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Identification of an Optimized Quinoa Supplementation Strategy that Protects Against High-Fat Diet-Induced Multi-Organ Injury in Rats: Integrated Biochemical, Bioenergetic and Histopathological Evidence

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ABSTRACT

Background: Obesity induced by chronic consumption of a high-fat diet (HFD) is associated with dyslipidemia, oxidative stress, mitochondrial dysfunction, and progressive multi-organ injury. Although *Chenopodium quinoa* Willd. is recognized as a nutrient-rich functional food with potent antioxidant and hypolipidemic properties, limited information is available regarding the comparative efficacy of different supplementation levels against obesity-associated metabolic disturbances. Objective: This study aimed to evaluate the protective effects of two supplementation levels of quinoa against HFD-induced obesity and to compare their efficacy with that of a reference hypolipidemic drug. **Methods:** Thirty male albino rats were randomly assigned to five experimental groups (n = 6/group): normal control, HFD-fed obese control, HFD supplemented with quinoa at the first dose level (HFQ1), HFD supplemented with quinoa at the second dose level (HFQ2), and HFD treated with a reference statin. Obesity was induced by HFD feeding for six weeks. Serum lipid profile, hepatic oxidative stress biomarkers [malondialdehyde (MDA), reduced glutathione (GSH), and oxidized glutathione (GSSG)], hepatic adenine nucleotides (ATP, ADP, and AMP), and histopathological alterations in the liver, kidney, and coronary arteries were evaluated using validated biochemical and histological methods. **Results:** HFD feeding induced marked dyslipidemia, enhanced lipid peroxidation, depletion of antioxidant defenses, impaired hepatic bioenergetics, and severe hepatic, renal, and vascular lesions compared with the normal control group. Both quinoa supplementation levels significantly ($P < 0.05$) attenuated these obesity-associated abnormalities; however, the higher supplementation level (HFQ2) produced greater improvements than HFQ1 in lipid homeostasis, oxidative status, tissue energy metabolism, and histopathological architecture. Although the reference statin significantly improved most measured parameters, HFQ2 exhibited comparable or superior protective effects in several biochemical and histopathological outcomes. **Conclusion:** Quinoa supplementation effectively ameliorated HFD-induced metabolic dysfunction and multi-organ injury through coordinated improvement of lipid metabolism, redox homeostasis, mitochondrial bioenergetics, and tissue integrity. The higher supplementation level (HFQ2) demonstrated the greatest protective efficacy, supporting its potential as a functional food strategy for preventing obesity-associated hepatic, renal, and cardiovascular complications.

Keywords: *Chenopodium quinoa*, high-fat diet, functional food, obesity, dyslipidemia, oxidative stress, mitochondrial bioenergetics and HP Inc. istopathology.

1. Introduction

Obesity has become one of the most challenging global health problems of the twenty-first century, with its prevalence increasing dramatically across both developed and developing countries. Beyond excessive adipose tissue accumulation, obesity is currently recognized as a chronic low-grade inflammatory disorder that predisposes individuals to metabolic syndrome, type 2 diabetes mellitus,

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non-alcoholic fatty liver disease (NAFLD), chronic kidney disease, and cardiovascular disorders. (WHO, 2024; Blüher, 2024) The excessive consumption of calorie-dense diets, particularly high-fat diets (HFDs), together with sedentary lifestyles, has accelerated the incidence of obesity and its associated metabolic complications worldwide. (Piché *et al*., 2020; Blüher, 2024)

Experimental HFD-induced obesity closely mimics many pathological characteristics observed in obese humans and therefore represents one of the most reliable experimental models for investigating obesity-associated metabolic disturbances and evaluating novel therapeutic interventions. (An *et al*., 2021) Chronic HFD consumption promotes adipocyte hypertrophy and dysfunction, resulting in excessive release of free fatty acids and adipokines that activate inflammatory signaling pathways, including nuclear factor-kappa B (NF-κB), thereby stimulating the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). (Furukawa *et al.,* 2004, Hotamisligil, 2017) Persistent activation of these inflammatory cascades contributes to insulin resistance, endothelial dysfunction, lipid abnormalities, and progressive tissue injury in multiple organs. (Hotamisligil, 2017; Saltiel and Olefsky, 2017).

Oxidative stress is another fundamental mechanism linking obesity with organ dysfunction. Excess nutrient intake markedly increases mitochondrial reactive oxygen species (ROS) production, overwhelming endogenous antioxidant defense systems and causing oxidative damage to lipids, proteins, and nucleic acids. (Furukawa *et al.,* 2004; Jones, 2006) Elevated lipid peroxidation products, particularly malondialdehyde (MDA), together with depletion of reduced glutathione (GSH) and disturbance of the GSH/GSSG redox balance, are consistently reported in obesity-induced hepatic, renal, and cardiovascular injury. (Jones, 2006; Sies, 2015) Oxidative stress further amplifies inflammatory signaling and accelerates apoptosis, fibrosis, and vascular remodeling, creating a vicious cycle that perpetuates metabolic deterioration. (Jones, 2006; Halliwell and Gutteridge, 2015)

The liver represents one of the primary organs affected by obesity because it plays a central role in lipid metabolism and energy homeostasis. Chronic exposure to excess dietary fat promotes hepatic triglyceride accumulation, hepatocyte ballooning, steatosis, inflammation, and progressive liver injury that may eventually evolve into metabolic dysfunction-associated steatotic liver disease (MASLD). (Rinella *et al.,* 2023; Younossi *et al*., 2024) Simultaneously, obesity-induced oxidative stress and systemic inflammation impair renal function through glomerular hypertrophy, tubular degeneration, and altered renal hemodynamics, while vascular endothelial dysfunction accelerates atherosclerotic changes and increases cardiovascular risk. (Alicic *et al*., 2017; Libby, 2021) Consequently, effective therapeutic strategies should ideally target multiple organs rather than correcting dyslipidemia alone. (Libby, 2021).

Growing interest has therefore focused on functional foods capable of simultaneously modulating oxidative stress, inflammation, lipid metabolism, and cellular bioenergetics. Functional foods contain naturally occurring bioactive compounds that provide health benefits beyond basic nutrition and may serve as complementary strategies for preventing chronic metabolic diseases. (Tang and Tsao, 2017; Granato, 2020) Numerous plant-derived phytochemicals, including phenolic acids, flavonoids, saponins, phytosterols, dietary fibers, and bioactive peptides, have demonstrated antioxidant, anti-inflammatory, hypolipidemic, and insulin-sensitizing properties in both experimental and clinical studies. (Tang and Tsao, 2017; Vilcacund, 2017).

Among these functional foods, quinoa (*Chenopodium quinoa* Willd.) has attracted considerable scientific attention owing to its exceptional nutritional composition and broad spectrum of biological activities. Unlike most cereal grains, quinoa contains high-quality proteins with a balanced essential amino acid profile, abundant dietary fiber, unsaturated fatty acids, vitamins, minerals, and numerous phytochemicals including phenolic compounds, flavonoids, betalains, phytosterols, saponins, and phytoecdysteroids. (Wang *et al*., 2022; Zhong *et al*., 2023) These bioactive constituents collectively contribute to potent antioxidant and anti-inflammatory activities that may counteract obesity-associated metabolic disturbances. (Wang *et al.,* 2022 , Gholamrezayi *et al*., 2025).

Recent experimental evidence suggests that quinoa supplementation improves lipid metabolism, enhances antioxidant enzyme activities, reduces lipid peroxidation, improves insulin sensitivity, and attenuates inflammatory responses in obesity and related metabolic disorders. (Kumpun *et al*., 2011; Zhong *et al*., 2023) Furthermore, quinoa-derived bioactive compounds have been shown to preserve mitochondrial function, improve cellular energy metabolism, and protect hepatic, renal, and cardiovascular tissues against oxidative injury. (Lafont and Dinan, 2003; Gholamrezayi *et al*., 2025)

These protective effects are believed to arise from the coordinated regulation of redox homeostasis, inflammatory signaling pathways, and mitochondrial bioenergetics, highlighting quinoa as a promising nutritional intervention for obesity-associated complications. (Lafont and Dinan, 2003; Gholamrezayi *et al.,* 2025).

