OPEN ACCESS

REVIEW

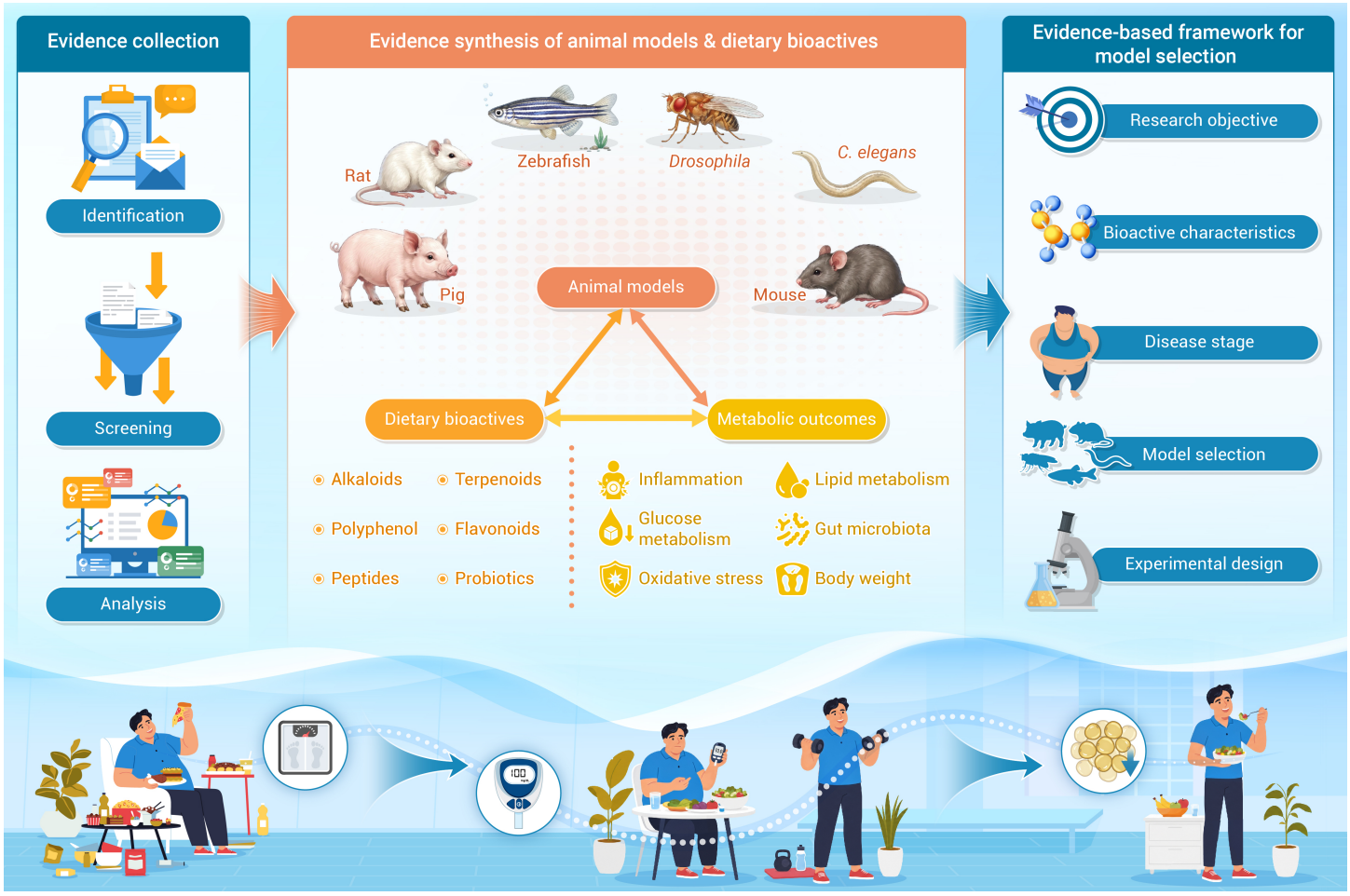
Animal models of obesity and diabetes mellitus for dietary bioactive component development: A systematic review

Nidesha Randeni,[1,2] Jinhai Luo,[1,2] and Baojun Xu[1,*]

*Correspondence: baojunxu@bnbu.edu.cn

Received: April 26, 2026; Accepted: July 15, 2026; Published Online: July 21, 2026; https://doi.org/10.59717/j.xinn-nutri.2026.100029

GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Diet-induced rodent models dominate bioactive research.
- Outcome reporting is skewed toward basic metabolic markers.
- Mechanistic depth varies and is often limited.
- Sex bias and design variability affect reproducibility.
- A framework is proposed to guide animal model selection.

REVIEW

www.the-innovation.org/the-innovation-nutrition

Animal models of obesity and diabetes mellitus for dietary bioactive component development: A systematic review

Nidesha Randeni,[1,2] Jinhai Luo,[1,2] and Baojun Xu[1,*]

[1]Food Science and Technology Program, Department of Life Sciences, Beijing Normal-Hong Kong Baptist University, Zhuhai 519087, China

[2]These authors have equal contributions to this paper

*Correspondence: baojunxu@bnbu.edu.cn

Received: April 26, 2026; Accepted: July 15, 2026; Published Online: July 21, 2026; https://doi.org/10.59717/j.xinn-nutri.2026.100029

Citation: Randeni N., Luo J. and Xu B. (2026). Animal models of obesity and diabetes mellitus for dietary bioactive component development: A systematic review. *The Innovation Nutrition* **1**:100029.

Animal models are widely used to investigate the metabolic effects of dietary bioactive compounds in obesity and type 2 diabetes mellitus (T2DM). However, unlike conventional drugs, dietary bioactives often exert pleiotropic and context-dependent effects that are strongly influenced by disease severity, dietary background, and experimental conditions. Consequently, model selection and methodological variability may affect the reproducibility and translational relevance of preclinical findings. This systematic review evaluated animal models used in dietary bioactive research, identified methodological trends and limitations, and proposed a framework to guide future model selection. A systematic search of PubMed, Scopus, Web of Science, ScienceDirect, SpringerLink, and Wiley Online Library was conducted for studies published within the last five years. Data were extracted on species, strain, model type, dietary induction, intervention characteristics, outcome measures, and mechanistic depth. A total of 194 studies were included. Diet-induced obesity models were the most frequently used (67.2%), followed by genetic, hybrid, and chemically induced models. Rodents, particularly mice and rats, predominated, while alternative models such as zebrafish and invertebrates were used infrequently. Outcome reporting focused mainly on body weight, glucose metabolism, and lipid profiles, whereas mechanistic investigations varied considerably. Substantial methodological heterogeneity was observed in diet composition, dosing strategies, study duration, and reporting quality, alongside a marked bias toward male animals. Overall, current preclinical research is characterized by model dominance, methodological variability, and inconsistent mechanistic validation. The proposed framework provides a structured approach to improve study design and enhance the translational relevance of dietary bioactive research in metabolic disease.

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are among the most significant global health challenges of the 21st century. The prevalence of these conditions has increased markedly over recent decades, affecting both developed and developing countries. According to the World Health Organization, more than 1 billion people worldwide were living with obesity in 2022, representing a substantial rise compared to previous decades.[1] In parallel, the International Diabetes Federation estimated that approximately 537 million adults were living with diabetes in 2021, with projections suggesting this number could reach 783 million by 2045.[2] These conditions are closely interconnected and share common underlying mechanisms, including insulin resistance, dysregulated glucose and lipid metabolism, chronic low-grade inflammation, and oxidative stress. As a result, obesity and T2DM are increasingly viewed as components of a broader metabolic disease spectrum rather than isolated disorders. This spectrum also includes related metabolic complications such as Metabolic dysfunction-associated steatotic liver disease (MASLD), which frequently coexists with obesity and insulin resistance and is increasingly recognized as a major component of cardiometabolic disease. Despite advances in pharmacological therapies, long-term management remains challenging, highlighting the need for effective and sustainable preventive strategies.[3]

In recent years, dietary bioactive compounds have attracted considerable attention as potential modulators of metabolic health. These compounds, which include polyphenols, flavonoids, peptides, probiotics, and other food-derived molecules, are widely present in plant- and animal-based foods.[4-5] Unlike conventional pharmaceuticals, dietary bioactives typically exert multi-target and pleiotropic effects, influencing multiple metabolic pathways. However, their mechanisms of action are often less specific, their bioavailability can be limited, and their effects may depend on dietary context and long-term exposure.[6] These characteristics make the evaluation of dietary bioactives fundamentally different from that of single-target pharmacological agents and require carefully designed experimental approaches. Importantly, the relatively modest and context-dependent effects of many dietary bioactives mean that experimental outcomes may be particularly sensitive to model selection, disease severity, dietary background, and intervention duration. Consequently, animal models that are optimized for rapid or severe disease induction may not always be optimal for evaluating nutritional interventions intended to produce gradual physiological improvements.

Animal models, therefore, remain central to the development of dietary bioactive interventions for obesity and diabetes. They allow controlled testing of efficacy, mechanism, dose, and treatment duration in ways that are difficult to achieve in human studies. However, animal models are not interchangeable. Diet-induced obesity (DIO) models are often preferred for mimicking the nutritional and environmental drivers of human metabolic disease, whereas chemically induced, genetic, and hybrid models capture different aspects of glucose dysregulation, insulin deficiency, insulin resistance, or obesity-related complications. As several recent reviews have pointed out, model choice strongly influences the phenotype observed, the pathways engaged, and the translational relevance of the findings.[7-8] This issue may be particularly important in dietary bioactive research, where excessively severe metabolic models, highly obesogenic diets, or extensive β-cell destruction may obscure, underestimate, or distort biologically relevant nutritional effects. Therefore, appropriate alignment between intervention characteristics and disease model selection is essential for generating meaningful and translatable findings.

Recent reviews have extensively summarized animal models of obesity and T2DM, focusing primarily on their physiological characteristics, induction methods, and relevance to human disease. These studies provide valuable insights into model validity and experimental design, but largely emphasize disease modeling rather than its application in specific research contexts. In particular, the use of these models in dietary bioactive research and how model selection influences intervention outcomes remains less systematically explored. Although numerous studies have examined dietary bioactives or specific animal models individually, there remains a lack of comprehensive, systematic analyses that integrate both aspects. In particular, there is limited understanding of how different animal models are used in dietary bioactive research, how model choice influences outcomes, and what methodological patterns and limitations exist across studies. Addressing these gaps is essential for improving the design, interpretation, and translational potential of future research. Accordingly, a critical evaluation of model selection is needed not only to catalogue current practices but also to identify potential mismatches between dietary bioactive interventions and experimental disease models that may limit translation to human nutrition and metabolic health.

Therefore, the objective of this systematic review was to analyze animal models of obesity and diabetes mellitus used for dietary bioactive component development over the past five years. Specifically, this review aimed to (i) characterize the types of animal models employed, (ii) examine the distribution and classes of dietary bioactive compounds investigated, (iii) assess

the metabolic outcomes and methodological features reported, and (iv) identify key limitations and translational challenges in current preclinical research.

METHODOLOGY

A systematic literature search was conducted to identify relevant studies published within the last five years. The electronic databases PubMed, Scopus, Web of Science, ScienceDirect, SpringerLink, and Wiley Online Library were searched. The search strategy combined keywords related to animal models, obesity, T2DM, and dietary bioactive compounds, along with metabolic outcomes. Representative search terms included "animal model", "mouse", "rat", or "zebrafish", combined with "obesity", "type 2 diabetes", "insulin resistance", or "metabolic syndrome", and further combined with "bioactive compound", "polyphenol", "flavonoid", "probiotic", "nutraceutical", or "dietary intervention", as well as terms related to "glucose metabolism" and "lipid metabolism". The search strategy was adapted for each database, and only studies published in English were considered. Additionally, manual searching of reference lists of included articles and relevant reviews was performed to identify further eligible studies. All studies identified through both database and manual searches were screened and evaluated based on the same inclusion and exclusion criteria. All retrieved records were exported and compiled into a master database, and duplicates were removed. The screening process was performed in three stages: title screening, abstract screening, and full-text assessment. Study selection was guided by predefined eligibility criteria and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Studies were included if they were conducted *in vivo* using animal models and investigated obesity, T2DM, insulin resistance, or metabolic syndrome. Eligible studies were required to evaluate a dietary bioactive compound or food-derived intervention and report at least one metabolic outcome related to body weight or adiposity, glucose metabolism (such as fasting glucose, oral glucose tolerance test, or insulin levels), or lipid metabolism. Studies were excluded if they were *in vitro, in silico*, or human-only studies, as well as review articles, meta-analyses, protocols, or conference abstracts. Studies that investigated pharmacological drugs only, without dietary bioactive components, or reported only mechanistic outcomes without assessing metabolic parameters were also excluded. Data from the included studies were extracted into a structured spreadsheet. Where studies used more than one animal model, each model was treated as a separate experimental entry while maintaining linkage to the original study. Extracted information included study characteristics (author, year, journal, DOI), animal model details (species, strain, sex, age or weight, model type, and induction method), intervention details (bioactive compound, class, form, dose, frequency, route, and duration), and experimental design (group number, sample size, and randomization). Outcomes extracted included body weight, adiposity, glucose metabolism, lipid profile, inflammation, oxidative stress, gut microbiota, and liver-related parameters. In addition, mechanistic depth was categorized based on the extent of molecular and functional analyses reported in each study. Studies reporting primarily phenotypic or biochemical outcomes were classified as having low mechanistic depth, while studies incorporating molecular pathway analyses, including gene or protein expression assessments, were classified as moderate mechanistic depth. Studies including functional validation approaches, such as pathway inhibition, genetic manipulation, or multi-level mechanistic investigations, were categorized as high mechanistic depth. A descriptive analytical approach was used to synthesize the data. Animal models were categorized into diet-induced, genetic, chemically induced, hybrid, and other models, while bioactive compounds were grouped into major classes such as polyphenols, flavonoids, probiotics or prebiotics, peptides, lipids, and mixed extracts. Quantitative summaries were generated to assess the distribution of animal models, types of bioactive compounds, reported outcomes, and key methodological characteristics across studies. Risk-of-bias assessment was conducted for the included studies using SYRCLE's risk-of-bias tool for animal studies. The assessment evaluated domains including random sequence generation, baseline characteristics, allocation concealment, blinding, incomplete outcome data, selective outcome reporting, and other potential sources of bias. Each domain was categorized as low, high, or unclear risk of bias.[9]

