

Development of a Standardized Thylakoid Phytochemical Combination: Phytochemical Profiling, Mechanistic Investigation, and Preclinical Evaluation for Weight Management

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ABSTRACT

Obesity is a multifactorial metabolic disorder characterized by excessive adiposity, dysregulated energy balance, and an increased risk of cardiovascular disease, type 2 diabetes, and other metabolic complications. Although lifestyle interventions and pharmacological therapies remain central to weight management, interest in plant-derived bioactive compounds and food-based interventions has increased because of their potential to modulate appetite, nutrient digestion, energy metabolism, and metabolic inflammation. Thylakoid membranes, which are pigment–protein-rich structures derived from the chloroplasts of green plants, have attracted attention for their potential effects on satiety and postprandial metabolism. Their proposed physiological actions include delayed lipid digestion, modulation of gastrointestinal satiety signals, and changes in food-reward responses. Combining thylakoids with selected phytochemicals may provide a complementary strategy for targeting multiple pathways involved in energy homeostasis. However, the efficacy, compatibility, and potential synergistic effects of such combinations require systematic investigation. This review discusses the scientific rationale for developing a standardized thylakoid–phytochemical formulation for weight management, with particular emphasis on source selection, phytochemical characterization, analytical standardization, formulation development, mechanistic investigation, and preclinical evaluation. Candidate phytochemicals, including catechins, chlorogenic acid, curcuminoids, and other plant-derived polyphenols, are examined in terms of their potential complementary biological activities and analytical requirements. The review also proposes an integrated quality-by-design framework incorporating critical quality attributes, risk assessment, chromatographic fingerprinting, stability studies, in vitro digestion models, cell-based assays, and animal studies. Safety, bioavailability, batch-to-batch variability, and translational limitations are considered essential determinants of product development. A standardized thylakoid–phytochemical combination represents a promising research direction, but its suitability for weight management must be established through reproducible analytical characterization, mechanistic validation, and appropriately designed preclinical studies before clinical efficacy can be claimed.

Keywords: *Thylakoid membranes; weight management; phytochemicals; obesity; phytochemical profiling; nutraceutical formulation.*

1. INTRODUCTION

Obesity has emerged as a major global public health concern because of its association with metabolic dysfunction, cardiovascular disease, type 2 diabetes

mellitus, certain cancers, and reduced quality of life. Its development reflects a complex interaction among dietary patterns, physical activity, genetic susceptibility, environmental influences, sleep, hormonal regulation,

and behavioural factors. Consequently, effective weight management requires approaches that address not only energy intake and expenditure but also appetite regulation, food preferences, metabolic health, and long-term adherence to lifestyle changes. Conventional weight-management strategies include dietary modification, physical activity, behavioural interventions, pharmacological treatment, and bariatric procedures in appropriately selected individuals. Although these interventions can produce clinically meaningful benefits, treatment accessibility, tolerability, long-term adherence, and weight regain remain important challenges. These limitations have encouraged investigations into plant-derived bioactive compounds and food-based interventions that may complement established approaches [1, 2].

Plant-based ingredients contain diverse bioactive constituents, including polyphenols, flavonoids, carotenoids, terpenoids, alkaloids, and other secondary metabolites. Depending on their chemical structures and biological properties, these compounds may influence digestive enzymes, nutrient absorption, oxidative stress, inflammatory signalling, insulin sensitivity, and energy metabolism. Nevertheless, their effects vary considerably with the source material, dose, formulation, bioavailability, and experimental model.

Thylakoids are specialized membrane structures located within plant chloroplasts. They contain chlorophyll, carotenoids, membrane lipids, proteins, and components of the photosynthetic machinery. Spinach-derived thylakoid preparations have received particular research attention because of their proposed effects on satiety, appetite, and lipid digestion. Their membrane-associated composition distinguishes them from conventional botanical extracts, which generally contain selected soluble or extractable phytochemicals. The development of a standardized thylakoid–phytochemical combination offers an opportunity to investigate whether the structural properties of thylakoid membranes can complement the biological activities of selected phytochemicals. For example, a formulation could be designed to investigate thylakoid-associated effects on gastrointestinal lipid processing alongside the potential metabolic activities of green tea catechins or chlorogenic acid. Such a combination could potentially target several processes associated with energy balance, although complementary mechanisms and improved efficacy must be demonstrated experimentally [3].

