Research Square

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Quercetin Intake and CKM Syndrome Risk: Dose-Response and Mechanistic Insights

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Abstract

Background/Objectives: Cardiovascular-kidney-metabolic (CKM) syndrome is a multisystem condition associated with oxidative stress. Flavonoids were studied individually, but not comprehensively in the CKM framework. This study aimed to explore associations between flavonoid intake and CKM risk and to investigate the underlying mechanisms.

Methods: This study assessed the associations between dietary flavonoid intake and CKM risk using dose–response modeling based on National Health and Nutrition Examination Survey (NHANES) dietary data. Network pharmacology and molecular docking were employed to predict quercetin targets and validate binding affinity with the hub oxidative stress genes.

Results: The advanced CKM group exhibited lower intakes of flavones, flavonols, kaempferol and quercetin compared to the nonadvanced group ($P < 0.05$). Higher total flavonoids, flavonols and quercetin intake was associated with lower odds of advanced CKM stage. Nonlinear associations were observed, with intake levels corresponding to the lowest observed risk estimated at 449 mg/d for total flavonoids, 31 mg/d for flavonols, and 19 mg/d for quercetin. Eleven quercetin targets were identified; MPO and NOX4 were identified as oxidative stress candidates. Docking predicted binding affinities of − 9.4 and − 8.4 kcal/mol.

Conclusions: Dietary quercetin intake is nonlinearly associated with lower odds of advanced CKM stage. These findings may suggest a potential role of quercetin in oxidative stress pathways via MPO and NOX4.

1. Introduction

1.1. CKM Syndrome Overview

CKM syndrome is defined by the American Heart Association(AHA), encompasses the pathological of metabolic risk factors, chronic kidney disease (CKD), and cardiovascular disease (CVD)[1]. This framework shifts the clinical paradigm from treating individual organ systems in isolation to recognizing a unified syndrome where metabolic dysfunction, such as obesity and diabetes, initiates a cascade leading to kidney injury and/or cardiovascular complications. Affecting nearly 90% of US adults and 15% met criteria for advanced stages, with progressive stages conferring higher mortality risk, frequent hospitalizations, representing a significant public health burden[2, 3].

Central to the pathogenesis of CKM syndrome is the concept of “cardiorenal metabolic risk,” driven by shared mechanisms including chronic inflammation, endothelial dysfunction, and, critically, oxidative stress[4]. Despite growing recognition of CKM as a unified entity, current preventive strategies often lack a holistic approach, underscoring the urgent need to identify modifiable lifestyle factors—such as dietary components—that can simultaneously target multiple nodes of this pathological network.

1.2. Flavonoids and Metabolic Health

Flavonoids are a diverse class of polyphenolic compounds abundant in plant-based foods, including fruits, vegetables, tea, and wine. Structurally, they are categorized into several subclasses, such as flavonols, flavones, flavanones, flavan-3-ols, anthocyanins, and isoflavones, each possessing distinct biological activities[5]. Among these, quercetin—the most abundant dietary flavonol found in fruits and vegetables—have demonstrated antioxidant, anti-inflammatory, and cardiorenal protective effects via the regulation of oxidative stress and modulation of multiple signaling pathways[6, 7].

Although previous studies have linked flavonoids intake to individual CKM components such as hypertension or diabetic kidney disease[8, 9], the integrated relationship between different flavonoid subclasses and CKM syndrome remains poorly characterized, particularly regarding dose-response relationships and optimal intake levels. While quercetin's antioxidative properties are well-established, the specific molecular targets underlying its effects on CKM progression have rarely been studied

1.3. Study Rationale and Objectives

Some study addressed several aspects that remain underinvestigated in the current literature. First, we used data from NHANES and Nutrient Database for Dietary Studies (FNDDS) to investigate the association between flavonoid intake and CKM syndrome risk as an integrated entity. Second, we characterized dose-response relationships using restricted cubic spline (RCS) models to identify intake levels corresponding to the lowest observed risk for total flavonoids, flavonols, and quercetin. Third, we employed network pharmacology and molecular docking as exploratory tools to generate hypotheses regarding potential mechanisms. Collectively, this study provides epidemiological evidence and in silico predictions that may inform flavonoid-based dietary strategies for CKM prevention.

2. Materials and Methods

2.1. Study Design and Population

Figure 1. Flow Diagram of the Screening and Enrollment of Study Participants.

This population-based study used data from NHANES, a complex, multistage probability sample assessing the health status. We pooled 30,210 participants from three NHANES cycles (2007–2008, 2009–2010, 2017–2018) and excluded pregnant individuals (n = 180), those aged < 20 or > 79 years (n = 16,251), and those with missing variables. A preliminary sample of 5,492 was identified; three participants were excluded for incomplete CKM staging, resulting in a final list of 5,489(Fig. 1).

2.2. Dietary Flavonoids Intake Assessment

Dietary intake data were collected using two 24-hour dietary recalls using the USDA Automated Multiple-Pass Method. The first recall was conducted in person at the Mobile Examination Center, and the second was conducted by telephone 3 to 10 days later. Only recalls determined as "complete and reliable" by NCHS staff were included. For the present analysis, the first 24-hour dietary recall was used as the primary exposure measure, following NHANES analytical guidelines which recommend the

in-person recall to minimize missing responses and ensure consistency with the physical examination data. Nutrient and food component values, including flavonoids, were derived from the FNDDS. Dietary intakes of total flavonoids and their subclasses were estimated from the first recall. While a single 24-hour recall is subject to within-person variation and may not reflect long-term habitual intake, this approach is widely used in most nutritional epidemiological studies and provides a valid estimate of population-level dietary exposure.

2.3. Definition and Staging of CKM Syndrome

CKM syndrome was defined and staged according to the 2023 AHA framework. Consistent with prior studies, participants were categorized into two groups for analysis to enhance statistical power and clinical interpretability: nonadvanced (Stages 0–2) and advanced (Stages 3–4)[10, 11]. Detailed staging criteria were as follows:

Stage 0: BMI 18.5–24.9 kg/m² and waist circumference < 88 cm (women) or < 102 cm (men), with no other CKM health risk factors.

Stage 1: Presence of excess weight (BMI ≥ 25 kg/m²), central obesity, or prediabetes (fasting glucose 100–124 mg/dL, HbA1c 5.7%–6.4%, or use of glucose-lowering medication).

Stage 2: Presence of metabolic risk factors (elevated triglycerides ≥ 135 mg/dL), hypertension, diabetes, metabolic syndrome, or moderate-to-high-risk CKD per Kidney Disease: Improving Global Outcomes (KDIGO) guidelines.

Stage 3: Very high-risk CKD was defined as eGFR < 30 mL/min/1.73 m² or urinary albumin-to-creatinine ratio (UACR) ≥ 300 mg/g or a high predicted 10-year CVD risk (≥ 20%).

Stage 4: Diagnosed clinical CVD (self-reported coronary heart disease, angina, myocardial infarction, heart failure, or stroke).

The 10-year CVD risk was estimated using the AHA PREVENT equations[12].

CKD staging was based on eGFR, calculated using the race-free Chronic Kidney Disease Epidemiology Collaboration 2021 creatinine equation, and the UACR[13].

2.4. Assessment of Covariates

Covariates were selected based on prior literature and biological plausibility. Multivariable logistic regression models were constructed with progressive adjustment to evaluate the robustness of the associations. Model 1 adjusted for basic demographics: age, gender, and ethnicity. Model 2 additionally accounted for socioeconomic and lifestyle factors: education level, ratio of family income to poverty (RIP), Body Mass Index (BMI), smoking status, alcohol consumption, and total energy intake. All models accounted for the complex survey design of NHANES by incorporating sampling weights, primary sampling units, and strata.

2.5. Statistical Analysis

All statistical analyses were performed using R software (version 4.1.3). Sampling weights, primary sampling units, and variance strata were incorporated into all analyses using the Taylor series linearization method. This approach ensured that all estimates were nationally representative and that variance estimation properly accounted for clustering and stratification.

The primary analysis employed weighted logistic regression to evaluate the association between quartiles of flavonoid intake and the odds of being in the Advanced CKM group. RCS models were used to visualize and test for nonlinear dose-response relationships. P-value of < 0.05(two-sided) was considered significant.

