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Dietary isoflavone intake is associated with oxidative stress biomarkers in individuals with cardiometabolic risk

La ingesta de isoflavonas en la dieta se asocia con biomarcadores de estrés oxidativo en individuos con riesgo cardiometabólico

María Lucía Baraquet[a,b*], Alejandra Mariel Canalis[b], Sebastián Del Rosso[c], Nilda Raquel Perovic[b], María Gabriela Ortega[a,d], María Daniela Defagó[b,e]

[a] Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA-CONICET), Córdoba, Argentina.
[b] Centro de Investigaciones en Nutrición Humana (CenINH), Escuela de Nutrición, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina.
[c] Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET), Córdoba, Argentina.
[d] Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.
[e] Instituto de Investigaciones en Ciencias de la Salud (INICSA-CONICET), Córdoba, Argentina.

* lucia.baraquet@unc.edu.ar

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ABSTRACT

Introduction: Oxidative stress plays a central role in the pathophysiology of cardiometabolic diseases (CMDs). Dietary flavonoids, particularly isoflavones, have been asociated with redox balance; however, clinical evidence in populations at cardiometabolic risk remains limited. This study aimed to evaluate the association between flavonoid intake and oxidative stress biomarkers in individuals at cardiometabolic risk.

Methodology: A cross-sectional study was conducted in a subsample of 45 adults aged 35–70 years with cardiometabolic risk factors. Dietary intake was assessed using a validated food-frequency questionnaire, and flavonoid consumption was estimated using USDA and Phenol-Explorer databases. Oxidative stress biomarkers—including antioxidant enzymes (SOD, CAT, GPx) and markers of oxidative damage (hydroperoxides, lipid peroxides, AOPPs, and sulfhydryl groups)—were measured in blood samples. Differences across tertiles of isoflavone intake were evaluated using ANOVA. Bivariate correlations and multivariable linear regression models adjusted for sex, age, and BMI were performed.

Results: Higher isoflavone intake was associated with lower markers of oxidative damage, particularly AOPP, hydroperoxides, and lipid peroxides, along with lower SOD and GPx activity. Hydroperoxides showed a decreasing trend, while catalase exhibited a nonlinear pattern.

Conclusions: These findings support the potential relevance of dietary flavonoids in the context of cardiometabolic health. However, given the study design, causal inferences cannot be drawn.

Keywords: flavonoids, isoflavones, oxidative stress, oxidative damage, cardiometabolic risk.

RESUMEN

Introducción: El estrés oxidativo es un mecanismo central en la fisiopatología de las enfermedades cardiometabólicas. Los flavonoides, en particular las isoflavonas, han sido asociadas con marcadores de estado redox, aunque la evidencia clínica en poblaciones en riesgo es limitada. El objetivo de este estudio fue evaluar la asociación entre la ingesta de flavonoides y biomarcadores de estrés oxidativo en individuos con riesgo cardiometabólico.

Metodología: Se realizó un estudio transversal en 45 adultos de 35–70 años con al menos un componente de síndrome metabólico. La ingesta dietaria se evaluó mediante un cuestionario de frecuencia alimentaria validado, y el consumo de flavonoides se estimó utilizando bases de datos internacionales. Se determinaron biomarcadores de estrés oxidativo, incluyendo enzimas antioxidantes (superóxido dismutasa, catalasa y glutatión peroxidasa) y marcadores de daño oxidativo (hidroperóxidos, peróxidos lipídicos, productos avanzados de oxidación proteica y grupos sulfhidrilo). Se aplicaron análisis de varianza según tertiles de consumo, correlaciones bivariadas y modelos de regresión lineal ajustados por sexo, edad e índice de masa corporal.

Resultados: Una mayor ingesta de isoflavonas se asoció con menores marcadores de daño oxidativo, en particular AOPP, hidroperóxidos y peróxidos lipídicos, junto con una menor actividad de SOD y GPx. Los hidroperóxidos mostraron una tendencia decreciente, mientras que la catalasa presentó un patrón no lineal.

Conclusiones: Estos hallazgos respaldan la relevancia potencial de los flavonoides dietéticos en el contexto de la salud cardiometabólica. Sin embargo, dado el diseño del estudio, no se pueden establecer inferencias causales.

Palabras clave: flavonoides, isoflavonas, estrés oxidativo, daño oxidativo, riesgo cardiometabólico.

