such as cancer. These positive effects are largely attributed to the complex chemical composition of coffee, which includes bioactive substances such as caffeine, diterpenes, polyphenolic compounds, caffeic acid, as well as volatile and heterocyclic molecules [1]. Previous investigations have demonstrated a negative association between coffee consumption and the development of type 2 diabetes mellitus [2]. Furthermore, the influence of coffee intake on non-alcoholic fatty liver disease has been examined, with reported evidence supporting a protective effect of coffee consumption against the progression of liver fibrosis [3].

Caffeine, the predominant active ingredient in coffee, functions as a non-selective antagonist of adenosine receptors. Distinct biological effects have been reported for caffeinated and decaffeinated coffee with respect to hepatic fibrosis. While caffeinated coffee has been shown to suppress liver fibrogenesis, similar effects have not been observed following the consumption of decaffeinated coffee [4]. Given the inconsistent findings related to coffee and caffeine intake in cardiovascular and metabolic disorders, further clarification of the underlying biological mechanisms remains necessary [5, 6].

Advances in genomics have enabled deeper investigation into the molecular consequences of coffee consumption. Barnung *et al*. analyzed the association between coffee intake and alterations in whole-blood gene expression in a post-genomic cohort study focusing on cancer outcomes in Norwegian women [7]. In parallel, bioinformatics tools have become essential components of genomic and proteomic research, and the biological effects of coffee have increasingly been explored using such computational approaches [8]. Protein–protein interaction (PPI) network analysis, in particular, has emerged as a powerful method for identifying functional molecular relationships and has been widely applied in medical, nutritional, and pharmacognostic studies. Our previous PPI-based analysis identified cholesterol metabolism as a principal pathway influenced by coffee consumption [9]. Similarly, Vafaee *et al*. employed PPI network analysis to investigate the chemopreventive properties of red propolis [10].

In the present study, publicly available gene expression data from the livers of high-fat diet–fed mice were analyzed to compare the molecular effects of caffeinated versus decaffeinated coffee consumption. Using PPI network analysis, the role of caffeine in the regulation of hepatic lipid metabolism was systematically explored.

Materials and Methods

Ethical approval

The study protocol was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.AEC.1401.044).

Data acquisition and processing

The GSE53131 dataset was obtained from the Gene Expression Omnibus to investigate caffeine-related effects on fatty liver disease. This dataset includes hepatic gene expression profiles from C57BL/6J mice fed a high-fat diet and treated with either caffeinated or decaffeinated coffee. Samples GSM282830–GSM282832 constituted the caffeinated coffee group, whereas GSM282833–GSM282835 represented the decaffeinated coffee group. Differential gene expression analysis was performed using GEO2R.

To confirm data comparability, box plot analysis was conducted on the selected samples. Genes showing statistically significant differences between the two groups were identified using a p-value threshold of ≤ 0.01 and a fold change ≥ 1.5. These differentially expressed genes were imported into the STRING database via protein query, and interaction networks were visualized using Cytoscape software. Undirected edges were used to construct the PPI network, which was subsequently analyzed with the Network Analyzer tool to determine topological and centrality parameters.

Functional enrichment analysis of the main connected component was performed using ClueGO to identify overrepresented biological processes. Enriched terms were clustered based on kappa statistics. Additionally, CluePedia was employed to examine potential regulatory interactions among network nodes, including binding, activation, inhibition, catalytic activity, post-translational modification, and reaction relationships.

Statistical criteria

Genes were considered differentially expressed at a significance level of $p \leq 0.01$ with a minimum fold change of 1.5. The PPI network was generated using a STRING confidence score cutoff of 0.40.

Results and Discussion

A comparison of hepatic gene expression profiles between mice receiving caffeinated coffee and those consuming decaffeinated coffee is shown in **Figure 1**. All datasets were normalized to the median and demonstrated appropriate comparability. Based on the predefined statistical criteria, 68 genes were identified as significantly differentially expressed and selected for downstream analysis.

Of these 68 genes, 62 were successfully mapped within the STRING database. Network analysis revealed that these genes were distributed among 29 isolated nodes, two interacting gene pairs, one three-node subnetwork, and a principal connected component consisting of 28 nodes **(Figure 2).** Gene ontology enrichment analysis of the main connected component identified multiple biological processes associated with lipid and metabolic regulation, which are summarized in **Figure 3.** In total, 19 functional groups corresponding to 19 nodes were identified.

Analysis of regulatory relationships within the main connected component demonstrated the presence of binding and activation interactions among specific nodes **(Figure 4).** Other examined interaction types, including inhibition, catalysis, reaction, and post-translational modification, were not detected.