RESULTS AND DISCUSSION

Study selection

A total of 10,394 records were identified through comprehensive database searching across PubMed, Scopus, Web of Science, ScienceDirect, SpringerLink, and Wiley Online Library. After removal of duplicate records (n = 2,356), the remaining records underwent an initial eligibility filtering step, during which titles were preliminarily reviewed to exclude clearly irrelevant publications based on predefined inclusion and exclusion criteria. This process excluded studies unrelated to metabolic dysfunction-associated steatotic liver disease (MASLD), non-animal studies, conference abstracts, reviews, editorials, and studies lacking dietary bioactive interventions, resulting in 696 records eligible for formal title screening. In parallel, an additional 154 records were identified through manual searching of reference lists from eligible articles and relevant review papers to ensure comprehensive literature coverage and minimize the risk of missing relevant studies. These manually identified records were screened using the same eligibility criteria and integrated with the database-derived records prior to subsequent screening stages. Following title screening, a total of 391 records were considered potentially relevant and subjected to abstract screening. After exclusion of 150 records at the abstract stage, 241 articles were assessed for full-text eligibility. Of these, 47 studies were excluded based on predefined inclusion and exclusion criteria, including non-animal study design, absence of dietary bioactive interventions, or lack of relevant metabolic outcomes. Ultimately, 194 studies were included in the final analysis. It should be noted that four publications contained multiple experimental comparisons; therefore, 194 studies contributed 198 study arms to the qualitative synthesis. The overall study selection process is illustrated in the PRISMA flow diagram (Figure 1). Additional methodological details, including the PRISMA checklist (Supplementary Table S1), detailed study selection methodolog, and complete database search strategies, are provided in the Supplementary Materials.

Risk-of-bias assessment of the included studies is presented in Supplementary Table S5 and Supplementary Figures 1–2. Overall, most studies demonstrated low risk for random sequence generation and selective outcome reporting. However, allocation concealment, blinding, and reporting of baseline characteristics were frequently rated as unclear due to insufficient methodological detail. Incomplete outcome data represented a potential source of bias in a subset of studies, indicating moderate variability in reporting quality across the included studies.

Characteristics of included studies

A total of 198 study arms from 194 studies were included in the final analysis, representing a broad range of experimental approaches investigating dietary bioactive compounds in animal models of obesity and T2DM. Detailed extracted data for all study arms are provided in Supplementary Tables S1–S3. For clarity, the term "studies" is hereafter used to refer to the 198 analyzed study arms unless otherwise specified. The studies were published within the last five years, reflecting the growing interest in the use of food-derived compounds for metabolic disease management. The majority of studies employed rodent models, with mice and rats being the most commonly used species, while a smaller number of studies utilized alternative models such as zebrafish or other organisms. Across the included studies, a wide variety of experimental designs were observed. Most studies involved diet-induced models of obesity or metabolic dysfunction, often using high-fat or high-energy diets to mimic human metabolic conditions. In addition, genetic models and chemically induced models, including those using streptozotocin (STZ), were also employed to investigate specific aspects of glucose dysregulation and insulin resistance. Some studies utilized combined or hybrid approaches to better replicate the complexity of metabolic disease. The interventions evaluated in these studies included a diverse range of dietary bioactive compounds, such as polyphenols, flavonoids, peptides, probiotics, prebiotics, and complex plant or food extracts. Both purified compounds and whole extracts were used, with considerable variation in dose, duration, and route of administration. Intervention periods ranged from short-term studies to longer experimental designs, reflecting differences in study objectives, including prevention and treatment approaches. In terms of outcomes, most studies assessed parameters related to body weight, glucose metabolism, and lipid profiles, which are central to obesity and

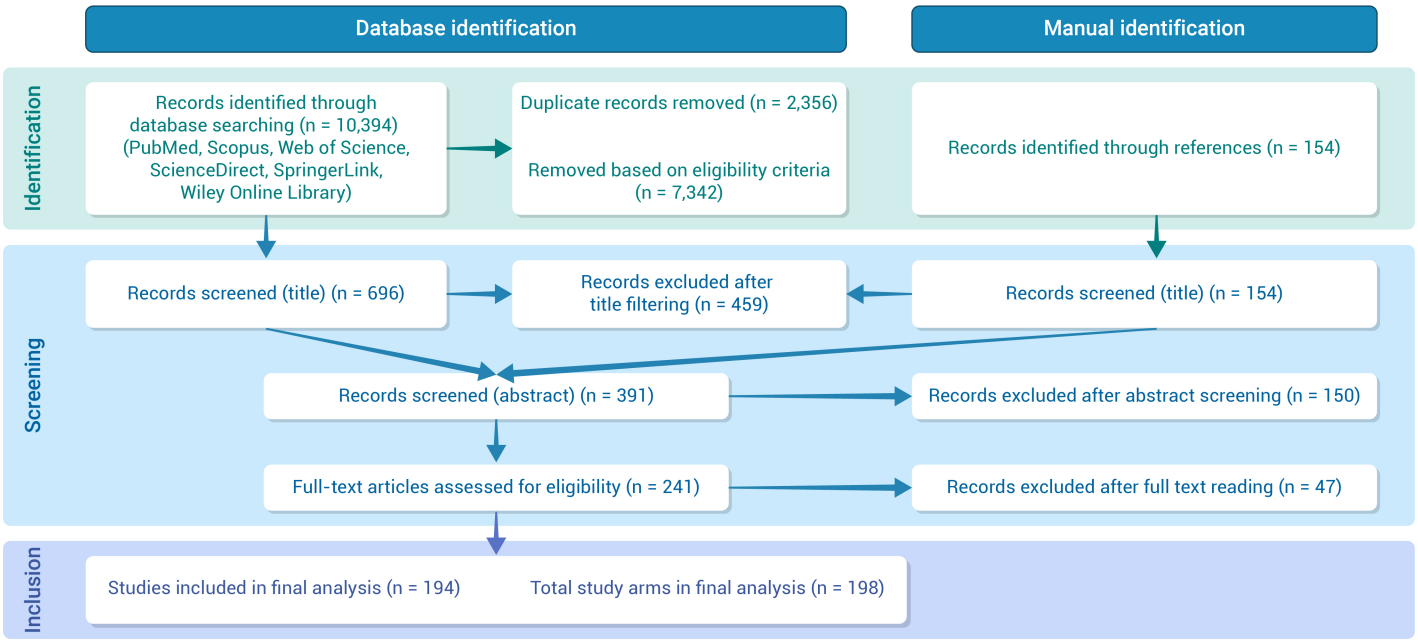


Figure 1. PRISMA flow diagram of the overall study process.

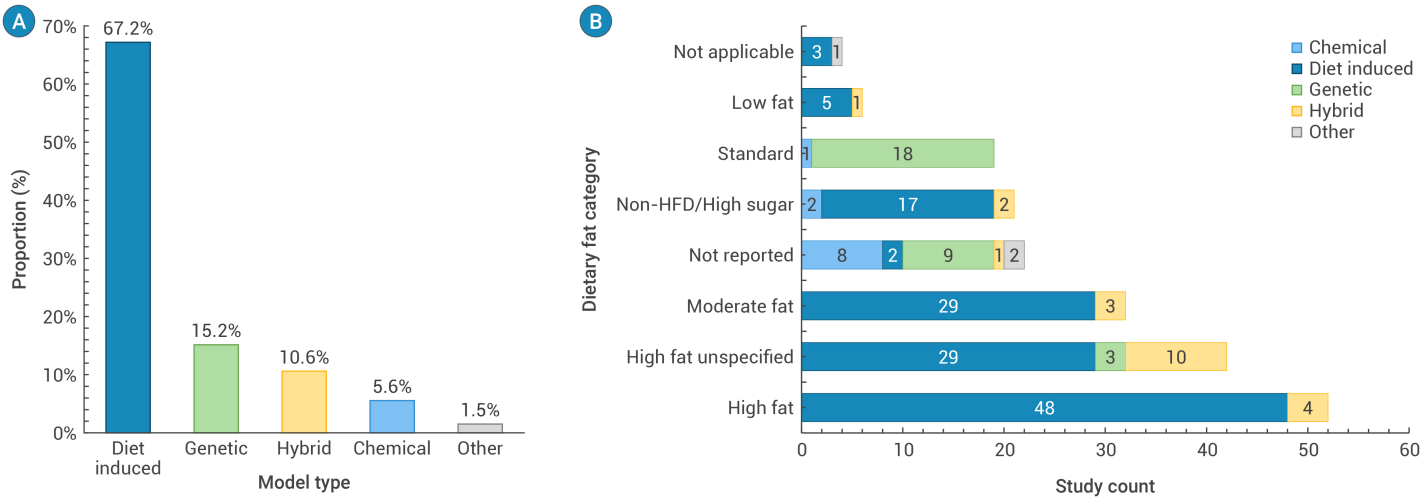


Figure 2. Distribution of animal models used in the included studies (A) Proportion of model types used across studies, (B) Distribution of dietary induction types within diet-induced models.

diabetes research. Additional outcomes, such as inflammation, oxidative stress, gut microbiota composition, and liver function, were also frequently reported, highlighting the multifactorial nature of metabolic disease and the diverse mechanisms through which dietary bioactives may exert their effects. Overall, the included studies demonstrate a high degree of heterogeneity in terms of animal models, experimental design, intervention strategies, and outcome measures. This variability underscores the need for systematic evaluation and comparison to better understand the strengths and limitations of current approaches and to improve the translational relevance of preclinical research in this field.

Animal model landscape

Distribution of model types. The distribution of animal models used across the included studies revealed a clear predominance of diet-induced approaches. DIO models accounted for 67.2% of all studies, followed by genetic models (15.2%), hybrid models (10.6%), and chemically induced models (5.6%), while other model types were rarely used (Figure 2A).

Predominance of diet-induced models. The pattern indicates a strong preference for diet-based induction of metabolic disorders in preclinical research on dietary bioactive compounds. This trend reflects practical considerations. These models are easier to establish, relatively inexpensive, and allow faster generation of metabolic phenotypes. These models reproduce key features such as weight gain, insulin resistance, and dyslipidemia through controlled dietary manipulation. Previous studies have emphasized that DIO models offer relatively high face validity for human metabolic disease, particularly in the context of overnutrition and sedentary behavior.[10,11] However, their construct and predictive validity remain variable, as the metabolic phenotype is highly dependent on diet composition, strain, and experimental conditions.

The marked dominance of DIO models in the present dataset likely reflects a combination of biological relevance and practical considerations. DIO models offer relatively high face validity for obesity and T2DM because they incorporate dietary and environmental drivers that contribute to human metabolic disease. However, their widespread use may also be influenced by accessibility, familiarity, relatively low cost, and ease of implementation compared with more specialized genetic, hybrid, or large-animal models. While this dominance has facilitated comparison across studies, excessive reliance on a single model type may inadvertently limit phenotypic diversity

and reduce exploration of alternative disease mechanisms. Given the heterogeneous nature of human metabolic disorders, greater use of complementary model systems may improve mechanistic understanding and translational interpretation.