Key message

- Higher dietary isoflavone intake is consistently associated with lower lipid and protein oxidative damage in individuals with cardiometabolic risk, supporting a potential role of diet in the association with redox status.
- Inverse associations with AOPPs remained significant after multivariable adjustment, highlighting protein oxidation as a sensitive biomarker of dietary isoflavone exposure.
- Lower SOD and GPx activity at higher intake levels suggests a possible association with endogenous antioxidant responses, although alternative regulatory mechanisms cannot be excluded.
- This study provides novel evidence from an Argentine population, contributing real-world data on flavonoid intake and its relationship with oxidative stress biomarkers in cardiometabolic risk settings.

INTRODUCTION

Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses, is recognized as a central mechanism in the pathophysiology of cardiometabolic diseases (CMDs)[1,2]. This redox imbalance not only damages cellular macromolecules but also modulates signaling pathways involved in inflammation, endothelial dysfunction, and metabolic regulation[3,4].

Diet plays a key role in the relationship between oxidative stress and cardiometabolic risk (CMR). Accordingly, international recommendations, including those of the World Health Organization (WHO) and the Argentine Dietary Guidelines (GAPA), emphasize the importance of consuming fruits and vegetables daily as a fundamental strategy for preventing chronic diseases[5]. These foods are a natural source of vitamins, minerals, and bioactive compounds with antioxidant properties, including flavonoids.

Flavonoids are a diverse group of polyphenolic compounds distributed in foods and plants that have attracted interest because of their potential antioxidant and anti-inflammatory effects[6]. To scavenging free radicals, flavonoids can modulate redox-sensitive pathways, including Nrf2 activation, NF-κB inhibition, and the regulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)[7]. Among them, isoflavones are of particular interest due to their structural similarity to estrogens and their potential vascular and metabolic effects[8].

Despite promising experimental evidence, human studies evaluating the association between dietary flavonoid intake and oxidative stress biomarkers remain limited, particularly among individuals at elevated CMR. Furthermore, most available evidence in humans comes from supplementation trials using flavonoid doses difficult to achieve through a typical diet, and only a few studies have integrated analyses of both enzymatic and non-enzymatic biomarkers of redox status in CMR populations.

Evidence from Latin American populations is especially scarce, despite the high prevalence of CMDs and the diversity of regional dietary patterns. Therefore, this study aimed to evaluate the association between dietary flavonoid intake and biomarkers of oxidative stress in adults with CMR factors. By examining both enzymatic and non-enzymatic markers of redox balance, this

study seeks to contribute evidence regarding the relationship between habitual flavonoid consumption and oxidative status in a real-world population.

METHODOLOGY

Study population

An observational cross-sectional study was conducted in a convenience subsample of 45 adults (35–70 years) recruited from a larger cohort of the Clinical-epidemiological approach to arterial hypertension based on biomarkers and food environment study (Secretary of Science and Technology, UNC; Res. No. 455–18). Participants were selected according to the availability of blood samples for oxidative stress biomarker analysis.

CMR was defined as the presence of at least one Adult Treatment Panel III metabolic syndrome component (abnormal waist circumference, fasting glucose, triglycerides, HDL cholesterol, or blood pressure). The study was approved by the HNC Ethics Committee (No. 177/18) and conducted according to the Declaration of Helsinki.

Body weight, height, waist circumference (WC), body mass index (BMI), blood pressure, HDL-c, triglycerides, and fasting glucose were assessed using standardized procedures. Blood pressure was measured according to American Heart Association recommendations, and biochemical analyses were performed at the HNC laboratory (Wiener Lab®)[9].

Flavonoids intake

Dietary flavonoid intake and food sources were assessed using a validated Argentine food frequency questionnaire (FFQ) together with a validated photographic atlas of portion sizes[10,11]. The data were analyzed using Interfood v.1.3 software. Flavonoid subclasses were estimated using the USDA database (USDA, 2003), whereas isoflavone intake was calculated using Phenol-Explorer.

Biological samples

After a 10–12 h fast, venous blood samples (10 mL) were collected, centrifuged, and separated into serum and erythrocyte fractions. Erythrocytes were washed, lysed, aliquoted, and stored until analysis. Oxidative stress biomarkers were measured in duplicate with internal controls.

Protein concentration was determined by the Lowry method. Blood collection, anthropometry, and dietary assessment were performed on the same day.