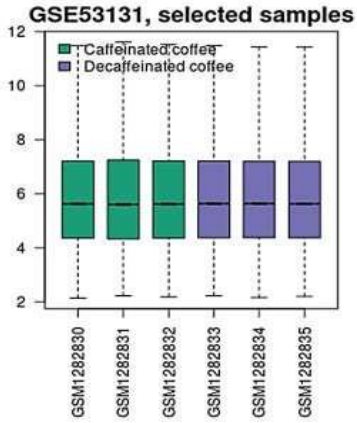

Figure 1. Box plot illustrating the distribution and comparability of the analyzed gene expression datasets.

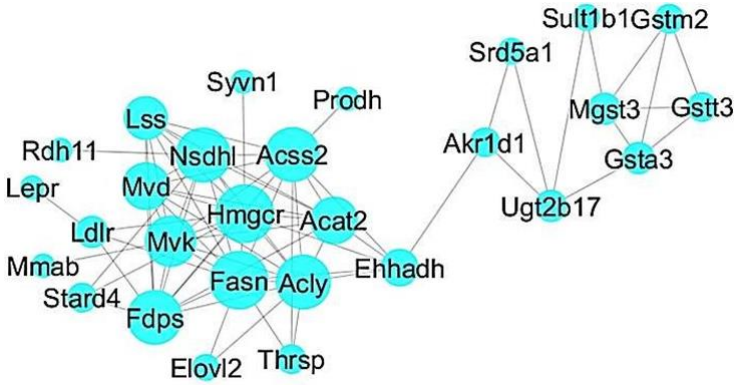

Figure 2. Primary connected subnetwork of the protein–protein interaction (PPI) analysis, with node positioning determined by degree centrality.

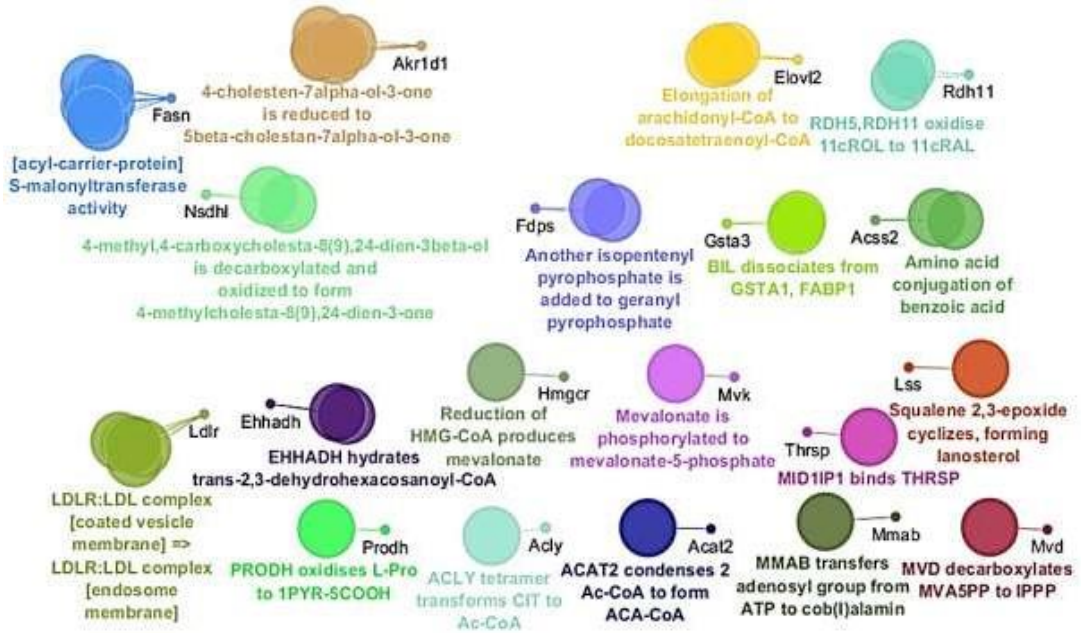

Figure 3. Nineteen functional clusters representing biological processes associated with the nodes in the main connected component; corresponding genes are highlighted in black.

