ORIGINAL ARTICLE

Metabolite Alteration Associated with Dabai Pulp Oil Supplementation in Hypercholesterolemic Rats

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ABSTRACT

Introduction: Metabolomic analyses have become paramount in unveiling the therapeutic capacities of bioactive agents. Dabai pulp oil (DPO) has emerged as a prospective agent against hypercholesterolemia. This investigation delineates the metabolic imprints of DPO's therapeutic actions using ^{1}H NMR-based urinary metabolomic profiling. **Methods:** Male Sprague-Dawley rats were first exposed to a high-cholesterol regimen to simulate hypercholesterolemia. Following this induction, they were transitioned to a DPO-infused diet. The ensuing metabolic variations were tracked using ^{1}H NMR-based urinary metabolomic analysis. **Results:** The metabolic landscape displayed discernible shifts post-DPO administration, underlining its therapeutic potential. There was a marked decrement in the concentrations of pivotal metabolites such as creatinine, succinate, pyruvate, acetate, TMAO, and choline (p<0.05). Notably, an augmented taurine concentration after DPO administration spotlighted the oil's antioxidative and anti-inflammatory prowess (p<0.05). These observations underscore DPO's proficiency in rectifying metabolic aberrations inherent to hypercholesterolemia, particularly affecting energy transduction and cardiovascular function. **Conclusion:** This empirical evidence bolsters the notion that DPO harbours potent therapeutic virtues for hypercholesterolemia amelioration. Nevertheless, in-depth explorations are quintessential to decoding its holistic therapeutic pathways, fortifying its role in future targeted interventions.
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INTRODUCTION

Canarium odontophyllum, locally known as "Dabai," is a unique tropical fruit-bearing tree found in the diverse rainforests of Southeast Asia, specifically Malaysia, Indonesia, Brunei, and the Philippines. Distinguished by its impressive stature of up to 30 meters, this member of the *Burseraceae* family produces a dark purple to black olive-shaped fruit. These fruits reaped during the monsoon season, are coveted for their unique flavour profile and potent nutritional content, establishing them as a highly sought-after delicacy in their native regions, such as Sibu and Kapit in Sarawak, Malaysia (1). The derivative of this fruit, dabai pulp oil (DPO), represents a valuable vegetable oil source and has kindled interest within the scientific community (2). Our previous study

revealed that the fatty acid profile of DPO, extracted using supercritical carbon dioxide (SC-CO2), bears a striking resemblance to that of palm oil. With a nearly balanced distribution of unsaturated fatty acids (UFA) and saturated fatty acids (SFA), DPO's fatty acid profile is composed of 39.37% oleic acid, 41.56% palmitic acid, and 4.31% stearic acid. Our study further highlighted the efficacy of DPO in alleviating hypercholesterolemia. This was evidenced by the reduction in total cholesterol, triglycerides, lipid peroxidation, HMG-CoA reductase, and inflammatory markers, paired with an enhancement in lipoprotein lipase activity and strengthened antioxidant defenses (3).

Hypercholesterolemia, characterized by elevated serum cholesterol levels, represents a notable health concern globally. Conventional treatments, primarily statins, though efficacious to an extent, are associated with a range of side effects such as muscle pain, hepatotoxicity, gastrointestinal disturbances, and potential cognitive effects (4). Given these limitations, there's an evident

demand for alternative therapeutic approaches (5). DPO, owing to its distinctive lipid profile, emerges as a potential therapeutic candidate in the management of hypercholesterolemia. Our study aims to explore the mechanistic roles of DPO in modulating hypercholesterolemia, potentially offering new therapeutic strategies and advancing the field of metabolic health research. Utilising sophisticated tools like nuclear magnetic resonance (NMR) spectroscopy, metabolomic analyses are poised to provide comprehensive insights into the complex metabolic responses elicited by DPO. NMR metabolomics, renowned for its non-destructive characterization, simultaneous quantification of multiple metabolites, and detailed molecular resolution is uniquely suited for capturing the nuanced therapeutic effects of DPO. (6,7). Central to our research is the identification and analysis of key metabolites involved in cholesterol metabolism post-DPO intervention, with an aim to decode the mechanistic roles of DPO in hypercholesterolemia modulation. Through this study, we aim to delineate the metabolomic shifts post-DPO administration in hypercholesterolemic models, thereby informing tailored therapeutic strategies for hypercholesterolemia. In essence, our work extends beyond merely elucidating DPO's bioactive potential; it underscores a novel approach to hypercholesterolemia management. The implications of our findings extend to potential therapeutic innovations and a deeper appreciation of metabolic dynamics, reaffirming DPO's potential in advancing metabolic health research.

