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Remodeling of macrophage lipid metabolism by phytosterol suppresses inflammation: Insights from targeted lipidomics

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ABSTRACT: Phytosterols are widely recognized bioactive compounds exhibiting anti-inflammatory effects. Macrophages adopt a distinct lipid metabolic state during inflammatory responses. However, the specific mechanisms by which phytosterols influence macrophage lipid metabolism and subsequently regulate inflammation through metabolic remodeling remain unclear. In this study, we elucidate how phytosterols reprogram macrophage lipid metabolism to attenuate inflammatory responses. Quantitative lipidomic analysis revealed that phytosterol-induced lipid remodeling predominantly affects long-chain triglycerides. Further mechanistic studies demonstrated that phytosterols increase lipid droplet accumulation and regulate their metabolism by suppressing adipose triglyceride lipase (ATGL), upregulating hypoxia inducible lipid droplet associated proteins (HILPDA), and reducing the production of pro-inflammatory intermediates, including arachidonic acid. Consequently, phytosterol treatment decreased prostaglandin E₂ (PGE₂) levels and reduced the secretion of pro-inflammatory cytokines such as IL-1 β . Collectively, these findings provide novel mechanistic insights into how phytosterols reduce macrophage-driven inflammation through lipid metabolic reprogramming.

Key words: phytosterols; inflammation; lipid metabolism.

1. Introduction

By 2030, chronic diseases—including cardiovascular disease, diabetes mellitus, and non-alcoholic fatty liver disease—are projected to account for a staggering 77% of the global disease burden^[1]. Emerging evidence indicates that systemic chronic inflammation plays a crucial role in the development of chronic diseases. This persistent inflammatory state not only disrupts immune tolerance but also induces profound dysregulation of lipid metabolism and glucose homeostasis^[2-4]. Macrophages are pivotal cells in the inflammatory process, driving sustained secretion of pro-inflammatory cytokines that contribute to the development of chronic inflammatory diseases^[2, 5]. While pharmacological interventions (e.g., metformin,

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nonsteroidal drugs) are commonly employed to suppress inflammation, their clinical utility is often limited by significant adverse effects [6, 7].

Consequently, dietary modulation has emerged as a promising adjunctive strategy for reducing the risk of chronic disease^[8]. Diet serves as a primary source of nutrition; its regulatory effects on metabolism and chronic inflammatory diseases vary significantly with dietary composition. For instance, diets high in saturated fat and sugar are associated with an increased risk of cardiovascular disease and type 2 diabetes; conversely, calorie-restricted diets are associated with a reduced risk of these conditions^[9, 10]. In this context, targeted nutritional supplementation represents a promising strategy to fine-tune inflammatory responses^[11, 12]. Phytosterols are natural bioactive compounds widely distributed in plants^[13]. It is well-recognized that phytosterols exhibit anti-inflammatory effects and thereby contribute to multiple biological processes in chronic diseases^[14–17].

Critically, macrophage function is intrinsically linked to metabolic reprogramming. Macrophages adopt distinct metabolic states to initiate inflammation, characterized by increased aerobic glycolysis, decreased oxidative phosphorylation^[18], and reprogrammed cholesterol metabolism^[19]. Notably, phytosterols modulate inflammation via the nuclear factor kappa B (NF- κ B) and AMPK signaling pathways^[20–22], both of which orchestrate energy metabolism homeostasis by balancing glycolysis and mitochondrial respiration^[23]. Recent advances have deepened our comprehension of the intricate interplay between diet, metabolic reprogramming, and various diseases is steadily deepening. Multiple studies have demonstrated the regulatory effect of phytosterols on lipid metabolism^[24]. Nevertheless, whether phytosterols regulate the inflammatory response via lipid metabolic remodeling remains unknown.

In this study, we systematically investigated the impact of phytosterols on macrophage lipidomic diversity and elucidated the relationship between lipid metabolism and the inflammatory response. This work provides mechanistic insights into the role of phytosterols in regulating macrophage-mediated inflammation.

2. Methods

2.1 Reagents and instruments.

β -sitosterol, stigmasterol, campesterol, β -cyclodextrin (β -CD) were obtained from Shanghai yuanye Bio-Technology. TNF- α , IL-1 β ELISA kit and RT-PCR primer (Table 1) were purchased from Sangon Biotechnology. RT-PCR reagents such as reverse transcriptase, random primer, oligo18 (dT), SYBR Green fluorescent was purchased from Genstar. BODIPY dye and *Hilpda* Mouse Pre-designed siRNA were purchased from MCE. FASN antibody was purchased from Cell Signaling. β -actin antibody was purchased from MDL Biotechnology. ATGL antibody, DMEM culture medium and fetal bovine serum were obtained from Thermo Fisher Scientific. Raw 264.7 macrophages were purchased from Procell Life Science & Technology. PGE₂ ELISA kit was obtained from Enzyme-linked Biotechnology. Free cholesterol test kit was obtained from Beyotime.

2.2 Cell culture and treatment

Phytosterols were nitrogen-dried to eliminate residual moisture, then combined with β -CD (10 mM) in DMEM at a 1:10 molar ratio. The solution was sonicated to complete dissolution and shaken at 37°C for 12 h to form sterol-cyclodextrin complexes. Notably, all subsequent references to β -sitosterol, stigmasterol, and campesterol specifically indicate these β -CD complexes.

Macrophages (1×10^6) were cultured in DMEM (high glucose) supplemented with 10% fetal bovine serum in a 6-well plate. 1 μ g/mL lipopolysaccharide (LPS) was added to induce inflammation response. The experimental groups were as follows: (1) Control; (2) LPS: treated with 1 μ g/mL lipopolysaccharide (LPS); (3) LPS+ β -sitosterol; (4) LPS+stigmasterol; and (5) LPS+campesterol. The last three groups were co-treated with 1 μ g/mL LPS and 10 μ M of the respective phytosterol (β -sitosterol, stigmasterol, or campesterol). The co-incubation time of phytosterols and LPS with macrophages was 12 h according to our previous study^[25].

2.3 Lipidomic analysis

Lipid compositions from the macrophages were extracted with extract solution (MTBE: MeOH=5:1) and spiked with internal standards. The samples were centrifuged, and the selected supernatants were dried in a vacuum concentrator at 37°C. The dried samples were resuspended in dichloromethane/methanol/H₂O (60:30:4.5 ratio). The mixed solution was centrifuged, and the supernatant was used for liquid chromatography–tandem mass spectrometry analysis (Biotree Biotech. Co.).

2.4 Detection of cholesterol

The cells were washed once with PBS followed by complete removal of residual liquid, and then pelleted by centrifugation. Isopropanol was added at a ratio of 100–200 μ L per $0.2\text{--}1 \times 10^6$ cells with gentle pipetting for homogenization. Cell lysis was performed using ice-cold ultrasonication at 300 W power with 2 s pulses and 3 s intervals for 3 min. The lysate was centrifuged at $12,000 \times g$ for 3–5 min at 4°C, and the resulting supernatant was collected and maintained on ice for subsequent analysis. For cholesterol detection, a 50 μ L free cholesterol working solution was prepared by mixing 46 μ L cholesterol assay buffer with 2 μ L Amplex Red reagent and 2 μ L Enzyme Mix. The samples were then mixed with the working solution at an appropriate ratio in a black 96-well plate, and fluorescence intensity was measured at excitation/emission wavelengths of 560/590 nm. The free cholesterol concentration was finally determined by comparison with a standard curve.