Determination of antioxidant enzymes

The method for SOD determination was based on the inhibition of adrenaline autoxidation in an alkaline medium, which is dependent on superoxide anion[12]. The change in absorbance was measured in the absence and presence of variable sample volumes (10, 15, and 20 µL), and the slope of $\Delta_{Abs/min}$ was calculated for each condition. One unit of SOD is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50%. The enzymatic activity was expressed as U SOD/mg protein.

The catalase detection method was based on the methodology described by Aebi (1984). The enzyme in samples was incubated with a known concentration of H_2O_2, and the reduction was monitored by following the reaction kinetics. The unit of measurement was expressed as U of catalase/mg of protein.

GPx activity was measured indirectly coupled reaction with glutathione reductase (Cayman Chemicals commercial kit)

Determination of oxidative damage biomarkers

Peroxides' aqueous and lipid fractions were studied in micromolar quantities[13]. Both were measured at 540 nm and expressed as absorbance after subtracting the corresponding blanks and standardizing for protein content.

AOPPs were determined spectrophotometrically at 340 nm under acidic conditions in the presence of potassium iodide. The results were expressed as mg chloramine T equivalents/mg protein.

Free sulfhydryl groups were measured by Ellman reagent, 5, 5'-dithio-bis- (2-nitrobenzoic acid) (Sigma-Aldrich Co., USA), which reacts with a –SH group of proteins under mild alkaline conditions to produce a deep yellow product measurable at 412 nm[14].

Statistical analysis

Descriptive statistics are presented as frequencies (%) or mean and standard deviation (SD).

To evaluate the markers according to different consumptions of flavonoids, exposure tertiles were calculated. Differences in oxidative stress biomarkers across tertiles of isoflavone intake

were evaluated using one-way analysis of variance (ANOVA). When a significant overall effect was detected, pairwise comparisons between tertiles were performed using Tukey's honestly significant difference (HSD) post-hoc test. Subsequently, bivariate correlation analyses were performed using Spearman coefficient according to variable distribution. Finally, multivariable regression analyses were conducted to assess these associations after adjustment for potential confounders (sex, age, and BMI). Covariates were selected a priori based on their biological relevance and their known associations with both oxidative stress and dietary intake. Given the small sample size, parsimonious models were prioritized to minimize overfitting. Sensitivity analyses additionally adjusted for selected metabolic syndrome components (Supplementary Table S1).

Model assumptions were evaluated using residual diagnostics, including the Shapiro–Wilk test. Analyses were performed with Stata v15.0 (StataCorp, College Station, TX, USA), and statistical significance was set at $p < 0.05$.

RESULTS

Table 1 shows the cardiometabolic characteristics of the study population (n = 45). According to BMI, a majority of participants were diagnosed with overweight or obesity, and with a significantly increased risk of developing CVD by WC value.

Table 1. Cardiometabolic characteristics of the study population

	Total sample (n=45)
Female sex, n (%)	31 (68.89)
Male sex, n (%)	14 (31.11)
BMI (kg/m²), mean (SD)	28.59 (6.14)
Overweight/obesity, n (%)	30 (66.66)
Elevated WC, n (%)	28 (62.22)
Elevated blood pressure, n (%)	15 (33.33)
Elevated fasting glucose, n (%)	15 (35.55)
Hypertriglyceridemia, n (%)	13 (28.89)
Low HDL-C, n (%)	4 (8.89)

The total intake of flavonoids reached a median of 321.41 mg/day, with the subclass of highest consumption being flavanones, followed by isoflavones and anthocyanins (Table 2).

Table 2. Average flavonoids by tertiles of consumption

	Total consumption, mean (SD)			
Subclass of flavonoid (mg/day)	**Total**	**Tertile 1**	**Tertile 2**	**Tertile 3**
Isoflavones	41.26 (38.64)	6.21 (4.66)	38.57 (12.48)	91.57 (30.53)
Flavones	2.74 (1.45)	1.33 (0.49)	2.62 (0.39)	4.38 (1.12)
Flavonols	21.48 (14.65)	9.61 (3.18)	17.65 (3.92)	38.04 (13.92)
Flavanones	71.61 (46.71)	25.16 (15.14)	68.30 (8.93)	124.68 (34.49)
Flavanols	22.71 (15.37)	9.20 (4.01)	19.99 (3.74)	39.88 (13.99)
Anthocyanins	30.93 (27.05)	10.95 (4.36)	24.06 (4.41)	59.20 (30.86)

Table 3 shows the distribution of oxidative stress biomarkers according to tertiles of dietary isoflavone intake. For visualization purposes, the corresponding graphical representation is provided as Supplementary Figures S1–S6. Significant differences among tertiles were observed for lipid peroxides (p=0.0002), hydroperoxides (p=0.0004), AOPPs (p=0.0008), SOD (p=0.007), GPx (p=0.017), and sulfhydryl groups (p=0.009). No significant differences were detected for CAT activity (p=0.791).