MATERIALS AND METHODS

SC-CO$_2$ Extraction of DPO
A meticulously selected fresh batch of dabai fruits was procured from the rich biomes of Sarikei, Sarawak, Malaysia. The preparation of the dabai pulp followed the method meticulously detailed in our previous research (2), affirming the consistency and accuracy of our processes. In brief, with precision and careful handling, 48.92 kg of freeze-dried dabai pulp underwent the process of SC-CO2 extraction. We meticulously maintained a pressure of 40 MPa and a steady temperature of 40 °C during the extraction process to ensure the quality and integrity of the resulting DPO. The acquired DPO was treated with the highest level of care during handling and storage to preserve its quality and potential therapeutic properties. It was carefully stored in a chiller, maintaining a temperature of 4 °C, safeguarding its stability and purity until further utilisation in our study.

Animal Experiment and ethics statement
Male Sprague-Dawley rats, aged four weeks and with weights ranging between 100 and 150 g, were obtained from Nomura Siam International Co., Ltd., Thailand. All rats were specific-pathogen-free (SPF) and individually housed in ventilated cages (IVC) within the Comparative Medicine and Technology Unit (COMeT), Universiti Putra Malaysia. The housing environment was regulated

to ensure a temperature of 21-23°C, relative humidity of 50-60%, and a 12-hour light-dark cycle. This meticulous control aimed to minimise environmental stress on the specimens. The study maintained rigorous adherence to approved ethical guidelines for the use and care of laboratory animals as per the Institutional Animal Care and Use Committee (IACUC) at Universiti Putra Malaysia (Approval number: IACUC R045/2015). After a two-week acclimatisation period, the rats were randomly divided into two groups: the Normal Group (NG, n = 5) and the Hypercholesterolemic Group (HG, n = 10). The NG was provided a Normal Diet (ND) while the HG was subjected to a High Cholesterol Diet (HCD) for a period of 30 days to induce hypercholesterolemia. Post-induction, the HG was further subdivided: the Dabai Pulp Oil (DPO) Treated Group (DTG, n = 5), receiving a DPO diet (DPOD), and the Hypercholesterolemic Continuation Group (HCG, n = 5), persisting with the HCD. The NG sustained its ND throughout this phase. This regimen was extended for another 30 days. Upon the completion of treatment on day 60, urine samples were procured from rats individually housed in plastic metabolic cages equipped with urine collection bottles. To inhibit microbial growth and ensure sample integrity, we fortified the collection bottles with 0.1% sodium azide. It is important to note that the urine samples used for °H-NMR urinary metabolomics analysis in this study were obtained from the same batch of male Sprague-Dawley rats as those used in our previous study (3). This ensures consistency in the sample population, effectively addressing potential concerns regarding batch-to-batch variations in disease induction and their impact on lipid profiles and metabolomic outcomes. The use of the same batch of animals aligns with our objective to comprehensively assess the metabolomic shifts induced by DPO supplementation in a hypercholesterolemic rat model. All collected biochemical samples were carefully preserved at -80°C until further analysis, ensuring the stability and integrity of all biochemical constituents. The cholesterol-free diet, formulated for our study, consisted of a blend of corn starch, sucrose, casein, cellulose, a mineral mix, a vitamin mix, DL-methionine, choline, corn oil, and ghee. For the preparation of this diet, ingredients were mixed thoroughly on a weekly basis, spread out in trays, sectioned into smaller portions, and then baked in a Binder ED23 oven (Tuttlingen, Germany) at a controlled temperature of 50–60 °C for a 24-hour period. In a similar manner, the high cholesterol diets were prepared by incorporating 1% cholesterol into the mixture. For the DPOD specifically, 2% of DPO oil was utilized, replacing the corn oil, to assess its dietary impact. A graphical representation of the experimental diet and protocol were depicted in Fig.1.