A major challenge is that combining two biologically active materials does not automatically produce synergistic effects. Interactions may be additive, synergistic, neutral, or antagonistic. Phytochemicals may also alter membrane integrity, pigment stability, dispersion characteristics, or the release of membrane-associated constituents. Therefore, formulation

development must integrate biological rationale with rigorous analytical characterization and mechanistic evaluation. This review examines the development of a standardized thylakoid–phytochemical combination for weight management, emphasizing the selection of compatible ingredients, phytochemical profiling, quality control, mechanistic investigation, and preclinical assessment [4].

2. Biological and Structural Characteristics of Thylakoids

2.1. Structure and composition

Thylakoids are flattened membranous compartments within the chloroplasts of plants and algae. They contain pigment–protein complexes, including photosystems I and II, light-harvesting complexes, and components involved in photosynthetic electron transport. Their membranes comprise a complex mixture of glycolipids, phospholipids, proteins, chlorophylls, and carotenoids. For nutraceutical development, the composition of thylakoid preparations depends on the plant species, cultivation conditions, maturity, harvesting practices, extraction procedure, and storage environment. Spinach is a commonly investigated source because it is widely available and contains chloroplast-rich green tissues. Other green plant materials may also be considered, but their suitability requires independent characterization. Thylakoid preparations should not be treated as chemically uniform materials. Depending on the manufacturing process, the final preparation may contain intact membrane fragments, partially disrupted membranes, residual soluble proteins, plant pigments, minerals, and other cellular components. Consequently, a standardized preparation should be defined through measurable structural, compositional, and functional characteristics [5].

2.2. Potential relevance to appetite regulation

Food intake is regulated by interconnected central and peripheral pathways. Gastrointestinal signals, circulating nutrients, adipose-derived hormones, and neural inputs collectively influence hunger, satiety, meal size, and food-reward responses. Thylakoid-containing preparations have been investigated for their potential to modify appetite and satiety. Proposed explanations include changes in the digestion and availability of dietary lipids, altered gastrointestinal signalling, and changes in the perceived reward associated with energy-dense foods. These mechanisms remain areas of investigation, and their relative contributions may depend on the preparation and experimental conditions. It is important to distinguish short-term appetite-related outcomes from sustained reductions in body weight. Changes in subjective hunger or satiety do not necessarily translate into lower long-term energy intake

or clinically meaningful weight loss. Accordingly, preclinical and clinical studies should evaluate these outcomes separately [6].

2.3. Potential influence on lipid digestion

Dietary lipids undergo emulsification, enzymatic hydrolysis, and intestinal absorption. Pancreatic lipase plays a central role in the hydrolysis of triglycerides into absorbable lipid products. Thylakoid preparations have been investigated for their capacity to interact with lipid digestion processes. Proposed mechanisms include interactions with lipid droplets, modification of the physical environment in which digestive enzymes act, and changes in the accessibility of dietary lipids to digestive enzymes. These possibilities require direct experimental verification. A reduction in apparent lipase activity in a laboratory assay may arise from genuine enzyme inhibition, substrate sequestration, altered emulsion characteristics, or optical interference caused by plant pigments. Therefore, multiple complementary assays should be used to determine whether a preparation genuinely modifies lipid digestion [7].

2.4. Factors influencing thylakoid functionality

Thylakoid functionality may be affected by:

- Membrane integrity and particle-size distribution.
- Lipid and protein composition.
- Chlorophyll and carotenoid content.
- Processing temperature and exposure to oxygen.

- pH, ionic strength, and digestive enzymes.
- Freeze–thaw cycles and storage conditions.
- Interactions with added phytochemicals and formulation excipients.

These factors should be incorporated into the product-development strategy because structural disruption or chemical degradation could alter the intended biological activity.

3. Scientific Rationale for Combining Thylakoids with Phytochemicals

3.1. Concept of a multi-component formulation

A thylakoid–phytochemical formulation is intended to combine a membrane-rich plant fraction with one or more chemically characterized bioactive compounds. The scientific rationale is to investigate complementary effects on appetite, digestive physiology, metabolic regulation, and associated biochemical pathways. Thylakoids may contribute membrane-associated effects during gastrointestinal digestion, whereas selected phytochemicals may exert biological effects through enzyme modulation, cellular signalling, or antioxidant and anti-inflammatory pathways. However, the magnitude and physiological relevance of these effects depend on the compound, concentration, route of exposure, and bioavailability. The formulation should therefore be developed around a defined biological hypothesis rather than a general assumption that combining plant ingredients necessarily enhances efficacy (Fig. 1) [8].