Post-hoc comparisons revealed that the lowest isoflavone intake tertile exhibited significantly higher concentrations of lipid peroxides, hydroperoxides, AOPPs, and sulfhydryl groups than the intermediate and highest tertiles. A similar pattern was observed for SOD and GPx activities, which were significantly higher in the lowest intake tertile.

Table 3: Distribution of oxidative stress biomarkers according to tertiles of dietary isoflavone intake

Biomarkers, mean (SD)	Tertil 1	Tertil 2	Tertil 3	p
SOD	1.982 (0.797)^{b}	0.942 (0.154)^{a,b}	0.577 (0.263)^{a}	**0.0074**
CAT	6.8×10^{-5} (1.48×10^{-5})	1.98×10^{-5} (1.08×10^{-5})	2.68×10^{-5} (1.78×10^{-5})	0.7911
GPx	9.678 (3.644)^{b}	3.183 (0.090)^{a}	2.982 (2.368)^{a}	**0.0171**
Lipid peroxides	0.047 (0.005)^{b}	0.010 (0.006)^{a}	0.013 (0.007)^{a}	**0.0002**
Hydroperoxides	0.014 (0.002)^{b}	0.006 (0.001)^{a}	0.007 (0.001)^{a}	**0.0004**
AOPPs	0.294 (0.061)^{b}	0.113 (0.035)^{a}	0.108 (0.034)^{a}	**0.0008**
Sulfhydryl groups	0.064 (0.030)^{b}	0.013 (0.008)^{a}	0.026 (0.008)^{a}	**0.0093**

Different superscript letters indicate statistically significant differences between tertiles according to post hoc comparisons. The graphical representation is available in Supplementary Figures.

Bivariate correlation analyses revealed significant inverse associations between dietary isoflavone intake and several oxidative stress biomarkers (Table 4). Isoflavone intake was negatively correlated with hydroperoxides (ρ = −0.63, p = 0.039), AOPPs (ρ = −0.85, p = 0.001), and sulfhydryl groups (ρ = −0.69, p = 0.019).

No significant correlations were observed between the remaining flavonoid subclasses (flavones, flavonols, flavanones, flavanols, and anthocyanins) and oxidative stress biomarkers.

Table 4: Bivariate correlations between flavonoid intake and oxidative stress biomarkers

Biomarker	Isoflavones		Flavones		Flavonols		Flavanones		Flavanols		Anthocyanins	
	r	p	r	p	r	p	r	p	r	p	r	p
SOD	-0.72	0.050	0.21	0.169	0.13	0.407	0.13	0.417	0.04	0.764	0.12	0.442
CAT	-0.40	0.223	0.19	0.208	-0.13	0.407	0.04	0.780	0.06	0.685	0.07	0.634
GPx	-0.68	0.061	0.09	0.549	-0.02	0.904	-0.22	0.156	0.07	0.657	0.21	0.176
Lipid peroxides	-0.58	0.060	-0.02	0.874	0.15	0.336	0.09	0.549	0.09	0.536	-0.01	0.935
Hydroperoxides	-0.63	**0.039**	0.15	0.344	0.17	0.254	0.03	0.802	0.13	0.410	0.12	0.425
AOPPs	-0.85	**0.001**	0.18	0.259	0.23	0.135	0.05	0.746	0.31	0.475	0.19	0.211
Sulfhydryl groups	-0.69	**0.019**	-0.01	0.947	0.05	0.733	-0.02	0.851	0.25	0.101	0.10	0.524

Correlation coefficients correspond to Spearman's rank correlation analyses.

Based on the results of these bivariate analyses, multivariate linear models adjusted for potential confounding factors were developed to explore these associations further.