^{1}H-NMR Urinary Metabolomics Analysis
NMR measurements were performed in adherence to the methodology outlined by Abu Bakar Sajak et al. (8) utilising a 500 MHz Varian INOVA NMR spectrometer (Varian Inc., Palo Alto, CA, USA), operating at 499.92
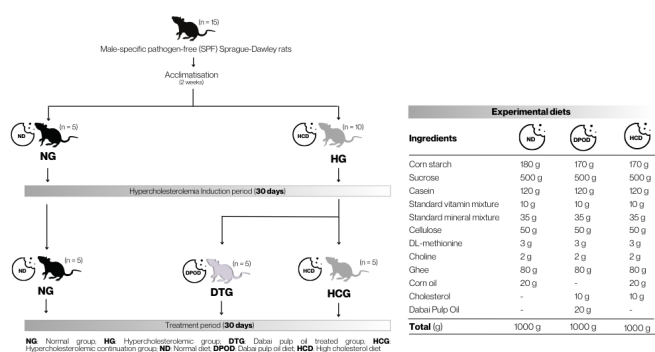
Experimental diets

Ingredients	ND	DPOD	HCD
Corn starch	180 g	170 g	170 g
Sucrose	500 g	500 g	500 g
Casein	120 g	120 g	120 g
Standard vitamin mixture	10 g	10 g	10 g
Standard mineral mixture	35 g	35 g	35 g
Cellulose	50 g	50 g	50 g
DL-methionine	3 g	3 g	3 g
Choline	2 g	2 g	2 g
Ghee	80 g	80 g	80 g
Corn oil	20 g	-	20 g
Cholesterol	-	10 g	10 g
Dabai Pulp Oil	-	20 g	-
Total (g)	**1000 g**	**1000 g**	**1000 g**

NG: Normal group. **HG**: Hypercholesterolemic group; **DTG**: Dabai pulp oil treated group, **HCG**: Hypercholesterolemic continuation group; **ND**: Normal diet, **DPOD**: Dabai pulp oil diet, **HCD**: High cholesterol diet

Figure 1: Experimental diet and protocol

MHz. The procedure involved mixing the thawed urine supernatant with a phosphate buffer solution (KH2PO4, pH 7.4, 0.1% TSP) prepared in deuterium oxide (D2O), in a 2:1 ratio. The prepared mixture was subsequently transferred into a 5 mm NMR tube for analysis. The focus on urinary metabolomes in this study is driven by the dynamic and all-encompassing nature of urine as a biological matrix. Recognized for its potential in non-invasively monitoring metabolic changes, particularly in conditions like hypercholesterolemia, urinary metabolomics offers insights into the affected biochemical pathways (9). Although the reliability of urine metabolites in predicting hypercholesterolemia requires further exploration, this approach is instrumental in understanding disease-related metabolic alterations. Our study aims to elucidate the metabolomic shifts following DPO administration in hypercholesterolemic models, thereby contributing to the development of targeted therapeutic strategies for managing hypercholesterolemia.

A proton NMR analysis was initially conducted for each sample, which was subsequently subjected to a standard water-suppression one-dimensional NMR, PRESAT sequence with 64 scans and an acquisition time of 206 seconds. Spectral processing, including phasing and binning, was executed using a uniform set of parameters in the Chenomx software (v. 8, Edmonton, AB, Canada). In this protocol, TSP was set as the reference peak (chemical shift indicator) at δ 0.00. A binning process, set to δ 0.04 per bin, was implemented on each spectrum, while selectively excluding the urea (δ 5.55–5.95) and water regions (δ 4.60–4.95), thereby resulting in a total of 235 bins. Normalisation of each bin was performed using the total area method, thereby facilitating a thorough and accurate comparative analysis of the metabolic changes induced by the DPOD supplementation.