2.5 BODIPY lipid staining

Dissolve 1 mg BODIPY 493/503 in 382 μ L DMSO to obtain a 10 mM stock solution. Fixed macrophages were incubated with 1 mL 10 μ M BODIPY solution at room temperature for 30 min. Washing the samples with PBS to remove free dye. The images were visualized using InvitrogenTMEVOSTM microscope and were quantified using Image J.

2.6 RT-qPCR analysis

Total RNA was isolated using a commercial extraction kit, followed by cDNA synthesis via reverse transcription. Inflammatory factor expression was quantified by SYBR Green RT-qPCR on a LightCycler

480II system (Roche), with β -actin serving as the housekeeping gene. The expression levels of targeted genes were normalized and calculated using the $2^{-\Delta\Delta CT}$ method.

Table1. RT-PCR primer sequence

Gene name	Forward (5'-3')	Reverse (5'-3')
β -actin	TCCTCCTGAGCGCAAGTACTCC	CATACTCCTGCTTGCTGATCCAC
Fasn	GCTGCGGAAACTTCAGGAAAT	AGAGACGTGTCACTCCTGGACTT
Hilpda	TCGTGCAGGATCTAGCAGCAG	GCCCAGCACATAGAGGTTCA
Pnpla2	CAACGCCACTCACATCTACGG	GGACACCTCAATAATGTTGGCAC

2.7 Western blot analysis

Protein extraction was performed using RIPA Lysis Buffer IV, with subsequent quantification via BCA assay. Samples were resolved on 4-12% gradient SDS-PAGE gels (100 V, 90 min) and transferred to PVDF membranes (100 V, 1 h). After blocking with 5% nonfat milk buffer, membranes were incubated with primary antibodies overnight at 4 °C and then incubated with HRP-conjugated secondary antibodies at room temperature for 1 h. Following PBST washes, chemiluminescent detection was performed, using β -actin as the loading control.

2.8 Detection the pro-inflammation cytokines and PGE₂.

After different treatments, culture supernatants were harvested and analyzed for TNF- α , IL-1 β and PGE₂ levels using commercial ELISA kits according to the manufacturer's protocols.

2.9 Hilpda gene silencing

Prepare the mixture of Solution A and Solution B according to the manufacturer's instructions. Two hours prior to transfection, remove the culture medium and add 400 μ L of serum-free medium to the cells. Add 100 μ L of the mixed solution (Solution A and B) to the cells, resulting in a final volume of 500 μ L. Gently rock the culture plate back and forth to mix. After 4 h, replace the serum-free medium with normal complete medium containing serum. Incubate the cells for 24 h, then collect samples to measure the relative expression level of *Hilpda*. The sequences of the three groups of siRNA are shown in Table 2.

Table2. *Hilpda* siRNA sequence

siRNA	Forward (5'-3')	Reverse (5'-3')
1	GCUUCCAUCUUUGUAGAGUTT	ACUCUAACAAAGAUGGAAAGCTT
2	GCUGGGCAUCAUGUUGACCCUTT	AGGGUCAACAUGAUGCCCAGCTT
3	GCCUCCUAAGGGCCUGCCAGATT	UCUGGCAGGCCCUCUAGGAGGCTT

3. Results and discussion

3.1 Phytosterols modify the lipid metabolism network of macrophages during inflammation.

Macrophages were incubated with phytosterols under optimal conditions, as established in our previous protocol^[25]. To investigate how the phytosterols influence lipid metabolism of macrophages, lipid profiles were determined by multiple-reaction monitoring LC–MS/MS. Principal component analysis (PCA) displayed clear separation between the three phytosterol-treated groups and the LPS group, indicating distinct intergroup differences (**Figure 1A**). Orthogonal projections to latent structures-discriminant analysis

(OPLS-DA) further confirmed robust discrimination between phytosterol-treated and LPS-treated macrophages (**Figures 1B-D**). The permutation test validated the model's predictive capability, with R^2Y values ≥ 0.9 and Q^2 values > 0.7 , suggesting that the OPLS-DA model was not overfitted and the separation between groups was statistically significant (**Figure 1E-H**).

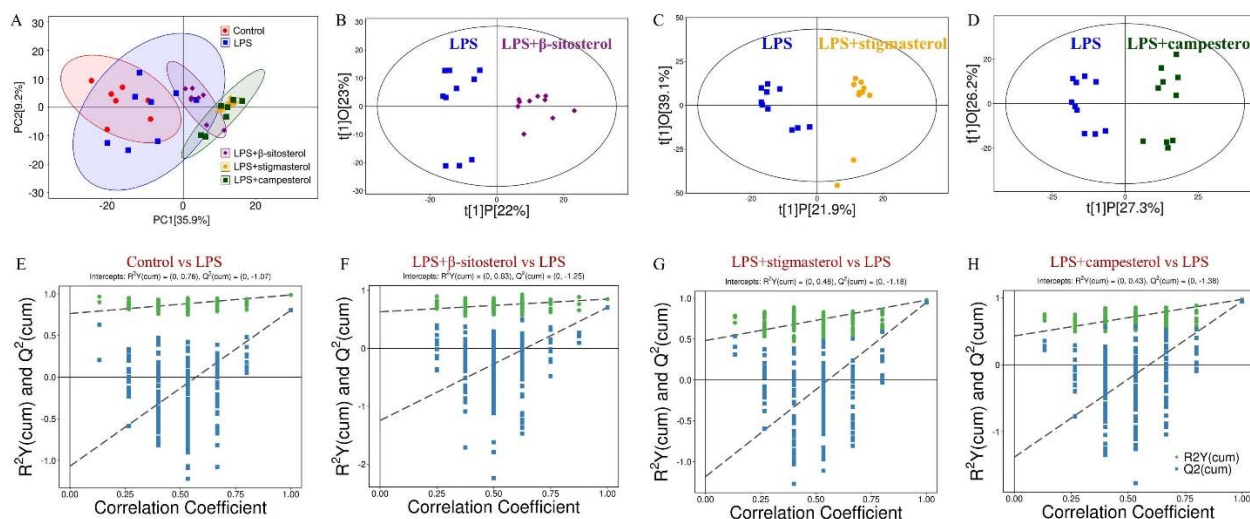


Figure 1. (A) Score plots of principal component analysis (PCA) based on control (n=7), LPS (n=8), LPS+ β -sitosterol (n=8), LPS+stigmaterol (n=7) and LPS+campesterol (n=7). (B-D) Score scatter plot of OPLS-DA model. (E-H) Permutation test of OPLS-DA model. The percentage of lipid subtypes derived from control (n=7), LPS (n=8), LPS+ β -sitosterol (n=8), LPS+stigmaterol (n=7) and LPS+campesterol (n=7).

