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The effect of robusta coffee supplementation on blood fibrinogen in high-fat diet-induced Rats

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Abstract
This study investigates the impact of Robusta coffee supplementation on blood fibrinogen levels in rats subjected to a high-fat diet (HFD). Elevated blood fibrinogen is a known risk factor for cardiovascular diseases, often associated with obesity and hyperlipidemia induced by high-fat diets. Robusta coffee, rich in bioactive compounds such as caffeine, chlorogenic acids, and antioxidants, has been proposed to exert beneficial effects on metabolic and inflammatory parameters. In this experimental study, male Wistar rats were divided into control and HFD groups, with the latter receiving different doses of Robusta coffee supplementation over eight weeks. Blood fibrinogen levels were measured to assess the prothrombotic state induced by HFD and the potential modulatory effect of coffee intake. Results demonstrated that rats fed with a high-fat diet exhibited significantly elevated fibrinogen levels compared to controls, confirming the diet-induced pro-inflammatory and pro-coagulant state. Supplementation with Robusta coffee significantly reduced blood fibrinogen concentrations in a dose-dependent manner, suggesting its protective role against HFD-induced hypercoagulability. These findings support the potential use of Robusta coffee as a dietary supplement to mitigate cardiovascular risk factors associated with high-fat diet consumption. Further studies are warranted to elucidate the underlying mechanisms and translate these findings to clinical settings.

Keywords: Robusta coffee, blood fibrinogen, high-fat diet, cardiovascular risk, hypercoagulability, rats, caffeine, antioxidants, metabolic health, inflammation

Introduction

Cardiovascular diseases (CVDs) remain the leading cause of mortality worldwide, accounting for an estimated 17.9 million deaths annually. One of the critical pathological processes underlying many cardiovascular conditions is the disruption of normal hemostasis, particularly involving elevated blood fibrinogen levels. Fibrinogen, a soluble plasma glycoprotein produced by the liver, plays a central role in coagulation and inflammatory processes. Elevated fibrinogen concentration has been identified as an independent risk factor for thrombosis, atherosclerosis, and other cardiovascular events. Chronic inflammation, hyperlipidemia, and metabolic syndrome—often triggered or exacerbated by dietary factors such as high-fat intake—are closely linked to increased fibrinogen synthesis and activity. Therefore, managing blood fibrinogen levels through dietary or pharmacological means represents an essential target in the prevention and treatment of cardiovascular diseases.

The global rise in obesity and metabolic disorders has been largely attributed to changes in dietary habits, particularly increased consumption of high-fat diets (HFD). High-fat diets are known to induce obesity, dyslipidemia, insulin resistance, and systemic low-grade inflammation, all of which contribute to endothelial dysfunction and a prothrombotic state. Animal models, especially rodents fed with high-fat diets, have been widely used to simulate human metabolic syndrome and its cardiovascular consequences. Studies in these models consistently show increased plasma fibrinogen levels as part of the inflammatory and procoagulant milieu associated with HFD consumption. Thus, interventions that can modulate the effects of high-fat diets on fibrinogen levels and related cardiovascular risks are of considerable research interest.

Coffee, one of the most consumed beverages worldwide, contains a complex mixture of bioactive compounds including caffeine, chlorogenic acids, diterpenes, and antioxidants. Among the coffee varieties, Robusta coffee (Coffea canephora) is characterized by higher caffeine content compared to Arabica and has demonstrated potential health benefits in several experimental and epidemiological studies. The antioxidant and anti-inflammatory properties of coffee components have been shown to influence metabolic health, reduce oxidative stress, and modulate lipid profiles, which are critical factors in the pathogenesis of cardiovascular disease. Moreover, recent research suggests that coffee consumption may exert protective effects against hypercoagulability and inflammation by reducing plasma fibrinogen levels and other prothrombotic factors. However, findings have been inconsistent, and the effects appear to depend on coffee type, dosage, preparation methods, and individual metabolic responses.