2.6. Network Pharmacology

2.6.1. Quercetin Targets Prediction

The three-dimensional structure of quercetin was obtained from the PubChem database. Potential quercetin targets were predicted using the SwissTargetPrediction and PharmMapper databases, with results merged and deduplicated.

2.6.2. CKM-related gene extraction

CKM syndrome-related targets were identified by searching the GeneCards and NCBI Gene databases using the keyword “cardiovascular AND kidney AND metabolic.” Genes retrieved from both databases were combined and deduplicated to generate a comprehensive set of CKM-associated targets.

2.6.3. Overlap discovery

The Venn diagram tool was used to intersect quercetin targets with CKM-related targets. These overlapping genes were considered as potential targets of quercetin in the context of CKM syndrome.

2.6.4. GO enrichment

Gene Ontology (GO) enrichment analysis of the intersecting targets was conducted using the DAVID database, focusing on Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Terms with an adjusted P < 0.05 were considered significant.

2.6.5. PPI construction

Protein-protein interaction (PPI) networks were constructed using the STRING database, with the species limited to Homo sapiens.

2.7. Molecular Docking Validation

The protein structures of key hub genes—MPO (PDB ID: 1CXP) and NOX4(AlphaFold ID: Q9NPH5)—were retrieved. The quercetin structure was prepared and converted to PDB format. Molecular docking was

performed using the CB-Dock2 server, which predicts binding pockets and performs docking with AutoVina. Binding affinity was evaluated using the Vina score (kcal/mol), with lower scores indicating more favorable binding. The top-ranked docking poses were visualized to identify potential interacting residues.

3. Results

3.1. Baseline Characteristics of Study Participants

Table 1 presents the baseline characteristics of the study participants stratified by CKM stage (nonadvanced vs. advanced). In unadjusted comparisons, individuals in the advanced group were significantly older, had a higher proportion of males, lower RIP, higher BMI, greater prevalence of smoking, and lower energy intake compared with the nonadvanced group. Distributions of ethnicity and education level also differed significantly between the two groups.

In terms of dietary flavonoid intake, the advanced group had significantly lower intakes of flavones, flavonols, kaempferol, and quercetin than the nonadvanced group ($P < 0.05$) in unadjusted analyses. No significant differences were observed for total flavonoid intake or other flavonoid subclasses. These descriptive differences should be interpreted with caution, as they do not account for potential confounding factors; the multivariable-adjusted associations are presented in subsequent analyses.

Table 1. Characteristics of NHANES participants

	Overall(n=5489)	Nonadvanced(n=4817)	Advanced(n=672)	p
Age (mean (SD))	48.64 (16.29)	46.04 (15.24)	67.26 (10.41)	<0.001
Gender = male (%)	2789 (50.8)	2379 (49.4)	410 (61.0)	<0.001
Ethnicity (%)				<0.001
black	999 (18.2)	854 (17.7)	145 (21.6)	
mexican	1019 (18.6)	940 (19.5)	79 (11.8)	
other	1169 (21.3)	1061 (22.0)	108 (16.1)	
white	2302 (41.9)	1962 (40.7)	340 (50.6)	
Education (%)				0.008
Below high school	2667 (48.6)	2305 (47.9)	362 (53.9)	
High school	1251 (22.8)	1099 (22.8)	152 (22.6)	
Above high school	1566 (28.5)	1408 (29.2)	158 (23.5)	
RIP (mean (SD))	2.56 (1.63)	2.60 (1.64)	2.29 (1.54)	<0.001
BMI (mean (SD))	28.20 (4.91)	28.04 (4.91)	29.37 (4.77)	<0.001
smoke = yes (%)	2470 (45.0)	2078 (43.1)	392 (58.3)	<0.001
alcohol (%)				<0.001
mild	3064 (55.8)	2575 (53.5)	489 (72.8)	
moderate	1205 (22.0)	1139 (23.6)	66 (9.8)	
heavy	1220 (22.2)	1103 (22.9)	117 (17.4)	
Energy intake (mean (SD))	2108.75 (982.03)	2147.73 (994.38)	1825.52 (834.81)	<0.001
total flavonoids (mean (SD))	205.01 (423.35)	206.41 (420.16)	194.81 (446.07)	0.515
isoflavones (mean (SD))	1.75 (11.63)	1.75 (10.81)	1.79 (16.45)	0.934
anthocyanidins (mean (SD))	11.65 (34.39)	11.72 (33.25)	11.10 (41.82)	0.667
flavan_3_ols (mean (SD))	159.17 (403.73)	160.26 (400.96)	151.23 (423.59)	0.595
flavanones (mean (SD))	13.44 (33.08)	13.40 (32.79)	13.75 (35.14)	0.799
flavones (mean (SD))	0.88 (1.38)	0.90 (1.39)	0.76 (1.26)	0.018
flavonols (mean (SD))	18.12 (19.59)	18.39 (19.53)	16.18 (19.94)	0.007
isorhamnetin (mean (SD))	0.90 (1.64)	0.90 (1.61)	0.84 (1.83)	0.389
kaempferol (mean (SD))	4.60 (6.59)	4.70 (6.61)	3.91 (6.45)	0.004
myricetin (mean (SD))	1.46 (2.60)	1.46 (2.59)	1.40 (2.66)	0.55
quercetin (mean (SD))	11.16 (11.90)	11.32 (11.88)	10.03 (12.00)	0.01

3.2. Association between favonoids intake and CKM syndrome

The forest plot(Figure 2)summarizes the multivariable-adjusted associations between intakes of various flavonoid subclasses and the odds of advanced CKM stage, estimated using weighted logistic regression models. Data are presented as odds ratios (ORs) with 95% confidence intervals (CIs).

Higher total flavonoid intake was associated with lower odds of advanced CKM stage (highest vs. lowest quartile: OR = 0.72; 95% CI: 0.53–0.978). Among flavonoid subclasses, flavonols demonstrated an inverse association at higher intake levels. Notably, quercetin—the predominant flavonol—showed a dose–response pattern across quartiles, with the highest quartile exhibiting a statistically significant inverse association compared with the lowest quartile (OR = 0.61; 95% CI: 0.45–0.83). The magnitude and direction of as-sociations varied across flavonoid subclasses, suggesting potential heterogeneity in their biological effects on CKM risk.

Figure 2. Forest plot of flavonoid intake–CKM associations from binomial logistic regression. Model 1 adjusted for basic demographics, model 2 additionally accounted for socioeconomic and lifestyle factors. Data are presented as ORs with 95% CIs. n/N, number of participants with CKM stage / total

participants per quartile. Q1–Q4, quartiles of intake. Blue lines: non-significant associations; red lines: significant protective associations (OR < 1, P < 0.05).

3.3. Dose–Response Relationship between Flavonoids Intake and CKM syndrome

Figure 3 presents the associations between total flavonoids, flavonols, and quercetin intake and the odds of advanced CKM stage, modeled using RCS regression. The RCS models demonstrated significant nonlinear dose–response relationships for all three exposures (P for nonlinearity < 0.05 for each).

Total flavonoid intake showed a nonlinear association with CKM odds, with the estimated odds gradually decreasing and reaching the lowest point at approximately 449 mg/day. Similarly, the lowest observed odds for flavonols and quercetin were at approximately 31 mg/day and 19 mg/day, respectively. Beyond these intake levels, the estimated odds tended to increase, consistent with a U-shaped pattern.

Figure 3. Dose–response associations of total flavonoids (a), flavonols (b), and quercetin (c) intake with risk of CKM syndrome, estimated from multivariable-adjusted RCS models. Solid lines denote adjusted ORs, and shaded areas represent corresponding 95% CIs. Vertical dashed lines indicate the optimal intake levels. A U-shaped association was observed across all three panels, suggesting that excessive intake may attenuate or reverse the beneficial effects. Data were adjusted to the following reference levels: age = 49 years, male sex, White ethnicity, below high school education, RIP = 2.4, BMI = 27.8 kg/m², non-smoker, mild alcohol consumption, and energy intake = 1940 kcal/d.(d) Flavonoid Intake Thresholds Graphic.