Despite the increasing evidence supporting the beneficial effects of quinoa, relatively few studies have systematically compared different quinoa formulations to identify the most effective preparation capable of providing comprehensive multi-organ protection. Most available investigations have focused on a single quinoa product or evaluated only limited biochemical outcomes without integrating metabolic, oxidative, and histopathological endpoints. (Kumpun *et al*., 2011; Wang *et al*., 2022) Therefore, identifying an optimized quinoa formulation with superior biological efficacy remains an important research priority.

Accordingly, the present study was designed to evaluate the protective efficacy of two dietary supplementation levels of *Chenopodium quinoa* Willd. against high-fat diet-induced obesity in rats and to compare their effects with those of a reference hypolipidemic drug. An integrated assessment of serum lipid profile, hepatic oxidative stress biomarkers, mitochondrial bioenergetics, and histopathological alterations in the liver, kidneys, and coronary arteries was performed to elucidate the mechanisms underlying quinoa-mediated protection and to provide experimental evidence supporting its application as a functional food for preventing obesity-associated multi-organ injury.

2. Materials and Methods

2.1 Chemicals and Reagents

Commercial rodent chow was obtained from the Animal Nutrition Unit, Faculty of Agriculture, Cairo University, Egypt. The high-fat diet (HFD) was freshly prepared using standard laboratory ingredients to provide approximately 25 % of total caloric intake from fat, thereby inducing obesity and its associated metabolic abnormalities as previously described in experimental obesity models (Buettner *et al*., 2007; Speakman *et al*., 2007). The reference hypolipidemic drug (commercial statin formulation) was obtained from a licensed pharmaceutical manufacturer and administered according to the recommended experimental dose (Rajyalakshmi *et al*., 2008).

Reduced glutathione (GSH), oxidized glutathione (GSSG), malondialdehyde (MDA), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) assay reagents were purchased from certified commercial suppliers and used according to the manufacturers' instructions. All solvents employed during biochemical analyses were of HPLC analytical grade, while all remaining chemicals and reagents were of analytical reagent grade with purity greater than 99%.

Fresh quinoa seeds formulations were prepared under identical laboratory conditions to minimize batch-to-batch variability throughout the experimental period.

2.2 Preparation of Quinoa Supplementation

Quinoa (*Chenopodium quinoa* Willd.) seeds were thoroughly cleaned to remove foreign materials, washed with distilled water, and air-dried at room temperature. The dried seeds were finely ground using a laboratory grinder to obtain a uniform powder, which was stored in airtight, light-protected containers until use. Immediately before administration, the required amount of quinoa powder was freshly suspended in distilled water and vortex-mixed to obtain a homogeneous suspension. The suspension was prepared daily at concentrations that enabled oral administration of 25 mg/kg body weight (HFQ1) and 50 mg/kg body weight (HFQ2) in a constant dosing volume adjusted according to the weekly body weight of each animal.

2.3 Animals and Experimental Design

The present study was conducted using healthy adult male albino rats weighing 170 ± 10 g. Animals were obtained from the animal breeding facility of the National Organization for Drug Control and Research (NODCAR), Egypt. Before the beginning of the experiment, all rats were clinically examined to ensure good health status and the absence of any abnormalities. The animals were housed in polypropylene cages under controlled laboratory conditions with a constant temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12-h light/12-h dark cycle. Rats had free

access to drinking water throughout the experimental period, and standard hygienic conditions were maintained.

Before starting the experimental procedures, rats were allowed to acclimatize for one week to minimize stress-related physiological variations. After acclimatization, rats were randomly assigned into five experimental groups (n = 6 rats per group). Animals were fed either a normal diet (ND) or a high-fat diet (HF) ad libitum for six weeks. The experimental groups were designed as follows:

Group I: Normal control rats received a normal diet (ND) throughout the experimental period.
Group II: High-fat diet (HF) rats received the high-fat diet alone to induce obesity and metabolic disturbances.
Group III: High-fat diet-fed rats supplemented with quinoa formulation at the low dose level HFQ1 (25 mg/kg rats).
Group IV: High-fat diet-fed rats supplemented with quinoa formulation at the high dose level HFQ2 (50 mg/kg rats).
Group V: High-fat diet-fed rats treated with the reference hypolipidemic drug statin (HFS) at a dose of 10 mg/kg body weight/day according to Rajyalakshmi *et al.* (2008).

The experimental diets and treatments were administered throughout the six-week experimental period. Food intake and dietary composition were monitored regularly and adjusted among groups to ensure comparable nutrient and energy intake. Body weight was recorded weekly using a calibrated digital balance. Animals were monitored daily for general health condition, food consumption, water intake, behavioral changes, and any signs of toxicity or treatment-related adverse effects.

At the end of the experimental period, rats were euthanized by decapitation under appropriate experimental conditions. Blood samples were collected immediately, and serum was separated by centrifugation at 3500 rpm for 12 min at 4°C. The obtained serum samples were stored at −20°C until biochemical analysis, including lipid profile determination.

Heart and liver tissues were rapidly removed, cleaned from adhering tissues, and weighed. Tissue samples were homogenized in cold suitable buffer and centrifuged at 5000 rpm for 20 min at 4°C. The prepared tissue homogenates were used for determination of oxidative stress markers, including malondialdehyde (MDA), reduced glutathione (GSH), oxidized glutathione (GSSG), and energy metabolism parameters (ATP, ADP, and AMP). Additional tissue samples were preserved for histopathological examination.

All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of NODCAR.

2.4 Determination of Serum Lipid Profile

Serum samples were analyzed for the assessment of lipid metabolism following completion of the experimental protocol. Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were determined using commercially available enzymatic colorimetric diagnostic kits according to the manufacturer's instructions. All assays were performed in duplicate using an automated spectrophotometer, and quality-control sera of known concentrations were included in each analytical run to ensure assay accuracy and precision (Allain *et al.,* 1974).

Low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C) concentrations were calculated using the Friedewald equation whenever serum triglyceride concentrations were within the recommended analytical range (Friedewald *et al*., 1972):

$$\text{LDL-C (mg/dL)} = \text{TC} - \text{HDL-C} - (\text{TG}/5)$$
$$\text{VLDL-C (mg/dL)} = \text{TG}/5$$

The atherogenic index (AI) and coronary risk index (CRI) were subsequently calculated to evaluate the degree of cardiovascular risk associated with obesity-induced dyslipidemia according to previously established equations (Dobiasova, 2004). These lipid-derived indices provide better prediction of cardiovascular complications than isolated lipid parameters and therefore were included in the present study.

2.5 Assessment of Oxidative Stress Biomarkers

Liver tissue homogenates were analyzed for oxidative stress biomarkers as indicators of cellular redox homeostasis. All procedures were carried out on ice to minimize artificial oxidation during sample preparation.

2.5.1 Determination of Malondialdehyde (MDA)

Lipid peroxidation was quantified by measuring malondialdehyde (MDA), one of the principal end products generated during oxidative degradation of membrane polyunsaturated fatty acids. MDA concentration was determined using the thiobarbituric acid reactive substances (TBARS) assay described by Ohkawa *et al*., (1979). Briefly, tissue homogenate was mixed with thiobarbituric acid reagent and heated in a boiling water bath to allow formation of the MDA-thiobarbituric acid complex. After cooling, the chromogen was extracted, and absorbance was measured spectrophotometrically at 532 nm. Results were expressed as nmol MDA per gram wet tissue.

2.5.2 Determination of Reduced Glutathione (GSH)

Reduced glutathione (GSH) concentration was determined according to the method originally described by Ellman (1959), based on the reaction between sulfhydryl groups and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) producing a yellow-colored compound measured spectrophotometrically at 412 nm. GSH levels were expressed as µmol/g tissue.

2.5.3 Determination of Oxidized Glutathione (GSSG)

Oxidized glutathione (GSSG) was quantified after selective masking of reduced glutathione using 2-vinylpyridine, followed by enzymatic recycling with glutathione reductase and NADPH according to the method of Griffith (1980). GSSG concentration was expressed as µmol/g tissue.