We next analyzed the lipid composition of macrophages following phytosterols incubation. Macrophages exhibited a similar profile in different groups, predominantly enriched in phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylinositol (PI) (**Figure 2A**). Notably, PE content was dramatically decreased in phytosterol-treated groups compared with the LPS group. In contrast, triglycerides (TAG) content was significantly elevated following phytosterol treatment. Relative lipid levels varied considerably between groups (**Figure 2B**). What's more, all three phytosterols treatments increased the relative content of phosphatidylinositol, consistent with previous report^[26]. As PI serves as key precursors of lysophosphatidylinositols (LPI)—mediators of cell proliferation and migration—we focused on PI(20:4/20:4), which is significantly increased in LPS activated resident peritoneal macrophages^[27]. Although we observed an increasing trend in PI(20:4/20:4) during inflammation, phytosterol treatment attenuated this trend, though the difference was not statistically significant (**Figure 2C**).

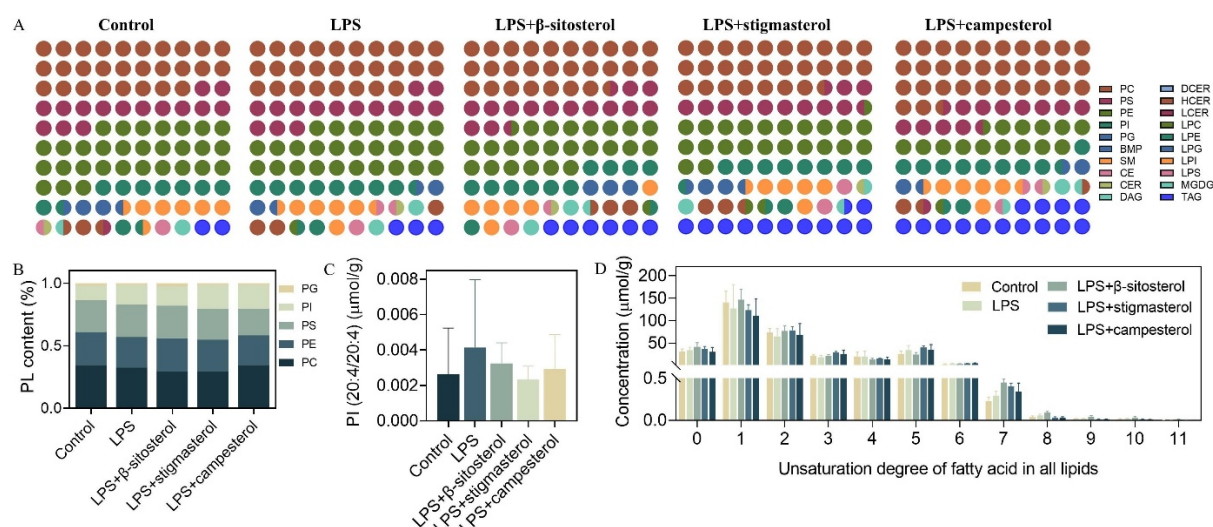


Figure 2. (A) Lipid profile of different groups. (B) Analysis of phospholipid content in control (n=7), LPS (n=8), LPS+β-sitosterol (n=8), LPS+stigmaterol (n=7) and LPS+campesterol (n=7) groups. (C) PI (20:4/20:4) content in different groups. (D) Unsaturation degree of fatty acid in all lipids of macrophages. Data are means ± s.d. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, analyzed by student's t-test.

Given our recent finding that phytosterols modulate macrophage mechanical properties^[25], and considering that fatty acid saturation profoundly influences membrane fluidity and cellular mechanics^[28], we systematically analyzed the degree of fatty acids unsaturation across all lipid classes. Lipidomic profiling revealed that the number of unsaturated double bonds in most lipid classes was fewer than 7, with no significant differences in concentration between phytosterol-treated and LPS-stimulated macrophages (Figure 2D).

These data indicate that phospholipid profiles and unsaturation degrees remained largely consistent across all treatment groups. However, it remains unclear whether this consistency extends to specific individual lipid species. Overall, distinct regulatory patterns in macrophage lipid metabolism were observed between the LPS group and the LPS + phytosterols groups. Therefore, subsequent investigations will focus on key differential lipid metabolites.

3.2 Phytosterols significantly increase long chain TAG content in macrophages

Matchstick analysis was conducted to further explore the differential metabolites with significant changes. These results suggest that LPS stimulation extensively altered the lipid composition of macrophages, including PC, PS, PE, PI, LPI, phosphatidylglycerol (PG), diacylglycerols (DAG), and TAG, compared to the control group. In contrast, the impact of phytosterols on lipid profiles was primarily observed in TAG (Figures 3A). Further analysis revealed that TAG molecules predominantly contained 50–56 total carbon atoms in their fatty acid chains, with palmitic acid (C16:0), palmitic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) as the major constituent fatty acids. These findings suggest that phytosterols remodel lipid metabolism through mechanisms distinct from those activated by LPS.

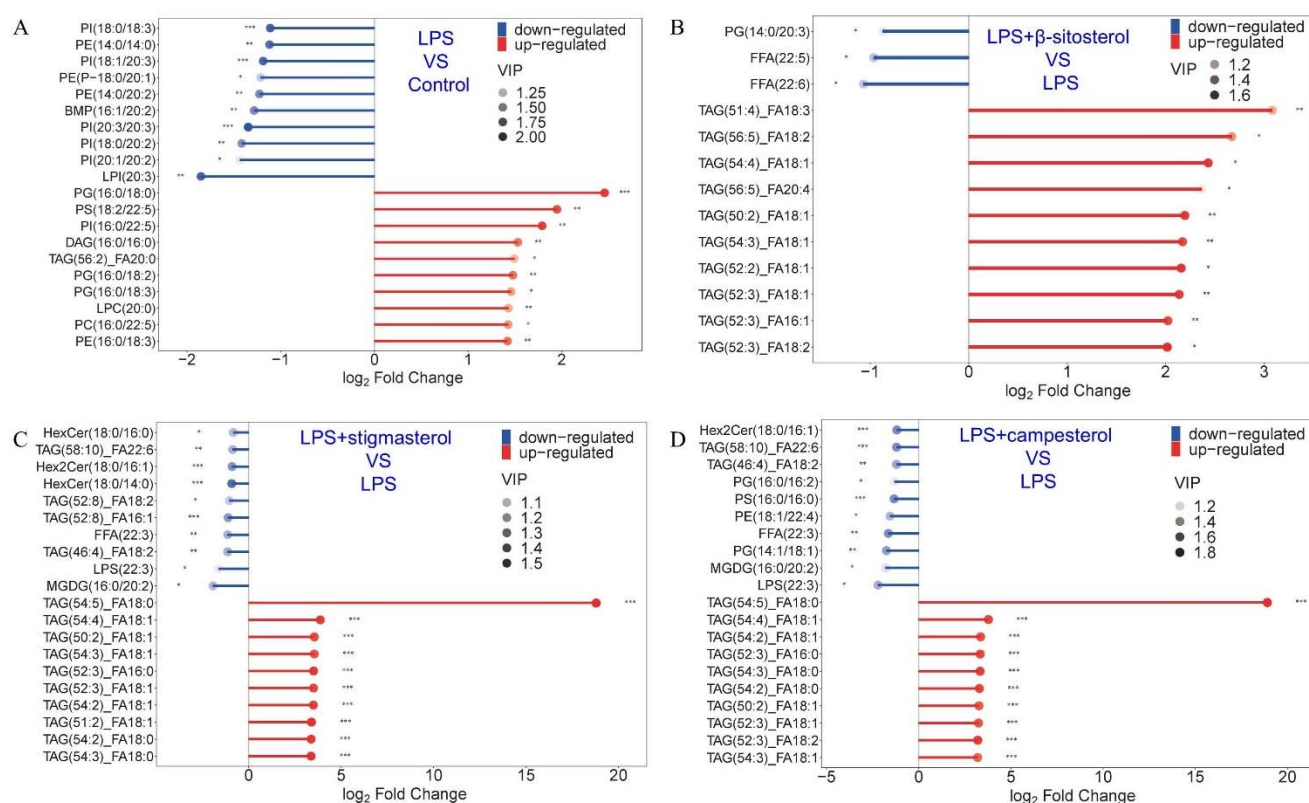


Figure 3. Matchstick analysis for (A) control vs LPS, (B) LPS+β-sitosterol vs LPS, (C) LPS+stigmasterol vs LPS and (D) LPS+campesterol vs LPS.