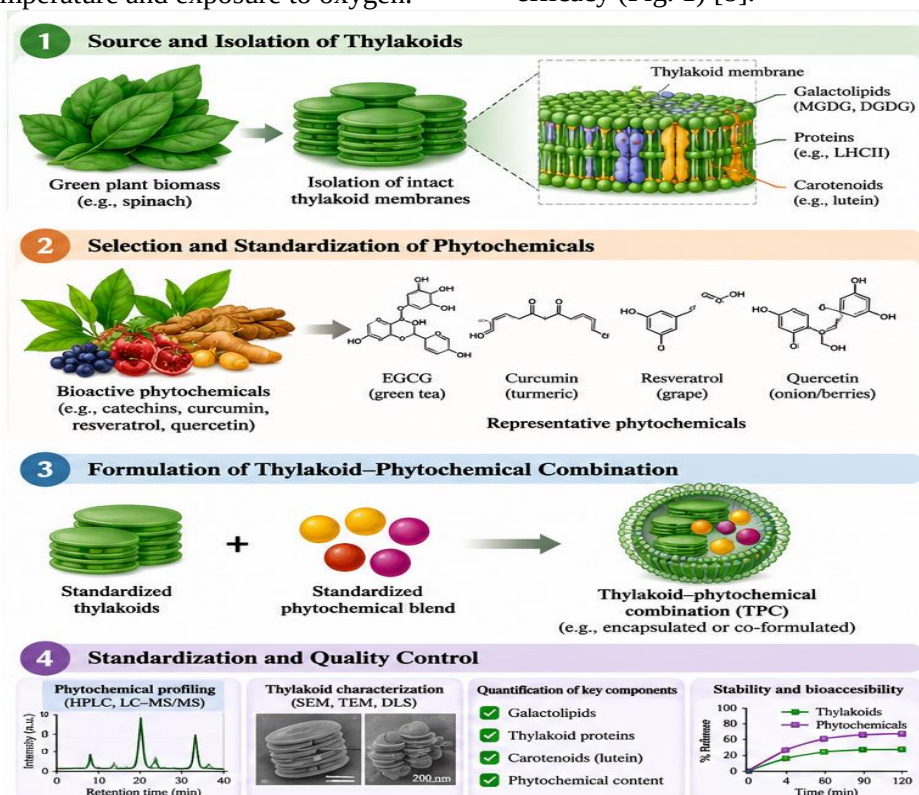


Fig. 1. Development and Standardization of a Thylakoid–Phytochemical Combination for Weight Management.

3.2. Selection of candidate phytochemicals

Potential candidates should be selected according to evidence supporting their biological activity, analytical detectability, chemical stability, safety, compatibility with thylakoids, and feasibility of standardization (Table 1).

Table 1. Potential phytochemical candidates for thylakoid-based formulation development

Candidate	Major chemical characteristics	Potential research rationale	Important development considerations
Green tea catechins, particularly EGCG	Flavan-3-ol polyphenols	Investigation of metabolic signalling, lipid handling, and energy expenditure	Oxidative stability, dose-related safety, and analytical selectivity
Chlorogenic acid	Caffeoylquinic acid derivative	Investigation of glucose and lipid metabolism	Isomer separation, stability, and source-dependent variability
Curcumin	Diarylheptanoid polyphenol	Investigation of inflammatory and metabolic signalling	Low aqueous solubility, degradation, and formulation compatibility
Quercetin	Flavonol	Investigation of cellular signalling and oxidative-stress pathways	Limited solubility, conjugative metabolism, and matrix effects
Resveratrol	Stilbene polyphenol	Investigation of metabolic and cellular signalling	Low systemic bioavailability and light sensitivity
Anthocyanins	Glycosylated flavonoid pigments	Investigation of metabolic and inflammatory pathways	Strong pH-dependent colour changes and degradation

These candidates are not interchangeable, and the table does not imply that any one of them has been validated in a thylakoid combination for weight management. Each candidate should be evaluated individually before selecting a lead compound.