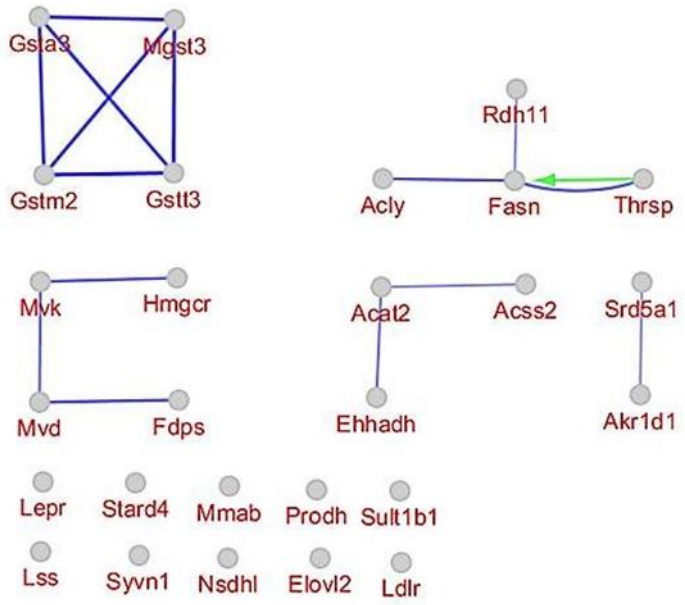

Figure 4. Interaction map depicting regulatory relationships among nodes within the main connected component. Binding and activation interactions are indicated by blue and green single-line edges, respectively.

Studies examining gene expression have highlighted the protective effects of coffee intake, including delayed hepatitis progression and reduced inflammation [11]. Accordingly, it is plausible that coffee consumption alters gene expression patterns in mice. **Figure 1** shows that the gene expression signatures in mice given caffeinated versus decaffeinated coffee are highly comparable, opening the door to additional investigations. The 28 nodes comprising the largest connected component appear particularly useful for differentiating mice exposed to caffeinated coffee from those receiving decaffeinated coffee.

Adan *et al*. conducted a study with 688 healthy undergraduate participants to compare caffeinated and decaffeinated beverages. Their data showed that caffeine consistently produced arousal effects at all measured time points after ingestion, while decaffeinated beverage effects were limited to the 10-minute interval. Gender-specific differences in responses were also noted [12].

Enrichment analysis using gene ontology terms identified 19 functional categories linked to the 19 nodes within the primary connected component. These categories are summarized below by shared biological roles: 1. “[acyl-carrier-protein] S-malonyltransferase activity” describes the transfer reaction malonyl-CoA + acyl carrier protein ⇌ CoA + malonyl-[acyl-carrier-protein], a key step in fatty acid biosynthesis [12].2. “4-cholesten-7alpha-ol-3-one is reduced to 5beta-cholestan-7alpha-ol-3-one” involves hepatic cholesterol 7α-hydroxylase activity [13].3. Likewise, “4-methyl,4-carboxycholesta-8(9),24-dien-3beta-ol is decarboxylated and oxidized to form 4-methylcholesta-8(9),24-dien-3-one” contributes to cholesterol metabolic pathways.4. “Elongation of arachidonyl-CoA to docosatetraenoyl-CoA” pertains to arachidonic acid processing [14]. 5. “Squalene 2,3-epoxide cyclizes, forming lanosterol” participates in cholesterol production [15].6. “LDLR:LDL complex [coated vesicle membrane] => LDLR:LDL complex [endosome membrane]” concerns low-density lipoprotein receptor dynamics [16].7. “EHHADH hydrates trans-2,3-dehydrohexacosanoyl-CoA” indicates EHHADH's involvement in hydration and dehydrogenation during β-oxidation of fatty acids [17].8. “Mevalonate is phosphorylated to mevalonate-5-phosphate” emphasizes mevalonate's contributions to sterol isoprenoids (including cholesterol) and non-sterol products like dolichol [18].9. “MVD decarboxylates MVA5PP to IPPP” also ties into the mevalonate pathway [19].10 “Reduction of HMG-CoA produces mevalonate” directly feeds into the processes described in items 8 and 9. 11 “Another isopentenyl pyrophosphate is added to geranyl pyrophosphate” supports terpenoid backbone synthesis [20].12 “MMAB transfers adenosyl group from ATP to cobalamin” relates to the MMAB-encoded enzyme that finalizes the conversion of vitamin B12 to adenosylcobalamin [21]. Reports further link MMAB to negative regulation of cholesterol balance [22].

The predominant theme across these terms is lipid handling, with a strong focus on cholesterol pathways. This points to distinct impacts of caffeinated versus decaffeinated coffee on cholesterol regulation. Node interactions in the core component are visualized in **Figure 4**, where most links represent binding interactions except for a single activation from THRSP to FASN. The relevant terms here are "MID1IP1 binds THRSP" and "[acyl-carrier-protein] S-malonyltransferase activity." THRSP-mediated activation of FASN drives substantial changes in fatty acid production [23]. Given the emphasis on binding in the protein-protein interaction network, other sub-networks in **Figure 4** received less detailed coverage.

Conclusion

Overall, liver gene expression data from mice indicate that caffeinated coffee specifically modulates a cluster of genes involved in lipid metabolism—particularly cholesterol synthesis—whereas decaffeinated coffee lacks this influence. These insights suggest potential applications for caffeine supplementation in dietary products to mitigate risks of fatty liver disease and liver cirrhosis.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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