3.4. Network Pharmacology Findings

3.4.1. Identification of Common Targets Between Quercetin and CKM

Target prediction and deduplication identified a total of 398 putative quercetin targets. A search of the GeneCards and NCBI Gene databases identified 14,613 CKM-related genes. Intersection analysis revealed 11 overlapping genes, which were considered as potential quercetin targets in the context of CKM syndrome for further exploratory analysis: Ribokinase (RBKS), NADPH oxidase 4 (NOX4), Glyoxalase I (GLO1), myeloperoxidase (MPO), Tyrosinase (TYR), Carboxypeptidase B2 (CPB2), Arylsulfatase A (ARSA), Arylsulfatase B (ARSB), Gigaxonin (GAN), Annexin A3 (ANXA3), and Transthyretin (TTR) (Fig. 4a). It should be noted that these 11 genes represent a subset of potential targets identified through in silico prediction; their biological relevance to CKM syndrome requires experimental validation.

3.4.2. GO Functional Annotation of Potential Targets

GO enrichment analysis indicated that these overlapping genes were significantly enriched in several biological processes (adjusted P < 0.05) (Fig. 4b). The predominant enriched terms included reactive

oxygen species (ROS) biosynthetic process, superoxide metabolic process (both related to oxidative stress), and animal organ regeneration. Notably, MPO and NOX4 were jointly involved in oxidative stress-related pathways, suggesting a potential link between quercetin targets and oxidative stress pathways.

3.4.3. PPI Network Analysis and Hub Gene Discovery

PPI network topology analysis identified five hub genes among the overlapping targets: MPO, NOX4, ARSA, ARSB, and GAN (Fig. 4c). MPO and NOX4 were identified as nodes common to both the GO enrichment and PPI analyses. Notably, the interaction between quercetin and MPO/NOX4 has been previously reported in experimental studies, and our findings are consistent with these reports, providing additional structural insights into potential binding modes.

Figure 4. Network pharmacology analysis of quercetin targets in CKM syndrome. (a) Venn diagram illustrating the intersection of putative quercetin targets and CKM-related targets, identifying 11 overlapping genes. (b) GO enrichment analysis of the intersecting targets, highlighting biological processes such as ROS biosynthetic process and superoxide metabolic process. (c)Hub genes (NOX4, MPO, ARSA, ARSB, and GAN) were identified from the PPI network.

3.5. Molecular Docking Validation

Molecular docking simulations predicted binding between quercetin and the target proteins (Fig. 5a). Quercetin showed binding to MPO (Fig. 5a), with the top-ranked pocket (C3) identified by CB-Dock2 yielding a Vina score of − 9.4 kcal/mol. Four additional pockets had calculated binding energies below − 6.5 kcal/mol (Fig. 5c), suggesting that quercetin could potentially interact with MPO at multiple sites. Similarly, docking with the AlphaFold-predicted structure of NOX4 indicated binding (Fig. 5b), with the highest-ranked pocket (C1) showing a Vina score of − 8.4 kcal/mol. Binding energies for the remaining pockets were consistently below − 7.0 kcal/mol (Fig. 5d), consistent with potential interactions between quercetin and NOX4.

Figure 5. Molecular docking simulations of quercetin with MPO and NOX4. (a) Three-dimensional binding pose of quercetin within the optimal binding pocket (C3) of MPO. (b) Three-dimensional binding pose of quercetin within the optimal binding pocket (C1) of NOX4. (c)Binding affinity scores (Vina scores) of quercetin across five predicted binding pockets of MPO. (d) Binding affinity scores of quercetin across five predicted binding pockets of NOX4.

4. Discussion

This population-based study evaluated the association between dietary flavonoid intake and CKM syndrome risk and explored potential mechanisms of quercetin through network pharmacology and molecular docking analyses. The key findings were three-fold(Fig. 6): (1) lower intakes of flavones, flavonols, and quercetin were associated with advanced CKM stage; (2) total flavonoids, flavonols, and quercetin exhibited nonlinear dose–response relationships with CKM odds, with intake levels corresponding to the lowest observed risk identified at approximately 449 mg/d, 31 mg/d, and 19 mg/d,

re-spectively—notably, individuals in the highest quercetin intake quartile had substan-tially lower odds of advanced CKM compared with the lowest quartile (OR = 0.61; 95% CI: 0.45–0.83), although the magnitude of this association should be interpreted with caution given potential residual confounding; and (3) quercetin may influence CKM progression through potential interactions with the oxidative stress-related genes MPO and NOX4, as suggested by in silico docking predictions.

Figure 6. Hypothesized mechanistic pathway linking quercetin intake to CKM syndrome. Based on the present findings, a potential pathway is proposed. After ingestion and absorption, quercetin may interact with MPO and NOX4, potentially modulating ROS generation. This could, in turn, influence pathways related to endothelial function, metabolic inflammation, and kidney stress, which are implicated in CKM pathogenesis.

4.1. Flavonoid Intake and CKM Risk: Quercetin Shows a Strong Inverse Association with Advanced CKM

In the present study, dietary intakes of flavonols and quercetin were significantly lower in the advanced CKM group compared with the nonadvanced group in unadjusted analyses. After multivariable adjustment, individuals in the highest quartile of quercetin intake had approximately 39% lower odds of advanced CKM (OR = 0.61; 95% CI: 0.45–0.83), consistent with previous reports demonstrating inverse associations between flavonoid consumption and cardiovascular disease as well as diabetes[14, 15]. These observed associations may be partly explained by the antioxidant, anti-inflammatory, and endothelium supporting properties of flavonoids that have been reported in experimental studies[5].

Although total flavonoid intake did not differ significantly between CKM stages in unadjusted analyses (Table 1), a significant inverse association emerged after multivariable adjustment, suggesting that covariates such as total energy intake and lifestyle factors may have masked or confounded the crude association. This pattern highlights the importance of accounting for dietary and lifestyle confounders when evaluating flavonoid–disease associations.

No significant differences were observed among other flavonoid subclasses, suggesting heterogeneity in the biological effects of different flavonoid types and pointing to a potentially more prominent role of flavonols and quercetin in CKM-related pathways. This specificity may be related to the chemical structure of quercetin, which has been associated with relatively higher bioavailability and radical-scavenging capacity com-pared with other subclasses in experimental systems[16], although these properties re-main to be confirmed in the context of CKM syndrome.

4.2. Intake Levels Corresponding to the Lowest CKM Risk

Restricted cubic spline analyses revealed significant nonlinear associations of total flavonoids, flavonols, and quercetin with CKM odds, with intake levels corresponding to the lowest observed risk identified at approximately 449 mg/d, 31 mg/d, and 19 mg/d, respectively. Beyond these intake levels, the estimated odds of advanced CKM tended to increase, a pattern that is consistent with U-shaped relationships

reported in other nu-tritional epidemiological studies exploring nutrient–disease associations[17], and may reflect prooxidant effects or interference with other metabolic pathways at higher doses[18]. However, given the cross-sectional design of the present study and the wide confidence intervals observed at higher intake levels, this apparent upturn should be interpreted cautiously and may be influenced by sparse data in the upper intake range rather than reflecting a true biological phenomenon.

The threshold estimates should be viewed as exploratory, given the measurement error inherent in 24-hour dietary recalls and the limited statistical precision at extreme intake levels. The apparent “optimal” points identified in the spline models are model-dependent and require validation in prospective cohort studies with more precise dietary assessment methods.

4.3. Integrated Analysis of the Potential Mechanisms of Quercetin

4.3.1. MPO and NOX4 were identified as key targets of quercetin

Through network pharmacology, we identified 11 potential target genes of quercetin. GO enrichment analysis revealed that these targets were significantly enriched in processes related to ROS biosynthesis and superoxide metabolism, suggesting that oxidative stress may play a role in the pathways linking quercetin targets to CKM-related pathophysiology[1].

PPI network analysis further identified five hub genes: MPO, NOX4, ARSA, ARSB, and GAN. Among them, MPO, released by activated neutrophils, catalyzes the formation of hypochlorous acid and has been implicated in vascular inflammation and oxidative injury[19]. NOX4, a member of the NADPH oxidase family, is highly expressed in the kidneys and vasculature; it catalyzes the production of hydrogen peroxide and has been associated with renal fibrosis and endothelial dysfunction[20].