3.3. Choosing a lead phytochemical

The selection process should consider the intended mechanism, anticipated gastrointestinal exposure, compatibility with membrane lipids and proteins, analytical feasibility, and toxicological profile. A green tea catechin such as epigallocatechin gallate (EGCG) could be investigated where the research hypothesis involves complementary effects on lipid handling and metabolic signalling. Chlorogenic acid may be appropriate when the emphasis is on dietary polyphenols and glucose-related metabolic pathways. Curcumin may be investigated when inflammatory signalling is a major mechanistic endpoint, although its physicochemical limitations require particular attention. A single lead phytochemical is preferable during initial formulation development because it simplifies interpretation of interactions and dose response relationships. Additional phytochemicals should only be introduced when their individual contributions and compatibility can be evaluated adequately [9].

4. Phytochemical Profiling and Chemical Characterization

4.1. Importance of comprehensive profiling

Phytochemical profiling establishes the chemical identity, composition, and reproducibility of the plant-derived materials used in a formulation. It is essential for ensuring batch consistency, identifying potentially active constituents, detecting degradation products, and

supporting safety evaluation. For a thylakoid–phytochemical combination, characterization should address both the membrane-rich plant fraction and the selected phytochemical. These materials have different chemical properties and should not be assessed using a single analytical method.

4.2. Characterization of the thylakoid fraction

The thylakoid fraction should be characterized using complementary physicochemical and analytical techniques [10].

Particle size and distribution: Dynamic light scattering may be used to estimate hydrodynamic size and polydispersity when the sample is suitable for this method. Because membrane fragments may be irregular and heterogeneous, the reported diameter should be interpreted in light of the measurement model and sample preparation [11].

Microscopic examination: Optical microscopy, transmission electron microscopy, or scanning electron microscopy may provide information about morphology and structural integrity. Electron microscopy requires suitable preparation and may introduce artifacts, so imaging results should be interpreted alongside other measurements [12].

Pigment profiling: Chlorophyll a, chlorophyll b, and selected carotenoids may be measured using spectrophotometric methods or chromatographic techniques. High-performance liquid chromatography with photodiode-array detection can provide greater selectivity for individual pigments than total-pigment measurements alone.

Lipid profiling: Lipid extraction followed by chromatographic analysis can characterize major membrane lipid classes and selected fatty acids. These measurements may be useful for monitoring processing-related changes in membrane composition.

Protein characterization: Total protein measurements may provide a general compositional indicator, while electrophoretic or proteomic methods can support more detailed characterization when justified by the research objectives.

Functional characterization: A standardized *in vitro* lipid-digestion assay may be included as a functional attribute. However, such an assay should not be used as a substitute for compositional analysis or as definitive proof of weight-management efficacy [13].

4.3. Characterization of the selected phytochemical

The selected phytochemical should be identified and quantified using a validated analytical method. Depending on the compound, suitable approaches may include HPLC–PDA, UHPLC–PDA, LC–MS/MS, or high-resolution mass spectrometry [14–16].

A practical analytical strategy may include:

5. Establishing the identity and purity of the reference standard.
6. Developing an extraction procedure that provides acceptable recovery from the plant matrix.
7. Optimizing chromatographic separation and detection.
8. Assessing specificity, linearity, accuracy, precision, and robustness.
9. Investigating matrix effects, recovery, and potential degradation.
10. Establishing acceptance limits for the marker compound.
11. Confirming the suitability of the method for routine batch testing.

Where the phytochemical is present as multiple isomers, glycosides, or related compounds, the analytical method should demonstrate adequate selectivity for the intended marker.

4.4. Chromatographic fingerprinting

A marker assay alone may not adequately characterize a complex botanical fraction. Chromatographic fingerprinting can provide a broader chemical profile and help detect unexpected differences among batches. HPLC–PDA can be useful for pigment-rich preparations, whereas LC–MS-based profiling may provide additional information on molecular features and potential degradation products. Untargeted metabolomic profiling can support exploratory comparisons, but identified features should not be described as confirmed compounds without appropriate evidence. Reference fingerprints should be established using representative batches, and similarity limits should be justified using observed manufacturing variability and biological relevance [17].

4.5. Quality-by-design-based standardization

A quality-by-design (QbD) approach can integrate formulation development and analytical control. The process begins by defining the quality target product profile (QTPP), including the intended dosage form, composition, stability, performance, and relevant safety characteristics [18, 19].

Potential critical quality attributes include:

- Identity and quantity of the selected phytochemical.
- Chlorophyll and carotenoid content.
- Particle-size distribution and polydispersity.
- Membrane-associated lipid and protein characteristics.
- Phytochemical recovery and distribution within the formulation.
- Microbial quality and relevant contaminant limits.
- Stability during storage and simulated gastrointestinal exposure.
- Reproducibility of a predefined *in vitro* functional assay.