As shown in Figure 2B, the dietary patterns in the included studies were dominated by high-fat diets (HFD), followed by moderate-fat and high-fat-unspecified categories. This is consistent with the wider DIO literature, where high-fat feeding is the most common strategy because it reliably induces weight gain, adiposity, and insulin resistance in rodents. Earlier reviews also note that diet-induced models are preferred because they capture the environmental component of obesity better than purely chemical or genetic models.[12–13] Not all HFDs are used for the same reason. In practice, moderate-fat diets are often chosen when researchers want a slower and potentially more physiologically relevant phenotype, whereas very-high-fat diets are used to accelerate obesity and metabolic dysfunction. This helps explain why our dataset contains both moderate-fat and high-fat categories. The choice is often a trade-off between translational relevance and speed. Several authors have argued that 45% kcal from fat may better approximate human Western diets, while 60% fat diets produce faster and stronger phenotypes but can be less representative of usual human intake.[14–15] The type of fat also matters. Many rodent studies use lard-based diets, not simply because lard is common, but because saturated-fat-rich diets tend to induce stronger insulin resistance and hepatic steatosis than many unsaturated-fat diets. Comparative work in rats has shown that the metabolic phenotype varies substantially with fatty acid composition, meaning that a "HFD" is not a single biological exposure. That is important for interpreting the high-fat unspecified group, because incomplete reporting of fat type and percentage reduces comparability across studies.[16–17]

Our results also support the discussion of mixed dietary patterns, especially high-fat, high-sucrose (HFHS) and high-fat, high-fructose (HFFr) models. These diets are usually selected when researchers want to move beyond obesity alone and generate a broader metabolic syndrome phenotype. HFHS diets are often described as closer to a modern Western dietary pattern because they combine excess fat with palatable sugar exposure, which worsens glucose dysregulation and metabolic stress. HFFr models are commonly used when the aim is to intensify hepatic steatosis, dyslipidemia, and insulin resistance, since fructose has a particularly strong effect on liver lipid metabolism.[18–19] There is also a mechanistic difference between the use of sucrose and fructose. Sucrose is often included to improve palatability and better reflect processed human diets, especially in HFHS models. Fructose, in contrast, is more often used when the goal is to aggravate liver fat accumulation and insulin resistance. That distinction helps explain why HFHS and HFFr should not be treated as equivalent categories in the discussion. They may both worsen metabolic disease, but they do so with different emphases.[19] By contrast, high-sugar-only models were less common in the dataset. This also fits the literature. Sugar-rich diets can induce glucose intolerance, dyslipidemia, and some features of metabolic syndrome, but they are generally less reliable than HFD for producing robust obesity, especially over shorter study periods. For that reason, sugar is often used in combination with fat rather than as a standalone obesogenic strategy.[20–21]

Although rodents dominate metabolic research, non-rodent models are also used to study DIO and diabetes, particularly when closer physiological similarity to humans is required. Species such as zebrafish, Drosophila, and non-human primates are employed with tailored dietary interventions that reflect their metabolic biology. For example, zebrafish models often use high-fat or high-cholesterol diets, as well as glucose-enriched feeding, to induce obesity, hyperglycemia, and hepatic steatosis in a relatively short time.[22] These models are advantageous for studying lipid metabolism and screening bioactive compounds due to their rapid response and transparency for imaging. Similarly, Drosophila models commonly utilize high-sugar or HFD to induce insulin resistance and lipid accumulation, making them useful for mechanistic and genetic studies, although their translational relevance is limited.[23] In contrast, non-human primates are typically fed Western-style diets rich in fat and sugar, which more closely mimic human dietary patterns and disease progression. These models develop obesity, insulin resistance, and metabolic syndrome over longer periods, offering higher translational value but at significantly greater cost and ethical complexity.[24] Overall, dietary selection in non-rodent models is strongly influenced by species-specific metabolism, with simpler organisms favoring high-sugar or high-fat formulations for rapid phenotype induction, while higher-order models aim to replicate human dietary exposure more closely.

Overall, the study suggests that dietary model selection is not random. Researchers appear to choose diets based on the phenotype they want to generate. HFDs are used for efficient induction of obesity and insulin resistance. Moderate-fat diets may be preferred for slower and less extreme disease development. HFHS diets are used to better mimic Western dietary exposure. HFFr diets are often selected when hepatic and metabolic injury need to be intensified. This makes dietary composition a mechanistic choice, not just a feeding protocol.

Representation of chemical-induced models. A major finding from the dataset is the heavy reliance on STZ-based models. These were used either alone or in combination with dietary interventions. STZ is widely recognized as one of the most preferred methods for inducing diabetes because it is simple, rapid, and cost-effective.[25] In most studies, STZ was used to induce hyperglycemia within a few days. This makes it highly attractive for intervention studies, especially when screening bioactive compounds. But this convenience comes with important limitations. STZ primarily works by destroying pancreatic β-cells. Therefore, STZ-alone models primarily reflect β-cell injury and insulin deficiency, whereas HFD combined with low-dose STZ models better represent the interaction between insulin resistance and partial β-cell dysfunction characteristic of T2DM. The resulting phenotype is closer to type 1 diabetes than T2DM. In contrast, human T2DM develops gradually, involving insulin resistance followed by progressive β-cell dysfunction. This mismatch is critical. Another issue is variability. The dose of STZ, number of injections, species, and even preparation methods differ widely between studies. These differences can significantly alter disease severity. In some cases, partial recovery of β-cell function may even occur, further complicating interpretation.[25] Taken together, while STZ models are efficient, they are biologically limited. Their widespread use raises concerns about how well findings translate to human metabolic disease.

Combination models: HFD + STZ and improved disease representation. To overcome the limitations of STZ alone, many studies in our dataset used combination models, particularly HFD combined with low-dose STZ. This approach attempts to mimic both key features of T2DM: insulin resistance and β-cell dysfunction. These models are often considered more relevant. The HFD induces obesity and metabolic stress, while STZ introduces controlled β-cell impairment. Together, they produce a phenotype that resembles human disease more closely than either component alone. However, these models are still not perfect. Their outcomes depend heavily on experimental conditions. Small changes in diet composition, duration, or STZ dose can lead to very different results. This lack of standardization is a major limitation and is widely reported in the literature. So while combination models are an improvement, they remain artificial and protocol-sensitive.

Taken together, these findings suggest that no single model adequately captures the full spectrum of human metabolic disease. DIO models better reproduce obesity-associated inflammation, gradual insulin resistance, and microbiota alterations, whereas STZ-based models provide rapid and reproducible hyperglycemia but limited metabolic complexity. Hybrid HFD + STZ models partially bridge this gap by incorporating both insulin resistance and β-cell dysfunction, although their outcomes remain highly protocol-dependent. These differences are important because model selection may directly influence interpretation of dietary bioactive efficacy, particularly for interventions targeting inflammation, gut microbiota, or insulin signaling pathways.

Use of genetic models and dietary considerations. Genetic models, although less frequently used compared to diet-induced or chemical models, play an important role in understanding specific mechanisms of obesity and diabetes. In our dataset, these models were typically maintained on standard laboratory diets, which generally contain around 16–17% fat. This is intentional. Unlike DIO models, where diet is used to induce metabolic dysfunction, genetic models such as ob/ob, db/db, or other mutation-driven systems already possess inherent defects in energy balance, leptin signaling, or insulin regulation. As a result, a standard diet is often preferred to avoid introducing additional dietary variables that could confound interpretation. Using a controlled baseline diet allows researchers to isolate the effects of the genetic

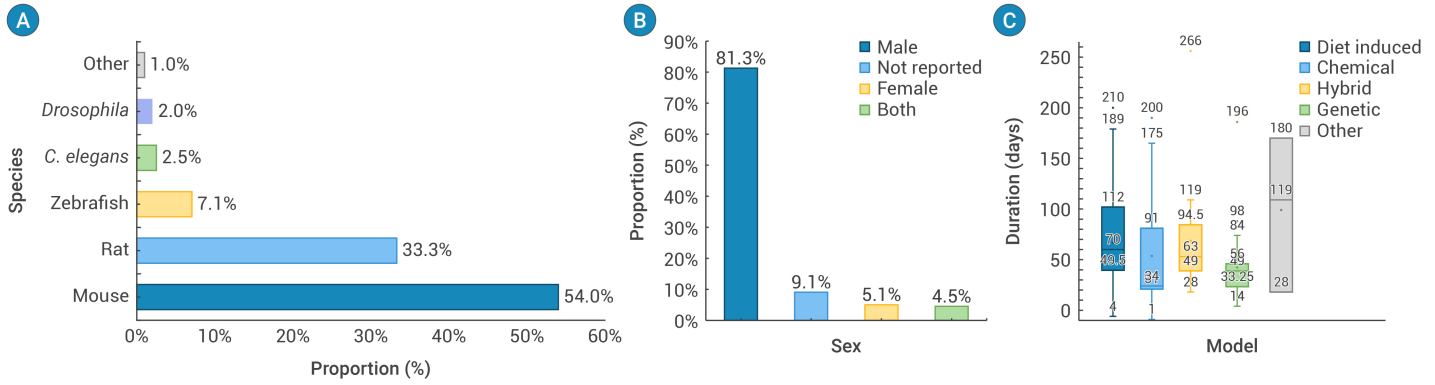


Figure 3. Distribution of animal models used in the included studies (A) Species distribution, (B) Sex distribution of animals used, (C) Study duration and experimental designs.

mutation itself, rather than combining it with external dietary stress. At the same time, some studies may still introduce high-fat feeding in genetic models to exacerbate the phenotype, but this is usually done selectively and with a clear mechanistic purpose.[26-28] Overall, the reliance on standard diets in genetic models reflects a different experimental goal, focusing on intrinsic biological pathways rather than environmental induction of disease.

Species and strain trends and their impact on metabolic outcomes. The distribution of species and strains across the included studies represents a major source of variability in experimental outcomes. Rodent models dominated the included studies, with mice accounting for 54.0% and rats for 33.3% of studies. However, non-rodent models such as zebrafish (7.1%), *Caenorhabditis elegans* (2.5%), and *Drosophila melanogaster* (2.0%) were used comparatively less frequently (Figure 3A). This imbalance reflects both practical considerations and long-standing methodological preferences in metabolic disease research. Rodents offer a favorable balance between experimental control, cost-effectiveness, and physiological relevance, whereas non-rodent models are often employed for specific purposes such as high-throughput screening or pathway discovery. However, each species presents distinct advantages and limitations that influence its suitability for different research objectives. A comparative overview of commonly used models, including their strengths, limitations, and optimal applications, is provided in Table 1.

Rodent models. Rodents remain the primary experimental systems for studying obesity and T2DM due to their favorable balance between experimental control, cost-effectiveness, and physiological relevance. Their relatively short lifespan, ease of handling, and well-characterized metabolic responses make them suitable for both mechanistic and intervention studies. In addition, the availability of standardized diets and protocols has contributed to their widespread adoption. However, despite their utility, rodents do not fully replicate human metabolic physiology. Differences in lipid metabolism, energy expenditure, and insulin dynamics can influence how results translate to clinical settings. As highlighted in previous reviews, no single animal model captures the full complexity of human metabolic disease, and findings in rodents should be interpreted within this context.[10,14]

Mice were the most frequently used species in the present dataset, reflecting their unique advantages in genetic manipulation and mechanistic research. The availability of transgenic, knockout, and knock-in models enables precise investigation of signaling pathways involved in metabolism, inflammation, and insulin resistance. This makes mice particularly valuable for mechanistic studies of dietary bioactive compounds. Among mouse strains, C57BL/6 is the most widely used due to its high susceptibility to DIO, insulin resistance, and hepatic steatosis. This susceptibility enhances the reproducibility of obesity-related phenotypes and makes it a preferred model for diet-induced studies. However, this same predisposition may also exaggerate disease progression compared to humans, potentially limiting translational relevance. Furthermore, mice differ from humans in several physiological aspects, including lipid metabolism, thermoregulation, and adipose tissue distribution. For example, mice have higher basal metabolic rates and distinct lipoprotein profiles, which may influence responses to dietary interventions. These differences should be carefully considered when extrapolating findings to human populations.[10]

Rats accounted for approximately one-third of the included studies and are often preferred for investigations requiring detailed physiological, pharmacokinetic, or behavioral assessments. Compared to mice, rats exhibit more stable metabolic responses and are considered more suitable for studies involving glucose tolerance, insulin sensitivity, and complex metabolic phenotyping. Commonly used strains such as Wistar and Sprague–Dawley rats are known for their responsiveness to dietary manipulation, although differences between strains can influence outcomes. Rats also offer practical advantages due to their larger size, which facilitates repeated blood sampling and surgical procedures. Despite these advantages, rats are less amenable to genetic manipulation compared to mice, which limits their use in mechanistic studies. Consequently, their role in metabolic research is often complementary rather than substitutive, with rats being used for physiological validation and mice for mechanistic exploration.[29]

Emerging non-rodent models: zebrafish and invertebrates. Non-rodent models such as zebrafish, *C. elegans*, and Drosophila were used less frequently but offer distinct advantages that are increasingly recognized in metabolic research. Zebrafish, in particular, have gained attention due to their rapid development, optical transparency, and suitability for high-throughput screening. They also share conserved metabolic pathways with mammals, making them useful for studying lipid metabolism and adipogenesis. Invertebrate models provide additional benefits, including low cost, rapid lifecycle, and genetic tractability. These systems are particularly valuable for identifying conserved metabolic pathways and conducting large-scale screening of bioactive compounds. However, these models also have important limitations. Differences in physiology, organ structure, and metabolic regulation restrict their ability to fully replicate human metabolic disease. In particular, their translational relevance for complex metabolic, endocrine, and reproductive physiology remains limited compared with mammalian systems. Consequently, these models are most appropriately used for early-stage screening and pathway discovery rather than as standalone systems for translational interpretation. Positive findings obtained from non-rodent models should therefore be further validated in rodent models before broader translational conclusions are drawn. As a result, they are often best used as complementary models for early-stage screening rather than as standalone systems for translational studies.[30–31]

Strain-dependent variability and reproducibility challenges. Strain selection represents a critical but often underappreciated determinant of metabolic outcomes in preclinical research. Within rodent models, strains such as C57BL/6 mice, Wistar rats, and Sprague–Dawley rats are most frequently used due to their well-characterized responses to dietary and pharmacological interventions. However, accumulating evidence indicates that metabolic phenotypes can vary substantially between strains, even when experimental conditions are identical. For example, C57BL/6 mice are highly susceptible to DIO, insulin resistance, and hepatic steatosis, making them a preferred model for studying obesity-related metabolic dysfunction. In contrast, other mouse strains, such as A/J or BALB/c, exhibit partial resistance to weight gain and metabolic impairment under similar dietary conditions. These strain-dependent differences reflect underlying genetic variation

Table 1. Advantages and limitations of common animal models in metabolic research.