The dataset was analyzed using the SIMCA-P software (Version 12.0, Umetrics, Umee, Sweden) for comprehensive multivariate data analysis. Initially, we analysed the dataset using Principal Component Analysis (PCA) to visually group the data based on metabolite differences. We then built a Partial Least

Squares-Discriminant Analysis (PLS-DA) model to distinguish and illustrate the defining metabolites of these groups. The PLS-DA model's efficiency was checked using R2 and Q2 values, considering it effective if these values were above 0.5 (10). Metabolites with a Variable Importance in the Projection (VIP) over 1.0 were identified as influential in group separations (11). We utilised Metaboanalyst 5.0 software (https://www.metaboanalyst.ca/) for pathway analysis, performing overrepresentation and pathway topology tests. Our pathway library was based on the Sprague–Dawley rat model, used in our experiments. Metabolic pathways with impact values over 0.1 were identified as potential pathways (12). The metabolomic profiling conducted in this study was untargeted in nature. Untargeted metabolomics allows for the broad screening of metabolites in a sample, providing an unbiased approach to discover novel biomarkers and pathways that might be implicated in hypercholesterolemia (13). Statistical analysis

The data are expressed as mean ± standard deviation (n = 5). Statistical analyses were performed using a one-way ANOVA, followed by the Least Significant Difference (LSD) post-hoc test for pairwise comparisons. Analyses were executed using the SPSS software (Chicago, IL, USA). A probability value of $p < 0.05$ was considered indicative of statistical significance.

RESULTS

Fig. 2 shows the representative NMR spectra of urine. The endogenous metabolites in the spectra were identified using Chenomx NMR suite 6.1. To verify their assignments, we compared their shape-splitting/coupling constants and chemical shifts within the expected range. Additionally, we confirmed and identified the metabolites by referencing the Human Metabolome Database (HMDB) and published assignments, considering the chemical shifts, and coupling constants. The urine sample contained a variety of metabolites, such as intermediates from the tricarboxylate cycle (TCA), amino acids, ketone bodies, organic acids, and others.

The DPO treatment model was constructed using the PLS-DA model, exhibiting an excellent fit with an R2Y = 0.971 and a Q2 = 0.745, as shown in Fig.3. In the score plot (Fig. 3A), the NG has distinctly separated from the HCG along the w*c[1] axis, suggesting the induction of hypercholesterolemia. This separation is further illustrated by the deviation of the DTG from the HCG, as it aligns with the NG in the same quadrant along w*c[1]. The observed distribution indicates that the administered treatments had a partial ameliorating effect on the hypercholesterolemic condition. The loading scatter plot (Fig.3B) complements the PLS-DA score by identifying the specific metabolites contributing to the separation among the groups. In the negative quadrant
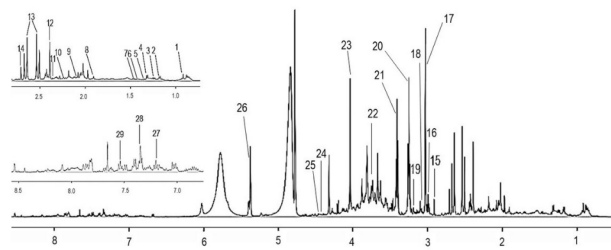
Urine spectra representative from 0.5 – 8.5 ppm.

1: Leucine, **2:** 3-hydroxybutyrate, **3:** Methylmalonate, **4:** Threonine, **5:** Lactate, **6:** Lysine, **7:** Alanine, **8:** Acetate, **9:** Acetone, **10:** Acetoacetate, **11:** Pyruvate, **12:** Succinate, **13:** Citrate, **14:** Dimethylamine, **15:** N,N-Dimethylglycine, **16:** 2-oxoglutarate, **17:** Creatine, **18:** cis-Aconitate, **19:** Choline, **20:** Trimethylamine N-oxide, **21:** Taurine, **22:** Glucose, **23:** Creatinine, **24:** Trigonelline, **25:** 1-Methylnicotinamide, **26:** Allantoin, **27:** 3-Indoxylsulfate, **28:** N-Phenylacetylglycine, **29:** Hippurate