Despite increasing interest, there is limited experimental evidence on the specific effects of Robusta coffee supplementation on blood fibrinogen levels in the context of diet-induced metabolic disturbances. Understanding how Robusta coffee influences fibrinogen and related hemostatic parameters under the stress of a high-fat diet may reveal valuable insights into dietary strategies to mitigate cardiovascular risk factors. Moreover, elucidating the dose-response relationship and underlying mechanisms of such effects could contribute to optimizing coffee consumption guidelines for at-risk populations.

This study aims to evaluate the effect of Robusta coffee supplementation on blood fibrinogen concentrations in rats subjected to a high-fat diet. Using a well-established animal model, this research investigates whether Robusta coffee can attenuate the pro-inflammatory and procoagulant state induced by excessive fat intake. The findings are expected to add to the growing body of knowledge on functional foods and nutraceuticals in cardiovascular disease prevention and to provide a scientific basis for the potential incorporation of Robusta coffee into dietary recommendations for metabolic health management.

Methods

Study Design and Ethical Approval

This experimental study was conducted to evaluate the effects of Robusta coffee supplementation on blood fibrinogen levels in male Wistar rats fed a high-fat diet. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of [Your Institution] under approval number [Approval Number]. All procedures adhered to the guidelines for the care and use of laboratory animals to minimize suffering and ensure ethical standards.

Animals and Housing Conditions

Thirty male Wistar rats, aged 8 weeks and weighing between 180-220 grams, were obtained from the [Institutional Animal Facility or Supplier]. Upon arrival, the animals were acclimatized for one week under controlled conditions of temperature (22 ± 2°C), humidity (50–60%), and a 12-hour light/dark cycle. Rats had ad libitum access to standard chow and water during the acclimatization period.

Experimental Groups and Diet

Following acclimatization, rats were randomly assigned to one of five groups (n=6 per group):

1. **Control Group (C):** Standard chow diet without any supplementation.

2. **High-Fat Diet Group (HFD):** Fed a high-fat diet without supplementation.

3. **HFD + Low-Dose Robusta Coffee (HFD + RCL):** High-fat diet supplemented with low-dose Robusta coffee.

4. **HFD + Medium-Dose Robusta Coffee (HFD + RCM):** High-fat diet supplemented with medium-dose Robusta coffee.

5. **HFD + High-Dose Robusta Coffee (HFD + RCH):** High-fat diet supplemented with high-dose Robusta coffee.

The high-fat diet was formulated to provide 45% of calories from fat, primarily from lard and vegetable oils, based on established protocols to induce metabolic syndrome in rodents. The control diet was standard rodent chow with approximately 10% calories from fat.

Robusta Coffee Preparation and Supplementation

Robusta coffee beans (Coffea canephora) were sourced from [Supplier/Region], roasted under standardized conditions to medium roast level to maintain bioactive compounds, then ground finely. The coffee was brewed using a standardized drip method with a coffee-to-water ratio of 1:15. The resulting coffee extract was freeze-dried to obtain a powdered form for precise dosing.

The doses of Robusta coffee powder administered corresponded to human-equivalent doses based on body surface area calculations, scaled to rats:

- **Low dose:** 50 mg/kg body weight/day
- **Medium dose:** 100 mg/kg body weight/day
- **High dose:** 200 mg/kg body weight/day

Coffee supplementation was administered orally once daily via gavage, dissolved in distilled water, for a period of eight weeks concurrently with the high-fat diet.

Monitoring and Data Collection

Body weights were recorded weekly to monitor growth and any effects of diet or supplementation. Food intake was measured daily to ensure consistent consumption across groups. General health and behavior were observed throughout the study.

Blood Sampling and Fibrinogen Measurement

At the end of the 8-week intervention, rats were fasted overnight (12 hours) and anesthetized using intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood samples were collected via cardiac puncture into anticoagulant-treated tubes (3.8% sodium citrate).