Species / Model	Major Strengths	Major Limitations	Best Use / Application
Mouse (general)	Extensive genetic tools; cost-effective; widely characterized	Physiological differences from humans	Mechanistic and transgenic studies
C57BL/6 mouse	High susceptibility to DIO and insulin resistance	May develop exaggerated metabolic phenotypes	DIO and obesity-related studies
Rat (general)	Stable physiology; larger sample collection capacity	Fewer genetic tools compared with mice	Pharmacokinetic and physiological studies
Wistar rat	Responsive to dietary interventions	Variable obesity susceptibility	Nutritional and long-term feeding studies
Sprague–Dawley rat	Suitable for chronic metabolic studies	Variable metabolic responses	Long-term intervention studies
Zucker diabetic rat	Strong insulin resistance phenotype	Monogenic/genetic artifact	Diabetes progression and insulin resistance
Zebrafish	High-throughput screening; conserved metabolic pathways	Limited physiological complexity	Early-stage metabolic screening
Drosophila *melanogaster*	Rapid lifecycle; strong genetic tractability	Limited translational relevance	Pathway discovery and genetic studies
C. elegans	Simple metabolic system; rapid experimentation	Lacks mammalian physiology	Basic metabolism and screening studies
DIO (HFD/HFHS)	High face validity for obesity and insulin resistance	Long induction duration; protocol variability	Obesity and metabolic syndrome
STZ	Rapid and reproducible diabetes induction	Limited insulin resistance phenotype	β-cell dysfunction and hyperglycemia
NA-STZ	Partial representation of T2DM	Variable induction severity	T2DM-related glucose dysregulation
HFD + STZ (Hybrid)	Combines insulin resistance and β-cell dysfunction	Complex and protocol-sensitive	Obesity-associated T2DM
Genetic (db/db, ob/ob)	Strong mechanistic and pathway specificity	Monogenic etiology limits translation	Mechanistic and signaling pathway studies

that influences energy balance, lipid metabolism, and insulin sensitivity.[10–11] Similarly, in rat models, differences between commonly used strains such as Wistar and Sprague–Dawley have been reported in terms of adiposity, glucose tolerance, and responsiveness to dietary interventions. These differences can influence both baseline metabolic status and the magnitude of response to bioactive compounds, thereby affecting the reproducibility and comparability of findings across studies.[29]

Importantly, strain effects extend beyond phenotypic variability to influence disease progression and mechanistic pathways. Experimental evidence demonstrates that susceptibility to obesity and diabetes varies widely across strains, even when animals are exposed to identical HFDs, highlighting the strong interaction between genetic background and environmental factors.[10] In genetic models, the influence of strain is even more pronounced. Phenotypes observed in models such as ob/ob and db/db mice are not solely determined by the underlying mutation but are significantly modified by the genetic background on which the mutation is expressed. As a result, identical mutations can produce different degrees of obesity, insulin resistance, and hyperglycemia depending on the strain context, complicating interpretation and cross-study comparison.[32]

These findings have important implications for reproducibility. Variability in strain selection across studies may contribute to inconsistent outcomes, even when experimental designs appear similar. Furthermore, inadequate reporting of strain details, as observed in several studies within this review, further limits the ability to interpret and replicate findings. This issue aligns with broader concerns in preclinical research, where lack of standardization and incomplete reporting have been identified as major barriers to reproducibility and translation.[33] Overall, the strong influence of genetic background suggests that strain selection should be considered a key experimental variable rather than a logistical choice. Greater standardization, along with explicit justification and reporting of strain selection, will be essential for improving reproducibility, comparability, and translational relevance in metabolic research.

Sex bias in animal models. A pronounced sex bias was observed across the included studies: 81.3% were conducted exclusively in male animals, compared to only 5.1% in females and 4.5% including both sexes; 9.1% did not report sex (Figure 3B). This male dominance is consistent with trends reported in preclinical metabolic research, where male rodents are often preferred to avoid variability associated with the estrous cycle. However, this approach may reduce biological and translational representation. Sex is a critical biological variable in metabolic regulation. Female animals often show partial protection against DIO and insulin resistance due to hormonal influences such as estrogen, whereas males tend to develop more severe metabolic dysfunction under similar conditions. Although this may partly explain the frequent use of male animals, it may also bias experimental findings toward more severe metabolic phenotypes.

Importantly, some models demonstrate sex-dependent differences in disease susceptibility and progression, where outcomes vary not only in magnitude but also in mechanism. As highlighted in rodent diabetes models, both strain and sex significantly influence metabolic phenotype and response to induction protocols.[25,34] Ignoring these differences limits the translational relevance of findings, particularly given that human obesity and diabetes also exhibit clear sex-specific patterns. Beyond differences in body weight and insulin sensitivity, sex also fundamentally influences endocrine signaling, adipose tissue distribution, inflammatory responses, and reproductive metabolism. Sex differences are also closely linked to immunometabolic regulation. Estrogen signaling has been shown to modulate adipose tissue inflammation, macrophage polarization, and insulin sensitivity, contributing to partial protection against obesity-associated metaflammation and metabolic dysfunction in females. Consequently, dietary bioactive compounds with anti-inflammatory or phytoestrogenic properties may exhibit sex-specific and potentially divergent biological effects that cannot be adequately captured in male-only study designs.[35] This is particularly relevant for female-specific metabolic conditions such as polyendocrine metabolic ovarian syndrome (PMOS) and obesity-associated reproductive dysfunction, where metabolic and hormonal disturbances are closely interconnected.[36] The underrepresentation of female animals, therefore, limits understanding of how dietary bioactive interventions may differentially affect female metabolic physiology and endocrine health. Overall, the strong male bias observed in this dataset

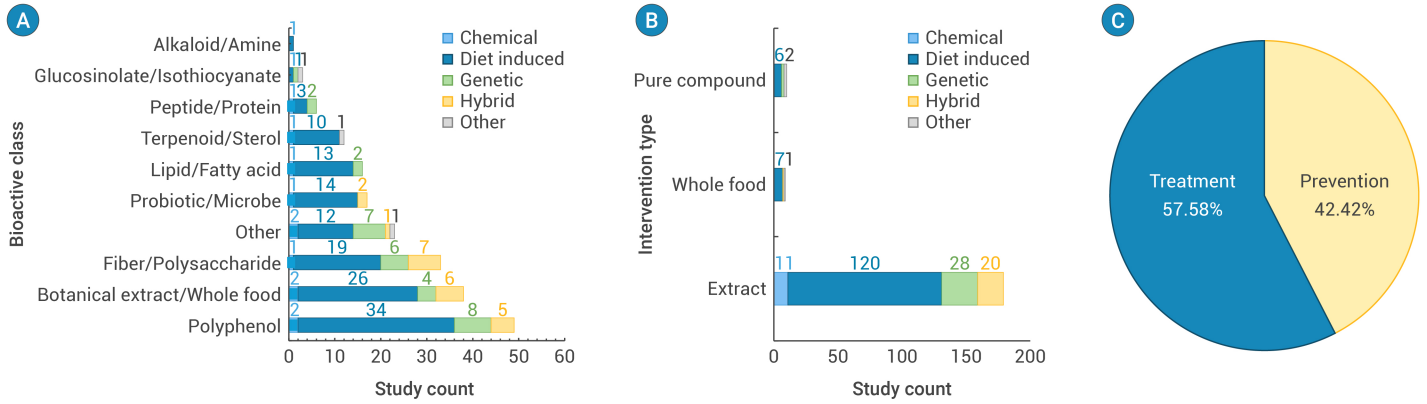


Figure 4. Distribution of dietary bioactive compounds investigated in the included studies (A) Classification of bioactive compounds by major classes, (B) Distribution of intervention types, (C) Relationship between bioactive class and model type.

suggests that many studies may overlook important biological variability. Increasing the inclusion of female animals and conducting sex-stratified analyses would improve the robustness and applicability of future research.

Influence of study duration and experimental design. Study duration varied considerably across the included models, reflecting differences in both experimental design and disease induction strategy. As shown in Figure 3C, diet-induced models generally required longer durations, with median values around ~70 days and a wide spread extending beyond 200 days. This is expected, as DIO models rely on gradual metabolic changes such as weight gain, insulin resistance, and adiposity, which develop over time. In contrast, chemical models showed shorter and more variable durations, often clustering around ~30–40 days, as agents like STZ rapidly induce hyperglycemia through β-cell damage. Hybrid models displayed intermediate durations, combining the slower onset of dietary induction with the accelerated effects of chemical intervention. Interestingly, genetic models also showed moderate to long durations, reflecting the progressive nature of phenotype development driven by inherent mutations.

These differences highlight an important trade-off. Shorter studies are efficient and suitable for screening interventions but may only capture acute metabolic changes. Longer studies, particularly in diet-induced and genetic models, better represent chronic disease progression but require greater time and resources. This variation in duration can significantly influence outcomes, including the severity of metabolic dysfunction and the observed efficacy of interventions. Therefore, study duration is not just a methodological detail but a critical factor shaping the phenotype and interpretability of results.

Bioactive compound landscape

Distribution of bioactive classes. The distribution of bioactive compounds across the included studies revealed a strong concentration within a limited number of classes (Figure 4A). Polyphenols represented the most frequently investigated group, followed by botanical extracts/whole-food-derived interventions and fiber/polysaccharide-based compounds. In contrast, lipid-derived compounds, probiotics, peptides, and other classes were comparatively less represented, while categories such as alkaloids and glucosinolates were only sporadically studied. This pattern reflects a clear research bias toward plant-derived phytochemicals, particularly polyphenols. The prominence of polyphenols is consistent with their well-documented antioxidant and anti-inflammatory properties, which have been extensively linked to metabolic health benefits. However, the disproportionate focus on this class raises concerns regarding mechanistic redundancy within the field. Many studies repeatedly investigate similar endpoints such as oxidative stress reduction and inflammatory modulation, without substantially advancing mechanistic understanding.

Formulation characteristics: Extract-dominated research. A striking feature of the dataset was the overwhelming dominance of extract-based interventions (Figure 4B). The majority of studies utilized crude or semi-purified extracts, whereas only a small proportion investigated pure compounds or whole-food-based interventions. While extract-based approaches may better reflect the complexity of dietary exposure, they introduce significant challenges related to standardization, reproducibility, and mechanistic interpretation. In many cases, the chemical composition of extracts is incompletely characterized, and batch-to-batch variability is rarely addressed. This makes it difficult to attribute observed biological effects to specific bioactive components.

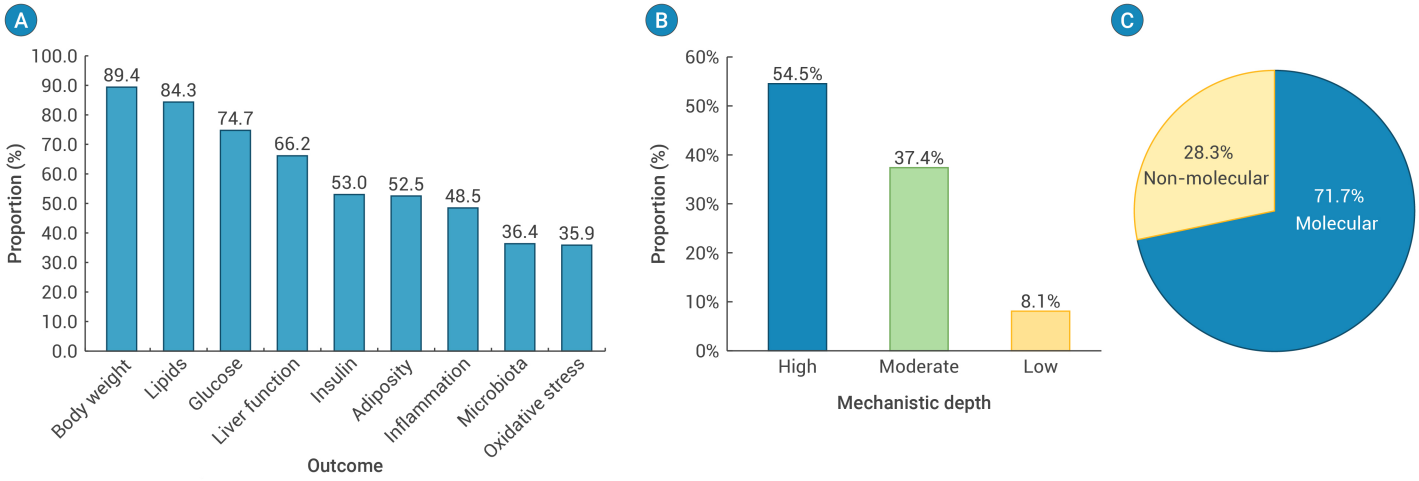
Furthermore, extract-based studies often operate at the interface between nutrition and pharmacology, yet without adhering to the rigor of either field. Unlike pharmaceutical compounds, extracts are frequently administered without precise characterization or dose normalization. At the same time, they are often delivered at concentrations that exceed typical dietary intake, raising concerns about translational relevance. Previous analyses have highlighted that poor standardization of botanical extracts is a major barrier to reproducibility and clinical translation in bioactive research.[37]