Detailed analysis of TAGs revealed that LPS stimulation had a minimal impact on their levels compared to the control. In contrast, phytosterols profoundly remodeled the TAG profile in LPS-stimulated macrophages, broadly altering species containing 46–56 carbon atoms (**Figure 4A–B**). Mechanistically, the observed accumulation of C46–C56 TAGs in phytosterol-treated macrophages likely reflects a key metabolic adaptation with functional consequences. This pattern resembles the lipidomic signature of M2 macrophages—a phenotype dependent on fatty acid oxidation—which supports this interpretation [29]. We postulate that this accumulation is an active process that may regulate macrophage polarization and thereby shape the inflammatory outcome. TAG biosynthesis occurs via diacylglycerol acyltransferase-mediated esterification of DAG derived from the Kennedy pathway. Furthermore, DAG is also generated during the initial hydrolysis of TAG within lipid droplets. Notably, phytosterol treatment significantly increased DAG concentrations in macrophages concomitant with elevated TAG accumulation (**Figure 4C**), suggesting a potential enhancement of de novo TAG synthesis and/or a reduction in TAG hydrolysis.

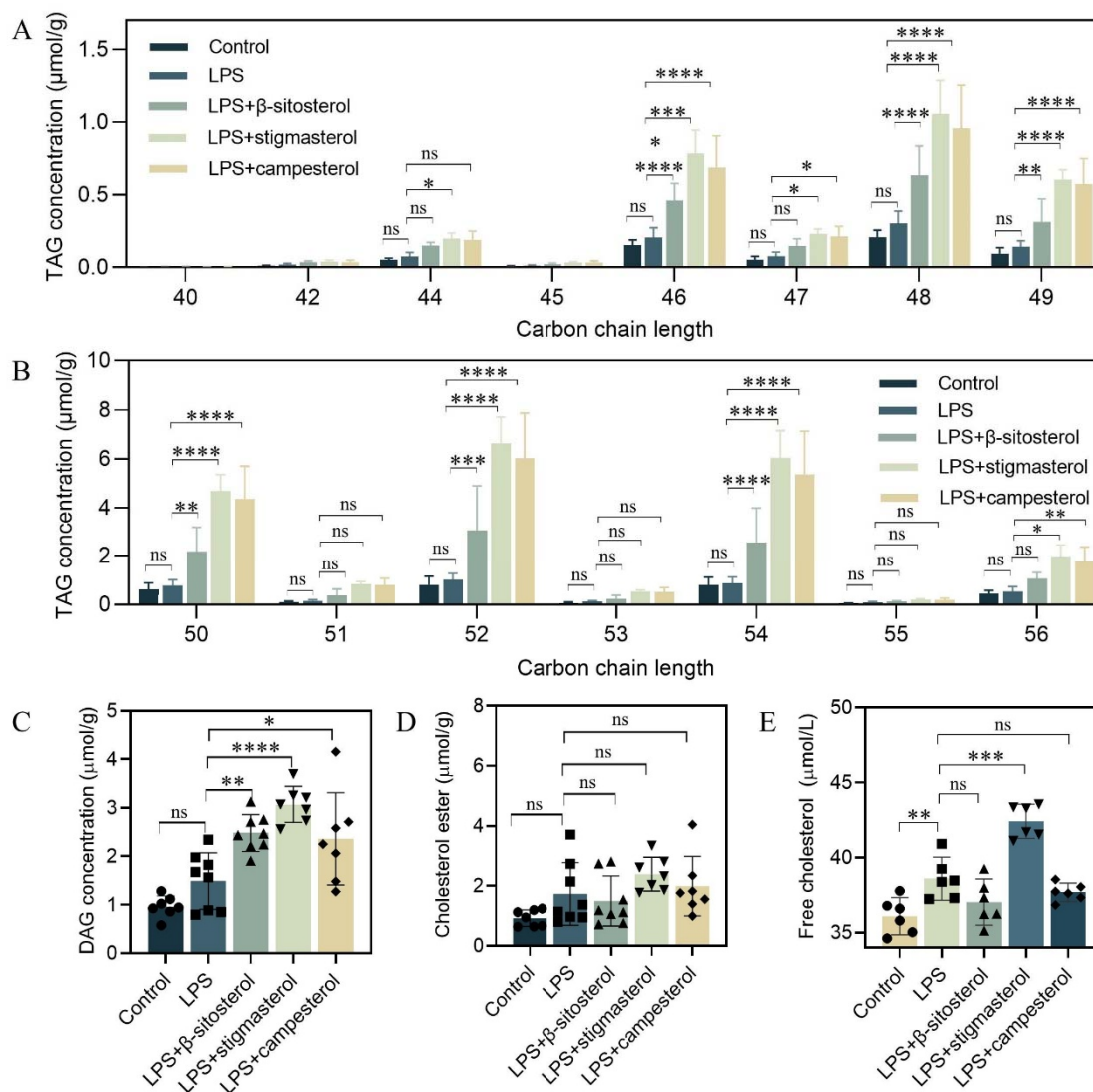


Figure 4. (A–B) Concentration of TAG with varied carbon chain length in control (n=7), LPS (n=8), LPS+ β -sitosterol (n=8), LPS+stigmasterol (n=7) and LPS+campesterol (n=7) groups. Concentration of (C) DAG and (D) cholesterol ester in control (n=7), LPS (n=8), LPS+ β -sitosterol (n=8), LPS+stigmasterol (n=7) and LPS+campesterol (n=7) groups. (E) Concentration of free cholesterol in different groups (n=6). Data are means $\pm$ s.d. * P < 0.05, ** P < 0.01, *** P < 0.001, analyzed by student's t-test.

As the analogue of cholesterol, the influence of phytosterols on cholesterol metabolism should not be ignored. Unlike free cholesterol, cholesterol esters are preferentially stored in lipid droplets rather than integrated into bilayer membranes [30]. Quantification revealed no significant effect of phytosterols on cholesterol ester levels (Figure 4D). Although both LPS and LPS+stigmasterol treatments increased free cholesterol concentrations, other phytosterols did not display this effect (Figure 4E). Collectively, these data indicate that phytosterols remodel macrophage lipid metabolism primarily through compositional changes in TAG species.