Risk assessment tools such as Ishikawa diagrams and failure mode and effects analysis can identify critical material attributes and process parameters. Design of experiments can then investigate the effects of extraction conditions, mixing, pH, temperature, and phytochemical concentration on the selected quality attributes. Standardization limits should be established from experimental data and manufacturing capability rather than assigned arbitrarily [20].

5. Mechanistic Investigation of Weight-Management Effects

5.1. Overview of the proposed mechanisms

The potential effects of a thylakoid–phytochemical formulation can be investigated through several mechanistic domains: gastrointestinal lipid processing,

satiety-related signalling, energy intake, metabolic regulation, and inflammatory pathways. These domains are interconnected. For example, changes in nutrient digestion may influence postprandial signalling, whereas alterations in energy intake can secondarily affect body weight and metabolic biomarkers. Mechanistic experiments should therefore be designed to distinguish direct effects from downstream consequences [21, 22].

5.2. Gastrointestinal lipid processing

The first mechanistic question is whether the combination alters the digestion of dietary lipids under physiologically relevant conditions. An *in vitro* digestion model can be used to evaluate changes in triglyceride hydrolysis, free-fatty-acid release, and the physical properties of lipid-containing digesta. A standardized simulated gastrointestinal digestion protocol, such as the INFOGEST framework, can help improve

reproducibility. Appropriate controls should include the digestion system without the test material, thylakoids alone, the phytochemical alone, and the combined formulation. A validated lipase inhibitor may be included as a positive control where appropriate. Because plant pigments and suspended particles may interfere with colorimetric or fluorescence-based assays, orthogonal measurements should be considered. pH-stat titration, chromatographic analysis of lipid products, or other suitable analytical approaches can help distinguish genuine changes in lipid hydrolysis from assay interference. An important limitation is that reduced lipid hydrolysis *in vitro* does not automatically establish reduced fat absorption *in vivo*. Physiological digestion, intestinal compensation, food composition, and gastrointestinal transit can alter the relationship between laboratory findings and systemic outcomes (**Fig. 2**) [23].