The ratio between reduced and oxidized glutathione (GSH/GSSG ratio) was calculated as an indicator of intracellular redox status. Decreased ratios indicate oxidative stress and impaired antioxidant defense capacity (Jones, 2008).

2.6. Determination of Cellular Energy Metabolites

To evaluate mitochondrial bioenergetics, hepatic ATP, ADP, and AMP concentrations were determined using validated high-performance liquid chromatographic (HPLC) methods previously described for adenine nucleotide analysis (Stocchi *et al*., 1985; Smolenski *et al*., 1990).

Frozen tissue samples were homogenized in ice-cold perchloric acid to terminate enzymatic degradation of adenine nucleotides. Following centrifugation, supernatants were neutralized with potassium hydroxide, filtered through 0.22-µm membrane filters, and injected into the HPLC system.

Chromatographic separation was achieved using a reverse-phase C18 analytical column maintained at ambient temperature. Ultraviolet detection was performed at 254 nm. Quantification was accomplished using external calibration curves generated from authentic ATP, ADP, and AMP reference standards.

Energy charge (EC), reflecting the overall metabolic state of the tissue, was calculated using the equation proposed by Atkinson (1968):

$$\text{Energy Charge} = (\text{ATP} + 0.5\ \text{ADP})/(\text{ATP} + \text{ADP} + \text{AMP})$$

Higher EC values indicate preserved mitochondrial energy metabolism, whereas lower values reflect impaired ATP production resulting from metabolic stress.

2.7. Histopathological Examination

Representative specimens from liver, kidney, and heart containing coronary blood vessels were immediately fixed in 10% neutral buffered formalin for at least 24 h. Fixed tissues were dehydrated through ascending concentrations of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 4–6 µm thickness using a rotary microtome, and stained with hematoxylin and eosin (H&E) according to the standard procedure described by Bancroft and Bancroft (2019). All slides were independently examined by an experienced pathologist blinded to treatment allocation using a light microscope.

2.7.1. Liver Histopathology

Hepatic lesions were graded according to the criteria described by Plaa and Charbonneau (Plaa (1994), where Grade 0 represented normal hepatic architecture, Grade I indicated hepatocellular swelling, Grade II represented ballooning degeneration, Grade III corresponded to hepatic steatosis, and Grade IV reflected apoptosis and hepatocellular necrosis.

2.7.2. Kidney Histopathology

Renal injury was evaluated according to the semi-quantitative scoring system of Zhang *et al*. (2008). Tubular degeneration, tubular epithelial necrosis, glomerular alterations, tubular casts, and apoptosis were examined and graded from 0 (normal kidney) to 5 (severe injury affecting more than 75% of renal tubules).

2.7.3. Coronary Blood Vessel Histopathology

Coronary arterial lesions were graded according to Rahimatul *et al.* (2016). Morphological assessment included endothelial integrity, foam cell accumulation, lipid deposition, vascular edema, and structural organization of the vascular wall. Scores ranged from 0 (normal vascular morphology) to 3 (severe endothelial damage associated with extensive lipid accumulation).

Semi-quantitative pathological scoring was used to compare tissue injury among all experimental groups.

2.8 Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM) (n = 6). Statistical analysis was performed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Effect of Quinoa Supplementation on Body Weight Gain

Data are presented as mean ± SEM (n = 6 rats per group). Values within the same row sharing different superscript letters (a–d) are significantly different according to Duncan's multiple range test at $P < 0.05$.

The effects of quinoa supplementation on body weight gain in HFD-fed rats are presented in Table 1. No statistically significant differences were observed among the experimental groups in their initial body weights ($P > 0.05$), confirming the successful randomization of animals before initiating the dietary intervention. Initial body weights ranged from 171.6 ± 2.5 to 172.4 ± 3.1 g, indicating that all animals started the experiment under comparable physiological conditions.

Table 1: Effect of quinoa supplementation on body weight of HFD-fed rats.

Group	Initial BW (g)	Final BW (g)	Body weight gain (g)
Normal Control	171.8 ± 2.9	282.4 ± 4.8^{d}	110.6 ± 3.7^{d}
HFD	172.4 ± 3.1	389.7 ± 5.6^{a}	217.3 ± 4.4^{a}
HFQ1	171.6 ± 2.5	332.8 ± 4.9^{b}	161.2 ± 3.5^{b}
HFQ2	172.2 ± 2.8	301.5 ± 4.2^{c}	129.3 ± 3.4^{c}
Statin	171.9 ± 2.7	309.6 ± 4.6^{c}	137.7 ± 3.6^{c}

Following six weeks of HFD feeding, a pronounced increase in final body weight was observed in the obese untreated group compared with the normal control group ($P < 0.05$). Rats receiving the HFD reached a final body weight of 389.7 ± 5.6 g, whereas normal control animals exhibited a final body weight of only 282.4 ± 4.8 g, representing an increase of approximately 38%. Likewise, body weight gain nearly doubled in the HFD group, reaching 217.3 ± 4.4 g compared with 110.6 ± 3.7 g in the normal control animals. These findings confirm the successful establishment of the experimental obesity model.

Administration of the reference statin significantly attenuated HFD-induced body weight gain. Animals receiving statin treatment exhibited a final body weight of 309.6 ± 4.6 g and a body weight gain of 137.7 ± 3.6 g, representing significant reductions compared with untreated obese rats ($P < 0.05$).

Quinoa supplementation also significantly limited excessive body weight gain in HFD-fed rats. Animals receiving HFQ1 demonstrated a final body weight of 332.8 ± 4.9 g and a body weight gain of 161.2 ± 3.5 g, corresponding to a marked improvement compared with the HFD group. A greater reduction was achieved with the higher supplementation level (HFQ2), which lowered final body weight to 301.5 ± 4.2 g and body weight gain to 129.3 ± 3.4 g. Although body weight values remained slightly higher than those recorded in normal control animals, HFQ2 produced significantly greater improvement than HFQ1 and yielded values statistically comparable with those of the statin-treated group.

Overall, both quinoa supplementation levels effectively suppressed HFD-induced body weight gain, with the higher supplementation level (HFQ2) demonstrating the greatest reduction in body weight among the quinoa-treated groups and approaching the physiological values observed in normal animals.

3.2 Effect of Quinoa Supplementation on Serum Lipid Profile

Data are presented as mean ± SEM (n = 6 rats per group). Values within the same row sharing different superscript letters (a–d) are significantly different according to Duncan's multiple range test at $P < 0.05$.

The effects of quinoa supplementation on serum lipid profile in HFD-fed rats are summarized in Table 2. Chronic consumption of the high-fat diet induced marked disturbances in lipid metabolism, as evidenced by significant increases in serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), atherogenic index (AI), and coronary risk index (CRI), together with a significant reduction in high-density lipoprotein cholesterol (HDL-C), compared with the normal control group ($P < 0.05$). These alterations confirmed the successful induction of obesity-associated dyslipidemia in the untreated HFD group.

Serum total cholesterol increased markedly from 74.5 ± 2.4 mg/dL in normal control rats to 168.7 ± 3.8 mg/dL in obese untreated animals. Treatment with the reference statin significantly reduced TC to 94.8 ± 2.5 mg/dL, whereas quinoa supplementation also produced significant cholesterol-lowering effects. Rats receiving HFQ1 exhibited a TC concentration of 119.4 ± 2.7 mg/dL, while HFQ2 further reduced TC to 87.6 ± 2.3 mg/dL, representing the lowest cholesterol level among all treated groups and approaching that of the normal control animals.

Table 2: Effect of quinoa supplementation on serum lipid profile.