3.3 Reduced lipolysis in lipid droplets inhibits production of pro-inflammatory mediators

Lipid droplets are ubiquitous organelles regulating TAG storage and mobilization. Emerging evidence indicates that beyond mediating energy homeostasis, lipid droplets are closely associated with innate inflammation [31]. Therefore, we first characterized the lipid storage dynamics in macrophages. As shown in

Figures 5A-B, LPS stimulation increased lipid droplets content, consistent with previous studies^[32, 33]. Hierarchical clustering analysis of TAG metabolism further demonstrated distinct LPS-induced reprogramming (**Figure 5C**), providing mechanistic context for the observed lipid storage fluorescence. Furthermore, phytosterol treatment, as evidenced by fluorescence intensity, surpassed LPS in potentiating LD formation and provoked more extensive TAG network remodeling, as visualized by hierarchical clustering heatmaps (**Figure S1**).

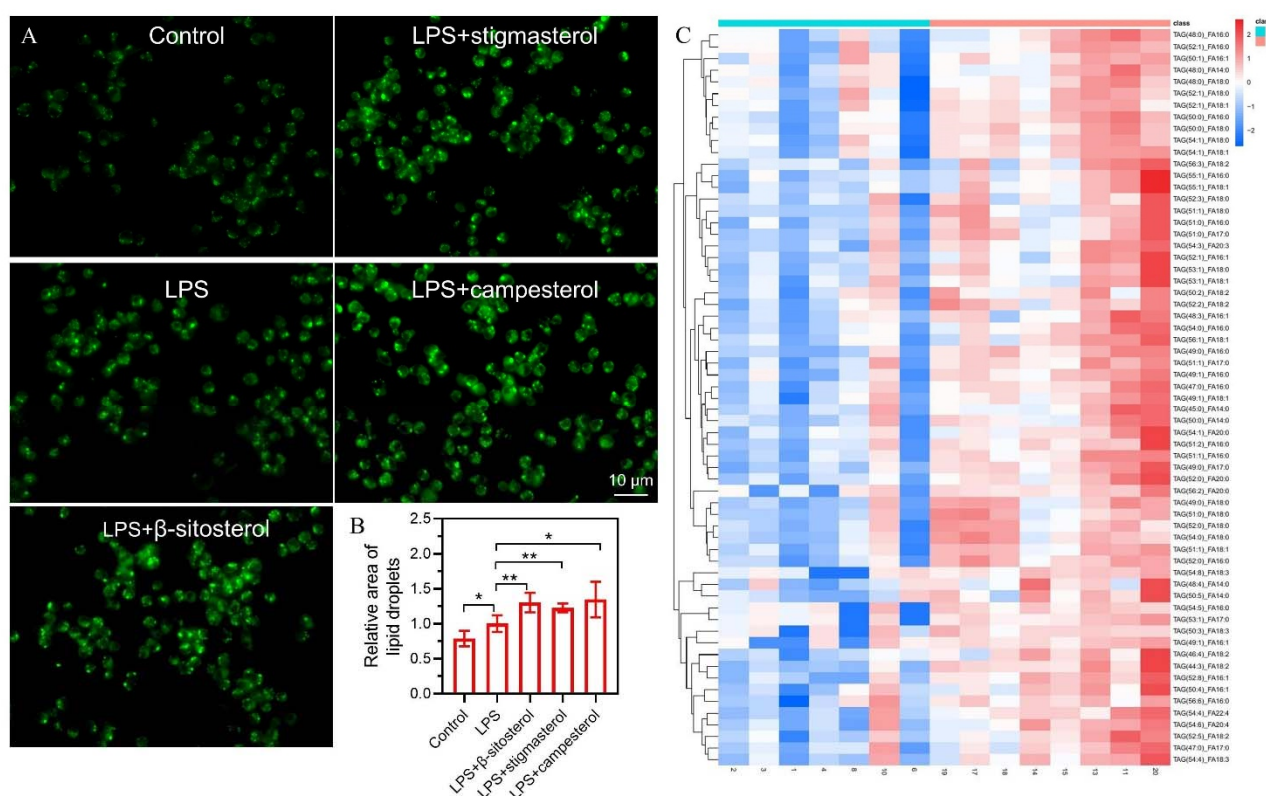


Figure 5. (A) Fluorescence images of lipid droplets in macrophages. (B) Relative area analysis of lipid droplets (n=5). (C) A hierarchical clustering heatmap of TAG between control and LPS group. Data are means $\pm$ s.d. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, analyzed by student's t-test.

To investigate how phytosterols influence lipolysis dynamic of lipid droplet, we analyzed the mRNA expression of key regulators of lipid-droplet homeostasis: adipose triglyceride lipase (*Atgl*), fatty acid synthase (*Fasn*) and hypoxia inducible lipid droplet associated proteins (*Hilpda*). RT-qPCR revealed that phytosterol treatment significantly downregulated *Fasn* and *Atgl* expression while upregulating *Hilpda* compared to LPS-stimulated macrophages (**Figure S2**). Consistent with these findings, western blot analysis confirmed reduced ATGL and FASN protein levels following phytosterol intervention (**Figures 6A**).

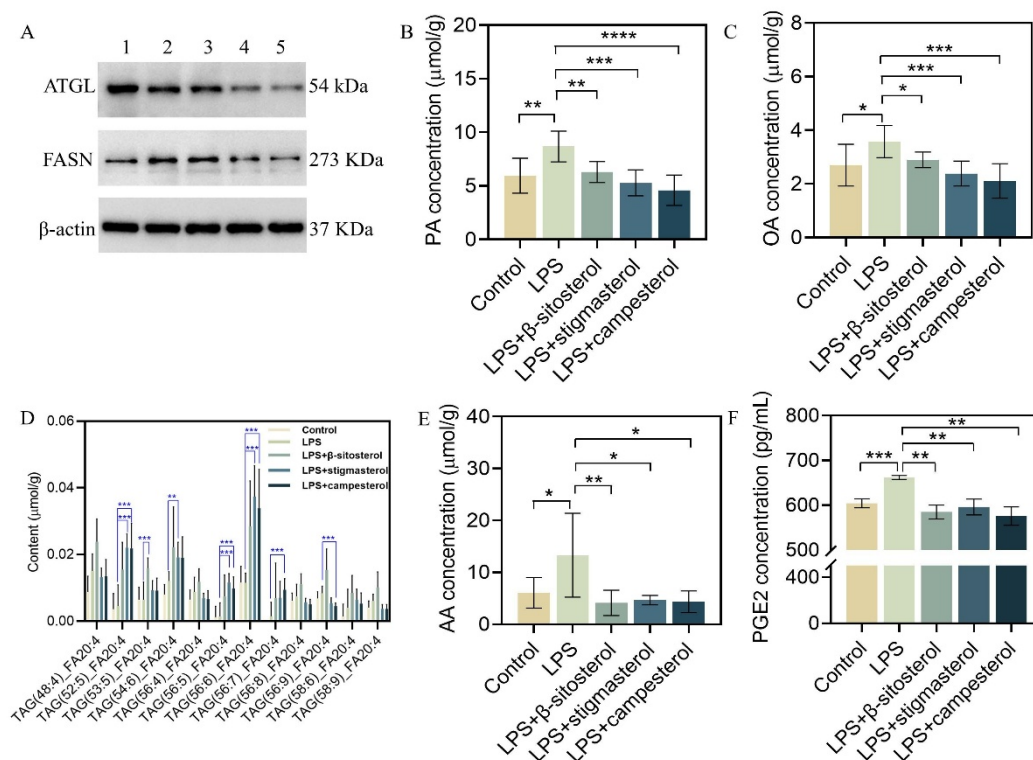


Figure 6. (A) ATGL and FASN levels quantified by western blot. (B-E) Concentration of (B) PA, (C) OA, (D) free AA and (E) esterified AA in control (n=7), LPS (n=8), LPS+ β -sitosterol (n=8), LPS+stigmasterol (n=7) and LPS+campesterol (n=7) groups. (F) PGE₂ concentration in macrophages after different treatments. Data are means $\pm$ s.d. * P < 0.05, ** P < 0.01, *** P < 0.001, analyzed by student's t-test.