Significant associations were observed between some markers of oxidative damage and isoflavone consumption (Table 5). Negative associations were detected between isoflavone consumption and the formation of AOPPs (β = -0.001; 95% CI = -0.003; -0.0004; p = 0.04), and the concentrations of sulfhydryl groups (β = -0.0005; 95% CI = -0.0009; -0.0005; p = 0.02). Although hydroperoxides tended to decrease across tertiles of isoflavone intake, this association did not remain statistically significant after adjustment.

Additional sensitivity analyses including selected metabolic syndrome components as additional covariates attenuated the statistical significance of some associations, although effect directions remained consistent.

Table 5. Multivariable linear regression models for the association between isoflavone consumption (mg/day) and oxidative damage markers.

Isoflavone intake (mg/day)			
Biomarker	**β (coef.)**	**95% CI**	**p**
Lipid peroxides	-0.0002	-0.000; 0.00001	0.16
Hydroperoxides	-0.00006	-0.006; 0.001	0.08
AOPPs	-0.001	-0.003; -0.00004	0.04
Sulfhydryl groups	-0.0005	-0.0009; -0.0001	0.02

(*) Models were adjusted for gender, age, and BMI.

DISCUSSION

Oxidative stress is a key mechanism underlying cardiometabolic diseases, contributing to endothelial dysfunction, chronic inflammation, and metabolic impairment[15]. Flavonoids have been extensively investigated for their antioxidant properties and their association with inflammation[16]. This study evaluated the association between dietary intake of flavonoids—particularly isoflavones—and oxidative stress biomarkers in individuals at CMR. The findings suggest that higher isoflavone intake is associated with a more favorable oxidative profile, characterized by lower levels of lipid peroxidation and protein oxidation markers, along with reduced activity of key antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). Regarding hydroperoxides, tertile-based analyses suggested lower levels with higher isoflavone intake, although this association was not confirmed in adjusted models. In contrast, sulfhydryl group levels showed an inverse pattern.

One of the most consistent findings was the inverse association between isoflavone intake and markers of oxidative damage, including AOPPs, which showed statistically significant differences across intake levels. These results are in line with previous studies suggesting that isoflavones may contribute to reducing oxidative damage through their capacity to scavenge reactive oxygen species[17-18]. Regarding AOPPs, these biomarkers are well-established indicators of oxidative stress in cardiometabolic conditions, showing positive correlations with waist circumference, fasting glucose, triglycerides, and insulin resistance[19].

Interestingly, higher isoflavone intake was also associated with lower activity of endogenous antioxidant enzymes such as SOD and GPx. This finding may reflect a reduced oxidative burden, leading to a lower requirement for endogenous antioxidant activity[20,21]. However, alternative explanations should also be considered, including potential alterations in antioxidant defense

regulation. Since lower enzyme activity was observed concurrently with lower levels of oxidative damage biomarkers, the former interpretation appears plausible, although the cross-sectional design precludes definitive conclusions regarding the underlying mechanisms. In contrast, CAT activity appeared to vary across tertiles of isoflavone intake, with lower values observed in the intermediate tertile and higher values in the highest tertile. Although this pattern may be biologically plausible, the present study was not designed to formally evaluate non-linear associations, and therefore this observation should be interpreted with caution. Experimental studies have shown that daidzein can increase catalase mRNA expression and that soy-derived compounds may enhance catalase activity and gene expression in animal models. Isoflavones have also been reported to activate regulatory pathways involved in antioxidant enzyme expression. Nevertheless, further studies specifically designed to evaluate dose-response relationships are needed to clarify the relevance of these mechanisms in humans[22-24].

An unexpected finding was the decrease in sulfhydryl group levels with increasing isoflavone intake. Sulfhydryl groups, which largely reflect reduced thiols such as glutathione, are typically considered markers of antioxidant capacity. Therefore, their reduction contrasts with the overall pattern of decreased oxidative damage observed in this study. This apparent inconsistency may reflect complex redox dynamics. Interestingly, a study in endothelial cells showed that genistein protection against oxidative stress was accompanied by decreases in intracellular glutathione levels, explained by the formation of glutathione conjugates with oxidized genistein metabolites25. However, other studies report that isoflavones can slightly increase reduced GSH levels (10-30% at 5 nM concentrations), and the meta-analysis showed significant increases in GSH (SMD: 0.51; 95% CI: 0.13, 0.88)[25]. Several mechanisms may explain the decrease in sulfhydryl groups observed with isoflavone intake. The oxidation of protein thiols is a process where cysteine residues can undergo reversible post-translational modifications, including S-glutathionylation which protects proteins from irreversible overoxidation[26,27]. Furthermore, isoflavones can modulate the expression of γ-glutamylcysteine synthetase, increasing intracellular GSH concentrations through antioxidant response elements present in the promoter regions of genes inducible by oxidative stress. Cysteine residues can also undergo a cascade of oxidations from reduced thiol to sulfenic, sulfinic, and sulfonic acids, where reversible

