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Effects of omega-3-enriched eggs on HDL cholesterol: an in vivo study

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ABSTRACT

Background: Low high-density lipoprotein (HDL) cholesterol remains an important risk factor for cardiovascular disease. Functional foods enriched with omega-3 fatty acids may offer a practical dietary approach to improving lipid profiles.

Purpose: To evaluate the effects of omega-3 enriched eggs on HDL cholesterol levels in Wistar rats compared with regular eggs and a standard diet.

Methods: Fifteen male Wistar rats were randomly assigned to three groups: control (standard diet), regular egg, and omega-3 enriched egg. Egg yolk preparations were administered orally via gastric gavage for 14 days. HDL cholesterol was measured before and after the intervention using an enzymatic colorimetric assay. Data were analyzed using paired t-tests for intragroup comparisons and one-way ANOVA with Tukey post-hoc testing for between-group differences.

Results: The omega-3 enriched egg group showed a significant increase in HDL cholesterol (from 39.83 ± 6.08 to 66.32 ± 4.35 mg/dL; $p = 0.001$), while increases in the regular egg ($p = 0.093$) and control groups ($p = 0.454$) were not significant. ANOVA indicated significant differences among groups ($F(2,12) = 6.308$, $p = 0.013$), with post-hoc analysis confirming higher HDL levels in the omega-3 group compared with the control group ($p = 0.011$).

Conclusion: Omega-3 enriched eggs elicited a substantial increase in HDL cholesterol compared with regular eggs and a standard diet, supporting their potential as a functional food for improving lipid profiles. Further studies with larger samples, extended intervention periods, and human participants are recommended to validate their clinical relevance.

INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of mortality globally and in Indonesia, with dyslipidemia—particularly low high-density lipoprotein (HDL) cholesterol—recognized as a major contributor to atherosclerosis progression. Simple, accessible dietary strategies capable of raising HDL levels are therefore increasingly important for population-level prevention efforts. HDL plays a central protective role through reverse cholesterol transport (RCT) and exerts antioxidant and anti-inflammatory effects, making both its concentration and functional quality essential for cardiovascular integrity.[1-4]

The burden of dyslipidemia continues to rise alongside unhealthy dietary patterns and sedentary lifestyles, especially in low- and middle-income settings.[5,6] Dietary modification is considered one of the most feasible approaches to improve lipid profiles, including the incorporation of functional foods enriched with bioactive compounds. Omega-3 fatty acids—particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—have been consistently shown to modulate lipid metabolism by enhancing apoA-I synthesis, activating HDL particles, and reducing inflammation and oxidative stress, ultimately improving HDL functionality.[7]

Omega-3-enriched eggs have emerged as an accessible functional food capable of delivering EPA and DHA in higher concentrations than regular eggs. Evidence suggests that such eggs may increase HDL cholesterol and reduce triglycerides, offering a practical source of omega-3 fatty acids for daily consumption.[8,9] However, despite extensive

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research on omega-3 supplementation, limited studies have directly compared the lipid-modulating effects of regular eggs versus omega-3-enriched eggs in an in vivo model. It also remains unclear whether omega-3 delivered through natural food matrices elicits HDL improvements comparable to purified omega-3 supplements. This gap mirrors broader questions in functional food research, where bioactive compounds such as aegeline and fucoxanthin have demonstrated mechanistic lipid effects but with limited translation into food-based interventions.[10,11]

Preclinical animal models such as Wistar rats are widely used to investigate dietary interventions due to their metabolic similarities to humans, particularly in lipid metabolism.[12,13] These models enable controlled examination of biological mechanisms and treatment effects before progressing to clinical studies. This study aimed to compare the effects of regular eggs and omega-3-enriched eggs on HDL cholesterol levels in Wistar rats. Findings from this research are expected to strengthen the evidence base for functional foods as practical dietary strategies in dyslipidemia management.

METHOD

Study Design

This study employed a quasi-experimental pre-test–post-test control group design to evaluate the effects of omega-3 enriched eggs on HDL cholesterol levels in male Wistar rats.[14] Three experimental conditions were established: a control group receiving standard laboratory chow, a group receiving regular egg yolk in addition to standard chow, and a group receiving omega-3 enriched egg yolk in addition to standard chow. HDL cholesterol levels were measured before and after the intervention to assess changes attributable to each treatment.