Parameter	Control	HFD	HFQ1	HFQ2	Statin
TC (mg/dL)	74.5 ±2.4^{d}	168.7 ±3.8^{a}	119.4 ±2.7^{b}	87.6 ±2.3^{c}	94.8 ±2.5^{c}
TG (mg/dL)	61.7 ±1.8^{d}	145.6 ±3.4^{a}	102.3 ±2.5^{b}	72.4 ±2.0^{c}	78.6 ±2.1^{c}
HDL-C (mg/dL)	48.6 ±1.3^{a}	24.5 ±1.1^{d}	35.8 ±1.2^{c}	45.3 ±1.1^{a}	41.9 ±1.2^{b}
LDL-C (mg/dL)	13.6 ±0.9^{d}	115.1 ±2.8^{a}	63.1 ±1.8^{b}	27.8 ±1.2^{c}	37.2 ±1.5^{c}
VLDL-C (mg/dL)	12.3 ±0.4^{d}	29.1 ±0.7^{a}	20.5 ±0.5^{b}	14.5 ±0.4^{c}	15.7 ±0.4^{c}
AI	0.53 ±0.03^{d}	5.89 ±0.15^{a}	2.33 ±0.09^{b}	0.93 ±0.04^{c}	1.26 ±0.05^{c}
CRI	1.53 ±0.05^{d}	6.88 ±0.19^{a}	3.34 ±0.11^{b}	1.93 ±0.06^{c}	2.26 ±0.07^{c}

A similar trend was observed for serum triglycerides. HFD feeding increased TG concentration from 61.7 ± 1.8 mg/dL in the normal control group to 145.6 ± 3.4 mg/dL in obese untreated rats. Administration of HFQ1 significantly decreased TG to 102.3 ± 2.5 mg/dL, whereas HFQ2 produced a greater reduction to 72.4 ± 2.0 mg/dL, showing values comparable to those obtained with statin treatment (78.6 ± 2.1 mg/dL).

Marked alterations were also observed in lipoprotein fractions. Serum HDL-C declined significantly from 48.6 ± 1.3 mg/dL in normal animals to 24.5 ± 1.1 mg/dL following HFD feeding. Both quinoa-treated groups significantly restored HDL-C concentrations, with HFQ2 (45.3 ± 1.1 mg/dL) demonstrating a greater increase than HFQ1 (35.8 ± 1.2 mg/dL) and exceeding the value recorded in the statin-treated group (41.9 ± 1.2 mg/dL). Conversely, LDL-C and VLDL-C increased markedly in obese untreated rats and were significantly reduced following quinoa supplementation. HFQ2 lowered LDL-C to 27.8 ± 1.2 mg/dL and VLDL-C to 14.5 ± 0.4 mg/dL, whereas HFQ1 reduced these parameters to 63.1 ± 1.8 mg/dL and 20.5 ± 0.5 mg/dL, respectively.

The calculated cardiovascular risk indices followed the same pattern. The HFD group exhibited the highest AI (5.89 ± 0.15) and CRI (6.88 ± 0.19), while both indices were significantly reduced in all treated groups ($P < 0.05$). HFQ2 produced the greatest reduction, lowering AI and CRI to 0.93 ± 0.04 and 1.93 ± 0.06, respectively, values that were comparable to those observed in the statin-treated animals and markedly lower than those recorded in the HFQ1 group. Collectively, these findings demonstrate that quinoa supplementation effectively ameliorated HFD-induced dyslipidemia, with the higher supplementation level consistently producing the most pronounced improvement across all lipid parameters.

3.3 Effect of Quinoa Supplementation on Hepatic Oxidative Stress Biomarkers

Data are presented as mean ± SEM (n = 6 rats per group). Values within the same row sharing different superscript letters (a–d) are significantly different according to Duncan's multiple range test at $P < 0.05$.

The effects of quinoa supplementation on hepatic oxidative stress biomarkers are presented in Table 3. HFD feeding induced marked oxidative stress in hepatic tissue, as evidenced by a significant increase in malondialdehyde (MDA) concentration accompanied by depletion of reduced glutathione (GSH), accumulation of oxidized glutathione (GSSG), and a marked reduction in the GSH/GSSG ratio compared with the normal control group ($P < 0.05$). These findings indicate a substantial disturbance in hepatic redox homeostasis following prolonged exposure to the high-fat diet.

Table 3: Effect of Quinoa Supplementation on Hepatic Oxidative Stress Biomarkers

Parameter	Control	HFD	HFQ1	HFQ2	Statin
MDA (nmol/g)	19.8 ±0.8^{d}	52.3 ±1.4^{a}	34.7 ±1.0^{b}	23.9 ±0.9^{c}	27.1 ±0.9^{c}
GSH (µmol/g)	8.92 ±0.25^{a}	4.13 ±0.16^{d}	6.84 ±0.19^{c}	8.27 ±0.21^{a}	7.76 ±0.18^{b}
GSSG (µmol/g)	1.18 ±0.05^{d}	3.96 ±0.12^{a}	2.54 ±0.08^{b}	1.49 ±0.06^{c}	1.71 ±0.06^{c}
GSH/GSSG	7.56 ±0.21^{a}	1.04 ±0.05^{d}	2.69 ±0.08^{c}	5.55 ±0.16^{a}	4.54 ±0.12^{b}

Hepatic MDA concentration increased markedly from 19.8 ± 0.8 nmol/g tissue in normal control rats to 52.3 ± 1.4 nmol/g tissue in the HFD group, representing an increase of approximately 164%. Administration of the reference statin significantly reduced MDA concentration to 27.1 ± 0.9 nmol/g tissue. Quinoa supplementation also markedly attenuated lipid peroxidation, with HFQ1 reducing MDA to 34.7 ± 1.0 nmol/g tissue, whereas HFQ2 further reduced MDA to 23.9 ± 0.9 nmol/g tissue, yielding values that approached those of the normal control animals and were lower than those observed in the statin-treated group.

Marked alterations were also observed in hepatic glutathione homeostasis. Reduced glutathione (GSH) decreased significantly from 8.92 ± 0.25 µmol/g tissue in the normal control group to 4.13 ± 0.16 µmol/g tissue following HFD feeding. Treatment with HFQ1 significantly restored GSH concentration to 6.84 ± 0.19 µmol/g tissue, whereas HFQ2 further increased GSH to 8.27 ± 0.21 µmol/g tissue, closely approaching the physiological value recorded in healthy animals. The statin-treated group exhibited an intermediate GSH concentration of 7.76 ± 0.18 µmol/g tissue.

In contrast, hepatic GSSG concentration increased markedly in obese untreated rats, rising from 1.18 ± 0.05 µmol/g tissue in the normal control group to 3.96 ± 0.12 µmol/g tissue. Both quinoa supplementation levels significantly reduced GSSG accumulation compared with the HFD group. HFQ1 lowered GSSG concentration to 2.54 ± 0.08 µmol/g tissue, whereas HFQ2 further reduced

GSSG to 1.49 ± 0.06 µmol/g tissue, a value closely comparable with that observed in the normal control animals. The reference statin reduced GSSG concentration to 1.71 ± 0.06 µmol/g tissue.

The calculated GSH/GSSG ratio further confirmed the restoration of intracellular redox balance following quinoa supplementation. HFD feeding caused a marked reduction in the ratio from 7.56 ± 0.21 in normal rats to 1.04 ± 0.05 in obese untreated animals. HFQ1 significantly improved the ratio to 2.69 ± 0.08, while HFQ2 restored it to 5.55 ± 0.16, representing the highest value among all treated groups. The statin-treated animals exhibited a ratio of 4.54 ± 0.12, indicating substantial but less pronounced improvement than that achieved with HFQ2. Overall, both quinoa supplementation levels significantly improved hepatic oxidative stress biomarkers, with the higher supplementation level consistently producing the greatest restoration of hepatic redox status across all evaluated parameters.3.4 Effect of Quinoa Supplementation on Hepatic Energy Metabolism. The effects of quinoa supplementation on hepatic energy metabolism are summarized in Table 4.

3.4. Effect of quinoa supplementation on hepatic energy metabolites.

Data are presented as mean ± SEM (n = 6 rats per group). Values within the same row sharing different superscript letters (a–d) are significantly different according to Duncan's multiple range test at $P < 0.05$.

Table 4: Effect of quinoa supplementation on hepatic energy metabolites.