modifications represent critical decision points in redox signaling pathways[28,29]. These mechanisms suggest that the decrease in sulfhydryl groups reflects a dynamic redox redistribution rather than a simple antioxidant depletion, where modified thiols may exert protective and signaling functions that are not detected by measuring free sulfhydryl groups, thus may help explain the apparent paradox between the decrease in thiols and the reduction in oxidative damage observed in this study.

Regarding hydroperoxides, although tertile-based analyses suggested lower levels with higher isoflavone intake, this association did not reach statistical significance in multivariable regression models. This discrepancy may be related to differences in statistical power, variability within groups, or the treatment of isoflavone intake as a continuous versus categorical variable. These inconsistencies highlight the need for cautious interpretation and are consistent with previous research showing variable effects of isoflavones on oxidative stress markers depending on study design and population characteristics.

From a dietary perspective, the levels of isoflavone intake observed in this population were relatively low compared to those reported in Asian populations, where soy-based foods are more commonly consumed. This suggests that even moderate intake levels, achievable within Western dietary patterns, may be associated with measurable differences in oxidative stress markers. Studies demonstrate beneficial effects with doses of 50-100 mg/day of isoflavones, achievable in Western diets with moderate soy consumption, although much lower than typical Asian intakes (25-50 mg/day vs >100 mg/day)[30].

The mechanisms that may underlie the associations observed between isoflavones and oxidative stress biomarkers are: isoflavones can directly bind to Keap1, facilitating Nrf2 activation. They also modify MAPK and PI3K pathways that regulate antioxidant enzyme expression. This hormetic mechanism, where low-dose oxidative stress activates endogenous defenses, may explain the complex patterns observed in our study30.

Several limitations should be acknowledged. The cross-sectional design precludes causal inference, and the hospital-based sample may limit generalizability.

The relatively small sample size and the stratification of participants into exposure tertiles may have limited statistical power, increasing the possibility of type II errors and unstable estimates.

Therefore, non-significant findings, particularly those observed for hydroperoxides in adjusted models, should be interpreted with caution. These results should be considered exploratory and hypothesis-generating. The sample size limited the number of covariates that could be included in the multivariable models without compromising their stability, so the possibility of residual confounding by unmeasured or incompletely adjusted cardiometabolic factors cannot be ruled out. Additionally, dietary intake was self-reported, which may introduce measurement error and misclassification bias.

A significant strength of this work is the focus on objective, quantifiable biomarkers of oxidative stress in a population at CM risk. Investigating how habitual flavonoid consumption influences these biomarkers provides evidence that may support dietary recommendations to reduce CM risk. From a local perspective, this work offers particular value. In Argentina, the availability of national population estimates of flavonoid intake is limited and fragmented. The lack of national intake data and longitudinal or interventional studies in our population hinders direct extrapolation of results from other contexts. Therefore, this study makes a significant contribution: it establishes an initial approach to the link between isoflavones and redox indicators in an Argentine sample, and highlights the need to develop specific dietary surveys, local databases of flavonoid content, and prospective or experimental studies that allow for the evaluation of the medium- and long-term impact on clinical outcomes.

CONCLUSIONS

Higher dietary isoflavone intake was associated with lower levels of selected biomarkers of oxidative damage and alterations in redox-related biomarkers among individuals with CMR. These findings contribute to the limited evidence available from Latin American populations and support the potential relevance of dietary flavonoids in the context of cardiometabolic health. However, given the cross-sectional design of the study, causal inferences cannot be established. Further longitudinal and mechanistic studies are needed to confirm these associations and better understand the underlying biological mechanism.