Research Setting

The experiment was conducted in the Laboratory of the Research, Publication, and Community Service Unit (UP3M), Faculty of Medicine, Universitas Muslim Indonesia, Makassar. All procedures were carried out in August 2025 under controlled laboratory conditions suitable for small-animal experimentation and biochemical analysis.

Animals, Housing, and Allocation

Fifteen male Wistar rats (Rattus norvegicus), aged 8–10 weeks and weighing 200–250 g, were used in this study. Animals were obtained from the institutional laboratory animal facility and screened to ensure they met the inclusion criteria, which included normal physical appearance, active behavior, and the absence of illness or prior experimental use. Rats exhibiting signs of illness during acclimatization or the intervention period were designated for exclusion; however, no exclusions occurred, and all animals completed the study. Sample size determination using Mead's resource equation yielded a value of E=13, indicating an adequate sample size for small-animal experimental research. Based on this calculation, the fifteen rats were divided evenly into three groups of five animals each.

All rats were housed in standard polypropylene cages containing wood-shaving bedding under controlled environmental conditions, including a temperature range of 22–25°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. Standard laboratory chow and drinking water were provided ad libitum. A seven-day acclimatization period preceded the intervention, during which animals were monitored daily for overall health and activity.

Following acclimatization, rats were randomly allocated to the three experimental groups using a simple random number generator. The control group received only standard chow, the regular egg group received regular egg yolk in addition to chow, and the omega-3 enriched egg group received omega-3 enriched egg yolk in addition to chow. Blinding was not implemented due to practical constraints associated with direct oral administration and monitoring of treatment adherence. The overall study timeline consisted of acclimatization, baseline blood collection on Day 7, a 14-day intervention from Day 8 to Day 21, and final blood collection on Day 22. Because only blood sampling was required and no tissue collection was performed, euthanasia was not conducted, and all animals were returned to the facility after the study.

Intervention and Dose Calculation

The intervention dose was calculated using allometric scaling based on human intake of approximately 100 grams of omega-3 enriched eggs per day (equivalent to two eggs). Applying the metabolic conversion factor of 0.018 for rats resulted in a rat-equivalent dose of 1.8 grams per 200 grams of body weight.[15] Considering the density of whole egg (approximately 1.035 g/mL), this dose corresponded to a daily volume of approximately 1.74 mL. The same dose and volume were used in both egg treatment groups to ensure comparability of energy intake and isolate the effects of omega-3 content. Egg yolks were homogenized and administered orally via gastric gavage once daily for 14 consecutive days. Throughout the intervention period, all animals were observed for changes in behavior, feeding patterns, and potential signs of distress.

Blood Collection and HDL Measurement

Blood samples were collected on Day 7 (baseline) and Day 22 (post-intervention) using retro-orbital venous sampling under light anesthesia. Approximately 1–1.5 mL of blood was drawn from each rat into EDTA tubes. Plasma was separated by centrifugation at 3000 rpm for five minutes. HDL cholesterol levels were measured using an enzymatic colorimetric assay involving precipitation of non-HDL lipoproteins with phosphotungstic acid, followed by spectrophotometric measurement at 500 nm using a UV–Vis spectrophotometer. All analyses were performed in accordance with the manufacturer's guidelines.[16]

Statistical Analysis

Data analysis was conducted using SPSS version 29.0. Normality of distribution was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene's test. Within-group pre- and post-intervention comparisons were performed using paired t-tests. Between-group differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to identify specific intergroup differences. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

All experimental procedures adhered to the ARRIVE guidelines and the NIH Guide for the Care and Use of Laboratory Animals (8th edition). Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muslim Indonesia (Approval No. 531/A.1/KEP-UMI/VIII/2025). All blood collection and handling procedures were performed by trained personnel, and all efforts were made to minimize discomfort and distress throughout the study.

RESULTS

A total of fifteen rats completed the study and were included in the analysis. Baseline HDL cholesterol levels were comparable across the three groups, indicating no initial differences prior to the intervention.