The co-occurrence of MPO and NOX4 in oxidative stress-related pathways, together with the predicted binding of quercetin to these proteins in molecular docking, offers a potential mechanistic hypothesis: quercetin could influence CKM-related pathways through interactions with MPO and NOX4. However, these in silico findings are exploratory and require experimental validation.

4.3.2. Quercetin Targets Key Oxidative Stress Mediators: MPO and NOX4

To explore the potential molecular interaction between quercetin and MPO, molecular docking simulations were performed using the CB Dock2 platform. Quercetin showed binding to all five predicted pockets of MPO, with calculated binding energies ranging from − 6.6 to − 9.4 kcal/mol. The top ranked pocket (C3) displayed a binding energy of − 9.4 kcal/mol, suggesting a possible interaction site. This multisite binding pattern suggests that quercetin could potentially interact with MPO, possibly through competitive or allosteric mechanisms, which may influence MPO activity. Given that the active site of

MPO contains heme and is critical for hypochlorous acid production, the predicted binding of quercetin to MPO raises the hypothesis that it could affect its peroxidase function, which might in turn influence oxidative pathways implicated in vascular injury. This hypothesis is supported by experimental evidence showing that quercetin inhibits MPO dependent hypochlorous acid formation and attenuates vascular endothelial injury[21, 22].

Similarly, molecular docking predicted binding between quercetin and NOX4, with calculated binding energies ranging from − 7.1 to − 8.4 kcal/mol across all five predicted pockets. The top-ranked pocket (C1) had a binding energy of − 8.4 kcal/mol, suggesting a potential interaction site that could correspond to a functionally relevant region of NOX4, such as the NADPH-binding domain. This multi-site binding pattern suggests that quercetin could potentially interact with NOX4, possibly through competitive or allosteric mechanisms, which may influence NOX4-mediated ROS generation. The potential role of NOX4 as a molecular target of quercetin is supported by experimental studies showing that quercetin treatment significantly downregulated NOX4 expression in cardiac injury models, and its derivative isoquercetin inhibited NOX4 expression in senescent chon-drocytes, thereby promoting redox homeostasis[23–25].

The interaction between quercetin and MPO/NOX4 has been previously reported in experimental studies. Consistent with these findings, our molecular docking simulations provide complementary in silico insights into the potential binding modes and affinities between quercetin and these enzymes. However, these computational predictions require experimental validation to confirm biological relevance.

4.4. Implications for Clinical Practice and Public Health

The findings of this study offer potential directions for both clinicians and public health practitioners in the context of CKM syndrome prevention. Regarding dietary targets, we identified intake levels corresponding to the lowest observed risk—approximately 19 mg/d for quercetin—which may serve as provisional nutritional goals for high-risk populations, pending confirmation in prospective studies.

To illustrate the feasibility of achieving this target through dietary sources, we provide a example based on average quercetin content values reported in the literature[26]: half a medium raw onion (≈ 50 g, ~ 10.15 mg quercetin), one small bowl of raw spinach (≈ 100 g, ~ 3.97 mg), one medium apple with skin (≈ 100 g, ~ 3.80 mg), and a serving of cooked buckwheat groats (≈ 50 g raw weight, ~ 1.74 mg), summing to ap-proximately 19.66 mg (Table 2). However, it should be noted that the quercetin content of foods varies considerably depending on cultivar, growing conditions, processing, and preparation methods; therefore, the values in Table 2 are estimates and should not be interpreted as exact compositional data. These examples are intended to illustrate dietary patterns that could contribute to quercetin intake rather than to provide precise dietary prescriptions.

Table 2. How to Achieve 19 mg Quercetin Daily

Food Source	Typical Quercetin Content	Example Serving	Contribution
Onions (raw)	20.30 mg/100 g	½ medium onion (≈50 g)	10.15mg
Spinach (raw)	3.97mg/100g	1 small bowl (≈100 g)	3.97mg
Apples (with skin)	3.80mg/100 g	1 medium apple	3.80mg
Buckwheat groats (raw)	3.47mg/100g	1 bowl (≈50 g)	1.74mg
Total			19.66 mg

These findings are broadly consistent with the AHA CKM framework, highlighting the potential role of dietary modification as part of early-stage CKM management strategies. Clinicians may consider these provisional intake thresholds as reference points for dietary counseling, although they should be interpreted with caution given the exploratory nature of the threshold estimates and the cross-sectional study design. Furthermore, the observed inverse association between flavonoid intake and advanced CKM stages may have implications for risk stratification; individuals with lower flavonoid intake could potentially be considered for more careful metabolic and renal function monitoring, although this suggestion remains speculative and requires validation in prospective studies.

4.5. Limitations

Several limitations of this study should be acknowledged. First, the cross sectional design precludes causal inference, and reverse causation cannot be ruled out. Second, the use of 24 hour dietary recalls is subject to recall bias and within person variation and may not accurately reflect long term habitual flavonoid intake. Third, the network pharmacology and molecular docking findings are computational predictions that require experimental validation to confirm biological relevance. Fourth, the absence of oxidative stress biomarkers in NHANES prevented direct verification of the proposed mechanistic pathways at the population level. Fifth, despite adjustment for multiple lifestyle and dietary factors, residual confounding cannot be entirely excluded. Flavonoid rich foods are components of overall healthy dietary patterns, and the observed inverse associations may partly reflect the cumulative benefits of such patterns rather than the specific effect of quercetin alone. Sixth, the wide confidence intervals at higher intake levels and the potential influence of sparse data in the upper intake range warrant cautious interpretation of the nonlinear dose–response relationships, particularly the upturn at high intakes.

5. Conclusions

These findings demonstrate that higher intakes of flavonols—particularly quercetin—are associated with lower odds of advanced CKM syndrome, with intake levels around 19 mg/d corresponding to the lowest observed risk in this study. The predictions suggest that quercetin may interact with MPO and NOX4, two key oxidative stress-related mediators, although these computational findings require experimental validation.

Abbreviations

AHA	American Heart Association
ARSA	Arylsulfatase A
ARSB	Arylsulfatase B
ANXA3	Annexin A3
BMI	body mass index
BP	Biological Process
CC	Cellular Component
CIs	confidence intervals
CKD	chronic kidney disease
CKM	Cardiovascular-kidney-metabolic
CPB2	Carboxypepti-dase B2
CVD	cardiovascular disease
eGFR	estimated glomerular filtration rate
FNDDS	Food and Nutrient Database for Dietary Studies
GAN	Gigaxonin
GLO1	Glyoxalase I
GO	Gene Ontology
KDIGO	Kidney Disease: Improving Global Outcomes
MCC	Maximal Clique Centrality
MF	Molecular Function
MPO	Myeloperoxidase
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NOX4	NADPH oxidase 4
ORs	odds ratios
RCS	restricted cubic spline
RIP	ratio of family income to poverty
RK	Ribokinase
ROS	reactive oxygen species

AHA	American Heart Association
TTR	Transthyretin
TYR	Tyrosinase
UACR	urinary albumin-to-creatinine ratio

Declarations

Author Contributions statement: Conceptualization, Data curation, methodology, software, writing—original draft preparation Jiao Quan; Visualization, Jingyi Hu; writing—review and editing, Qian Hou. All authors have read and approved the final version of the manuscript.

Competing interests policy The authors declare no competing interests.

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Permission to Use Third-Party Material: All figures, tables, and text passages presented in this manuscript were created by the authors. No third-party copyrighted material was used in this submission.

Data Availability: The data used in this study are publicly available from NHANES database and FNDDS database. All NHANES data are freely accessible at https://www.cdc.gov/nchs/nhanes/, FNDDS Flavonoid data are freely accessible at https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds-flavonoid-database/. No additional original data were generated during this study. All findings are based on secondary analysis of publicly available NHANES and FNDDS data.

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Ethics statement: The NHANES protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board, and all participants provided written informed consent prior to participation. As FNDDS is a publicly accessible food composition data-base without individual human identifiers, no separate ethical review was needed beyond the ap-proved NHANES protocol and participant informed consent.