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AUTHOR CONTRIBUTIONS

MLB: writing–original draft, writing– review and editing, conceptualization, data curation, formal analysis, investigation, methodology. AMC: writing–original draft, writing– review and editing, formal analysis, investigation, methodology, validation, and visualization. SDR: writing–original draft, writing– review and editing, data curation, formal analysis, investigation, methodology, validation, and visualization. NRP: writing–original draft, writing– review and editing, funding acquisition, project administration, resources, supervision, and visualization.: MGO: writing–original draft, writing– review and editing, data curation, formal analysis, MDD: writing– original draft, writing– review and editing, conceptualization, data curation, formal analysis, funding acquisition, project administration, resources, supervision, and visualization.

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COMPETING INTERESTS

The authors declare that they have no commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCIAS

1. Ji LL, Yeo D. Oxidative stress: an evolving definition. Fac Rev. 2021;10:13. doi: 10.12703/r/10-13.
2. Caturano A, Rocco M, Tagliaferri G, Piacevole A, Nilo D, Di Lorenzo G, Iadicicco I, Donnarumma M, Galiero R, Acierno C, Sardu C, Russo V, Vetrano E, Conte C, Marfella R, Rinaldi L, Sasso FC. Oxidative Stress and Cardiovascular Complications in Type 2 Diabetes: From Pathophysiology to Lifestyle Modifications. Antioxidants. 2025;14(1):72. doi: 10.3390/antiox14010072.
3. De Araújo FF, de Paulo Farias D, Neri-Numa IA, Pastore GM. Polyphenols and their applications: An approach in food chemistry and innovation potential. Food Chem. 2021;338:127535. doi: 10.1016/j.foodchem.2020.127535.
4. Peng ML, Fu Y, Wu CW, Zhang Y, Ren H, Zhou SS. Signaling Pathways Related to Oxidative Stress in Diabetic Cardiomyopathy. Front Endocrinol. 2022;13:907757. doi: 10.3389/fendo.2022.907757.
5. Guías Alimentarias para la Población Argentina (GAPA) [Internet]. Buenos aires: Ministerio de Salud de la Nación; 2016. Available at: https://www.inti.gob.ar/assets/uploads/files/certificaciones/manipuladores-de-alimentos/Guias-alimentarias-poblacion-argentina.pdf
6. Bustos PS, Echeverría J, Páez PL, Ortega MG. Evaluation of the protective effect of quercetin and luteolin against ciprofloxacin- and chloramphenicol-induced oxidative stress in blood cells and their impact on the microbiological activity. Front Pharmacol. 2025;16:1626058. doi: 10.3389/fphar.2025.1626058.
7. Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. Arch Toxicol. 2024;98(5):1323-1367. doi: 10.1007/s00204-024-03696-4.
8. Messina M, Barnes S, Setchell KD. Perspective: Isoflavones-Intriguing Molecules but Much Remains to Be Learned about These Soybean Constituents. Adv Nutr. 2025;16(5):100418. doi: 10.1016/j.advnut.2025.100418.

9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.
10. Perovic NR, Defago MD, Aguinaldo A, Joekes S, Actis AB. Validación y reproducibilidad de un cuestionario de frecuencia de consumo alimentario para valorar la ingesta de lípidos y fitoquímicos [Validity and reproducibility of a food frequency questionnaire to assess lipid and phytochemical intake]. Rev Fac Cien Med Univ Nac Cordoba. 2015;72(2):69-77.
11. Vazquez M, Witriw A. Modelos Usuales de Alimentos & Tablas de relación peso/volumen, 1st ed., Buenos Aires, 1997.
12. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170-5.
13. Soria EA, Goleniowski ME, Cantero JJ, Bongiovanni GA. Antioxidant activity of different extracts of Argentinian medicinal plants against arsenic-induced toxicity in renal cells. Hum Exp Toxicol. 2008;27(4):341-6. doi: 10.1177/0960327108092192.
14. Boyne AF, Ellman GL. A methodology for analysis of tissue sulfhydryl components. Anal Biochem. 1972;46(2):639-53. doi: 10.1016/0003-2697(72)90335-1.
15. Abrashev H, Abrasheva D, Nikolov N, Ananiev J, Georgieva E. A Systematic Review of Endothelial Dysfunction in Chronic Venous Disease-Inflammation, Oxidative Stress, and Shear Stress. Int J Mol Sci. 2025;26(8):3660. doi: 10.3390/ijms26083660.
16. Ayyadurai VAS, Deonikar P, Bannuru RR. Attenuation of low-grade chronic inflammation by phytonutrients: A computational systems biology analysis. Clin Nutr ESPEN. 2022;49:425-435. doi: 10.1016/j.clnesp.2022.03.010.
17. Cristani M, Speciale A, Saija A, Gangemi S, Minciullo PL, Cimino F. Circulating Advanced Oxidation Protein Products as Oxidative Stress Biomarkers and Progression Mediators in Pathological Conditions Related to Inflammation and Immune Dysregulation. Curr Med Chem. 2016;23(34):3862-3882. doi: 10.2174/0929867323666160902154748..
18. Klisic A, Kavaric N, Ninic A, Kotur-Stevuljevic J. Oxidative stress and cardiometabolic biomarkers in patients with non-alcoholic fatty liver disease. Sci Rep. 2021;11(1):18455. doi: 10.1038/s41598-021-97686-6.