Descriptive and Intragroup Analysis

Changes in HDL cholesterol levels before and after the 14-day intervention are summarized in Table 1. The omega-3 enriched egg group demonstrated a marked increase in HDL levels, rising from 39.83 ± 6.08 mg/dL to 66.32 ± 4.35 mg/dL, representing a statistically significant improvement ($p = 0.001$). The regular egg group showed a non-significant increase from 46.90 ± 7.96 mg/dL to 59.72 ± 18.29 mg/dL ($p = 0.093$), while the control group exhibited only minimal change (37.43 ± 12.10 mg/dL to 40.99 ± 7.21 mg/dL, $p = 0.454$).

Table 1. HDL Cholesterol Levels (mg/dL) Before and After the Intervention

Group	Pre-test	Post-test	% Change	p-value
Omega-3 Egg	39.83 ± 6.08	66.32 ± 4.35	+66.5%	0.001
Regular Egg	46.90 ± 7.96	59.72 ± 18.29	+27.3%	0.093
Control	37.43 ± 12.10	40.99 ± 7.21	+9.5%	0.454

Between-group Analysis

A one-way ANOVA revealed a significant difference in post-intervention HDL levels among the three groups ($F(2,12) = 6.308$, $p = 0.013$), with a large effect size ($\eta^2 = 0.512$), indicating that the intervention accounted for a substantial proportion of variance in HDL outcomes (Table 2).

Table 2. One-way ANOVA Results for Post-test HDL Levels

Analysis	Statistic Value	p-value	Description
ANOVA (F (2,12))	6.308	0.013	Significant difference
Eta squared (η^2)	0.512	–	Large effect size

Post-hoc Analysis

Tukey's post-hoc test showed that the omega-3 enriched egg group had significantly higher post-intervention HDL levels compared with the control group ($p = 0.011$). No significant differences were observed between the regular egg and control groups or between the regular egg and omega-3 egg groups (Table 3).

Table 3. Tukey Post-hoc Test for Pairwise Comparisons of Post-test HDL Levels

Comparison	Mean Diff (mg/dL)	Adjusted p-value
Control vs Omega-3 Egg	–22.921	0.011
Control vs Regular Egg	–9.259	0.359
Omega-3 Egg vs Regular Egg	+13.663	0.131

Changes in HDL Levels

Figure 1 illustrates the change in HDL cholesterol levels across the three groups, with the omega-3 enriched egg group showing the largest increase.

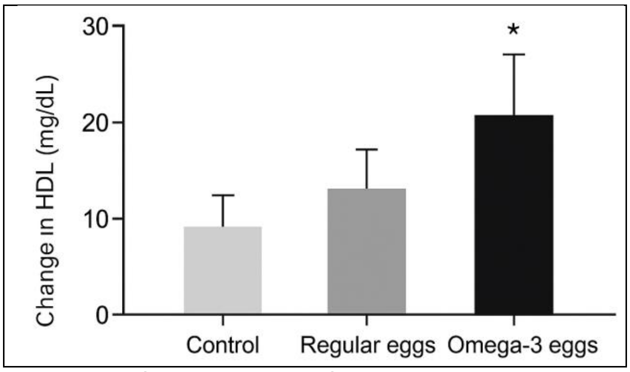

Figure 1. Change in HDL Cholesterol Levels Across Difference Group

DISCUSSION

This study demonstrated that omega-3 enriched eggs produced a significant elevation in HDL cholesterol levels in Wistar rats compared with both regular eggs and the control group. The omega-3 group exhibited a 66.5% increase in HDL concentrations, whereas the regular egg group showed a modest, non-significant rise of 27.3%, and the control group experienced minimal change. These findings indicate that dietary enrichment with omega-3 fatty acids confers a substantially greater lipid-modulating effect than standard eggs or a normal diet within the 14-day intervention period.