Parameter	Control	HFD	HFQ1	HFQ2	Statin
ATP (µmol/g)	3.96 ±0.10^{a}	1.61 ±0.06^{d}	2.82 ±0.08^{c}	3.63 ±0.09^{a}	3.29 ±0.08^{b}
ADP (µmol/g)	1.22 ±0.04^{a}	0.63 ±0.03^{d}	0.91 ±0.03^{c}	1.15 ±0.04^{a}	1.05 ±0.03^{b}
AMP (µmol/g)	0.34 ±0.02^{d}	1.19 ±0.04^{a}	0.74 ±0.03^{b}	0.42 ±0.02^{c}	0.51 ±0.02^{c}
Energy Charge	0.89 ±0.01^{a}	0.63 ±0.01^{d}	0.78 ±0.01^{c}	0.87 ±0.01^{a}	0.84 ±0.01^{b}

The effects of quinoa supplementation on hepatic energy metabolism are presented in Table 4. Chronic HFD feeding markedly impaired hepatic bioenergetic status, as evidenced by significant alterations in adenine nucleotide concentrations together with a pronounced reduction in adenylate energy charge compared with the normal control group ($P < 0.05$). These findings indicate substantial impairment of hepatic energy homeostasis in obese untreated animals.

Hepatic ATP concentration decreased significantly from 3.96 ± 0.10 µmol/g tissue in the normal control group to 1.61 ± 0.06 µmol/g tissue following HFD feeding. Treatment with the reference statin significantly restored ATP concentration to 3.29 ± 0.08 µmol/g tissue. Likewise, quinoa supplementation significantly improved hepatic ATP content, reaching 2.82 ± 0.08 µmol/g tissue in the HFQ1 group and 3.63 ± 0.09 µmol/g tissue in the HFQ2 group. The ATP concentration observed in the HFQ2 group was statistically comparable to that of the normal control animals and represented the highest value among all treated groups.

A similar pattern was observed for hepatic ADP concentration. HFD feeding significantly reduced ADP levels from 1.22 ± 0.04 µmol/g tissue in normal rats to 0.63 ± 0.03 µmol/g tissue in obese untreated animals. Both quinoa supplementation levels significantly restored ADP concentration, with HFQ1 reaching 0.91 ± 0.03 µmol/g tissue, whereas HFQ2 further increased ADP to 1.15 ± 0.04 µmol/g tissue, closely approaching the normal physiological value. The statin-treated group exhibited an intermediate ADP concentration of 1.05 ± 0.03 µmol/g tissue.

Conversely, hepatic AMP concentration increased markedly following HFD administration, rising from 0.34 ± 0.02 µmol/g tissue in the normal control group to 1.19 ± 0.04 µmol/g tissue in obese untreated rats. Administration of HFQ1 significantly reduced AMP accumulation to 0.74 ± 0.03 µmol/g tissue, while HFQ2 further decreased AMP concentration to 0.42 ± 0.02 µmol/g tissue, representing the lowest value among the treated groups. The reference statin also significantly reduced hepatic AMP concentration to 0.51 ± 0.02 µmol/g tissue.

The calculated adenylate energy charge showed a similar trend. Obese untreated rats exhibited a significant reduction in energy charge from 0.89 ± 0.01 in normal animals to 0.63 ± 0.01 following HFD feeding. Both quinoa supplementation levels significantly improved hepatic energy charge, reaching 0.78 ± 0.01 in the HFQ1 group and 0.87 ± 0.01 in the HFQ2 group, while the statin-treated group recorded a value of 0.84 ± 0.01. Overall, HFQ2 consistently produced the greatest restoration of

hepatic bioenergetic parameters, with ATP, ADP, AMP, and energy charge values approaching those observed in the normal control group and exceeding the improvements achieved with the reference statin.

3.5 Histopathological Findings

Histopathological examination of the liver, kidney, and coronary blood vessels provided morphological evidence supporting the biochemical findings observed in the present study. Representative photomicrographs are presented in Figures 1–5. Overall, high-fat diet (HFD) feeding induced marked structural alterations in the examined organs, whereas quinoa supplementation at different levels resulted in variable degrees of tissue protection.

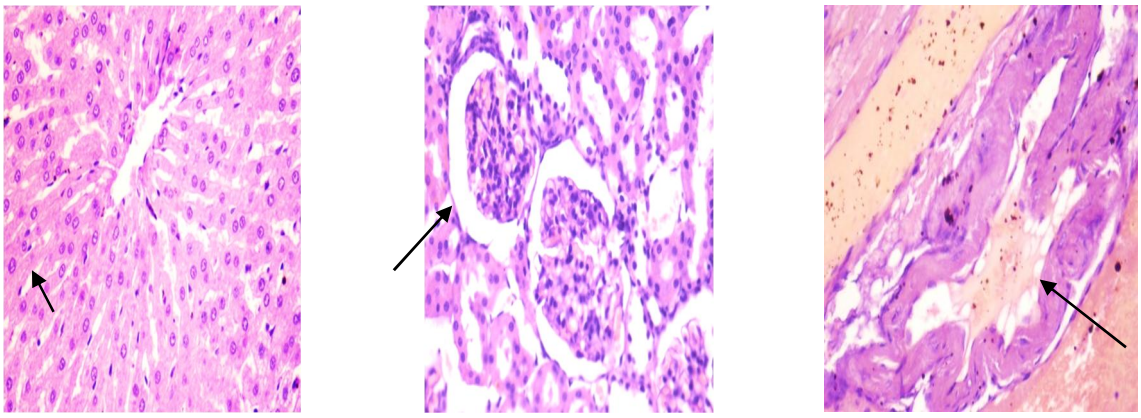

Fig. 1: Representative photomicrographs of the normal control group showing (a) liver with normal hepatic lobular architecture composed of radiating cords of polygonal hepatocytes (arrow), (b) kidney with normal histological appearance of glomeruli and renal tubules (arrow), and (c) coronary blood vessel exhibiting intact tunica intima, normal endothelial lining, internal elastic lamina, and external elastic lamina (arrow). Hematoxylin and eosin (H&E), ×200.

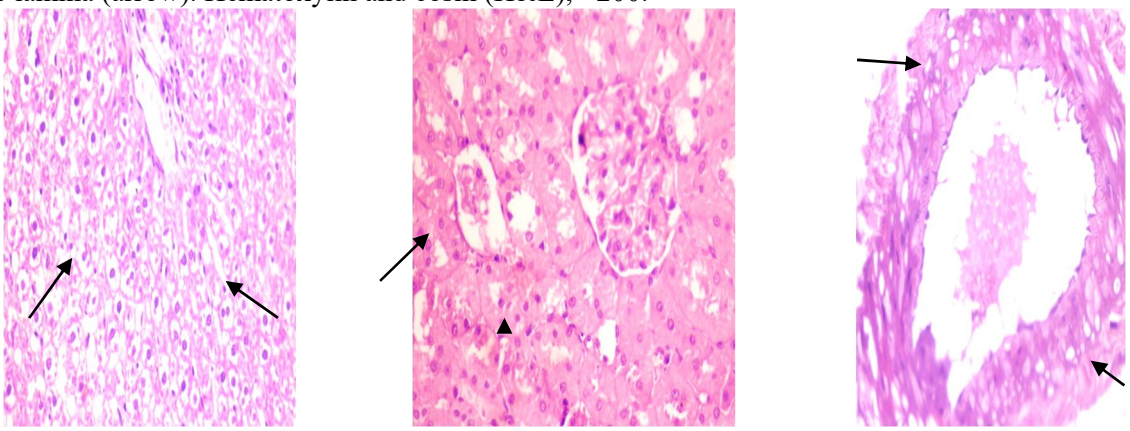

Fig. 2: Representative photomicrographs of the high-fat diet (HFD) group showing (a) liver with marked disorganization of hepatic cords and necrobiotic changes of hepatocytes (arrow), (b) kidney exhibiting shrinkage of glomerular capillary tufts with widening of Bowman's space (arrow) and degeneration of renal tubular epithelial cells (arrowhead), and (c) coronary blood vessel showing endothelial damage with foam cell infiltration (arrow). H&E, ×200.