FASN, a key enzyme in *de novo* lipogenesis, has been shown to negatively regulate M1 macrophage activation when overexpressed [34]. ATGL catalyzes the initial step of triglyceride hydrolysis and modulates immune responses; its deficiency is known to suppress inflammation [35]. Upregulation of *Hilpda* is closely associated with enhanced lipid storage and functions as a physiological ATGL inhibitor [36, 37]. Collectively, downregulation of ATGL and upregulation of HILPDA promote TAG accumulation. These results align with matchstick analysis, indicating that although LPS broadly remodels macrophage lipid metabolism, phytosterols primarily drive TAG accumulation.

Palmitic acid (C16:0) and stearic acid (C18:0) represent major triglyceride components within lipid droplets [38]. Their decreased levels further support the attenuation of TAG hydrolysis upon phytosterol treatment (Figures 6B-C). However, the precise role of lipid droplet accumulation in mediating the anti-inflammatory effects of phytosterols remains unclear. While lipid droplet formation typically functions as a cytoprotective mechanism against lipotoxicity generated by lipid intermediates or free fatty acids, LDs simultaneously serve as enzymatic platforms for eicosanoid biosynthesis from arachidonic acid (AA) [32, 39]. Therefore, we analyzed AA distribution pattern in macrophages. The results demonstrated that phytosterols significantly reduced free AA levels (Figure 6E) while increasing esterified AA within TAG (Figure 6D).

AA acts as a precursor for diverse bioactive lipid metabolites that serve as intracellular messengers [40]. Among these, prostaglandin E₂ (PGE₂) is a major AA-derived lipid mediator critically associated with macrophage inflammatory responses [41]. It is well established that PGE₂ directly regulates cytokine release.

Our results indicated that phytosterol treatment markedly reduced PGE₂ concentration (**Figure 6F**), consistent with the decreased availability of free AA.

To assess whether phytosterols inhibit lipid droplet hydrolysis through dual regulation of ATGL suppression and HILPDA induction, we performed *Hilpda* silencing using small interfering RNA (siRNA). Transfection efficiency was confirmed in **Figure 7A**. RT-qPCR analysis demonstrated that siRNA-2 and siRNA-3 effectively reduced *Hilpda* mRNA expression (**Figure 7B**). We further measured the pro-inflammatory cytokines in macrophages transfected with siRNA-3. As shown in **Figures 7C-D**, phytosterol treatment resulted in higher production of TNF- α and IL-1 β in *Hilpda*-silenced macrophages compared with non-silenced controls. Notably, phytosterols retained anti-inflammatory activity even upon *Hilpda* silencing, indicating that HILPDA-mediated regulation of lipid droplet dynamics is not the sole pathway through which phytosterols exert anti-inflammatory effects.

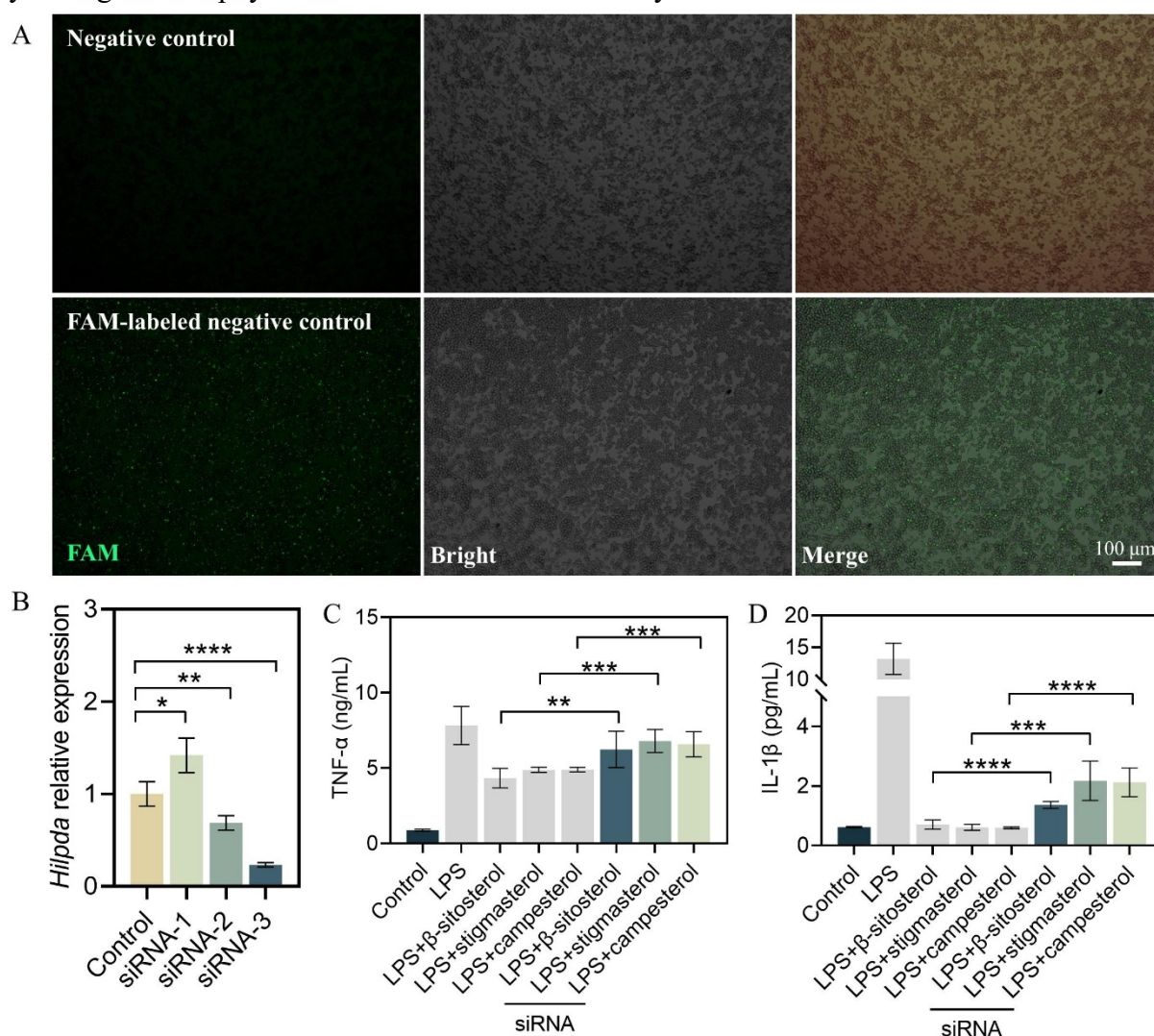


Figure 7. (A) Fluorescence images of macrophages after transfection of siRNA. (B) Relative expression of *Hilpda* in macrophages after transfection of siRNA. (C-D) The concentration of pro-inflammatory cytokines in macrophages after transfected with *Hilpda* siRNA. Data are means $\pm$ s.d. * P < 0.05, ** P < 0.01, *** P < 0.001, analyzed by student's t-test.