Competing Interests: The authors declare no conflicts of interest.

References

1. Ndumele CE, Rangaswami J, Chow SL, et al. Cardiovascular-Kidney-Metabolic Health: A Presidential Advisory From the American Heart Association. Circulation. 2023;148:1606–35. https://doi.org/10.1161/CIR.0000000000001184.
2. Aggarwal R, Ostrominski JW, Vaduganathan M. Prevalence of Cardiovascular-Kidney-Metabolic Syndrome Stages in US Adults, 2011–2020. JAMA. 2024;331:1858. https://doi.org/10.1001/jama.2024.6892.
3. Gunnarsson S, Vito O, Unwin RJ. Cardiovascular-kidney-metabolic syndrome: prevalence, risks, disease trajectories, and early-stage management. Am J Physiology-Cell Physiol. 2026;330:C1–8. https://doi.org/10.1152/ajpcell.00499.2025.
4. Mutruc V, Bologa C, Șorodoc V et al. (2025) Cardiovascular–Kidney–Metabolic Syndrome: A New Paradigm in Clinical Medicine or Going Back to Basics? JCM 14:2833. https://doi.org/10.3390/jcm14082833
5. Perez-Vizcaino F, Duarte J. Flavonols and cardiovascular disease. Mol Aspects Med. 2010;31:478–94. https://doi.org/10.1016/j.mam.2010.09.002.
6. Jomova K, Alomar SY, Valko R, et al. Flavonoids and their role in oxidative stress, inflammation, and human diseases. Chemico-Biol Interact. 2025;413:111489. https://doi.org/10.1016/j.cbi.2025.111489.
7. Alharbi HOA, Alshebremi M, Babiker AY, Rahmani AH. The Role of Quercetin, a Flavonoid in the Management of Pathogenesis Through Regulation of Oxidative Stress, Inflammation, and Biological Activities. Biomolecules. 2025;15:151. https://doi.org/10.3390/biom15010151.
8. Niu Y, Chen X, Xiao L, et al. The relationship between dietary flavonoid intake and hypertension: a cross-sectional study from NHANES. Front Cardiovasc Med. 2025;12:1518549. https://doi.org/10.3389/fcvm.2025.1518549.
9. Wang Q, Deng W, Yang J, et al. Association of Dietary Flavonoids Intake With All-Cause and Cardiovascular Disease Mortality in Diabetic Kidney Disease: A Cohort Study From the NHANES Database. J Diabetes Res. 2024;2024:8359294. https://doi.org/10.1155/2024/8359294.
10. Xie D, You F, Yang L, et al. The role of oxidative balance score in Cardiovascular-Kidney-Metabolic syndrome progression and mortality: insights from NHANES 1999–2020. Front Nutr. 2025;12:1597693. https://doi.org/10.3389/fnut.2025.1597693.
11. Zhu R, Wang R, He J, et al. Prevalence of Cardiovascular-Kidney-Metabolic Syndrome Stages by Social Determinants of Health. JAMA Netw Open. 2024;7:e2445309. https://doi.org/10.1001/jamanetworkopen.2024.45309.
12. Khan SS, Matsushita K, Sang Y, et al. Development and Validation of the American Heart Association's PREVENT Equations. Circulation. 2024;149:430–49. https://doi.org/10.1161/CIRCULATIONAHA.123.067626.

13. Inker LA, Eneanya ND, Coresh J, et al. New Creatinine- and Cystatin C–Based Equations to Estimate GFR without Race. N Engl J Med. 2021;385:1737–49. https://doi.org/10.1056/NEJMoa2102953.
14. Goetz ME, Judd SE, Safford MM, et al. Dietary flavonoid intake and incident coronary heart disease: the REasons for Geographic and Racial Differences in Stroke (REGARDS) study. Am J Clin Nutr. 2016;104:1236–44. https://doi.org/10.3945/ajcn.115.129452.
15. Liu Y-J, Zhan J, Liu X-L, et al. Dietary flavonoids intake and risk of type 2 diabetes: A meta-analysis of prospective cohort studies. Clin Nutr. 2014;33:59–63. https://doi.org/10.1016/j.clnu.2013.03.011.
16. Boyer J, Brown D, Liu RH. Uptake of Quercetin and Quercetin 3-Glucoside from Whole Onion and Apple Peel Extracts by Caco-2 Cell Monolayers. J Agric Food Chem. 2004;52:7172–9. https://doi.org/10.1021/jf030733d.
17. Aune D, Keum N, Giovannucci E, et al. Nut consumption and risk of cardiovascular disease, total cancer, all-cause and cause-specific mortality: a systematic review and dose-response meta-analysis of prospective studies. BMC Med. 2016;14:207. https://doi.org/10.1186/s12916-016-0730-3.
18. Bjelakovic G, Nikolova D, Gluud LL, et al. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. (ed) Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2008. p. CD007176. The Cochrane Collaboration.
19. Zhang R, Brennan M-L, Shen Z, et al. Myeloperoxidase Functions as a Major Enzymatic Catalyst for Initiation of Lipid Peroxidation at Sites of Inflammation. J Biol Chem. 2002;277:46116–22. https://doi.org/10.1074/jbc.M209124200.
20. Bedard K, Krause K-H. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. Physiol Rev. 2007;87:245–313. https://doi.org/10.1152/physrev.00044.2005.
21. Lu N, Sui Y, Tian R, Peng Y-Y. Inhibitive Effects of Quercetin on Myeloperoxidase-Dependent Hypochlorous Acid Formation and Vascular Endothelial Injury. J Agric Food Chem. 2018;66:4933–40. https://doi.org/10.1021/acs.jafc.8b01537.
22. Shiba Y, Kinoshita T, Chuman H, et al. Flavonoids as Substrates and Inhibitors of Myeloperoxidase: Molecular Actions of Aglycone and Metabolites. Chem Res Toxicol. 2008;21:1600–9. https://doi.org/10.1021/tx8000835.
23. Sanchez M, Lodi F, Vera R, et al. Quercetin and Isorhamnetin Prevent Endothelial Dysfunction, Superoxide Production, and Overexpression of p47phox Induced by Angiotensin II in Rat Aorta. J Nutr. 2007;137:910–5. https://doi.org/10.1093/jn/137.4.910.
24. Mostafa EMA, Atta R, Maher SA, et al. Quercetin and its potential therapeutic effects on aluminum phosphide-induced cardiotoxicity in rats: Role of NOX4, FOXO1, ERK1/2, and NF-κB. Tissue Cell. 2024;91:102622. https://doi.org/10.1016/j.tice.2024.102622.
25. Zhou S, Liao F, Wen H. Isoquercetin alleviates osteoarthritis via regulating the NOX4/Nrf2 redox imbalance in senescent chondrocytes. Int J Biol Macromol. 2025;306:141562. https://doi.org/10.1016/j.ijbiomac.2025.141562.

26. Sequeira I, Poppitt S. Unfolding Novel Mechanisms of Polyphenol Flavonoids for Better Glycaemic Control: Targeting Pancreatic Islet Amyloid Polypeptide (IAPP). Nutrients. 2017;9:788. https://doi.org/10.3390/nu9070788.