The pronounced elevation in HDL levels in the omega-3 group is consistent with the established molecular actions of EPA and DHA, which modulate lipid metabolism through multiple biological pathways. Omega-3 fatty acids activate peroxisome proliferator-activated receptor gamma (PPAR-γ), thereby enhancing insulin sensitivity, regulating adipocyte differentiation, and improving hepatic lipid handling.[17,18] Additionally, EPA and DHA have been shown to inhibit cholesteryl ester transfer protein (CETP), a key regulator of HDL remodeling, leading to reduced CE transfer from HDL to apoB-containing lipoproteins and resulting in higher circulating HDL concentrations.[19] Their anti-inflammatory and antioxidant properties further support HDL stability and function by suppressing cytokine-mediated oxidative lipid disruption.[20,21] Collectively, these mechanisms provide strong biological plausibility for the substantial increase in HDL observed in the omega-3 enriched egg group.

Although both egg groups received additional lipid sources, the divergent outcomes may reflect differences in omega-3 content, bioavailability, and dose adequacy. Regular eggs contain only small amounts of EPA and DHA, which may be insufficient to produce measurable metabolic changes over a short intervention period. This interpretation aligns with evidence indicating that higher doses and longer durations of omega-3 intake yield more robust lipid responses.[22] Furthermore, enriched eggs deliver EPA/DHA in a natural food matrix, which may influence digestion and incorporation into lipoprotein pathways differently than synthetic supplementation. Previous studies have highlighted that omega-3 enriched eggs can improve HDL profiles and contribute to functional lipoprotein improvements.[23] When compared with omega-3 supplements, enriched eggs may offer advantages in dietary acceptability and sustained adherence, although supplements can achieve greater triglyceride reduction due to higher EPA/DHA concentrations.[24–27]

The translational relevance of these findings must be considered within the context of rodent physiology. Rats exhibit inherently high HDL levels and lack CETP activity, a fundamental difference from human lipoprotein metabolism.[28] These physiological characteristics may exaggerate HDL responses and limit direct extrapolation to humans. The genetically homogeneous population and controlled laboratory conditions further reduce variability but may also limit generalizability to free-living populations.[29] Nevertheless, animal models remain valuable for elucidating mechanistic effects prior to clinical studies, particularly when dietary functional foods are being evaluated.

Several limitations should be acknowledged. The sample size was relatively small, and the intervention period was limited to 14 days, which may underestimate longer-term lipid adaptations. Additionally, only HDL concentrations were measured; future studies should incorporate assessments of HDL functionality, such as cholesterol efflux capacity, antioxidant activity, and anti-inflammatory potential, given their established relevance to cardiovascular risk reduction.[1–4] Moreover, the study did not evaluate triglycerides or LDL parameters, which would provide a more complete picture of omega-3–mediated lipid modulation.

Despite these limitations, the findings provide important preliminary evidence supporting the potential of omega-3 enriched eggs as a functional food for modulating HDL levels. Given their affordability, accessibility, and cultural acceptability, omega-3 enriched eggs may offer a practical dietary strategy for populations at risk of dyslipidemia. Future research should focus on longer intervention durations, dose–response evaluations, and randomized clinical trials in humans to determine whether the HDL-elevating effects observed in this model translate into meaningful cardiovascular benefits.

CONCLUSIONS AND RECOMMENDATION

This study demonstrated that omega-3 enriched eggs produced a significant elevation in HDL cholesterol levels in Wistar rats compared with regular eggs and a standard diet. The substantial 66.5% increase in HDL observed in the omega-3 group indicates that food-based delivery of EPA and DHA can exert meaningful lipid-modulating effects within a relatively short intervention period. These findings provide preliminary evidence supporting omega-3 enriched eggs as a functional dietary option for improving HDL profiles and potentially contributing to cardiovascular risk reduction.

Future studies should employ larger sample sizes, longer intervention durations, and broader lipid assessments—including HDL functionality, LDL subfractions, and triglyceride responses—to obtain a more comprehensive understanding of the cardiometabolic impact of omega-3 enriched eggs. Human clinical trials are particularly warranted to evaluate dose–response relationships, long-term safety, and their comparative effectiveness relative to conventional omega-3 supplementation. Investigations into consumer acceptability and dietary adherence may further support the integration of omega-3 enriched eggs into public health nutrition strategies

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