Figure 2: Urine spectra representative from 0.5 – 8.5 ppm
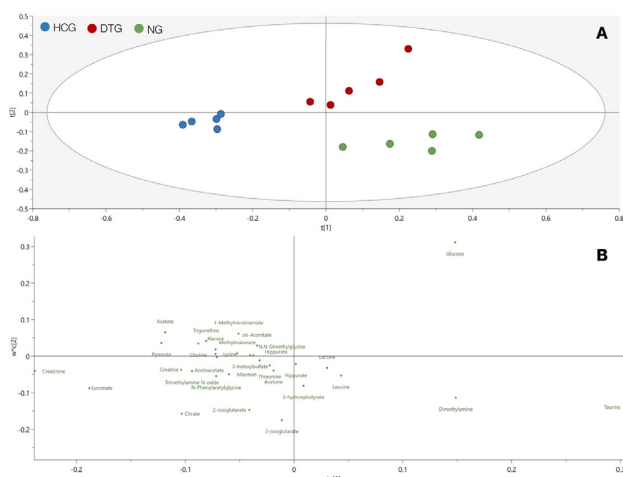
Figure 3: PLS-DA Model (A) Score plot (B) Loading scatter plot. HCG: hypercholesterolemic continuation group; DTG: DPO-treated group; NG: normal group

of w*c[1], the high abundance of creatinine, choline, succinate, pyruvate, acetoacetate, acetate, and alanine was primarily associated with HCG. In the lower side of the positive quadrant at w*c[1], the observed presence of metabolites such as taurine, dimethylamine, leucine, lactate, 3-hydroxybutyrate, and hippurate may be associated with NG.

Among the metabolites that were previously identified, only those possessing VIP values exceeding 1.0 were evaluated as potential biomarkers. A total of 9 such biomarkers were subsequently identified. Taurine emerged with the highest VIP value, exceeding 3, and was followed in importance by creatinine, succinate, pyruvate, acetate, TMAO (Trimethylamine N-oxide), choline, alanine, and acetoacetate. In a focused effort to detail the alteration trends of these potential biomarkers, an in-depth statistical analysis was undertaken, the findings of which are presented in Table I. After 60 days of consuming HCD, the HCG exhibited a significant increase in the levels of creatinine, succinate, pyruvate, acetate, TMAO, choline, alanine, and acetoacetate when compared with NG ($p < 0.05$). Incorporating DPOD supplementation led to a significant decrement in the levels of creatinine, succinate, pyruvate, acetate,

Table I: Potential biomarkers and fold changes among groups

Metabolites	VIP	HCG/NG	DTG/HCG
Taurine	4.58	0.53***	1.77***
Creatinine	3.57	1.64***	0.66***
Succinate	2.81	1.44***	0.68***
Pyruvate	1.82	1.45**	0.81**
Acetate	1.77	1.62*	0.82*
TMAO	1.40	1.32*	0.75*
Choline	1.31	1.31**	0.76**
Alanine	1.07	1.28*	0.86
Acetoacetate	1.06	1.25*	0.84

NG: Normal group; HCG: Hypercholesterolemic continuation group; DTG: DPO-treated group. The notation XXX/YYY represents the quotient obtained by dividing the integral of a metabolite in the XXX group by that in the YYY group. A ratio exceeding 1.00 signifies an increase, while a ratio falling below 1.00 suggests a decrease. One-way analysis of variance was conducted, followed by a Least Significant Difference (LSD) test. Significance levels were denoted as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$.

TMAO, and choline in the DTG in comparison with HCG ($p < 0.05$). Concurrently, there was a significant elevation in taurine level when compared with HCG ($p < 0.05$).