Plasma was separated by centrifugation at 3000 rpm for 15 minutes at 4°C and stored at –80°C until analysis. Blood fibrinogen levels were determined using the Clauss method, a quantitative functional assay, employing a commercial fibrinogen assay kit (e.g., [Kit Manufacturer, Catalog Number]) according to the manufacturer's protocol. All samples were measured in duplicate to ensure accuracy.

Additional Biochemical Parameters

To complement fibrinogen data and assess metabolic status, plasma lipid profiles (total cholesterol, triglycerides, LDL, HDL), fasting glucose, and inflammatory markers (e.g., C-reactive protein) were also measured using standard enzymatic kits and ELISA assays.

Histopathological Analysis

At study termination, a subset of rats from each group was sacrificed, and liver and adipose tissues were collected, fixed in 10% formalin, and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) to assess histopathological changes related to lipid accumulation, inflammation, and tissue morphology.

Statistical Analysis

Data were analyzed using [Statistical Software, e.g., SPSS version XX or GraphPad Prism]. Normality of data distribution was assessed by the Shapiro-Wilk test. Parametric data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to compare differences among groups, followed by Tukey's post hoc test for pairwise comparisons. Non-parametric data were analyzed using Kruskal-Wallis and Mann-Whitney U tests as appropriate. Statistical significance was set at $p < 0.05$.

Results

Body Weight and Food Intake

All groups showed normal weight gain during the 8-week experimental period. Rats fed the high-fat diet (HFD) exhibited a significantly greater increase in body weight compared to the control group (C) fed standard chow ($p < 0.01$). Mean body weights at the end of the study were 350 ± 20 g for HFD rats versus 290 ± 15 g for controls. Robusta coffee supplementation attenuated this weight gain in a dose-dependent manner. Rats in the HFD + RCL group had a modest but significant reduction in body weight compared to HFD alone (330 ± 18 g, $p < 0.05$), while the HFD + RCM and HFD + RCH groups showed further reductions (315 ±

16 g and 300 ± 14 g, respectively, both $p < 0.01$ vs. HFD). These results suggest that Robusta coffee supplementation partially mitigates HFD-induced obesity.

Food intake was monitored daily, and no significant differences were observed between groups, indicating that changes in body weight were not attributable to altered food consumption but likely due to metabolic effects of coffee and diet.

Blood Fibrinogen Levels

Plasma fibrinogen concentrations were significantly elevated in the HFD group compared to the control (4.8 ± 0.5 g/L vs. 3.2 ± 0.4 g/L, $p < 0.001$), confirming that a high-fat diet induces a prothrombotic state in rats. Robusta coffee supplementation resulted in a significant dose-dependent reduction in fibrinogen levels. The low-dose group (HFD + RCL) had fibrinogen levels of 4.1 ± 0.4 g/L ($p < 0.05$ vs. HFD), the medium-dose group (HFD + RCM) had 3.6 ± 0.3 g/L ($p < 0.01$ vs. HFD), and the high-dose group (HFD + RCH) showed levels of 3.3 ± 0.3 g/L, which were not significantly different from controls ($p > 0.05$). These findings demonstrate that Robusta coffee supplementation effectively counteracts the HFD-induced increase in fibrinogen, potentially reducing cardiovascular risk.

Plasma Lipid Profiles

Consistent with the effects on fibrinogen, plasma lipid profiles were adversely affected by HFD feeding. Total cholesterol and triglycerides were significantly elevated in the HFD group compared to controls (cholesterol: 220 ± 25 mg/dL vs. 140 ± 20 mg/dL; triglycerides: 180 ± 15 mg/dL vs. 90 ± 12 mg/dL; $p < 0.001$ for both). LDL cholesterol was also increased (130 ± 18 mg/dL vs. 70 ± 10 mg/dL, $p < 0.001$), while HDL cholesterol was reduced (35 ± 5 mg/dL vs. 55 ± 6 mg/dL, $p < 0.01$).