Figures

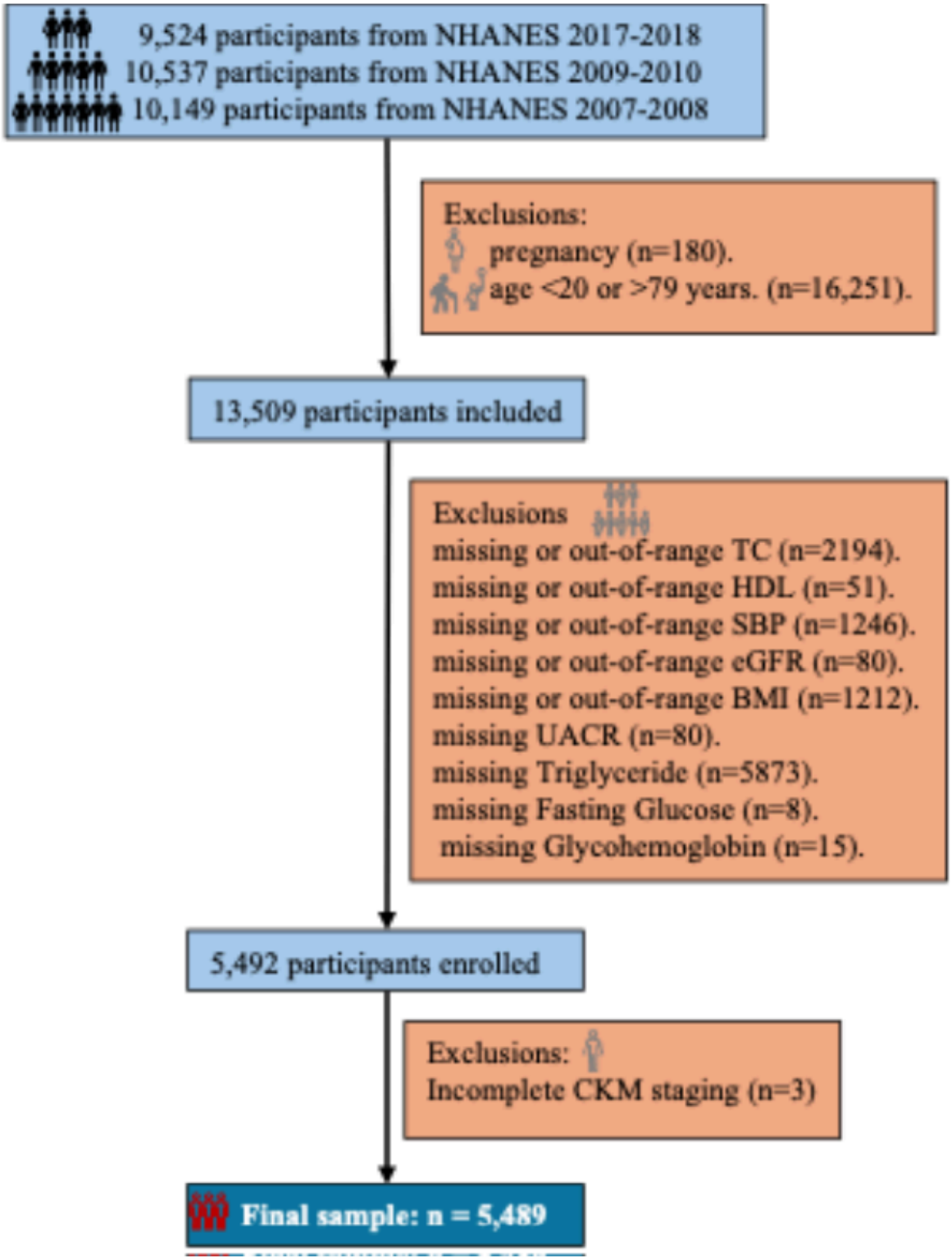


Figure 1

Flow Diagram of the Screening and Enrollment of Study Participants.

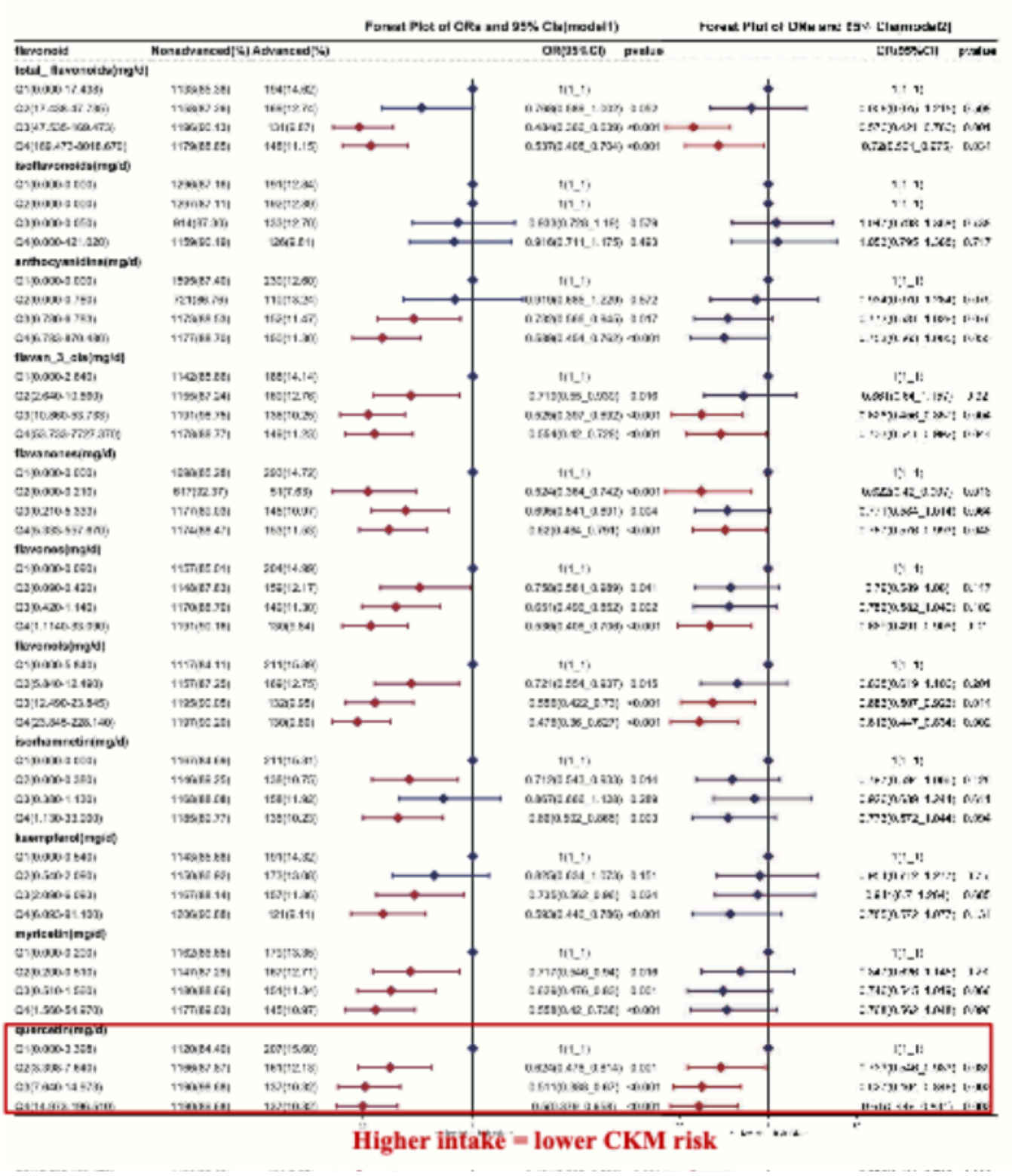


Figure 2

Forest plot of flavonoid intake–CKM associations from binomial logistic regression. Model 1 adjusted for basic demographics, model 2 additionally accounted for socioeconomic and lifestyle factors. Data are presented as ORs with 95% CIs. n/N, number of participants with CKM stage / total participants per

quartile. Q1–Q4, quartiles of intake. Blue lines: non-significant associations; red lines: significant protective associations (OR < 1, P < 0.05).