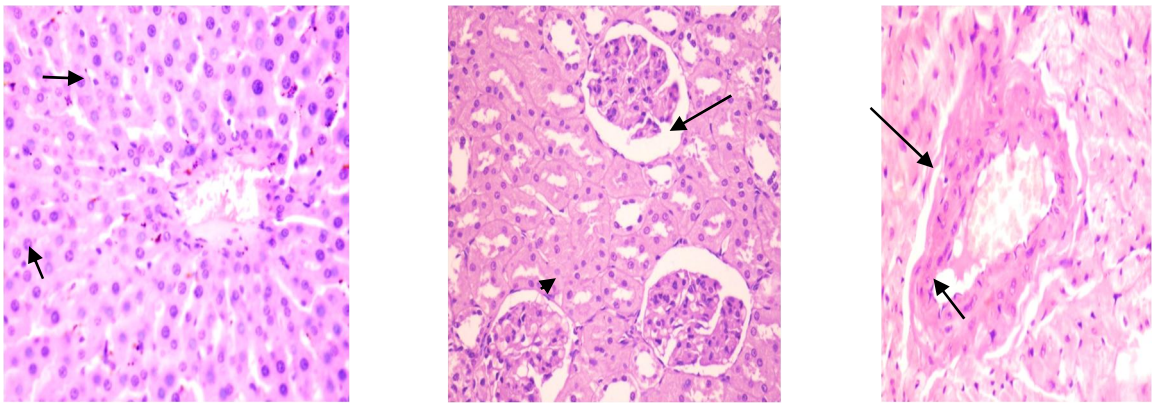

Fig. 3: Representative photomicrographs of the statin-treated group showing (a) liver with mild disorganization of hepatic cords and focal necrobiotic changes of hepatocytes (arrow), (b) kidney with mild shrinkage of glomerular capillary tufts, slight widening of Bowman's space (arrow), and minimal degeneration of tubular epithelial cells (arrowhead), and (c) coronary blood vessel demonstrating mild endothelial damage (arrow). H&E, ×200.

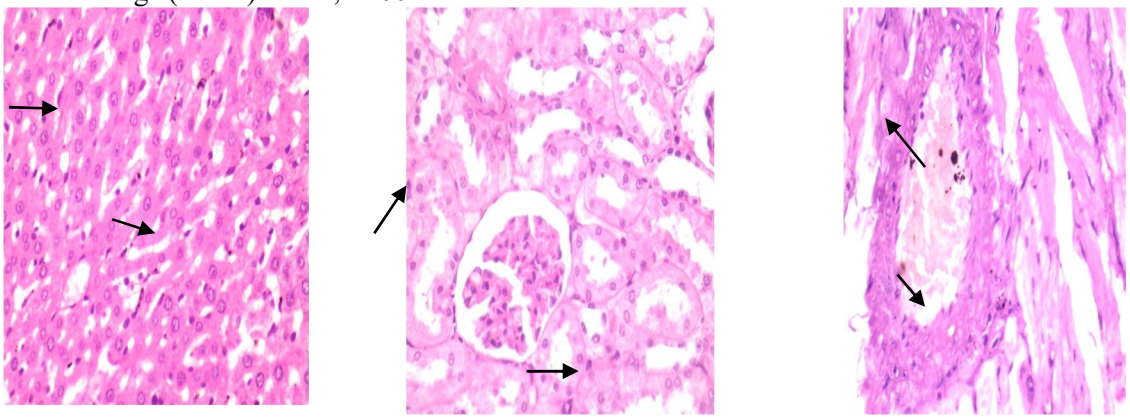

Fig. 4: Representative photomicrographs of the HFQ1-treated group showing (a) liver with nearly normal hepatic lobular architecture (arrow), (b) kidney exhibiting mild swelling of renal tubular epithelial cells (arrow), and (c) coronary blood vessel showing slight endothelial cell damage (arrow). H&E, ×200.

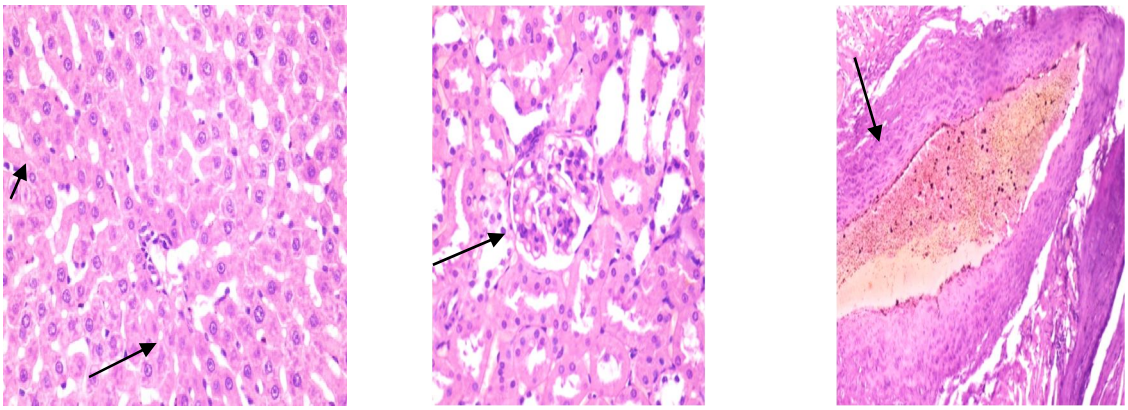

Fig. 5: Representative photomicrographs of the HFQ2-treated group showing (a) liver with preserved normal hepatic lobular architecture composed of radiating cords of polygonal hepatocytes (arrow), (b) kidney with normal histological appearance of glomeruli and renal tubules (arrow), and (c) coronary blood vessel exhibiting intact endothelial lining and well-preserved vascular wall architecture (arrow). H&E, ×200.

Figures (1-5): Histopathological examination of the liver, kidney, and coronary blood vessels.

The normal control group exhibited preserved tissue architecture in all examined organs (Fig. 1). Liver sections showed normal hepatic lobules composed of well-organized radiating hepatic cords with normal hepatocytes, intact sinusoids, and regular distribution of Kupffer cells. Kidney sections demonstrated normal glomeruli and renal tubules with preserved architecture. Coronary blood vessels showed intact endothelial lining with normal tunica intima, internal elastic lamina, and external elastic lamina. No pathological alterations were detected, and all examined organs received zero pathological scores.

In contrast, the HFD-induced obese group showed severe histopathological alterations (Fig. 2). Liver sections revealed disorganization of hepatic cords accompanied by hepatocellular degeneration, necrobiotic changes, and fatty alterations. Kidney sections showed shrinkage of glomerular capillary tufts, widening of Bowman's space, and degeneration of tubular epithelial cells. Coronary blood vessels exhibited endothelial damage with lipid accumulation and foam-cell infiltration, indicating obesity-associated vascular injury.

Treatment with the reference hypolipidemic drug (Statin group) markedly improved tissue morphology compared with the untreated HFD group (Fig. 3). Hepatic sections showed partial restoration of hepatic architecture with mild hepatocellular changes. Renal tissues demonstrated improved glomerular and tubular structure with slight residual alterations. Coronary blood vessels showed only mild endothelial damage while maintaining general vascular organization, indicating the protective effect of statin against HFD-induced tissue injury.

Quinoa supplementation produced significant histological improvement. Animals treated with the first quinoa level (HFQ1) showed considerable preservation of tissue architecture (Fig. 4). Liver sections demonstrated nearly normal hepatic lobules with minimal alterations. Kidney sections revealed only mild tubular epithelial swelling and slight glomerular changes. Coronary blood vessels showed slight endothelial alterations without severe lipid deposition, suggesting moderate protective activity.

The highest protective effect was observed in the second quinoa-treated group (HFQ2) (Fig. 5). Liver sections showed normal hepatic lobules with intact hepatocytes, preserved sinusoids, and absence of degenerative changes. Kidney sections exhibited normal glomerular and tubular structures without detectable pathological lesions. Coronary blood vessels maintained normal endothelial integrity and vascular organization without foam-cell infiltration or lipid accumulation. The pathological scores of this group were comparable to the normal control group.