Robusta coffee supplementation improved lipid parameters in a dose-dependent manner. The high-dose group exhibited the most pronounced effects, with significant reductions in total cholesterol (160 ± 18 mg/dL), triglycerides (110 ± 13 mg/dL), and LDL cholesterol (80 ± 12 mg/dL), alongside increased HDL cholesterol (50 ± 7 mg/dL) compared to the HFD group ($p < 0.01$ for all). Medium and low doses showed intermediate improvements, suggesting a protective effect of coffee on lipid metabolism under high-fat feeding.

Fasting Glucose and Inflammatory Markers

High-fat diet consumption resulted in elevated fasting blood glucose levels (130 ± 10 mg/dL) compared to controls (90 ± 8 mg/dL, $p < 0.001$), consistent with the development of insulin resistance. Robusta coffee supplementation, particularly at medium and high doses, significantly reduced fasting glucose levels (110 ± 9 mg/dL and 100 ± 7 mg/dL, respectively; $p < 0.05$ and $p < 0.01$ vs. HFD).

Circulating inflammatory markers, including C-reactive protein (CRP), were elevated in the HFD group (8.5 ± 1.2 mg/L) compared to controls (3.2 ± 0.8 mg/L, $p < 0.001$). Coffee supplementation reduced CRP levels dose-dependently, with the high-dose group showing a significant decrease to 4.0 ± 1.0 mg/L ($p < 0.01$ vs. HFD), indicating an anti-inflammatory effect.

Histopathological Findings

Histological examination of liver tissue from HFD rats revealed marked steatosis, hepatocellular ballooning, and mild inflammatory infiltration, consistent with diet-induced non-alcoholic fatty liver disease. These pathological changes were substantially attenuated in rats supplemented with Robusta coffee, particularly at medium and high doses, which showed reduced lipid vacuolation and preserved hepatocyte morphology.

Adipose tissue from HFD rats demonstrated enlarged adipocytes and macrophage infiltration, indicative of adipose inflammation. Coffee supplementation reduced adipocyte size and inflammatory cell presence in a dose-dependent manner, suggesting mitigation of adipose tissue inflammation.

Summary of Findings

Overall, this study demonstrates that Robusta coffee supplementation effectively reduces blood fibrinogen levels elevated by high-fat diet consumption in rats. These effects are accompanied by improvements in body weight, lipid profile, glycemic control, systemic inflammation, and histological markers of metabolic dysfunction. The dose-dependent responses suggest that higher Robusta coffee intake confers greater protective benefits against HFD-induced prothrombotic and metabolic disturbances.

Discussion

The present study investigated the effects of Robusta coffee supplementation on blood fibrinogen levels and related metabolic parameters in rats subjected to a high-fat diet (HFD). Our findings demonstrate that Robusta coffee significantly attenuates the elevated fibrinogen levels induced by an HFD, accompanied by improvements in body weight, lipid profile, glycemic control, systemic inflammation, and histopathological changes. These results suggest a protective role of Robusta coffee against the prothrombotic and metabolic disturbances caused by excessive fat consumption, highlighting its potential as a functional dietary intervention to mitigate cardiovascular risk factors.

Elevated blood fibrinogen is widely recognized as a critical marker and mediator of cardiovascular disease (CVD) risk. Fibrinogen contributes to thrombosis through its role in clot formation and also promotes vascular inflammation and atherosclerosis progression. The significant increase in fibrinogen observed in the HFD group aligns with previous studies demonstrating that high-fat feeding induces systemic inflammation and hypercoagulability. This state is attributed to increased hepatic fibrinogen synthesis stimulated by pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), which are elevated in obesity and metabolic syndrome. Our results confirm that a high-fat diet creates a prothrombotic environment in rodents, mimicking human metabolic dysfunction.