Further, to uncover the specific metabolic pathways associated with the identified potential biomarkers, the MetaboAnalyst software—a pathway analysis tool—was employed. This enabled the illumination of the possible metabolic pathways disrupted in hypercholesterolemic rats due to a high-cholesterol diet, as well as the alterations introduced by DPO supplementation, as depicted in Fig. 4. Table II outlines five metabolic pathways that are intertwined with the identified biomarkers. These include pathways connected to lipid metabolism (specifically the synthesis and degradation of ketone bodies), energy metabolism-related pathways (such as pyruvate metabolism, and glycolysis/gluconeogenesis), and other pathways like butanoate metabolism and taurine and hypotaurine metabolism.
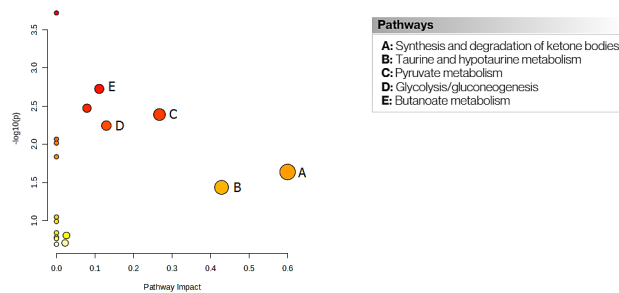

Figure 4: Pathways analysis in the DPO treatment model

Table II: Metabolic pathways analysis in the DPO treatment model

Metabolic pathways	P value	Impact value
Synthesis and degradation of ketone bodies	0.023	0.60
Taurine and hypotaurine metabolism	0.037	0.43
Pyruvate metabolism	0.004	0.27
Glycolysis/gluconeogenesis	0.006	0.13
Butanoate metabolism	0.002	0.11

Pathway analysis was conducted by using Metaboanalyst 4.0 software. Impact value is the pathway impact value calculated from pathway topology analysis.

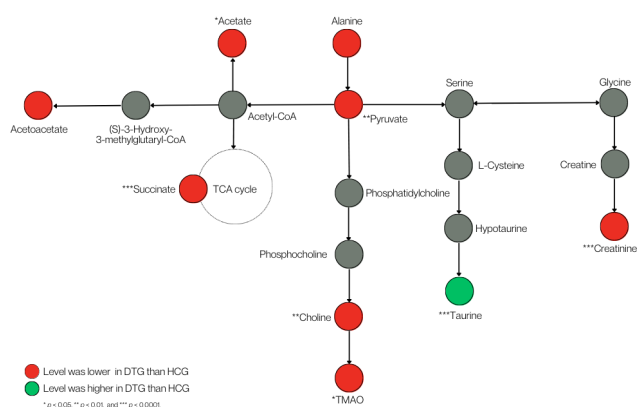Further, the therapeutic mechanisms underlying DPOD supplementation are depicted in Fig. 5.

Figure 5: Therapeutic mechanisms underlying DPOD supplementation

DISCUSSION

Urine metabolomics, especially when employing 1H-NMR-based analysis, has emerged as a significant method in elucidating the intricacies of cholesterol metabolism. This approach has been observed to be effective in various studies focused on hyperlipidemia, where it aided in the comprehensive profiling of metabolites in biological samples. It is important to note that these findings indicate a correlation, not necessarily causation, between metabolomic profiles and cholesterol metabolism. Such quantitative metabolomic profiling suggests the method's capacity to unravel complex metabolic pathways. The capacity of this technique to quantify a broad spectrum of metabolites may enable an in-depth understanding of the metabolic disturbances linked to cholesterol metabolism and related disorders. The application of urine metabolomics has been associated with substantial metabolic changes induced by diets rich in fat and cholesterol. These dietary alterations appear to lead to notable shifts in lipid accumulation and inflammatory processes, detectable through urine metabolomic analysis (14,15). However, these observations should be interpreted cautiously, as they are associative and further research is needed to establish a direct causal relationship. This indicates that, while urine metabolomics provides a global overview, further studies are needed for comparing these findings with serum/plasma or tissue-specific data to achieve a more comprehensive understanding of cholesterol dysregulation. These observations suggest the method's sensitivity in identifying nuanced yet meaningful metabolic variations, positioning it as a potentially potent tool for early detection and monitoring of diseases.