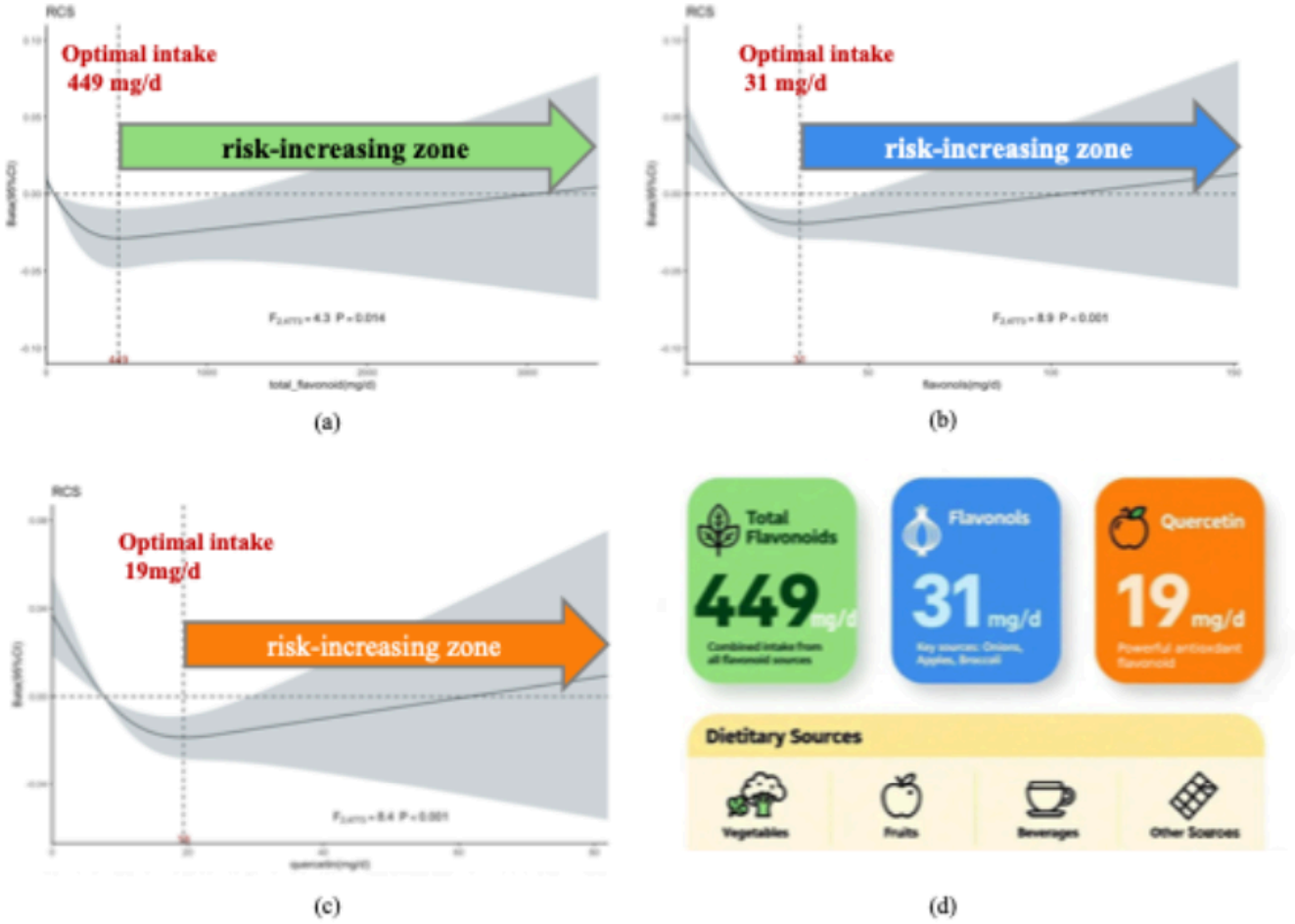


Figure 3

Dose–response associations of total flavonoids (a), flavonols (b), and quercetin (c) intake with risk of CKM syndrome, estimated from multivariable-adjusted RCS models. Solid lines denote adjusted ORs, and shaded areas represent corresponding 95% CIs. Vertical dashed lines indicate the optimal intake levels. A U-shaped association was observed across all three panels, suggesting that excessive intake may attenuate or reverse the beneficial effects. Data were adjusted to the following reference levels: age = 49 years, male sex, White ethnicity, below high school education, RIP = 2.4, BMI = 27.8 kg/m², non-smoker, mild alcohol consumption, and energy intake = 1940 kcal/d.(d) Flavonoid Intake Thresholds Graphic.

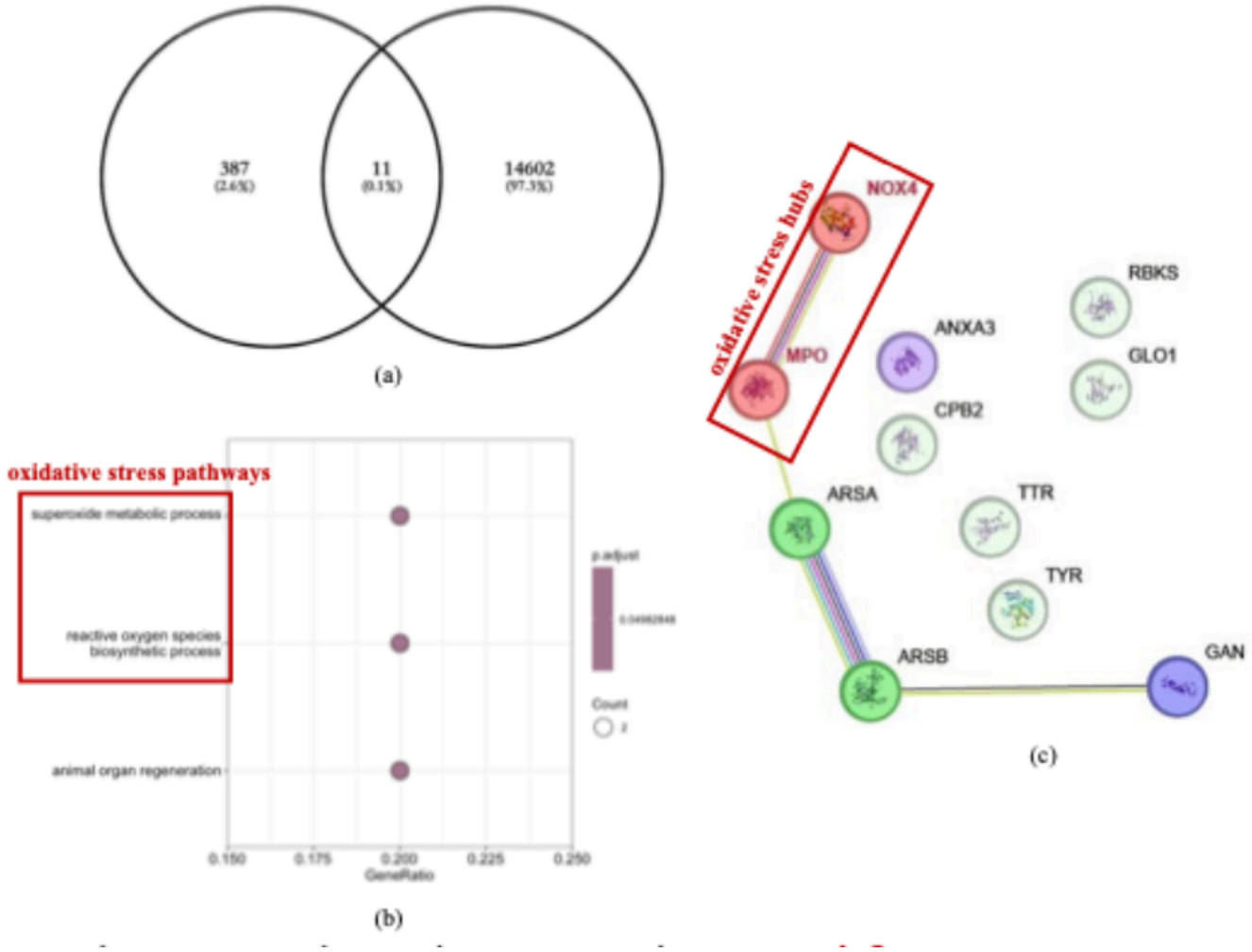
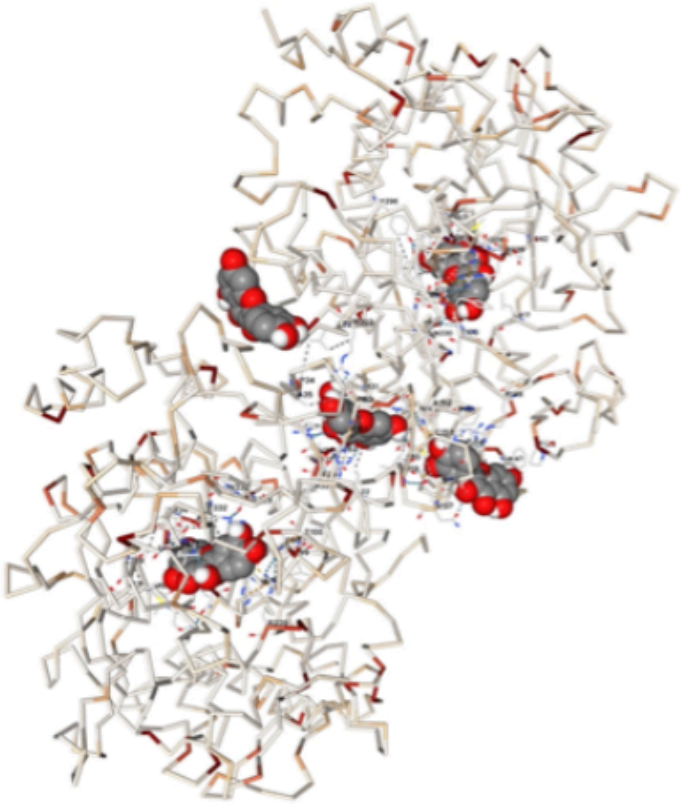
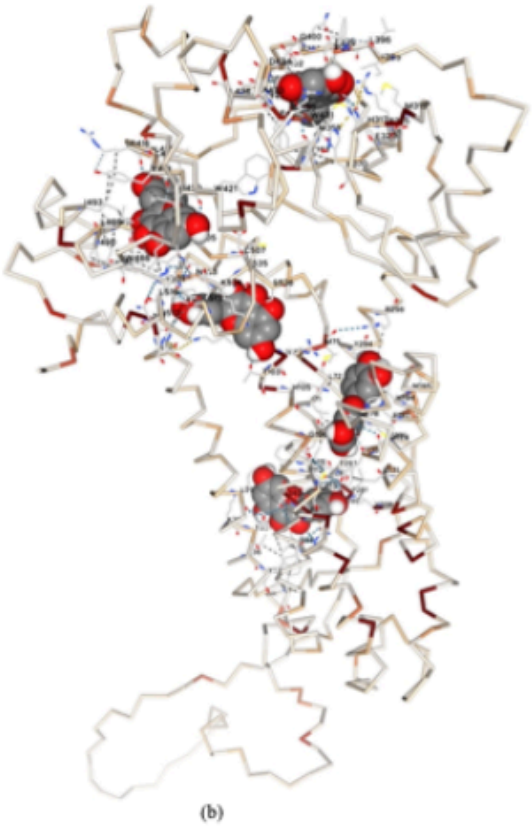