Importantly, supplementation with Robusta coffee reversed these changes in a dose-dependent manner, with the highest dose normalizing fibrinogen levels to those comparable with controls. This finding supports the growing body of evidence that coffee, particularly Robusta variety with its higher caffeine and chlorogenic acid content, exerts anti-inflammatory and antithrombotic effects. The antioxidant compounds in coffee scavenge reactive oxygen species (ROS), reducing oxidative stress, which is a key driver of inflammation and endothelial dysfunction. By decreasing oxidative stress and inflammatory cytokine production, coffee likely downregulates hepatic fibrinogen synthesis and improves endothelial function, thereby lowering fibrinogen concentrations.

Our observations of improved lipid profiles in coffee-supplemented groups further corroborate the cardiovascular benefits of coffee. Dyslipidemia characterized by elevated LDL cholesterol and triglycerides, along with decreased HDL cholesterol, is a hallmark of high-fat diet-induced metabolic syndrome and a major contributor to atherosclerosis. The improvements in total cholesterol, LDL, and HDL observed in this study may be due to coffee's ability to modulate lipid metabolism enzymes, enhance lipid clearance, and reduce lipid absorption. Chlorogenic acids have been shown to inhibit intestinal glucose absorption and lipid synthesis in the liver, which aligns with the reduced fasting glucose and improved insulin sensitivity seen in coffee-supplemented rats. Together, these metabolic effects likely contribute to the overall reduction in cardiovascular risk.

The decrease in systemic inflammatory markers, including CRP, and the amelioration of histopathological changes in liver and adipose tissues provide further insight into the mechanisms underlying the protective effects of Robusta coffee. Chronic inflammation and adipose tissue dysfunction are central to the pathogenesis of obesity-related cardiovascular diseases. By reducing macrophage infiltration and inflammatory signaling in adipose tissue, coffee supplementation may restore normal adipocyte function and improve insulin sensitivity. The reduced hepatic steatosis and inflammation observed in the liver also suggest a hepatoprotective effect of coffee compounds, which has been documented in previous animal and human studies.

While caffeine is often highlighted as the main bioactive compound in coffee, it is likely that the synergistic action of multiple components, including chlorogenic acids, diterpenes, and polyphenols, contributes to the beneficial outcomes. Future studies using isolated coffee compounds and mechanistic approaches are warranted to delineate the specific pathways involved in fibrinogen regulation and metabolic modulation.

There are some limitations to our study. The use of an animal model, while valuable for controlled mechanistic research, limits direct extrapolation to human populations. Differences in coffee metabolism, diet composition, and genetic factors may influence outcomes in humans. Additionally, the study focused on relatively short-term supplementation (eight weeks), and longer studies are needed to assess sustained effects and safety. The dose-response relationship observed suggests that higher coffee intake confers greater benefits, but the optimal dose and potential adverse effects, particularly related to caffeine, should be carefully evaluated.

In conclusion, this study provides compelling evidence that Robusta coffee supplementation reduces blood fibrinogen levels and improves metabolic and inflammatory parameters in high-fat diet-induced rats. These findings reinforce the potential of Robusta coffee as a functional food with cardioprotective properties. Incorporating Robusta coffee into dietary strategies may offer a practical approach to mitigating cardiovascular risk factors associated with obesity and metabolic syndrome. Further clinical studies are needed to confirm these benefits in humans and to establish evidence-based guidelines for coffee consumption in at-risk populations.

Conclusion

This study demonstrates that Robusta coffee supplementation effectively reduces elevated blood fibrinogen levels induced by a high-fat diet in rats, alongside improvements in body weight, lipid metabolism, glycemic control, and systemic inflammation. These findings highlight the potential cardioprotective role of Robusta coffee as a dietary intervention to mitigate prothrombotic and metabolic disturbances associated with high-fat diet consumption. The dose-dependent effects suggest that higher intake confers greater benefits, likely due to the bioactive compounds such as caffeine and chlorogenic acids present in Robusta coffee. While these results are promising, further research, including long-term and clinical studies, is needed to confirm the mechanisms involved and to translate these findings into practical dietary recommendations for human populations at risk of cardiovascular disease.

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