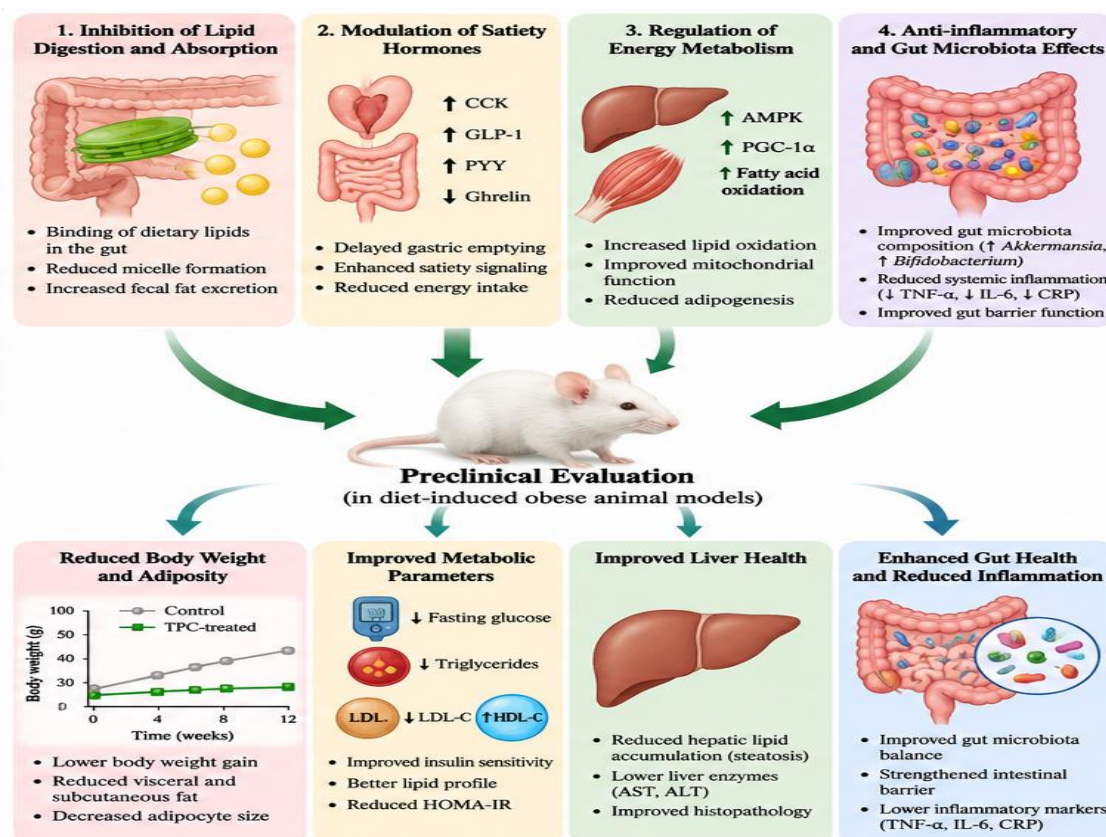


Fig. 2. Mechanistic Pathways and Preclinical Evaluation of a Thylakoid-Phytochemical Combination in Weight Management.

Appetite regulation involves several gastrointestinal peptides, including cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin. Their responses depend on nutrient composition, gastrointestinal physiology, and the broader metabolic state. The formulation could be investigated for its effects on these pathways through suitable cellular systems or animal studies. However, an observed change

in circulating hormone concentrations should not automatically be interpreted as proof that the hormone mediates a reduction in food intake. Mechanistic studies should establish temporal relationships, include appropriate controls, and where feasible investigate whether blocking or modifying a proposed pathway changes the observed response. *In vitro* studies using enteroendocrine cell models may provide initial

evidence, but the physiological relevance of the model, the concentration of the test material, and the possibility of cytotoxicity must be considered [24-2].

6. Safety, Toxicology, and Translational Considerations

6.1. Safety of the individual components

The safety of the combined formulation cannot be inferred solely from the fact that its components originate from edible plants. Concentrated extracts may differ substantially from customary dietary exposure, and the toxicity profile of a phytochemical may depend on its dose, purity, metabolism, and duration of administration. The selected phytochemical should undergo an appropriate review of existing toxicological evidence. For example, concentrated green tea extracts containing EGCG have been associated with liver injury in some circumstances, making dose selection and hepatic safety monitoring particularly important. The thylakoid fraction should also be evaluated for microbial contamination, pesticide residues, heavy metals, relevant environmental contaminants, and potential allergenic components, as appropriate to the source material and intended product [27].

6.2. Combination-related risks

Combining two materials can alter their stability, absorption, metabolism, and gastrointestinal tolerability. The formulation may produce effects that are not predictable from the individual components. Safety assessment should therefore consider the complete formulation, including excipients and processing residues. Where indicated, the evaluation should include clinical observations, food consumption, body weight, haematology, clinical chemistry, and histopathology. Potential interactions with medicines should be considered during development, particularly when a candidate phytochemical may influence drug-metabolizing enzymes, transporters, or clinically relevant physiological pathways [28, 29].

6.3. Gastrointestinal tolerability

A formulation intended to influence lipid digestion or gastrointestinal signalling may affect stool characteristics, gastrointestinal comfort, or nutrient handling. These outcomes should be monitored rather than assuming that an effect on digestion is necessarily beneficial. Longer-term studies may be required to evaluate whether sustained changes in nutrient handling affect fat-soluble nutrient availability or other aspects of nutritional status [30].

Strengths of the Review

This review provides an integrated overview of the development and standardization of thylakoid–

phytochemical combinations for weight management. It discusses phytochemical selection, analytical characterization, chromatographic fingerprinting, quality-by-design approaches, proposed biological mechanisms, and safety considerations. Another strength is its emphasis on distinguishing the potential effects of individual ingredients from the effects of their combined formulation. The review also highlights the importance of reproducibility, formulation compatibility, and appropriate preclinical evaluation before making claims regarding efficacy.

Future Perspectives

Future research should focus on developing and characterizing standardized thylakoid–phytochemical formulations using validated analytical methods and reproducible manufacturing procedures. Further studies are needed to assess ingredient compatibility, membrane stability, phytochemical bioavailability, and potential interactions between formulation components. Well-designed *in vitro* studies and controlled preclinical investigations should evaluate lipid digestion, appetite-related responses, metabolic parameters, and safety. Comparative studies involving thylakoids alone, individual phytochemicals, and their combination may help determine whether the formulation produces additive, synergistic, or antagonistic effects. Clinical investigations may be considered only after adequate evidence of formulation quality, safety, and preclinical performance has been established.