19. Hernandez-Montes E, Pollard SE, Vauzour D, Jofre-Montseny L, Rota C, Rimbach G, Weinberg PD, Spencer JP. Activation of glutathione peroxidase via Nrf1 mediates genistein's protection against oxidative endothelial cell injury. Biochem Biophys Res Commun. 2006;346(3):851-9. doi: 10.1016/j.bbrc.2006.05.197.
20. Barbosa AC, Lajolo FM, Genovese MI. Effect of free or protein-associated soy isoflavones on the antioxidant status in rats. J Sci Food Agric. 2011;91(4):721-31. doi: 10.1002/jsfa.4242.
21. Röhrdanz E, Ohler S, Tran-Thi QH, Kahl R. The phytoestrogen daidzein affects the antioxidant enzyme system of rat hepatoma H4IIE cells. J Nutr. 2002;132(3):370-5. doi: 10.1093/jn/132.3.370.
22. Siow RC, Mann GE. Dietary isoflavones and vascular protection: activation of cellular antioxidant defenses by SERMs or hormesis? Mol Aspects Med. 2010;31(6):468-77. doi: 10.1016/j.mam.2010.09.003.
23. Liang F, Cao W, Huang Y, Fang Y, Cheng Y, Pan S, Xu X. Isoflavone biochanin A, a novel nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response element activator, protects against oxidative damage in HepG2 cells. Biofactors. 2019;45(4):563-574. doi: 10.1002/biof.1514.
24. Guo Q, Rimbach G, Moini H, Weber S, Packer L. ESR and cell culture studies on free radical-scavenging and antioxidant activities of isoflavonoids. Toxicology. 2002;179(1-2):171-80. doi: 10.1016/s0300-483x(02)00241-x.
25. Mieyal JJ, Chock PB. Posttranslational modification of cysteine in redox signaling and oxidative stress: Focus on s-glutathionylation. Antioxid Redox Signal. 2012;16(6):471-5. doi: 10.1089/ars.2011.4454.
26. Zhang J, Ye ZW, Singh S, Townsend DM, Tew KD. An evolving understanding of the S-glutathionylation cycle in pathways of redox regulation. Free Radic Biol Med. 2018;120:204-216. doi: 10.1016/j.freeradbiomed.2018.03.038.
27. Fujimoto A, Masuda T. Chemical interaction between polyphenols and a cysteinyl thiol under radical oxidation conditions. J Agric Food Chem. 2012;60(20):5142-51. doi: 10.1021/jf3008822.

28. Nakamura Y, Ishii T, Abe N, Murata Y. Thiol modification by bioactivated polyphenols and its potential role in skin inflammation. Biosci Biotechnol Biochem. 2014;78(6):1067-70. doi: 10.1080/09168451.2014.905190.
29. Trost P, Fermani S, Calvaresi M, Zaffagnini M. Biochemical basis of sulphenomics: how protein sulphenic acids may be stabilized by the protein microenvironment. Plant Cell Environ. 2017;40(4):483-490. doi: 10.1111/pce.12791.
30. Amaral AL, Mendonça AM, Giolo JS, Costa JG, Mariano IM, de Souza TCF, Batista JP, Rodrigues ML, de Souza AV, Caixeta DC, Peixoto LG, de Oliveira EP, Espindola FS, Puga GM. The effects of isoflavone supplementation plus combined exercise on salivary markers of oxidative stress in postmenopausal women. J Clin Biochem Nutr. 2020;66(1):43-48. doi: 10.3164/jcbn.19-44.