Source and conceptual framing of "dietary" bioactives. The predominance of botanical extracts and phytochemical-rich compounds suggests that the field is heavily oriented toward plant-based interventions. However, this raises an important conceptual issue: many of these interventions are framed as "dietary" bioactives, yet are tested under conditions that resemble pharmacological treatment rather than normal dietary exposure. For example, concentrated plant extracts administered via oral gavage or high doses may not reflect realistic human consumption patterns. This creates a disconnect between experimental design and real-world applicability. While such approaches may be useful for mechanistic exploration, they risk overstating the practical relevance of findings. This issue has been widely recognized in nutrition research, where the distinction between dietary exposure and pharmacological dosing is critical for interpretation. Studies that do not account for this distinction may contribute to inflated expectations regarding the efficacy of bioactive compounds in human populations.[38]

Intervention strategy: Prevention versus treatment. The analysis revealed that treatment-oriented studies (57.6%) were more common than prevention-based approaches (42.4%) (Figure 4C). This distribution suggests that most studies focus on evaluating the therapeutic potential of bioactive compounds in established disease models rather than their role in disease prevention. While treatment studies are important, prevention-based approaches are particularly relevant in the context of dietary interventions, where long-term modulation of metabolic risk factors is a primary goal. The relatively lower representation of prevention studies may therefore represent a gap in the literature. Additionally, prevention studies often involve concurrent administration of bioactives during model induction, which may not accurately reflect real-world scenarios where interventions are introduced after disease onset. Conversely, treatment studies may better simulate clinical conditions but are often conducted over relatively short durations, limiting their ability to capture long-term effects. This imbalance highlights the need for more longitudinal and well-designed studies that integrate both preventive and therapeutic perspectives.

Outcome reporting patterns

Predominance of core metabolic outcomes. Across the included studies,



D

Model	Glucose metabolism	Lipid metabolism	Inflammation	Oxidative stress	Gut microbiota modulation
Chemical	9	4	3	6	1
Diet induced	99	121	68	45	52
Genetic	28	28	15	8	12
Hybrid	20	19	8	12	6
Other	2	3	2	0	1

Figure 5. Outcome reporting patterns and mechanistic depth across studies (A) Frequency of reported outcomes, (B) Distribution of mechanistic depth, (C) Proportion of studies incorporating molecular evidence compared to non-molecular assessments, (D) Heatmap showing the relationship between model type and reported outcomes.

outcome reporting was strongly centered on a relatively narrow set of core metabolic endpoints. Body weight was the most frequently reported variable (89.4%), followed by lipid-related outcomes (84.3%), glucose metabolism (74.7%), and liver function (66.2%) (Figure 5A). Insulin and adiposity were reported in roughly half of the studies, whereas inflammation, oxidative stress, and especially gut microbiota were less consistently assessed. This pattern indicates that most studies remain focused on readily measurable phenotypic indicators of metabolic improvement rather than building a broader and more integrated assessment of disease biology. This trend is understandable. Body weight, fasting glucose, oral glucose tolerance, and serum lipids are widely accepted indicators of metabolic dysfunction in animal models and remain central to preclinical obesity and diabetes research. At the same time, reliance on these endpoints alone can oversimplify the evaluation of dietary bioactive compounds. A reduction in body weight or fasting glucose does not necessarily indicate meaningful correction of insulin resistance, β-cell dysfunction, adipose tissue remodeling, or organ-specific pathology. Recent methodological reviews have similarly emphasized that preclinical studies of bioactive compounds in metabolic disease often generate only a limited range of information, and that conclusions about efficacy are highly dependent on the breadth and quality of the selected outcome measures.[7,10,39]

In the context of dietary bioactive development, this is particularly important. Unlike single-target drugs, dietary bioactives are typically expected to act across multiple physiological systems. If studies assess only body weight or glucose-related endpoints, the resulting evidence may appear positive but remain too shallow to support mechanistic interpretation or translational prioritization. In other words, outcome selection itself can shape the apparent success of an intervention.

Integration of secondary outcomes. A second pattern in the dataset was the partial but inconsistent incorporation of secondary outcomes such as inflammation, oxidative stress, microbiota composition, and liver-related parameters. These outcomes are biologically relevant because obesity and T2DM are not isolated glycemic disorders; they involve chronic low-grade inflammation, redox imbalance, altered gut–host interactions, and tissue-specific dysfunction. However, in many studies, these measures appeared as supplementary endpoints rather than being integrated into a coherent mechanistic framework. This distinction matters. Measuring inflammatory cytokines or oxidative stress markers can strengthen interpretation, but only when these outcomes are linked clearly to the disease model and intervention hypothesis.

In the present dataset, liver function was reported more frequently than many other secondary endpoints, suggesting growing recognition of hepatic steatosis and liver injury as central features of metabolic disease. By contrast, gut microbiota-related outcomes were among the least frequently reported, despite the increasing emphasis on microbiota-mediated mechanisms in nutrition and bioactive research. Similarly, endocrine and reproductive outcomes were rarely evaluated across the included studies, despite increasing recognition that obesity and T2DM profoundly influence hypothalamic-pituitary-gonadal (HPG) axis regulation, sex hormone signaling, fertility, and reproductive health.[36,40] Recent advances in immunometabolism and gut–host interaction research further emphasize the importance of integrating inflammatory, microbial, endocrine, and metabolic endpoints when evaluating dietary bioactive efficacy.[41–42] Furthermore, interpretation of microbiota-related outcomes should consider the bioavailability characteristics of many dietary bioactive compounds. Numerous polyphenols and flavonoids exhibit limited absorption in the upper gastrointestinal tract and may reach the colon largely intact, where they undergo extensive microbial biotransformation into secondary metabolites that can contribute substantially to biological activity. Consequently, observed metabolic effects may be mediated not only by the parent compounds but also by microbiota-derived metabolites. This highlights the need for more rigorous mechanistic approaches beyond descriptive microbiota profiling, including functional analyses, fecal microbiota transplantation, antibiotic-depletion models, or germ-free systems capable of establishing causal relationships between microbial changes and host metabolic outcomes.[42] This uneven distribution suggests that the field is beginning to broaden beyond classical metabolic markers, but has not yet achieved methodological consistency in how secondary outcomes are selected or interpreted. Importantly, variability in diet composition and model selection may substantially influence microbiota composition, inflammatory signaling, and metabolic phenotype, complicating direct comparison across studies. The wider literature supports this concern. Reviews of obesity- and diabetes-related preclinical models have repeatedly noted that experimental

outcomes are highly sensitive to model design, feeding conditions, housing, and endpoint selection, and that overinterpretation is common when a limited set of biomarkers is used to support broad mechanistic claims.[10,43]

The molecular depth of outcomes. One of the more encouraging findings from the present study is that molecular evidence was incorporated in a substantial proportion of studies. Based on the final dataset, 71.7% of studies included molecular-level analysis, whereas 28.3% remained non-molecular (Figure 4C). Likewise, most studies were classified as having high or moderate mechanistic depth, with only a small minority categorized as low-depth studies (Figure 5B). Mechanistic depth classification was based on the extent of molecular and functional analyses reported in each study, including the use of pathway-focused molecular assessments and functional validation approaches. This suggests that the field is not limited to phenotype-level screening alone and that many investigators are attempting to connect metabolic outcomes to underlying pathways.

However, this should not be interpreted uncritically. The presence of molecular evidence does not automatically indicate strong mechanistic inference. In many preclinical studies, mechanistic claims are based on a small number of gene-expression or protein-expression markers, often without pathway perturbation, inhibitor studies, loss-of-function validation, or tissue-specific functional testing. Accordingly, molecular measurements such as gene or protein expression changes should be interpreted primarily as supportive or correlative evidence unless accompanied by functional experiments capable of establishing causal pathway involvement. Although many studies incorporated molecular analyses, mechanistic interpretation should be approached with caution, as a substantial proportion relied primarily on correlational gene or protein expression data without functional validation experiments to establish causal relationships. In such cases, molecular data provide useful clues, but not definitive evidence of causality. A change in AMPK, AKT, GLUT4, or inflammatory marker expression may be consistent with a proposed mechanism, yet still fall short of proving that the pathway mediates the observed phenotype. Therefore, although many studies demonstrated moderate or high mechanistic depth according to the predefined classification criteria, the strength of causal inference varied considerably across the included literature.

Nevertheless, several mechanistic patterns emerged repeatedly across the included studies. Improvements in inflammatory signaling pathways, including reductions in TNF-α, IL-6, NF-κB activation, and oxidative stress markers, were among the most consistently reported findings across diverse intervention classes. Multiple studies also reported modulation of insulin-signaling pathways such as AMPK, AKT, and GLUT4, suggesting convergence toward improved metabolic homeostasis. In contrast, mechanisms involving gut microbiota modulation, bile acid signaling, neuro-metabolic regulation, and immunometabolic interactions were less consistently investigated and often relied on associative rather than functionally validated evidence. Consequently, while inflammatory and insulin-signaling pathways appear comparatively well supported across studies, several emerging mechanistic pathways remain preliminary and require more rigorous causal validation. This is a broader issue in preclinical animal research. Recent methodological discussions emphasize that the robustness, reliability, and translational value of bioactive research depend not only on whether mechanistic endpoints are included but on how convincingly those endpoints are linked to functional outcomes and experimental design. In this sense, the present dataset suggests a field that is moving toward greater mechanistic sophistication, but where mechanistic claims may still outpace experimental validation.[10,33,39]

Outcome selection across model types. The model–outcome heatmap Figure 5D provides an especially important insight: outcome reporting was not uniform across model classes. Diet-induced models were associated with the largest number of reported outcomes across all categories, including glucose metabolism, lipid metabolism, inflammation, oxidative stress, and gut microbiota modulation. This is expected because diet-induced models dominated the dataset numerically and are commonly used to evaluate broad metabolic phenotypes. Genetic and hybrid models also showed relatively wide outcome coverage, whereas chemically induced models tended to be more narrowly represented.

This pattern has important implications. Not all animal models are equally suited to all outcome domains. For example, diet-induced models are particularly appropriate for studying adiposity, insulin resistance, dyslipidemia, and diet–microbiota interactions, whereas chemically induced models are often more focused on hyperglycemia and pancreatic injury. If a study uses a given model but measures only a subset of the outcomes most relevant to that model, important biological information may be missed. The question is therefore not simply whether outcomes are measured, but whether the chosen outcomes are appropriately aligned with model biology. This is well supported by the broader literature. Recent reviews stress that no single animal model captures the full spectrum of human obesity or T2DM, and that the phenotype of a model can differ substantially depending on induction strategy, feeding conditions, and duration. As a result, outcome selection should be model-informed rather than routine or formulaic.[7,43]

Implications for dietary bioactive development. Taken together, the outcome-reporting patterns identified in this review reveal both progress and persistent limitations. On the positive side, most studies now go beyond simple weight change and include a mixture of glycemic, lipid, inflammatory, oxidative, hepatic, microbial, or molecular outcomes. This reflects increasing recognition that obesity and diabetes are multisystem disorders and that dietary bioactives may act through more than one biological route. At the same time, the field still appears skewed toward commonly measured, low-barrier endpoints, while deeper physiological integration remains inconsistent. Several gaps remain clear: insulin-related measures are less common than body weight or lipids, gut microbiota outcomes are still relatively underused, and mechanistic studies do not always achieve true causal depth. For the development of dietary bioactive compounds, these weaknesses matter directly. A candidate compound that lowers body weight but lacks convincing evidence for improved insulin signaling, adipose remodeling, hepatic metabolism, or durable pathway engagement may appear promising in early-stage research yet fail to support meaningful translation.

From a methodological perspective, this reinforces the importance of more standardized and hypothesis-driven outcome selection. Reporting frameworks such as ARRIVE 2.0 were developed precisely because inadequate reporting and incomplete methodological detail reduce reproducibility and make cross-study comparison difficult. For future preclinical work in dietary bioactives, stronger integration of phenotypic, biochemical, and molecular endpoints matched appropriately to model type will likely be necessary to improve both interpretability and translational confidence.[33]

Methodological quality and trends

Study design characteristics and internal validity. Across the included studies, considerable variability was observed in key elements of experimental design, including randomization, blinding, and group allocation. While most studies employed controlled experimental settings, explicit reporting of randomization procedures and blinding was inconsistent. In many cases, these methodological details were either not described or only briefly mentioned, making it difficult to assess the internal validity of the findings. The absence of clearly reported randomization and blinding raises concerns regarding potential bias in outcome assessment and data interpretation. These factors are well recognized as essential components of rigorous experimental design, particularly in preclinical research where investigator influence may inadvertently affect measurements such as histological scoring, behavioral assessment, or endpoint selection. Previous methodological analyses have highlighted that the lack of randomization and blinding is a common limitation in animal studies and can contribute to inflated estimates of treatment effects.[33]

Incomplete reporting and transparency limitations. A recurring issue across the dataset was incomplete reporting of key experimental parameters. Critical details such as diet composition, dose justification, duration of intervention, and even basic animal characteristics (e.g., sex or age) were not consistently reported. In some cases, studies referred to "HFD" or "bioactive supplementation" without providing sufficient detail to allow replication. This lack of transparency represents a major barrier to reproducibility and cross-study comparison. Without standardized reporting of experimental conditions, it becomes difficult to determine whether differences in outcomes are attributable to the intervention itself or to variations in study design.