CONCLUSION

The development of a standardized thylakoid–phytochemical combination represents a scientifically relevant research direction for investigating complementary mechanisms involved in weight management. Thylakoid membranes provide a structurally complex plant-derived matrix that may influence gastrointestinal lipid processing and appetite-related responses, while selected phytochemicals offer additional opportunities to investigate metabolic and cellular signalling pathways. Nevertheless, these proposed effects must be established for the specific combination rather than inferred from studies of individual ingredients. A robust development strategy should integrate source-material qualification, phytochemical profiling, chromatographic fingerprinting, physicochemical characterization, compatibility testing, and quality-by-design-based optimization. *In vitro* digestion studies and mechanistic assays should establish biological plausibility before appropriately powered and controlled preclinical studies evaluate food intake, body composition, metabolic biomarkers, and safety. Factorial experimental designs and suitable interaction models are particularly

important for determining whether the combined formulation produces additive, synergistic, or antagonistic effects. The major challenges include batch-to-batch variability, membrane stability, phytochemical bioavailability, assay interference, long-term safety, and the limited ability of preclinical outcomes to predict human efficacy. These limitations can be addressed through standardized analytical procedures, transparent reporting, independent confirmation, and a staged translational strategy. Overall, a well-characterized thylakoid–phytochemical combination could provide a useful platform for investigating plant-derived approaches to weight management. Its ultimate value, however, will depend on reproducible evidence demonstrating that the formulation is safe, mechanistically credible, and more useful than its individual components or appropriate controls.

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REFERENCES

1. World Health Organization. Obesity and overweight. WHO Fact Sheet. Geneva: World Health Organization. Consult the current WHO publication for updated epidemiological estimates and guidance.
2. Montelius C, Erlandsson D, Vitija E, Stenblom EL, Egecioglu E, Erlanson-Albertsson C. Thylakoid-rich spinach extract reduces hunger, increases satiety and reduces cravings for palatable food in overweight women. *Appetite*. 2014;81:295–304. Verify the bibliographic details against the publisher's record before submission.
3. Emek SC, Szilagyi A, Akerlund HE, Albertsson PA. A large-scale method for preparation of spinach thylakoids for use in dietary supplements. Verify the full bibliographic record, including journal, year, volume, and pages, before citing this work in a submitted manuscript.
4. Hall KD, Ayuketah A, Brychta R, et al. Ultra-processed diets cause excess calorie intake and weight gain: an inpatient randomized controlled trial of ad libitum food intake. *Cell Metabolism*. 2019;30(1):67–77.e3.
5. Williamson G. The role of polyphenols in modern nutrition. *Nutrients*. 2017;9(10). Verify relevance to the specific phytochemical and mechanistic claims before use.
6. Del Rio D, Rodriguez-Mateos A, Spencer JPE, Tognolini M, Borges G, Crozier A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling*. 2013;18(14):1818–1892.
7. Lampe JW. Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. *The American Journal of Clinical Nutrition*. 1999;70(3 Suppl):475S–490S.
8. Minekus M, Alminger M, Alvito P, et al. A standardised static in vitro digestion method suitable for food—an international consensus. *Food & Function*. 2014;5:1113–1124.
9. Brodkorb A, Egger L, Alminger M, et al. INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*. 2019;14:991–1014.
10. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLOS Biology*. 2020;18(7).
11. Tomizioli M, Lazar C, Brugière S, Burger T, Salvi D, Gatto L, Moyet L, Breckels LM, Hesse AM, Lilley KS, Seigneurin-Berny D. Deciphering thylakoid sub-compartments using a mass spectrometry-based approach. *Molecular & Cellular Proteomics*. 2014 Aug 1;13(8):2147–67.
12. Koochak H, Puthiyaveetil S, Mullendore DL, Li M, Kirchhoff H. The structural and functional domains of plant thylakoid membranes. *The Plant Journal*. 2019 Feb;97(3):412–29.
13. Danielsson R, Albertsson PÅ, Mamedov F, Styring S. Quantification of photosystem I and II in different parts of the thylakoid membrane from spinach. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*. 2004 Jan 30;1608(1):53–61.
14. Trotta A, Gunell S, Bajwa AA, Paakkari V, Fujii H, Aro EM. Defining the heterogeneous