4. Discussion

Obesity is currently recognized as one of the leading global health challenges because of its close association with dyslipidemia, oxidative stress, mitochondrial dysfunction, chronic low-grade inflammation, and progressive multi-organ damage. Chronic consumption of a high-fat diet (HFD) reproduces many of the metabolic abnormalities observed in human obesity, making it a well-established experimental model for investigating the efficacy of nutritional interventions against obesity-associated complications (Shapiro and Wilk, 1965; Duncan, 1955). In the present study, HFD feeding successfully induced significant body weight gain, severe disturbances in serum lipid profile, impairment of hepatic redox homeostasis, deterioration of mitochondrial bioenergetics, and pronounced histopathological alterations affecting the liver, kidney, and coronary arteries. Collectively, these findings confirm successful establishment of the obesity model and demonstrate that metabolic dysfunction induced by chronic HFD exposure extends beyond excessive fat accumulation to involve multiple interconnected pathological pathways.

Dietary supplementation with quinoa markedly attenuated these metabolic abnormalities. Both supplementation levels significantly improved body weight gain, serum lipid profile, oxidative stress biomarkers, hepatic adenine nucleotide concentrations, and tissue architecture compared with untreated obese animals. However, the higher supplementation level (HFQ2) consistently demonstrated greater efficacy than HFQ1 and, in several measured parameters, produced protective effects comparable with or exceeding those achieved by the reference statin. These findings indicate that quinoa exerts broad multi-target biological activity rather than acting through a single metabolic mechanism.

One of the earliest manifestations of HFD-induced metabolic dysfunction observed in the present study was the marked increase in body weight. Excessive dietary fat intake promotes a sustained

positive energy balance, resulting in adipocyte hypertrophy and expansion of adipose tissue mass (Kruskal and Wallis, 1952). As adipose tissue enlarges, it undergoes profound endocrine and metabolic alterations characterized by increased secretion of pro-inflammatory adipokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and resistin, together with reduced production of protective adiponectin (Kasai *et al.,* 2023). This imbalance contributes to systemic insulin resistance, chronic inflammation, and progressive metabolic dysfunction. In agreement with these mechanisms, untreated HFD-fed rats in the present study exhibited substantial body weight gain accompanied by severe biochemical and histopathological abnormalities.

The significant reduction in body weight observed following quinoa supplementation may be attributed to the unique nutritional composition of quinoa. Unlike many cereal grains, quinoa provides high-quality proteins, abundant dietary fiber, resistant starch, unsaturated fatty acids, vitamins, minerals, and diverse phytochemicals that collectively influence appetite regulation and energy metabolism (Blüher, 2024). Increased dietary fiber delays gastric emptying, enhances satiety, and reduces overall caloric intake, while quinoa-derived peptides have been reported to stimulate satiety-related hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (Rosen and Spiegelman, 2014). In addition, growing evidence suggests that quinoa favorably modulates gut microbiota composition, increasing short-chain fatty acid production and improving whole-body energy expenditure, thereby contributing to reduced adiposity (Ouchi *et al.,* 2011). These complementary mechanisms likely explain the superior reduction in body weight observed in the HFQ2 group.

Obesity-induced dyslipidemia represented another major pathological feature successfully reproduced in the present investigation. HFD feeding significantly elevated serum total cholesterol, triglycerides, LDL-C, and VLDL-C while reducing HDL-C, indicating profound impairment of lipid homeostasis. These findings are consistent with previous reports demonstrating that excessive dietary fat stimulates hepatic de novo lipogenesis through activation of sterol regulatory element-binding protein-1c (SREBP-1c) while simultaneously suppressing fatty acid oxidation, ultimately promoting hepatic lipid accumulation and hyperlipidemia (Saltiel and Olefsky, 2017; Vilcacundo and Hernández-Ledesma, 2017).

Quinoa supplementation markedly improved all measured lipid parameters, with HFQ2 consistently producing greater improvement than HFQ1. The hypolipidemic activity of quinoa is probably mediated through several complementary mechanisms. Soluble dietary fibers reduce intestinal cholesterol absorption by enhancing bile acid excretion, whereas phytosterols compete directly with dietary cholesterol for intestinal uptake, thereby lowering circulating cholesterol concentrations (Graf *et al.,* 2015). Furthermore, quinoa polyphenols and flavonoids activate AMP-activated protein kinase (AMPK), suppress hepatic lipogenesis, and promote fatty acid β-oxidation, leading to simultaneous reductions in serum cholesterol and triglycerides (Wang *et al*., 2022). Restoration of HDL-C together with reductions in LDL-C and VLDL-C observed in the present study therefore reflects comprehensive improvement of lipid metabolism rather than isolated cholesterol lowering.

An important observation was that improvement in serum lipid profile occurred concurrently with attenuation of oxidative stress and restoration of hepatic energy metabolism. This integrated response supports the concept that obesity is a systemic metabolic disorder involving extensive crosstalk between lipid metabolism, mitochondrial function, and cellular redox balance rather than isolated disturbances within individual metabolic pathways (Klop *et al*., 2013). Consequently, nutritional interventions capable of simultaneously targeting these interconnected mechanisms are likely to provide greater therapeutic benefit than approaches directed toward a single metabolic target.

Oxidative stress represents one of the principal mechanisms linking obesity with progressive tissue injury and metabolic dysfunction. Chronic HFD feeding markedly increased hepatic lipid peroxidation, as evidenced by elevated MDA concentrations, together with depletion of reduced glutathione and disruption of the GSH/GSSG redox balance. These findings indicate exhaustion of endogenous antioxidant defenses following prolonged exposure to excessive dietary fat and are consistent with previous reports demonstrating that obesity accelerates reactive oxygen species (ROS) generation through mitochondrial overload and activation of redox-sensitive inflammatory pathways (Shimano and Sato, 2017; Goldstein and Brown, 2015). Persistent ROS production promotes oxidative modification of lipids, proteins, and nucleic acids, ultimately impairing cellular function and accelerating the progression of obesity-associated organ injury.

Quinoa supplementation effectively attenuated oxidative stress, as reflected by reduced MDA concentrations, restoration of GSH levels, decreased GSSG accumulation, and normalization of the GSH/GSSG ratio. The higher supplementation level (HFQ2) consistently demonstrated greater antioxidant efficacy than HFQ1, suggesting a dose-dependent enhancement of antioxidant capacity. The antioxidant activity of quinoa is likely attributable to its rich content of phenolic acids, flavonoids, tocopherols, carotenoids, and other phytochemicals that directly scavenge free radicals while simultaneously enhancing endogenous antioxidant defense systems (Soliman, 2019). In addition, several quinoa-derived polyphenols have been reported to activate the Nrf2 signaling pathway, resulting in increased expression of antioxidant enzymes including glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase, thereby providing sustained cellular protection against oxidative injury (Jones and AbuMweis, 2009).

The observed restoration of glutathione homeostasis is particularly important because glutathione constitutes the major intracellular antioxidant responsible for maintaining redox equilibrium. Preservation of the GSH/GSSG ratio following quinoa supplementation indicates recovery of intracellular reducing capacity and improved resistance to oxidative damage. These findings support the hypothesis that quinoa functions not only as a direct antioxidant but also as a regulator of endogenous antioxidant defense mechanisms, thereby providing prolonged protection against HFD-induced oxidative stress.

Another major finding of the present study was the significant improvement in hepatic energy metabolism following quinoa supplementation. Mitochondrial dysfunction has emerged as a central feature of obesity and metabolic syndrome, with impaired oxidative phosphorylation leading to reduced ATP production, increased AMP accumulation, and diminished cellular energy charge (Hardie, 2015). In the present study, obese untreated animals exhibited marked reductions in ATP and ADP concentrations accompanied by increased AMP levels, confirming profound impairment of hepatic bioenergetics. Such alterations compromise hepatocellular function by limiting energy availability for protein synthesis, membrane transport, antioxidant regeneration, and cellular repair processes.

Treatment with quinoa significantly restored adenine nucleotide concentrations and improved hepatic energy charge, indicating preservation of mitochondrial function. The greater improvement observed in the HFQ2 group suggests that higher quinoa supplementation more effectively maintains mitochondrial integrity during chronic metabolic stress. This beneficial effect may be explained by the close interaction between oxidative stress and mitochondrial function. Excessive ROS production damages mitochondrial membranes and respiratory chain complexes, thereby reducing ATP synthesis. Consequently, attenuation of oxidative stress by quinoa is likely to preserve mitochondrial structure and respiratory efficiency, allowing recovery of cellular energy production (Tang *et al.,* 2016).