Reproducibility challenges and experimental heterogeneity. The findings of this review highlight substantial heterogeneity across studies in terms

of model selection, dietary composition, intervention strategies, and outcome measurement. While some degree of variability is expected in biological research, the extent observed here suggests a lack of standardization that may limit reproducibility. For example, diet-induced models varied widely in fat content and composition, intervention durations ranged from short-term to long-term designs, and dosing strategies for bioactive compounds were often inconsistent or poorly justified. These factors can significantly influence metabolic outcomes, making it difficult to compare results across studies or to draw generalized conclusions about the efficacy of specific bioactive compounds. Such variability has been widely recognized as a major challenge in preclinical metabolic research. Even small differences in experimental conditions, such as diet formulation, animal strain, or housing environment, can lead to divergent results, underscoring the need for more standardized and well-characterized experimental frameworks.[10]

An additional but often overlooked source of variability is the selection of control diets. In many studies, HFDs were compared with standard chow diets, despite substantial differences extending beyond fat content alone. Chow diets typically contain complex ingredient mixtures, variable fiber sources, and naturally occurring bioactive compounds, whereas purified experimental diets are more compositionally defined. Consequently, observed metabolic effects may reflect multiple dietary differences rather than fat content alone, complicating interpretation and cross-study comparison. The use of matched low-fat purified control diets may improve experimental rigor by minimizing confounding dietary variables and enhancing reproducibility across studies.[44]

Translational limitations of current preclinical approaches. The methodological limitations identified in this review collectively contribute to the reduced translational relevance of preclinical findings. Several recurring issues are particularly important in this context. First, many studies utilized experimental conditions that do not reflect typical human exposure, including extremely HFDs or supraphysiological doses of bioactive compounds. Second, the strong bias toward male animals limits the generalizability of findings, given well-established sex differences in metabolic regulation. Third, relatively short study durations may fail to capture the chronic and progressive nature of obesity and T2DM. In addition, while many studies reported positive metabolic outcomes, these findings were often based on a limited set of endpoints or supported by relatively shallow mechanistic evidence. As a result, the predictive value of these studies for human interventions remains uncertain. This issue is not unique to dietary bioactives; it reflects broader challenges in translating preclinical findings into clinical success. Analyses of translational failure have consistently shown that promising results in animal models frequently do not replicate in human trials, often due to differences in physiology, experimental design, and outcome relevance.[45]

A broader issue emerging from this review is the potential mismatch between the biological characteristics of dietary bioactive interventions and the severity of some experimental disease models. Unlike conventional pharmacological agents, dietary bioactives often exert pleiotropic, multi-target, and context-dependent effects that may require prolonged exposure to produce measurable physiological benefits. Consequently, highly aggressive disease models, including severe obesogenic diets or extensive β-cell destruction, may underestimate, obscure, or distort the efficacy of dietary interventions. This highlights the importance of model–mechanism matching, where the choice of animal model should be aligned not only with the disease phenotype of interest but also with the expected magnitude and mechanism of action of the dietary bioactive under investigation. Improving this alignment may enhance both biological interpretation and translational relevance.[10]

Emerging improvements and future methodological directions. Despite these limitations, several positive trends were also observed. An increasing number of studies have begun to incorporate molecular analyses, microbiota assessments, and multi-system outcome measures, reflecting a shift toward more comprehensive evaluation of metabolic function. There is also growing awareness of the importance of model selection, disease relevance, and experimental design in improving the quality of preclinical research. Moving forward, several key improvements are needed to strengthen the field. These include more consistent application of randomization and blinding, improved reporting of experimental details in line with established guidelines, and greater standardization of diets and dosing strategies. In addition, incorporating both sexes, extending study duration where appropriate, and integrating mechanistic validation with phenotypic outcomes will be critical for enhancing translational relevance. Ultimately, improving methodological rigor is essential not only for reproducibility but also for the successful development of dietary bioactive compounds as evidence-based interventions for metabolic disease. Addressing these challenges will help bridge the gap between preclinical findings and clinical application, ensuring that promising results are both reliable and meaningful.

Model selection and experimental design framework for future studies

The findings of this review highlight substantial variability in animal model selection, outcome reporting, and methodological rigor across studies investigating dietary bioactive compounds. While this diversity reflects the complexity of metabolic diseases, it also underscores the absence of a standardized, objective-driven approach to model selection. To address this gap, a structured framework is proposed to guide researchers in selecting appropriate animal models based on study objectives, disease characteristics, and practical considerations. Table 2 and Figure 6 together provide a practical framework linking study objectives, model selection, dosing considerations, comparator interventions, and outcome assessment strategies for future dietary bioactive research.

Objective-driven model selection. The choice of animal model should be primarily determined by the specific research objective. As demonstrated in the present analysis, diet-induced models dominate the field and are particularly suitable for studies focusing on obesity, adiposity, and diet-related metabolic dysfunction. These models offer high face validity and closely mimic environmental and dietary drivers of human metabolic disease.[14] In contrast, studies targeting hyperglycemia or β-cell dysfunction may require chemically induced or genetic models. STZ-based models, including nicotinamide-STZ combinations, are widely used for inducing pancreatic β-cell damage and are appropriate for investigating insulin deficiency and glucose regulation.[46] However, these models may not fully capture the complexity of obesity-associated insulin resistance.[10] For studies aiming to investigate combined metabolic phenotypes, such as obesity-associated T2DM, hybrid models (e.g., HFD combined with low-dose STZ) provide a more comprehensive representation of disease pathology. Similarly, genetic models such as db/db and ob/ob mice are particularly useful for mechanistic studies, as they allow investigation of specific signaling pathways and gene–phenotype relationships, although their translational relevance may be limited due to monogenic etiology.[47]

Alignment of the model with disease phenotype and stage. A critical consideration in model selection is the alignment between the chosen model and the targeted stage or phenotype of disease. Obesity and T2DM develop progressively, involving transitions from adiposity and insulin resistance to β-cell dysfunction and systemic metabolic impairment. No single model captures all aspects of this progression.[10] Diet-induced models are most appropriate for early-stage metabolic disturbances, including weight gain, dyslipidemia, and insulin resistance. In contrast, chemical and genetic models are better suited for studying later-stage features such as hyperglycemia and impaired insulin secretion. Hybrid models offer an intermediate approach by combining features of both, enabling the study of multi-system metabolic dysfunction. This distinction is essential because mismatches between model characteristics and study objectives can lead to misleading conclusions. For example, evaluating anti-obesity interventions in models primarily characterized by β-cell toxicity, or assessing insulin resistance in models lacking dietary induction, may limit the biological relevance of the findings.

Mechanism-oriented model selection. In addition to disease phenotype, model selection should consider the primary biological mechanism targeted by the intervention. As observed in this review, dietary bioactive compounds often exert pleiotropic effects, including modulation of inflammation, oxidative stress, gut microbiota, and metabolic signaling pathways. Diet-induced models are particularly suitable for studying inflammation and microbiota-related mechanisms, as these models involve chronic exposure to obesogenic diets that directly influence immune responses and gut microbial composition. Conversely, genetic models provide controlled systems for investigating specific signaling pathways, such as insulin signaling or lipid

Table 2. Recommended animal models and experimental considerations according to research objectives.

Research Objective	Recommended Model	Key Strength	Major Limitation	Suggested Outcomes	Positive Control / Comparator	Dose Consideration
Obesity / adiposity	DIO (HFD/HFHS)	High face validity	Long induction duration	Body weight, adiposity, insulin resistance	Metformin Orlistat	Prefer physiologically relevant dietary doses
Insulin resistance	HFD + low-dose STZ	Combined metabolic phenotype	Protocol variability	OGTT, insulin sensitivity, HOMA-IR	Metformin	Dose normalization by body weight and intake
β-cell dysfunction	STZ / NA-STZ	Rapid hyperglycemia induction	Limited obesity phenotype	Fasting glucose, insulin secretion	Metformin Insulin	Avoid supraphysiological exposure
NAFLD/ MASLD	HFHS/HFFr diets	Hepatic steatosis progression	Long-term feeding required	Liver histology, ALT/AST, lipid accumulation	Pioglitazone Metformin	Consider dietary matrix composition
Inflammation / immunometabolism	DIO or hybrid models	Chronic inflammatory phenotype	Variable inflammatory severity	TNF-α, IL-6, NF-κB, macrophage markers	Anti-inflammatory comparator	Match dose to achievable nutritional exposure
Microbiome-focused studies	DIO + defined diet	Gut-host interaction relevance	Highly diet-sensitive	Microbiota profiling, bile acids, SCFAs	Standardized diet comparator	Strict control of background diet
Mechanistic pathway studies	Transgenic/genetic mouse models	Strong mechanistic precision	Reduced translational generalizability	Molecular and functional validation	Pathway-specific controls	Include functional validation

Adapted and synthesized from outside[10–11,14,46–47] and related studies discussed in this review.

metabolism, due to their well-defined molecular defects. Chemical models are useful for studying β-cell function and glucose homeostasis, but may be less appropriate for investigating complex systemic mechanisms. Aligning model selection with the hypothesized mechanism of action enhances the interpretability of results and reduces the risk of overgeneralizing findings from inappropriate experimental systems.[14]

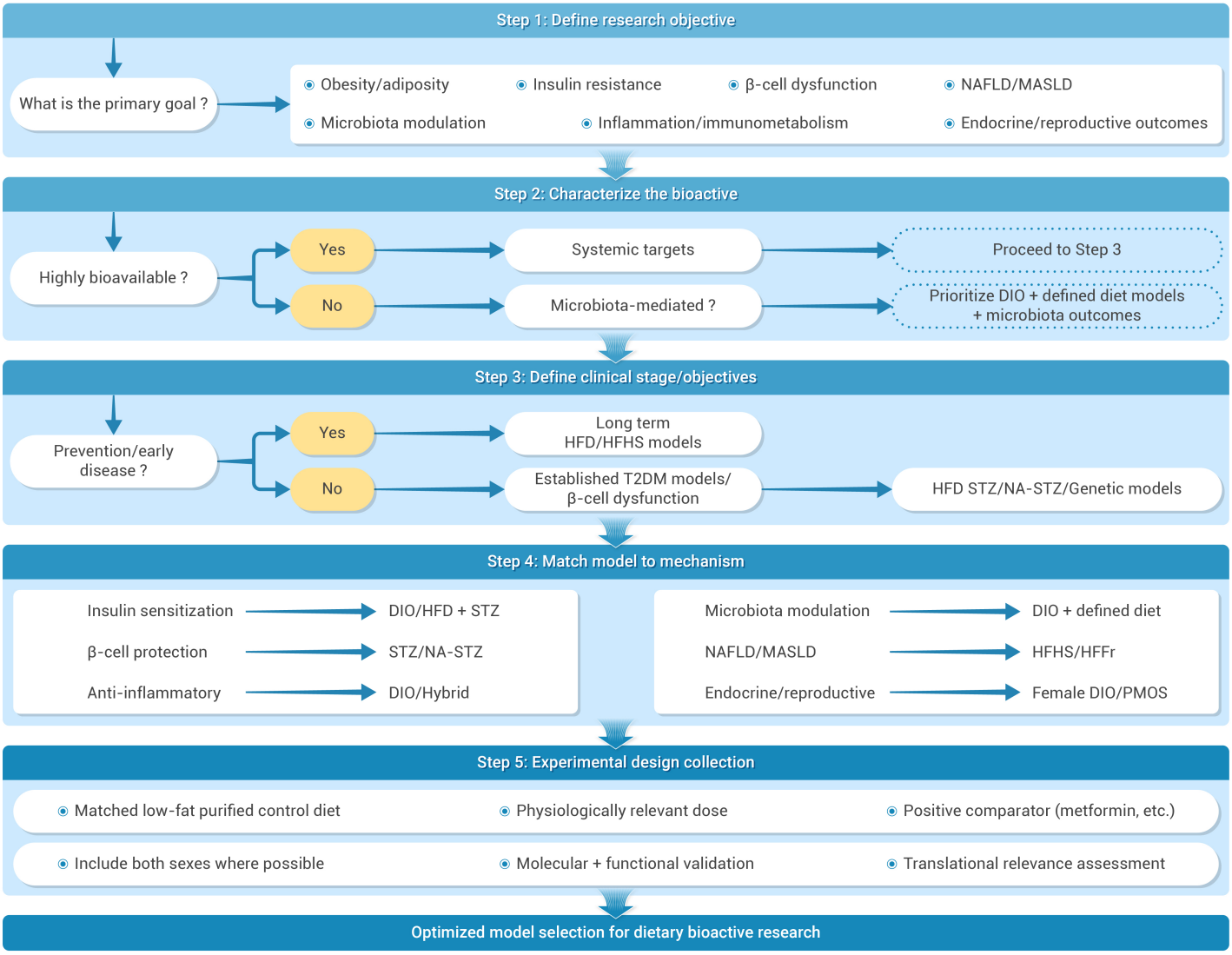
Beyond the depth of molecular analysis, effective mechanistic investigation requires alignment between the proposed mechanism of action of a dietary bioactive and the underlying pathology of the selected animal model. For example, compounds intended to improve insulin sensitivity are most appropriately evaluated in models exhibiting robust insulin resistance, whereas interventions targeting β-cell preservation may be better assessed using STZ-based or hybrid models. Similarly, microbiota-targeted bioactives require dietary models that preserve gut–host interactions, while anti-inflammatory interventions are best evaluated in models characterized by chronic metabolic inflammation. Failure to align intervention mechanisms with model pathology may reduce interpretability and translational relevance, even when extensive molecular analyses are performed. To facilitate mechanism-oriented study design, Table 3 summarizes the alignment between major dietary bioactive mechanisms of action and animal models most suitable for evaluating these pathways.