- composition of Arabidopsis thylakoid membrane. *The Plant Journal*. 2025 Feb;121(3):e17259.
15. Svoboda V, Oung HM, Koochak H, Yarbrough R, Mckenzie SD, Puthiyaveetil S, Kirchhoff H. Quantification of energy-converting protein complexes in plant thylakoid membranes. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*. 2023 Apr 1;1864(2):148945.
 16. LaBrant EW, Smith CN, Torres-Gerena AD, Ishimwe J, Huang F, Tullis A, Litterer L, Modi BF, Naldrett MJ, Altartouri B, Roston RL. Lipid metabolism and contact site homologs are present at the chloroplast envelope-thylakoid interface. *Nature Communications*. 2026 May 19.
 17. Zhang J, Gong D, Lan L, Zheng Z, Pang X, Guo P, Sun G. Comprehensive evaluation of Loblolly fruit by high performance liquid chromatography four wavelength fusion fingerprint combined with gas chromatography fingerprinting and antioxidant activity analysis. *Journal of Chromatography A*. 2022 Feb 22;1665:462819.
 18. Muppayyanamath A, Harish DR, Mastiholimath V, Patil PP, Patil VS, Hegde HV, Roy S. Quality by design-based optimization and HP-TLC densitometric standardization of Theobroma cacao L. extract as a nutraceutical supplement. *Frontiers in nutrition*. 2025 Apr 9;12:1537963.
 19. Mohapatra D, Kumar DN, Shreya S, Pandey V, Dubey PK, Agrawal AK, Sahu AN. Quality by design-based development and optimization of fourth-generation ternary solid dispersion of standardized Piper longum extract for melanoma therapy. *Drug Delivery and Translational Research*. 2023 Dec;13(12):3094-131.
 20. Mohapatra D, Kumar DN, Shreya S, Panigrahi D, Agrawal AK, Sahu AN. Quality-by-design-based development of ultradeformable nanovesicular transgelosome of standardized Piper longum extract for melanoma. *Nanomedicine*. 2023 Jun 1;18(14):963-85.
 21. Flanagan EW, Spann R, Berry SE, Berthoud HR, Broyles S, Foster GD, Krakoff J, Loos RJ, Lowe MR, Ostendorf DM, Powell-Wiley TM. New insights in the mechanisms of weight-loss maintenance: summary from a Pennington symposium. *Obesity*. 2023 Dec;31(12):2895-908.
 22. Rosenbaum M, Foster G. Differential mechanisms affecting weight loss and weight loss maintenance. *Nature metabolism*. 2023 Aug;5(8):1266-74.
 23. Liu H, Li B, Liu L, Ying W, Rosen CJ. Weight loss induced bone loss: mechanism of action and clinical implications. *Bone research*. 2025 Dec 2;13(1):99.
 24. Della Guardia L, Shin AC. Obesity-induced tissue alterations resist weight loss: A mechanistic review. *Diabetes, Obesity and Metabolism*. 2024 Aug;26(8):3045-57.
 25. Liao YH, Wang LK, Tsai FM. Mechanistic Insights into Selected Traditional Chinese Medicine Formulas and Their Key Herbs for Weight Management. *Natural Product Communications*. 2026 Feb;21(2):1934578X261418804.
 26. Bates S, Norman P, Breeze P, Brennan A, Ahern AL. Mechanisms of action in a behavioral weight-management program: latent growth curve analysis. *Annals of Behavioral Medicine*. 2022 Jan 1;56(1):64-77.
 27. Das A, Pathak K, Saikia R, Pathak MP, Gogoi U, Das D, Sahariah JJ, Gogoi S, Sonowal S. Future perspectives and challenges: clinical translation and regulatory aspects, scale-up and manufacturing, ethical considerations. *Nanocarriers for nucleic acids and proteins*. 2025 Aug 11:343-70.
 28. Edelman IS, Ovchinnikov SG, Suhag D, Thakur A, Thakur P. Nanotoxicology and safety considerations. In *Advancements in Nanobiology* 2025 Mar 25 (pp. 318-344). CRC Press.
 29. Moffit JS, Blanset DL, Lynch JL, MacLachlan TK, Meyer KE, Ponce R, Whiteley LO. Regulatory consideration for the nonclinical safety assessment of gene therapies. *Human gene therapy*. 2022 Nov;33(21-22):1126-41.
 30. Korde A, Mikolajczak R, Kolenc P, Bouziotis P, Westin H, Lauritzen M, Koole M, Herth MM, Bardiès M, Martins AF, Paulo A. Practical considerations for navigating the regulatory landscape of non-clinical studies for clinical translation of radiopharmaceuticals. *EJNMMI Radiopharmacy and Chemistry*. 2022 Jul 19;7(1):18.