Moreover, the broader implementation of 1H-NMR-based urine metabolomics across different studies, including those in various metabolic disorders, highlights its adaptability. In the context of developing targeted therapies, understanding the specific changes in urine metabolites can potentially inform the development of interventions tailored to the metabolic disturbances identified, possibly guiding the use of DPO in specific subtypes of hypercholesterolemia. This potential application, however, remains speculative and requires further validation through comprehensive studies. The ability to identify specific metabolic pathways and biomarkers in these contexts demonstrates the potential of urine metabolomics beyond cholesterol metabolism, encompassing a diverse array of metabolic diseases (15). Hence, metabolomics may offer insights into cholesterol dysregulation, urine metabolomics, particularly 1H-NMR-based, is considered a non-invasive, comprehensive, and sensitive method. It is proficient in detecting metabolic alterations, identifying potential biomarkers, and contributing to the understanding of the etiology and progression of disorders related to cholesterol metabolism. Nevertheless, these insights must be integrated with other biological data for a holistic understanding of metabolic disorders.

In our previous investigation (16), we identified coumaric acid and anisic acid as the primary phenolic acids in the oil. These compounds are renowned for their antioxidative properties and are likely to play a key role in the protective effects against hypercholesterolemia-induced metabolic changes (17,18). This understanding of DPO's phenolic composition sets the stage for examining its therapeutic implications. Our subsequent research (3), complements this by providing detailed lipid profiles of hypercholesterolemic rats supplemented with DPO. The connection established here between DPO's chemical makeup and its biological impact, while compelling, must be further explored to confirm its therapeutic implications. Linking these pivotal findings to our current study's metabolomic observations, we observe that the influence of DPO extends beyond lipid profile modulation, inducing comprehensive metabolic alterations in hypercholesterolemic rats. This suggests a broader biological impact of DPO, yet these findings should be cautiously interpreted until replicated and validated in further studies.

Building on the foundation laid by our investigation, recent human studies suggest the predictive power of urine metabolomics in hypercholesterolemia. A key study on the urine metabolic profiling of hyperlipidemia patients identified 22 potential biomarkers, encompassing amino acid, fatty acid, nucleotide, steroid hormone, and intestinal flora metabolism, linked to inflammatory reactions and oxidative stress (19). While these biomarkers may provide a potential link between urine metabolomic profiles and cholesterol metabolism dysregulation, this association requires further investigation to establish its clinical relevance. These findings resonate with our observations, where the modulation of similar metabolic pathways was observed in hypercholesterolemic conditions treated with DPO. It is crucial to note that while the alterations in amino

acid and fatty acid metabolism markers in the human study align with our findings, these parallels should be approached with caution until more conclusive evidence is available. This alignment of rat model findings with human data provides insights into the translational potential of our findings and contributes to a more nuanced understanding of DPO's therapeutic applications in cholesterol metabolism. However, the direct applicability of these rat model findings to human pathology requires careful consideration and validation through further research.

Comparing the HCG to NG, observed variations in metabolite levels are concisely supported by preceding research (11,20–22). Specifically, in HCG, pathways linked to energy homeostasis, amino acids metabolism, synthesis and degradation of ketone bodies, and gut microbiota metabolism are noticeably upregulated. Conversely, the metabolism of taurine and hypotaurine is downregulated. Succinate serves as a pivotal intermediary in the tricarboxylic acid (TCA) cycle, predominantly localised within the mitochondria. The TCA cycle remains integral for cellular energy provision (23). Pyruvate, derived from glycolysis, acts as a bridge between glycolysis and the TCA cycle, facilitating energy production (22). A noticeable increase in both succinate and pyruvate in HCG implies that a high-cholesterol diet profoundly influences the TCA cycle, disrupting cellular energy balance. This implication, while supported by our findings, should be further explored to understand its full implications in hypercholesterolemia (24). Conversely, a marked reduction in succinate and pyruvate in DTG hints at a return to metabolic balance. The potential of DPO in rectifying these metabolic perturbations is a promising avenue for future research, although these observations are preliminary and require further substantiation.

Significant increment levels of the amino acid alanine in HCG suggest modifications in amino acid metabolism, potentially driven by acetyl-CoA conversion and suppressed gluconeogenesis (11). Creatinine, an indicator of renal function, exhibited elevated levels under hypercholesterolemia, underscoring renal implications in this pathology (14). However, the interpretation of these changes in creatinine levels should be made cautiously, as they are indicative rather than definitive of renal function under hypercholesterolemic conditions. Remarkably, DTG exhibited diminished creatinine levels, perhaps alluding to improved renal functionality and reduced oxidative stress (25), in tandem with the antioxidant potential of DPO (3,16).