Integration of outcome measures and experimental design parameters. Appropriate model selection must be complemented by careful selection of outcome measures and experimental design. As highlighted in Section 3.5, many studies rely heavily on core metabolic outcomes such as body weight and glucose levels, while deeper mechanistic and system-level assessments are inconsistently included. The proposed framework emphasizes the importance of integrating multiple outcome layers, including phenotypic, biochemical, and molecular endpoints. Core outcomes should be supplemented with measurements of insulin sensitivity, inflammatory markers, oxidative stress, and, where relevant, gut microbiota and liver function. Importantly, molecular validation should be incorporated to support mechanistic claims. Experimental design considerations are equally critical. These include the use of appropriate control groups, inclusion of both sexes where feasible, selection of biologically relevant doses, and ensuring sufficient study duration to capture chronic disease progression. Failure to address these factors may compromise both internal validity and translational relevance.[33]

A critical but often underreported aspect of experimental design is the selection of appropriate intervention doses and the inclusion of suitable control groups. In the context of dietary bioactive compounds, considerable variability exists in dosing strategies, with many studies employing doses that exceed physiologically relevant levels. While such approaches may demonstrate efficacy, they can limit translational applicability. Therefore, future studies should prioritize dose selection based on dietary relevance, pharmacokinetic considerations, and, where possible, dose–response relationships. Equally important is the inclusion of positive control groups using established pharmacological agents, such as metformin or other standard antidiabetic drugs. The absence of such controls, as observed in a proportion of studies within this dataset, restricts the ability to contextualize the magnitude and relevance of observed effects. Incorporating both negative and positive controls, alongside appropriate vehicle groups, is essential for improving interpretability, benchmarking efficacy, and enhancing the overall rigor of experimental design.

Practical considerations and translational relevance. Beyond biological and mechanistic factors, practical constraints play a significant role in model selection. These include study duration, cost, facility availability, and feasibility of sample size. For example, diet-induced and genetic models often require longer study durations and higher costs, whereas chemical models provide rapid induction but may sacrifice physiological relevance. Balancing these constraints with scientific objectives is essential. Short-term models may be appropriate for initial screening, but longer-term and more physiologically relevant models are necessary for evaluating translational potential. Translational interpretation may also be influenced by dietary matrix composition, as differences in lipid and carbohydrate sources can alter gut microbiota, bile acid metabolism, and the biological activity of dietary bioactives. In addition, the limited use of clinically relevant comparator interventions in many studies makes it difficult to benchmark the translational significance of reported effects. Recent advances in metabolic animal research have also expanded toward neuro-metabolic and systems-level mechanisms involved in obesity and T2DM. For example, next-generation GLP-1 receptor agonists have been shown to modulate brain reward circuitry and hedonic feeding pathways in mouse models, highlighting increasing integration between metabolic and neurobehavioral research.[48] Other emerging approaches include microbiota-targeted models, humanized models, and multi-organ metabolic phenotyping strategies designed to improve translational relevance and better capture the complexity of human metabolic disease.[10,42] Such developments further emphasize the importance of model selection strategies that integrate metabolic, behavioral, endocrine, and tissue-specific outcomes.

In parallel, alternative experimental platforms such as organoids, multicel-



-Low relevance -Moderate relevance -High relevance

Model	Obesity	Diabetes	Microbiota relevance	Mechanistic depth	Duration requirement	Translational relevance	Bioactive suitability
DIO (HFD)	●●●	●●	●●●	●●	Long	●●●	●●●
HFHS (High fat + sucrose)	●●●	●●	●●●	●●	Long	●●●	●●●
HFFr (High fat + fructose)	●●●	●●	●●●	●●	Long	●●●	●●●
STZ	●	●●●	●	●●	Short	●	●●
Nicotinamide-STZ (NA-STZ)	●	●●●	●	●●	Medium	●●	●●
HFD + STZ (Hybrid)	●●●	●●●	●●	●●	Medium	●●●	●●●
Genetic (db/db, ob/ob)	●●●	●●●	●	●●●	Long	●●	●
Zebrafish (overfeeding)	●●	●●	●●	●	Short	●●	●●
Zebrafish (glucose immersion)	●	●●	●	●	Very short	●	●
Drosophila (high sugar)	●	●●	●	●	Very short	●	●
C. elegans (glucose)	●	●	●	●	Very short	●	●

Figure 6. Framework for selection of animal models in dietary bioactive research for metabolic disorders.

lular *in vitro* systems, and advanced humanized models are increasingly being explored to complement conventional animal studies.[49-50] These approaches offer several potential advantages, including improved mechanistic control, higher-throughput screening capacity, reduced experimental variability, and partial reduction of animal use in accordance with the principles of replacement, reduction, and refinement (3Rs). Humanized and organoid-based systems may also provide greater human-specific physiological relevance for investigating tissue-specific metabolic responses, drug metabolism, gut–host interactions, and personalized therapeutic strategies. In particular, organ-on-chip and multicellular co-culture systems are increasingly being utilized to model complex inter-organ communication and microenvironmental interactions that are difficult to isolate in conventional

Table 3. Mechanism–model matching matrix for dietary bioactive research in obesity and T2DM.

Dietary Bioactive Mechanism	Key Biological Target	Recommended Animal Models	Less Suitable Models	Rationale
Insulin sensitization	AMPK, AKT, GLUT4, IRS-1 signaling	DIO, HFD + low-dose STZ	High-dose STZ alone	Requires established insulin resistance phenotype
β-cell protection or regeneration	β-cell survival, insulin secretion	STZ, NA-STZ, HFD + STZ	DIO alone	Allows direct assessment of β-cell preservation and function
Anti-inflammatory effects	TNF-α, IL-6, NF-κB, macrophage infiltration	DIO, HFD + STZ	Acute chemical models	Chronic low-grade inflammation resembles human metabolic disease
Antioxidant and mitochondrial modulation	ROS pathways, Nrf2, mitochondrial biogenesis	DIO, genetic models	Severe β-cell destruction models	Oxidative stress is prominent and measurable
Gut microbiota modulation	Microbiota composition, SCFAs, bile acid metabolism	DIO with defined diets	STZ-only models	Preserves diet–microbiota interactions and host metabolic responses
Lipid metabolism and NAFLD/MASLD	Hepatic lipid accumulation, lipogenesis, β-oxidation	HFHS, HFFr, DIO	STZ-only models	Better representation of hepatic disease progression
Appetite regulation and energy balance	Leptin, ghrelin, hypothalamic signaling	DIO, ob/ob, db/db	STZ-only models	Allows evaluation of feeding behavior and adiposity
Endocrine and reproductive regulation	HPG axis, sex hormones, fertility	Female DIO models, PMOS models	Male-only models	Captures sex-specific endocrine and metabolic interactions
Immunometabolic regulation	Innate immunity, adipose inflammation	DIO, hybrid models	Simple acute hyperglycemia models	Better reflects chronic metabolic inflammation
Multi-target nutritional interventions	Combined metabolic pathways	DIO, HFD + STZ	Highly specialized genetic models	More closely reflects complexity of human dietary exposure

animal models. However, despite these advances, important limitations remain. Most alternative platforms cannot yet fully reproduce the systemic interactions involved in obesity and T2DM, including neuroendocrine regulation, immune responses, behavioral influences, long-term disease progression, and whole-body metabolic adaptation. Consequently, these emerging systems are best considered complementary translational tools that may enhance mechanistic investigation and improve translational precision, rather than complete replacements for *in vivo* metabolic models.[10] Additionally, the widespread use of male animals, as observed in this review, highlights the need to consider sex as a biological variable in future studies.

Proposed framework and its implications. The decision-making framework presented in Figure 6 integrates these considerations into a structured workflow, guiding researchers from defining research objectives to selecting appropriate models, outcomes, and experimental design parameters. By incorporating both biological relevance and practical constraints, this framework aims to improve consistency, reproducibility, and translational value in dietary bioactive research. Importantly, this framework does not prescribe a single "optimal" model but instead emphasizes context-dependent decision-making. Given the multifactorial nature of obesity and T2DM, the use of complementary models and multi-layered outcome assessment may be necessary to generate robust and translatable findings. Future studies would also benefit from greater adherence to standardized reporting frameworks such as ARRIVE 2.0 guidelines, particularly regarding randomization, blinding, sample size justification, and experimental transparency. Improved reporting consistency would strengthen reproducibility, facilitate cross-study comparison, and enhance the translational reliability of preclinical dietary bioactive research.[33]

Limitations of the current evidence base and this review

Despite the growing body of research investigating dietary bioactive compounds in animal models of metabolic disease, several important limitations should be acknowledged. A strong reliance on DIO models was observed across the included studies. Although these models offer relatively high face validity for obesity-associated metabolic dysfunction, they may not fully capture the heterogeneity and progression of human obesity and T2DM. Similarly, the predominant use of male animals limits the broader applicability of findings, given well-established sex differences in metabolism, hormonal inflammation, and endocrine regulation.

Considerable heterogeneity was also identified in experimental design, including differences in diet composition, intervention duration, dosing strategies, and outcome selection. This heterogeneity complicates cross-study comparison and limits reproducibility and translational interpretation. Moreover, the term "dietary bioactives" encompasses a wide range of interventions, including whole-food interventions, purified compounds, concentrated extracts, and high-dose gavage administration. Consequently, not all included studies reflected physiologically achievable dietary exposures, and some interventions may more closely resemble pharmacological rather than nutritional exposure models. This distinction should be considered when interpreting translational relevance to human dietary practice. In addition, differences in dietary matrix composition, including lipid and carbohydrate sources, may alter gut microbiota composition, bile acid metabolism, nutrient absorption, and metabolic signaling, thereby potentially modifying the efficacy and biological activity of dietary bioactives. Variability in background diet composition across studies may therefore contribute to differences in reported outcomes and translational interpretation. Furthermore, relatively few studies incorporated clinically relevant comparator interventions, such as established pharmacological therapies or standardized dietary interventions, making it difficult to benchmark the magnitude and clinical relevance of reported bioactive effects.

Third, although many studies reported positive metabolic outcomes, the depth of mechanistic investigation was often limited. Mechanistic conclusions were frequently based on limited gene or protein expression markers without functional validation, reducing confidence in causal pathway interpretation. Species and strain selection also represented important but inconsistently addressed sources of variability, as genetic background can substantially influence susceptibility to metabolic dysfunction and responsiveness to interventions.

Several limitations of the present review should also be considered. Although multiple databases were systematically searched, the inclusion of only studies meeting predefined criteria may have excluded relevant work. The review was limited to recent publications, which may overlook earlier foundational studies. Furthermore, due to the heterogeneity of study designs, interventions, and outcome measures, a quantitative meta-analysis was not feasible, and findings are therefore based on qualitative synthesis. Finally, the analysis relied on reported data, and incomplete reporting in primary studies may have influenced the interpretation of methodological quality and outcomes. Taken together, these limitations highlight the need for improved standardization, reporting transparency, and mechanistic rigor in future preclinical studies. Addressing these challenges will be essential for enhancing reproducibility and improving the translation of dietary bioactive research into clinically meaningful outcomes.

Future directions

Future research should focus on improving both the diversity and rigor of animal model-based studies in metabolic disease. Greater use of complementary models, including hybrid and non-rodent systems, may help capture the multifactorial nature of obesity and T2DM more effectively. In parallel, standardization of experimental design, particularly diet composition, dosing strategies, and study duration, will be essential to enhance reproducibility and cross-study comparability. Incorporating both sexes and adopting physiologically relevant dosing approaches should be prioritized to improve translational relevance. Additionally, future studies should move beyond descriptive outcomes and emphasize mechanistic validation through integrated molecular and functional approaches. Given the close interaction between metabolic and endocrine dysfunction, particularly in conditions such as obesity-associated reproductive dysfunction and PMOS, future preclinical studies should more comprehensively incorporate reproductive and endocrine endpoints when assessing long-term dietary bioactive interventions. Finally, aligning model selection, outcome measures, and study design with clearly defined research objectives will be critical for generating more reliable and clinically meaningful evidence.