Previous studies have established that phytosterols alleviate inflammation by modulating classical signaling pathways such as the NLRP3 inflammasome and NF- κ B. Our study provides novel insight by demonstrating that phytosterols suppress the inflammatory response by downregulating AA production—a

key pro-inflammatory precursor—through regulation of ATGL and HILPDA expression. Substantial evidence indicates that AA and its pro-inflammatory derivatives are potent activators of both NLRP3 and NF- κ B signaling^[41–43]. Consequently, our findings uncover an upstream key mechanism that precedes the modulation of these classical inflammatory pathways.

4. Conclusion

These findings substantially deepen our understanding of the complex interactions among phytosterols, lipid metabolism, and inflammation in macrophages. By establishing a direct mechanistic connection—whereby phytosterols modulate key lipolytic enzymes (ATGL and HILPDA) to control lipid droplet breakdown—this work provides a crucial molecular explanation for the observed lipid remodeling, characterized by reduced generation of pro-inflammatory arachidonic acid and concurrent accumulation of TAG. More importantly, this study unravels a previously unrecognized pathway through which dietary or supplemental phytosterols exert immunomodulatory effects: by regulating ATGL-mediated lipolysis, they limit the production of the potent inflammatory mediator PGE₂ and subsequent cytokine secretion. Therefore, our results support the potential of phytosterol supplementation as a promising nutritional strategy for inflammatory disorders associated with dysregulated macrophage lipid metabolism, such as atherosclerosis and certain autoimmune diseases. In a broader context, this research underscores how specific dietary components can shape fundamental inflammatory signaling cascades through metabolic reprogramming.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data supporting this article have been included as part of the Supplementary Information.

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Reference

- [1] J. Shu, W. Jin, Prioritizing non-communicable diseases in the post-pandemic era based on a comprehensive analysis of the GBD 2019 from 1990 to 2019, *Sci. Rep.* 13 (2023) 13325. <https://doi.org/10.1038/s41598-023-40595-7>.
- [2] D. Furman, J. Campisi, E. Verdin, et al., Chronic inflammation in the etiology of disease across the life span, *Nat. Med.* 25 (2019) 1822–1832. <https://doi.org/10.1038/s41591-019-0675-0>.

- [3] V.N. Sukhorukov, A.N. Orekhov, Molecular aspects of inflammation and lipid metabolism in health and disease: The role of the mitochondria, *Int. J. Mol. Sci.* 25 (2024) 6299. <https://doi.org/10.3390/ijms25126299>.
- [4] Y.A.-O. Lee, J. Olefsky, Chronic tissue inflammation and metabolic disease, *Genes Dev.* 35 (2021) 307-328. <https://doi.org/10.1101/gad.346312>.
- [5] J.N. Fullerton, D.W. Gilroy, Resolution of inflammation: a new therapeutic frontier, *Nat. Rev. Drug Discovery.* 15 (2016) 551-567. <https://doi.org/10.1038/nrd.2016.39>.
- [6] M. Foretz, B. Guigas, B. Viollet, Metformin: update on mechanisms of action and repurposing potential, *Nat. Rev. Endocrinol.* 19 (2023) 460-476. <https://doi.org/10.1038/s41574-023-00833-4>.
- [7] Z. Salis, A. Sainsbury, Association of long-term use of non-steroidal anti-inflammatory drugs with knee osteoarthritis: a prospective multi-cohort study over 4-to-5 years, *Sci. Rep.* 14 (2024) 6593. <https://doi.org/10.1038/s41598-024-56665-3>.
- [8] P. Wang, M. Song, A.H. Eliassen, et al., Optimal dietary patterns for prevention of chronic disease, *Nat. Med.* 29 (2023) 719-728. <https://doi.org/10.1038/s41591-023-02235-5>.
- [9] X. Xiao, Z. Qin, X. Lv, et al., Dietary patterns and cardiometabolic risks in diverse less-developed ethnic minority regions: results from the China Multi-Ethnic Cohort (CMEC) Study, *The Lancet Regional Health Western Pacific.* 15 (2021) 100252. <https://doi.org/10.1016/j.lanwpc.2021.100252>.
- [10] E. Ahmad, S. Lim, R. Lamprey, et al., Type 2 diabetes, *The Lancet.* 400 (2022) 1803-1820. [https://doi.org/10.1016/S0140-6736\(22\)01655-5](https://doi.org/10.1016/S0140-6736(22)01655-5).
- [11] Y. Xiao, Y. Gong, Y. Qi, et al., Effects of dietary intervention on human diseases: molecular mechanisms and therapeutic potential, *Signal Transduction and Targeted Ther.* 9 (2024) 1-34. <https://doi.org/10.1038/s41392-024-01771-x>.
- [12] S.H. Ley, O. Hamdy, V. Mohan, et al., Prevention and management of type 2 diabetes: dietary components and nutritional strategies, *The Lancet.* 383 (2014) 1999-2007. [https://doi.org/10.1016/S0140-6736\(14\)60613-9](https://doi.org/10.1016/S0140-6736(14)60613-9).
- [13] M. Chang, Z. Wang, T. Zhang, et al., Characterization of fatty acids, triacylglycerols, phytosterols and tocopherols in peony seed oil from five different major areas in China, *Food Res. Int.* 137 (2020) 109416. <https://doi.org/10.1016/j.foodres.2020.109416>.
- [14] M. Prasad, S. Jayaraman, M.A. Eladl, et al., A comprehensive review on therapeutic perspectives of phytosterols in insulin resistance: A mechanistic approach, *Molecules.* 27 (2022) 1595. <https://doi.org/10.3390/molecules27051595>.
- [15] M. Shen, L. Yuan, J. Zhang, et al., Phytosterols: Physiological functions and potential application, *Foods.* 13 (2024) 1754. <https://doi.org/10.3390/foods13111754>.
- [16] Y. Li, Y. Liu, J. Qiao, et al., Foxtail millet prolamin-pectin nanoparticles enhanced the stability and bioavailability of β -sitosterol, *Food Res. Int.* 205 (2025) 115998. <https://doi.org/10.1016/j.foodres.2025.115998>.
- [17] Y. Chen, L. Fu, Y. Wang, et al., Cholesterol-lowering effects of phytosterol combined with phosphatidylcholine and their mechanism of action in hypercholesterolemic rats, *Food Funct.* 16 (2025) 7858-7879. <https://doi.org/10.1039/d5fo02852c>.
- [18] E. Vassiliou, R. Farias-Pereira, Impact of lipid metabolism on macrophage polarization: implications for inflammation and tumor immunity, *Int. J. Mol. Sci.* 24 (2023) 12032. <https://doi.org/10.3390/ijms241512032>.
- [19] M.-S. Lee, S.J. Bensinger, Reprogramming cholesterol metabolism in macrophages and its role in host defense against cholesterol-dependent cytotoxins, *Cell. Mol. Immunol.* 19 (2022) 327-336. <https://doi.org/10.1038/s41423-021-00827-0>.
- [20] F. Jie, X. Yang, B. Yang, et al., Stigmasterol attenuates inflammatory response of microglia via NF- κ B and NLRP3 signaling by AMPK activation, *Biomed. Pharmacother.* 153 (2022) 113317. <https://doi.org/10.1016/j.biopha.2022.113317>.
- [21] S. Jayaraman, N. Devarajan, P. Rajagopal, et al., β -Sitosterol circumvents obesity induced inflammation and insulin resistance by down-regulating IKK β /NF- κ B and JNK signaling pathway in adipocytes of type 2 diabetic rats, *Molecules.* 26 (2021) 26072101. <https://doi.org/10.3390/molecules26072101>.
- [22] N. El Omari, S. Bakrim, A. Khalid, et al., Unveiling the molecular mechanisms: dietary phytosterols as guardians against cardiovascular diseases, *Natural Products and Bioprospecting.* 14 (2024) 38722432. <https://doi.org/10.1007/s13659-024-00451-1>.
- [23] C. Mauro, S.C. Leow, E. Anso, et al., NF- κ B controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration, *Nat. Cell Biol.* 13 (2011) 1272-1279. <https://doi.org/10.1038/ncb2324>.