In addition to its antioxidant properties, quinoa contains several bioactive compounds capable of regulating intracellular energy metabolism. Previous experimental studies have demonstrated that quinoa polyphenols activate AMP-activated protein kinase (AMPK), a master regulator of cellular energy homeostasis that promotes fatty acid oxidation while suppressing hepatic lipogenesis (Zhong *et al*., 2023). Activation of AMPK improves mitochondrial efficiency, enhances ATP generation, and reduces intracellular lipid accumulation. Therefore, the concurrent improvement of serum lipid profile, oxidative stress biomarkers, and adenine nucleotide concentrations observed in the present study probably reflects coordinated regulation of multiple metabolic pathways rather than isolated biochemical effects.

The biochemical improvements were strongly supported by histopathological observations. Untreated obese animals exhibited severe hepatic injury characterized by hepatocellular degeneration, steatosis, ballooning, inflammatory infiltration, and focal necrosis, confirming the detrimental impact of prolonged HFD consumption on liver structure. Similar pathological alterations have been consistently reported in experimental models of obesity and represent characteristic features of metabolic dysfunction-associated steatotic liver disease (MASLD) (Libby, 2021). In contrast, quinoa-treated animals demonstrated marked preservation of hepatic architecture, with reduced steatosis, minimal inflammatory changes, and restoration of normal hepatic organization, particularly in the HFQ2 group. These morphological findings closely paralleled the improvements observed in oxidative stress biomarkers and mitochondrial bioenergetics, highlighting the central role of oxidative damage in obesity-induced liver injury.

Renal histopathological examination revealed relatively milder alterations than those observed in the liver, which is expected because hepatic tissue represents the primary site of lipid metabolism and therefore is affected earlier during nutritional obesity. Nevertheless, untreated HFD-fed rats developed tubular degeneration and glomerular alterations that were substantially ameliorated following quinoa supplementation. Similarly, preservation of coronary arterial architecture together with reduced endothelial injury further supports the cardioprotective potential of quinoa. These protective effects are likely secondary to improved lipid metabolism, attenuation of oxidative stress, and preservation of endothelial function, collectively limiting the development of obesity-associated vascular injury (Förstermann and Sessa, 2012).

Taken together, the biochemical and histopathological findings clearly demonstrate that the protective effects of quinoa are mediated through multiple complementary mechanisms. Improvement of lipid metabolism was accompanied by restoration of antioxidant defenses and preservation of mitochondrial bioenergetics, ultimately resulting in maintenance of normal tissue architecture. Such coordinated responses emphasize that obesity should be regarded as a systemic metabolic disorder requiring interventions capable of simultaneously targeting interconnected pathological pathways rather than isolated biochemical abnormalities. The present findings therefore support quinoa as a promising functional food capable of providing broad metabolic protection against HFD-induced multi-organ injury.

An important strength of the present study lies in its comprehensive evaluation of obesity-associated metabolic dysfunction using multiple complementary endpoints. Rather than relying exclusively on serum lipid profile or individual oxidative stress markers, the present investigation integrated anthropometric measurements, lipid metabolism, hepatic redox status, mitochondrial bioenergetics, and detailed histopathological examination of three major target organs. This integrated experimental approach enabled a more comprehensive understanding of the biological effects of quinoa supplementation and demonstrated a high degree of consistency between biochemical alterations and morphological findings. The close agreement between restoration of lipid homeostasis, improvement of antioxidant status, preservation of hepatic energy metabolism, and normalization of tissue architecture considerably strengthens the biological significance of the present findings.

Another notable aspect of this study is the direct comparison between two different quinoa supplementation levels using the same experimental model. Although numerous previous investigations have demonstrated beneficial effects of quinoa in obesity and metabolic syndrome, relatively few studies have evaluated whether increasing the level of supplementation provides additional biological benefits (Piché *et al.,* 2020). The consistently greater efficacy observed with HFQ2 suggests that the protective effects of quinoa may be influenced by the administered level, emphasizing the importance of optimizing dietary supplementation to maximize its metabolic benefits. Such findings may contribute to the development of evidence-based nutritional strategies utilizing quinoa as a functional food for the prevention and management of obesity-associated disorders.

The observed improvements are unlikely to be explained by a single active constituent. Instead, the biological activity of quinoa probably results from synergistic interactions among its diverse nutritional and phytochemical components, including dietary fiber, high-quality proteins, unsaturated fatty acids, phenolic compounds, flavonoids, vitamins, minerals, phytosterols, and naturally occurring antioxidants. These constituents influence multiple metabolic pathways simultaneously, improving lipid metabolism, preserving mitochondrial function, reducing oxidative injury, and maintaining cellular redox balance. Consequently, quinoa represents a multi-target nutritional intervention capable of addressing several pathological mechanisms involved in obesity rather than acting through a single pharmacological target.

Despite these encouraging findings, several limitations should be acknowledged. First, although the present study demonstrated significant biochemical and histopathological improvements, the underlying molecular mechanisms were not directly investigated. Evaluation of signaling pathways involved in lipid metabolism, oxidative stress, and mitochondrial regulation, including AMPK, Nrf2, NF-κB, PPARα, and SREBP-1c, would provide further mechanistic insight into the protective effects of quinoa. Second, circulating inflammatory mediators such as TNF-α, IL-6, IL-1β, leptin, and adiponectin were not determined, limiting assessment of the inflammatory component of obesity. Third, gut microbiota composition was not evaluated despite increasing evidence suggesting that

modulation of intestinal microbial communities contributes substantially to the metabolic benefits of quinoa (Furukawa and Fujita, 2004). Finally, the present investigation was performed in an experimental animal model; therefore, extrapolation of these findings to humans should be undertaken with caution until confirmed by well-designed clinical studies.

Future investigations should focus on integrating molecular biology, metabolomics, and microbiome analyses to provide a more detailed understanding of the mechanisms underlying quinoa-mediated protection. Determination of gene and protein expression related to mitochondrial biogenesis, oxidative stress, inflammation, and lipid metabolism would further clarify the signaling pathways responsible for the observed biological effects. In addition, long-term intervention studies evaluating different quinoa doses, processing methods, and dietary formulations are warranted to establish optimal nutritional recommendations. Carefully designed randomized clinical trials will also be essential to determine whether the metabolic improvements observed in experimental animals can be translated into obese human populations (Sies, 2015).

Overall, the present findings demonstrate that quinoa supplementation exerts broad multi-target protective effects against HFD-induced obesity through coordinated improvement of lipid metabolism, antioxidant defense, mitochondrial bioenergetics, and tissue integrity. The consistency observed between biochemical, metabolic, and histopathological outcomes provides strong evidence that the beneficial actions of quinoa extend well beyond simple cholesterol lowering. The higher supplementation level (HFQ2) consistently exhibited superior efficacy compared with HFQ1 across the evaluated biochemical and histopathological parameters, highlighting the importance of optimizing quinoa supplementation to maximize its biological activity. Collectively, these findings support the growing concept that *Chenopodium quinoa* may serve as an effective functional food capable of complementing conventional therapeutic approaches for reducing obesity-associated hepatic, renal, and cardiovascular complications while improving overall metabolic homeostasis.

5. Conclusion

Dietary supplementation with *Chenopodium quinoa* effectively mitigated high-fat diet-induced metabolic dysfunction and multi-organ injury in rats. Quinoa significantly improved body weight gain, corrected dyslipidemia, restored hepatic redox homeostasis, preserved mitochondrial bioenergetics, and ameliorated histopathological alterations in the liver, kidney, and coronary arteries. The higher supplementation level (HFQ2) consistently exhibited greater efficacy than HFQ1 and demonstrated protective effects comparable to, and in some parameters exceeding, those of the reference statin. These findings highlight quinoa as a promising functional food with multi-target biological activity and support its potential as a complementary nutritional strategy for preventing obesity-associated metabolic, hepatic, renal, and cardiovascular complications.

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