CONCLUSION

This review provides a comprehensive evaluation of animal models used to investigate dietary bioactive compounds in obesity and T2DM. The findings highlight a strong reliance on diet-induced rodent models, with comparatively limited use of alternative or complementary systems. While these models offer practical advantages and high face validity, substantial variability in species, strain, experimental design, and outcome selection contributes to inconsistencies across studies. A key observation is that many studies prioritize basic metabolic outcomes, with relatively fewer incorporating deeper mechanistic validation or integrated, multi-system assessments. In addition, methodological limitations, including sex bias, lack of standardization, and inconsistent reporting, further restrict reproducibility and translational relevance. These challenges collectively underscore the need for a more structured and objective-driven approach to preclinical study design. To address these gaps, this review proposes a model selection framework that aligns research objectives with appropriate animal models, outcome measures, and experimental design considerations. By integrating biological relevance with practical constraints, this framework aims to improve consistency, interpretability, and translational potential in dietary bioactive research. Overall, advancing the field will require greater model diversity, improved methodological rigor, and stronger integration of mechanistic and physiological outcomes. Such efforts are essential to bridge the gap between preclinical findings and clinical application, and to support the development of dietary bioactive compounds as effective interventions for metabolic disease.

REFERENCES

1. World Health Organization. (2022). Https://www.Who.Int/News-Room/Fact Sheets/Detail/Obesity-and-Overweight.
2. Genitsaridi I., Salpea P., Salim A., et al. (2026). 11th edition of the IDF diabetes atlas: Global, regional, and national diabetes prevalence estimates for 2024 and projections for 2050. *Lancet Diabetes Endocrinol.* **14**:149–156. DOI:10.1016/S2213-8587(25)00299-2
3. Donath M.Y. and Drucker D.J. (2025). Obesity, diabetes, and inflammation: Pathophysiology and clinical implications. *Immunity* **58**:2373–2382. DOI:10.1016/j.immuni.2025.09.011
4. Aguerd O., Elhrech H., El Fessikh M., et al. (2025). Dietary bioactive compounds for type 2 diabetes: A comprehensive review of molecular interactions and mechanistic insights. *J. Funct. Foods* **126**:106705. DOI:10.1016/j.jff.2025.106705
5. Sayed S.M.U.F., Moshawih S., Goh H.P., et al. (2023). Natural products as novel anti-obesity agents: Insights into mechanisms of action and potential for therapeutic management. *Front. Pharmacol.* **14**:1182937. DOI:10.3389/fphar.2023.1182937
6. Jia H., Ren F. and Liu H. (2024). Evaluation of bioaccessibility and bioavailability of dietary bioactives and their application in food systems. *Food Biosci.* **62**:105428. DOI:10.1016/j.fbio.2024.105428
7. Athmuri D.N. and Shiekh P.A. (2023). Experimental diabetic animal models to study diabetes and diabetic complications. *MethodsX* **11**:102474. DOI:10.1016/j.mex.2023.102474
8. de Moura e Dias M., dos Reis S.A., da Conceição L.L., et al. (2021). Diet-induced obesity in animal models: Points to consider and influence on metabolic markers. *Diabetol. Metab. Syndr.* **13**:47. DOI:10.1186/s13098-021-00647-2
9. Hooijmans C.R., Rovers M.M., De Vries R.B.M., et al. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* **14**:43. DOI:10.1186/1471-2288-14-43
10. Kleinert M., Clemmensen C., Hofmann S.M., et al. (2018). Animal models of obesity and diabetes mellitus. *Nat. Rev. Endocrinol.* **14**:140–162. DOI:10.1038/nrendo.2017.161
11. Speakman J., Hambly C., Mitchell S., et al. (2008). The contribution of animal models to the study of obesity. *Lab. Anim.* **42**:413–432. DOI:10.1258/la.2007.006067
12. Bastías-Pérez M., Serra D. and Herrero L. (2020). Dietary options for rodents in the study of obesity. *Nutrients* **12**:3234. DOI:10.3390/nu12113234
13. Hariri N. and Thibault L. (2010). High-fat diet-induced obesity in animal models. *Nutr. Res. Rev.* **23**:270–299. DOI:10.1017/S0954422410000168
14. Speakman J.R. (2019). Use of high-fat diets to study rodent obesity as a model of human obesity. *Int. J. Obes.* **43**:1491–1492. DOI:10.1038/s41366-019-0363-7
15. Stott N.L. and Marino J.S. (2020). High fat rodent models of type 2 diabetes: From rodent to human. *Nutrients* **12**:3650. DOI:10.3390/nu12123650
16. Buettner R., Parhofer K.G., Woenckhaus M., et al. (2006). Defining high-fat-diet rat models: Metabolic and molecular effects of different fat types. *J. Mol. Endocrinol.* **36**:485–501. DOI:10.1677/JME.1.01909
17. Saleh N.E.H., Ibrahim M.Y., Saad A.H., et al. (2024). The impact of consuming different types of high-caloric fat diet on the metabolic status, liver, and aortic integrity in rats. *Sci. Rep.* **14**:68299. DOI:10.1038/s41598-024-68299-6
18. Ivić V., Zjalić M., Blažetić S., et al. (2023). Elderly rats fed with a high-fat high-sucrose diet developed sex-dependent metabolic syndrome regardless of long-term metformin and liraglutide treatment. *Front. Endocrinol.* **14**:1181064. DOI:10.3389/fendo.2023.1181064
19. Softic S., Gupta M.K., Wang G.X., et al. (2017). Divergent effects of glucose and fructose on hepatic lipogenesis and insulin signaling. *J. Clin. Invest.* **127**:4059–4074. DOI:10.1172/JCI94585
20. Fisher S.L., Campbell G.J., Senior A. and Bell-Anderson K. (2024). The effect of high-sugar feeding on rodent metabolic phenotype: A systematic review and meta-analysis. *NPJ Metab. Health Dis.* **2**:43. DOI:10.1038/s44324-024-00043-0
21. Preguiça I., Alves A., Nunes S., et al. (2020). Diet-induced rodent models of obesity-related metabolic disorders—A guide to a translational perspective. *Obes. Rev.* **21**:e13081. DOI:10.1111/obr.13081
22. Jin Y., Kozan D., Young E.D., et al. (2024). A high-cholesterol zebrafish diet promotes hypercholesterolemia and fasting-associated liver steatosis. *J. Lipid Res.* **65**:100637. DOI:10.1016/j.jlr.2024.100637
23. Musselman L.P., Fink J.L., Narzinski K., et al. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila* . *Dis. Model. Mech.* **4**:842–849. DOI:10.1242/dmm.007948
24. Nakamura T., Fujiwara K., Saitou M., et al. (2021). Non-human primates as a model for human development. *Stem Cell Rep.* **16**:1093–1103. DOI:10.1016/j.stemcr.2021.03.021
25. Rashmi P., Urmila A., Likhit A., et al. (2023). Rodent models for diabetes. *3 Biotech* **13**, 89. DOI:10.1007/s13205-023-03488-0.
26. Liu R., Wang J., Zhao Y., et al. (2024). Study on the mechanism of modified Gegen Qinlian decoction in regulating the intestinal flora-bile acid-TGR5 axis for the treatment of type 2 diabetes mellitus based on macro genome sequencing and targeted metabonomics integration. *Phytomedicine* **132**:155329. DOI:10.1016/j.phymed.2023.155329
27. Wang X., Zhang L., Qin L., et al. (2022). Physicochemical properties of the soluble dietary fiber from *Laminaria japonica* and its role in the regulation of type 2 diabetes mice . *Nutrients* **14**:329. DOI:10.3390/nu14020329
28. Zhang X., Wang H., Xie C., et al. (2022). Shenqi compound ameliorates type 2 diabetes mellitus by modulating the gut microbiota and metabolites. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **1194**:123189. DOI:10.1016/j.jchromb.2022.123189
29. King A.J. (2012). The use of animal models in diabetes research. *Br. J. Pharmacol.* **166**:877–894. DOI:10.1111/j.1476-5381.2012.01911.x
30. Pandey U.B. and Nichols C.D. (2011). Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery . *Pharmacol. Rev.* **63**:411–436. DOI:10.1124/pr.110.003293
31. Sharchil C., Vijay A., Ramachandran V., et al. (2022). Zebrafish: A model to study and understand the diabetic nephropathy and other microvascular complications of type 2 diabetes mellitus. *Vet. Sci.* **9**:312. DOI:10.3390/vetsci9070312
32. Martins T., Castro-Ribeiro C., Lemos S., et al. (2022). Murine models of obesity. *Obesities* **2**:127–147. DOI:10.3390/obesities2020012
33. du Sert N.P., Hurst V., Ahluwalia A., et al. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* **18**:e3000410. DOI:10.1371/journal.pbio.3000410.
34. Maric I., Krieger J.P., van der Velden P., et al. (2022). Sex and species differences in the development of diet-induced obesity and metabolic disturbances in rodents. *Front. Nutr.* **9**:828522. DOI:10.3389/fnut.2022.828522
35. Mauvais-Jarvis F., Manson J.A.E., Stevenson J.C., et al. (2017). Menopausal hormone therapy and type 2 diabetes prevention: Evidence, mechanisms, and clinical implications. *Endocr. Rev.* **38**:173–188. DOI:10.1210/er.2016-1146
36. Escobar-Morreale H.F. (2018). Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. *Nat. Rev. Endocrinol.* **14**:270–284. DOI:10.1038/nrendo.2018.24

37. Williamson G. (2017). The role of polyphenols in modern nutrition. *Nutr. Bull.* **42**:226–235. DOI:10.1111/nbu.12278
38. Scalbert A., Johnson I.T. and Saltmarsh M. (2005). Polyphenols: Antioxidants and beyond. *Am. J. Clin. Nutr.* **81**:215S–217S. DOI:10.1093/ajcn/81.1.215S
39. Aranaz P., Clavel-Millan M., Gil-Cardoso K., et al. (2025). Preclinical research in obesity-associated metabolic diseases using *in vitro*, multicellular, and non-mammalian models . *J. Physiol. Biochem.* **81**:1225–1255. DOI:10.1007/s13105-025-01130-6
40. Mauvais-Jarvis F. (2015). Sex differences in metabolic homeostasis, diabetes, and obesity. *Biol. Sex Differ.* **6**:14. DOI:10.1186/s13293-015-0033-y
41. Hotamisligil G.S. (2017). Foundations of immunometabolism and implications for metabolic health and disease. *Immunity* **47**:406–420. DOI:10.1016/j.immuni.2017.08.009
42. Fan Y., and Pedersen O. (2021). Gut microbiota in human metabolic health and disease. *Nat Rev Microbiol.* **19**:55–71. DOI:10.1038/s41579-020-0433-9
43. Lutz T.A. (2023). Mammalian models of diabetes mellitus, with a focus on type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **19**:350–360. DOI:10.1038/s41574-023-00818-3
44. Pellizzon M.A. and Ricci M.R. (2018). Effects of rodent diet choice and fiber type on data interpretation of gut microbiome and metabolic disease research. *Curr. Protoc. Toxicol.* **77**:e55. DOI:10.1002/cptx.55
45. Bart van der Worp H., Howells D.W., Sena E.S., et al. (2010). Can animal models of disease reliably inform human studies. *PLoS Med.* **7**:e1000245. DOI:10.1371/journal.pmed.1000245
46. Kottaisamy C.P.D., Raj D.S., Prasanth Kumar V., et al. (2021). Experimental animal models for diabetes and its related complications—a review. *Lab. Anim. Res.* **37**:23. DOI:10.1186/s42826-021-00101-4
47. Suriano F., Vieira-Silva S., Falony G., et al. (2021). Novel insights into the genetically obese (ob/ob) and diabetic (db/db) mice: Two sides of the same coin. *Microbiome* **9**:147. DOI:10.1186/s40168-021-01097-8
48. Godschall E.N., Gungul T.B., Sajonia I.R., et al. (2026) A brain reward circuit inhibited by next-generation weight loss drugs. *Nature* DOI: 10.1038/s41586-026-10444-4.
49. Fatehullah A., Tan S.H. and Barker N. (2016). Organoids as an in vitro model of human development and disease. *Nat. Cell Biol.* **18**:246–254. DOI:10.1038/ncb3312
50. Song X., Chen X., Chen Q., et al. (2026). Organoids in drug development: From predictive models to regulatory integration. *Drug Discov. Today* **31**:104608. DOI:10.1016/j.drudis.2026.104608

FUNDING AND ACKNOWLEDGMENTS

This work was supported by Guangdong and Hong Kong Universities "1+1+1" Joint Research Collaboration Scheme, project No. 2025A0505000013, UICR0800014-24A, and Guangdong Higher Education Upgrading Plan (2021-2025) with No. of UICR0400015-24B & UICR0400016-24B at Beijing Normal-Hong Kong Baptist University, Zhuhai, PR China. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

NR: Design study, Investigation, Data analysis, Software, Writing- Original draft preparation. JL: Investigation, Data analysis, Software, Writing- Original draft preparation. BX: Conceptualization. All authors contributed to and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

No new data were created or analyzed in this study. All data supporting the findings of this review are available within the article and its Supplementary Materials.