- [24] X. Chen, L.S. Memory Kunda, X. Li, et al., A comprehensive review of beneficial effects of phytosterols on glycolipid metabolism and related mechanisms, *J. Agric. Food Chem.* 73 (2025) 3826-3841. <https://doi.org/10.1021/acs.jafc.4c10375>.
- [25] X. Wang, Y. Liu, F. Du, et al., Phytosterol-mediated disturbance of the lipid packing order in the plasma membrane regulates inflammatory response, *Food Funct.* 16 (2025) 6150-6159. <https://doi.org/10.1039/D5FO01767J>.
- [26] J. Masquelier, M. Alhouayek, R. Terrasi, et al., Lysophosphatidylinositols in inflammation and macrophage activation: Altered levels and anti-inflammatory effects, *Biochim. Biophys. Acta.* 1863 (2018) 1458-1468. <https://doi.org/10.1016/j.bbalip.2018.09.003>.
- [27] L. Gil-de-Gómez, A.M. Astudillo, C. Meana, et al., A phosphatidylinositol species acutely generated by activated macrophages regulates innate immune responses, *J. Immunol.* 190 (2013) 5169-5177. <https://doi.org/10.4049/jimmunol.1203494>.
- [28] M.F. Renne, R. Ernst, Membrane homeostasis beyond fluidity: control of membrane compressibility, *Trends Biochem. Sci.* 48 (2023) 963-977. <https://doi.org/10.1016/j.tibs.2023.08.004>.
- [29] P. Yang, H. Qin, Y. Li, et al., CD36-mediated metabolic crosstalk between tumor cells and macrophages affects liver metastasis, *Nat. Commun.* 13 (2022) 5782. <https://doi.org/10.1038/s41467-022-33349-y>.
- [30] E. Ikonen, Cellular cholesterol trafficking and compartmentalization, *Nat. Rev. Mol. Cell Biol.* 9 (2008) 125-138. <https://doi.org/10.1038/nrm2336>.
- [31] M. Bosch, M. Sánchez-Álvarez, A. Fajardo, et al., Mammalian lipid droplets are innate immune hubs integrating cell metabolism and host defense, *Science.* 370 (2020) <https://doi.org/10.1126/science.aay8085>.
- [32] A. Castoldi, L.B. Monteiro, N. van Teijlingen Bakker, et al., Triacylglycerol synthesis enhances macrophage inflammatory function, *Nat. Commun.* 11 (2020) 4107. <https://doi.org/10.1038/s41467-020-17881-3>.
- [33] W.-Y. Hsieh, Q.D. Zhou, A.G. York, et al., Toll-Like receptors induce signal-specific reprogramming of the macrophage lipidome, *Cell Metab.* 32 (2020) 128-143. <https://doi.org/10.1016/j.cmet.2020.05.003>.
- [34] Y. Xiao, Y. Yang, H. Xiong, et al., The implications of FASN in immune cell biology and related diseases, *Cell Death Differ.* 25 (2024) 88. <https://doi.org/10.1038/s41419-024-06463-6>.
- [35] E. Aflaki, N.A. Balenga, P. Luschnig-Schratl, et al., Impaired Rho GTPase activation abrogates cell polarization and migration in macrophages with defective lipolysis, *Cell. Mol. Life Sci.* 68 (2011) 3933-3947. <https://doi.org/10.1007/s00018-011-0688-4>.
- [36] K.A.-O. Padmanabha Das, L. Wechselberger, M. Liziczai, et al., Hypoxia-inducible lipid droplet-associated protein inhibits adipose triglyceride lipase, *J. Lipid Res.* 59 (2018) 531-541. <https://doi.org/10.1194/jlr.M082388>.
- [37] X.A.M.H. van Dierendonck, M.A. de la Rosa Rodriguez, A. Georgiadi, et al., HILPDA uncouples lipid droplet accumulation in adipose tissue macrophages from inflammation and metabolic dysregulation, *Cell Rep.* 30 (2020) 1811-1822. <https://doi.org/10.1016/j.celrep.2020.01.046>.
- [38] C. Yew Tan, S. Virtue, S. Murfitt, et al., Adipose tissue fatty acid chain length and mono-unsaturation increases with obesity and insulin resistance, *Sci. Rep.* 5 (2015) 18366. <https://doi.org/10.1038/srep18366>.
- [39] M.A. Bermúdez, M.A. Balboa, J. Balsinde, Lipid droplets, phospholipase a2, arachidonic acid, and atherosclerosis, *Biomedicines*, *Biomedicines.* 9 (2021) 1891. <https://doi.org/10.3390/biomedicines9121891>.
- [40] B. Wang, L. Wu, J. Chen, et al., Metabolism pathways of arachidonic acids: mechanisms and potential therapeutic targets, *Signal Transduction Targeted Ther.* 6 (2021) 94. <https://doi.org/10.1038/s41392-020-00443-w>.
- [41] F.A. Kuehl, R.W. Egan, Prostaglandins, arachidonic acid, and inflammation, *Science.* 210 (1980) 978-984. <https://doi.org/doi:10.1126/science.6254151>.
- [42] Y. Ma, J. Jiang, C. Zhao, et al., Arachidonic acid metabolism in metabolic dysfunction-associated steatotic liver disease and liver fibrosis, *Hepatol. Commun.* 9 (2025) e0802. https://journals.lww.com/hepcomm/fulltext/2025/09010/arachidonic_acid_metabolism_in_metabolic.18.aspx
- [43] T. Wang, X. Fu, Q. Chen, et al. Arachidonic acid metabolism and kidney inflammation. *Int. J. Mol. Sci.* 20. 2019:3683. <https://doi.org/doi: 10.3390/ijms20153683>.