Figure 4

Network pharmacology analysis of quercetin targets in CKM syndrome. (a) Venn diagram illustrating the intersection of putative quercetin targets and CKM-related targets, identifying 11 overlapping genes. (b) GO enrichment analysis of the intersecting targets, highlighting biological processes such as ROS biosynthetic process and superoxide metabolic process. (c) Hub genes (NOX4, MPO, ARSA, ARSB, and GAN) were identified from the PPI network.



(a)



(b)

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
C3	-9.4	1447	17, -14, -4	21, 21, 21	View
C1	-9.0	2824	21, 7, 39	21, 21, 21	View
C2	-8.5	2196	29, -2, 18	28, 21, 21	View
C5	-7.7	608	7, 8, 14	21, 21, 21	View
C4	-6.6	616	26, -9, 32	21, 21, 21	View

(c)

CurPocket ID	Vina score	Cavity volume (Å³)	Center (x, y, z)	Docking size (x, y, z)	Contact residues
C1	-8.4	4539	3, 3, 4	28, 29, 21	View
C2	-8.3	3640	-18, -5, 18	21, 31, 21	View
C3	-8.1	1882	32, 2, -11	21, 35, 21	View
C4	-8.1	1720	10, 6, -25	21, 35, 21	View
C5	-7.1	1625	7, -9, -15	21, 21, 21	View

(d)

Figure 5

Molecular docking simulations of quercetin with MPO and NOX4. (a) Three-dimensional binding pose of quercetin within the optimal binding pocket (C3) of MPO. (b) Three-dimensional binding pose of quercetin within the optimal binding pocket (C1) of NOX4. c Binding affinity scores (Vina scores) of quercetin across five predicted binding pockets of MPO. (d) Binding affinity scores of quercetin across five predicted binding pockets of NOX4.

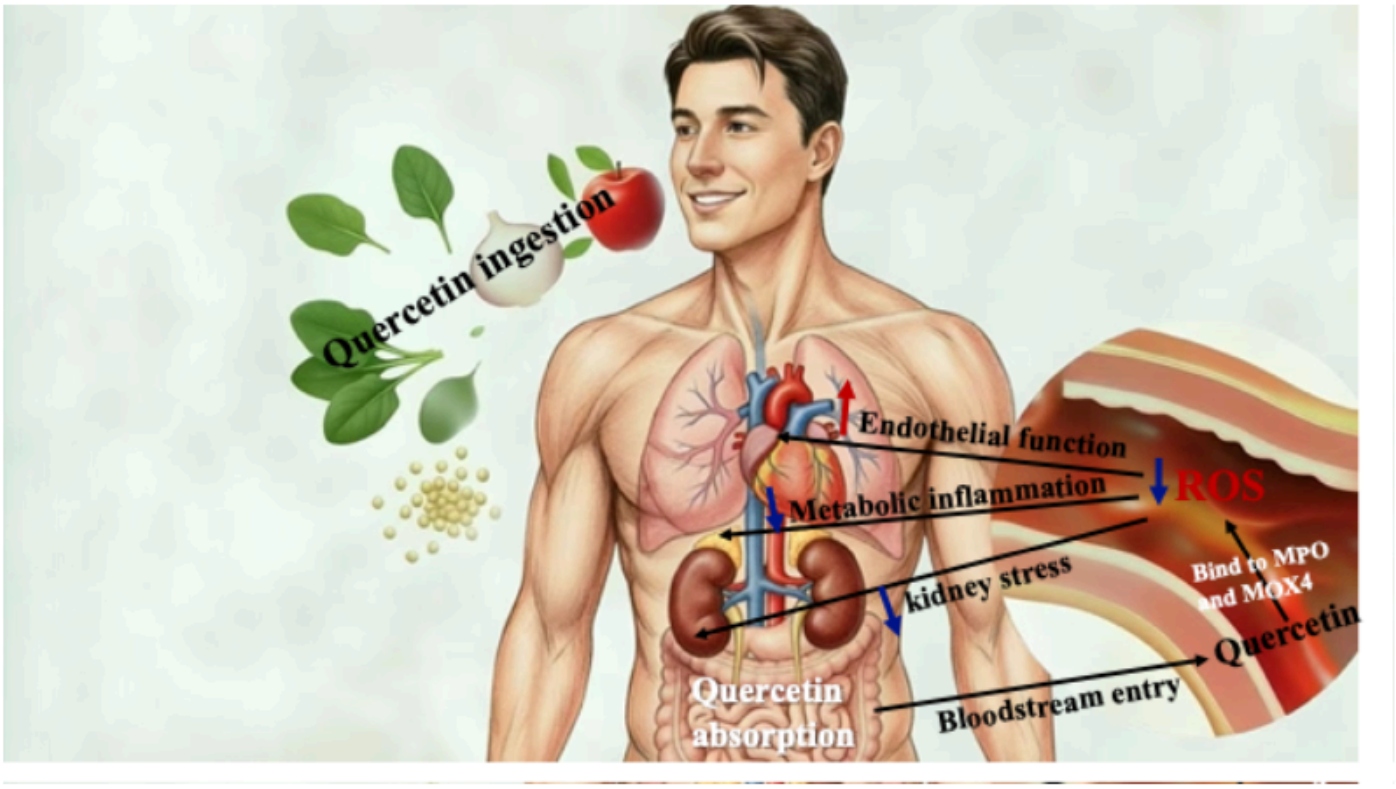


Figure 6

Hypothesized mechanistic pathway linking quercetin intake to CKM syndrome. Based on the present findings, a potential pathway is proposed. After ingestion and absorption, quercetin may interact with MPO and NOX4, potentially modulating ROS generation. This could, in turn, influence pathways related to endothelial function, metabolic inflammation, and kidney stress, which are implicated in CKM pathogenesis.