Acetate is predominantly formed during fatty acid oxidation. Elevated acetate in HCG, compared to NG, implies escalated fatty acid β-oxidation, leading to ketone body production. As the liver processes these ketone bodies, they are converted to acetyl-CoA (26) subsequently entering the TCA cycle. Their accumulation can signal liver damage (27). The observed decline in acetate in DTG suggests a metabolic re-balancing of lipid pathways, yet this observation requires further study to fully understand its implications.

Choline and TMAO are intricately linked to lipid transport and gut microbiota, respectively, both factors heightening cardiovascular disease risks (28,29). Their elevation in HCG accentuates the nuanced metabolic shifts in hypercholesterolemia. It's important to consider these changes in the context of overall metabolic health and to explore their implications in further studies. Notably, a decrease in these metabolites in DTG heralds potential therapeutic interventions for cardiovascular health (30,31). However, the causal relationship between these metabolite changes and cardiovascular health remains to be fully elucidated.

Taurine exhibits multifaceted physiological roles, ranging from antioxidation to modulating inflammation (32). The therapeutic potential of taurine in hypercholesterolemia is reinforced by its capability to attenuate associated adversities (33). The role of taurine, particularly in the context of DPO treatment, is intriguing but requires more research to confirm its effectiveness in clinical settings. The prominence of taurine, especially in the DPO treatment context, suggests its cardinal role in ameliorating oxidative stress and inflammation, while also improving endothelial health (34,35). The observed inverse correlation between specific metabolites and taurine levels during DPO treatment is an area of interest, but the exact nature of this relationship should be investigated further to determine its clinical significance.

The broader clinical implications of these findings, particularly in the context of developing DPO as a functional food for managing hypercholesterolemia, warrant further exploration. Future investigations, including human clinical trials, are essential to validate the efficacy of DPO in real-world settings. Moreover, the potential of DPO to serve as a natural alternative to conventional lipid-lowering agents, especially for individuals intolerant to statins, could be a significant advancement, although more comprehensive studies are required to establish its safety and effectiveness. It is also essential to delve deeper into the potential interactions between DPO and gut microbiota. Recent studies suggest a significant interplay between gut microbiome composition and lipid metabolism (21), but the exact mechanisms and implications of this relationship need to be more thoroughly understood. Understanding this relationship could provide valuable insights into personalized dietary interventions for hypercholesterolemia. In conclusion, while DPO shows promise in impacting hypercholesterolemia-induced metabolic disturbances, it is imperative to approach these findings with caution. The disparities between rat and human physiology, the complexity of metabolomic

interplay (considering pathway interconnectivity, feedback systems, and external influencers), and limited DPO research necessitate more rigorous scientific scrutiny.

CONCLUSION

In summary, the utilization of metabolomics in investigating hypercholesterolemia offers a comprehensive perspective on the systemic alterations associated with this disorder. The observed changes in concentrations of key metabolites such as pyruvate, succinate, creatinine, acetate, choline, and TMAO, coupled with the increased levels of taurine following Dabai Pulp Oil (DPO) administration, indicate potential therapeutic effects of this oil. However, these findings should be interpreted with caution, as they are preliminary and require further validation. Our study underscores the potential of DPO as a novel approach in managing hypercholesterolemia, as evidenced by the observed metabolomic shifts. Moving forward, clinical trials are essential to confirm DPO's efficacy in humans, thereby enhancing our understanding of its role in therapy. The development of DPO as a functional food or supplement presents a promising avenue, offering a natural alternative to traditional treatments for hypercholesterolemia, especially for those intolerant to standard medications. However, comprehensive mechanistic explorations are imperative to pinpoint the exact biochemical pathways affected by DPO. Such investigations are crucial not only to validate the oil's therapeutic effects but also to guide the development of more targeted and refined treatment strategies. This approach is critical in ensuring that the potential benefits of DPO are realized in a clinical setting and contribute meaningfully to the broader scope of hypercholesterolemia management.

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