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# Comparative Study of Effects of Cod Liver Oil and Quail (Coturnix japonica) Egg Yolk Hexane Extract in Remedizing Dyslipidemia in the Liver and Heart

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**Abstract**
The biochemical modulations by fatty acids as a major components of lipids cannot be over-emphasized. The apolipoprotein-containing (LDL, VLDL and TG) and nonapolipoprotein-containing (HDL) lipid biomolecules. Cod liver oil has since gained acceptance as reliable lipid source compared to other sources of lipids that had been reported by researchers to have hypocholesterolemic activities. This study was aimed at comparing quail egg yolk hexane extract with cod liver oil. This research was the proceed of the antidyslipidemic activities of quail egg yolk hexane extract and the decision to compare with other acceptable fatty acid source. The comparism between these samples was evaluated by analytical processes carried out by Gas Chromatography coupled with Mass Spectrophotometer (GC-MS) and clinical lipid profile assays-HDL,TChol and TG, VLDL, LDL. atherogenic coefficient and index as well as coronary risk index, HDL/LDL-C and some other parameters that define dyslipidemia were calculated. Competitive activities were inferred between quail egg yolk hexane extract and cod liver oil, however, the cod liver oil had more antidyslipidemic effect than quail egg yolk. The n-6:n-3 ratio showed a more viable codliver oil than quail egg yolk extract-0.386 and 0.525 respectively.
**Keywords:** Fatty acids, LDL,VLDL, Total Cholesterol, HDL, Atherogenicity, Dyslipidemia, Cod liver oil, Quail egg yolk lipid

**Abbreviations:** High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Total Cholesterol (TChol), Very Low Density Lipoprotein (VLDL), Triglyceride (TG), Omega 6 (n-6), Omega 3 (n-3), Cholesterol (C), Polyunsaturated Fatty Acid (PUFA), Cardiovascular Disease (CVD), Cholesteryl Ester Transfer Protein (CETP), Lipoprotein Lipase (LPL), LDL Receptor (LDLR), Microsomal Triglyceride Transfer Protein (MTP)

## INTRODUCTION

Fatty acids are the building blocks from which lipids are made. It has been estimated that less than 8% of alpha linoleic acid (ALA) is metabolised to eicosapentaenoic (EPA), and the capacity for the body to synthesize docosahexaenoic acid (DHA) appears to be particularly limited. It is estimated that only between 0.02% and 4% of ALA is metabolised to DHA [1-3], with women having a greater capacity for DHA synthesis than men [4]. Feedback inhibition of enzymes involved in *n*-3 and *n*-6 fatty acid synthesis occurs; for example, if the amount of DHA in the diet increases, there is reduced metabolism of ALA to EPA. Furthermore, *n*-3 and *n*-6 fatty acids compete for the enzymes involved in fatty acid elongation and desaturation, with certain enzymes having greater affinity for *n*-3 fatty acids and others having greater affinity for the *n*-6 series [5].

Essential components of lipids which include n-3 PUFAs have been established to possess antioxidant properties which have been shown to have lipid peroxidation inhibition activities. High cholesterol in the liver, a condition known as fatty liver or steatosis, has been attributed to hyperlipidemia, which as a result of electrically unpaired radical form of cholesteryl ester is able to activate degeneration of biomolecular components of these organs leading to inflammation and oxidative stress as a whole.

Lipid profile consists of a group of biochemical testes often used in predicting, diagnosing and treating lipid related disorders for example, atherosclerosis. Prospective epidemiologic studies have shown that blood levels of low-density lipoprotein cholesterol (LDL-C) significantly predict incident atherosclerotic cardiovascular disease (CVD), and LDL-C-lowering therapy has been repeatedly demonstrated in many populations to reduce CVD risk [6]. This has led to the formulation of risk prediction algorithms for identification of high-risk individuals and specific LDL-C goals to be achieved with lifestyle and pharmacological interventions [7]. Generally, hyperlipidemias are of interest to the physician in the context of risk factors for Ischemic heart disease (IHD) and peripheral vascular disease. The first step in diagnosis of hyper- and hypolipoproteinaemias is to define the lipoprotein pattern by chemical analysis of the plasma lipid and lipoproteins [8]. Accumulated evidences relating the concentrations of lipids (TChol and TG) and their associated blood transporting lipoproteins (HDL-C, LDL-C,VLDL-C) with the occurrence of atherosclerosis in general and Cardiovascular diseases (CVD) in particular [9].

Several deaths associated with tumor and disorders of the metabolic organ (liver) and organ responsible for blood circulation (heart) have been attributed to dyslipidemia. Atherogenic dyslipidemia is a condition induced genetically or via diet. The basis for genetics and atherogenic dyslipidemia is that the atherogenic

lipoprotein phenotype has numerous genes that influence LDL concentration, these genes include, CETP genes, LDLR genes, LPL, MTP and the apolipoprotein genes-APO5,APOB,APOC3 and APOE [6]. Natural alterations of these genes leads to dyslipidemia in the organ system irrespective of the cell involved. The perspective of diet related dyslipidemia is in line with hypercholesterolemic or high fat diets, which can increase apolipoprotein containing molecules (LDL, TG and VLDL) in the circulatory and organ systems.

Many individuals with normal LDL-C levels nevertheless develop CVD [10], particularly in older age groups [6]. Recurrent dyslipidemic diseases have prompted researchers to proffering solutions that will attenuate dyslipidemic conditions and thus sustaining better living. The comparative attenuation of dyslipidemia by cod liver oil and quail egg yolk lipid extract are evaluated but not cognitive study, using the blood, liver and heart as subject of study.

## MATERIALS AND METHODS

### Reagents and Chemicals

Reagents and Chemicals used in this experiment were obtained from different sources such as British Drug House (BDH) and were all of good analytical grades and analytical kits were purchased from local distributors of Randox Laboratories Limited. All the solutions, buffers and reagents were prepared using glass distilled water. Cod liver oil used in this experiment as standard omega-3 supplement (reference drug) was obtained from Martadol pharmaceutical shop, Akure, Ondo State. Nigeria and was NAFDAC registered.

### Lipid Extraction

Lipid from the *Coturnix japonica* egg yolk was extracted using AOAC (1990) with slight modification, methods for lipid extraction and methylation. The yolk sample (up to 50g) is dissolved in chloroform (100ml) in a test tube fitted with a condenser, and 1% sulfuric acid in methanol (200ml) was added, before the mixture was refluxed for 2 hours (or alternatively the mixture could be left overnight in a stoppered tube at 50°C). Water (50ml) containing sodium chloride (5%) is added and the required esters were extracted with hexane (2 x 15ml), using Pasteur pipettes to separate the layers. The hexane layer was washed with water (40ml) containing potassium bicarbonate (2%) and dried over anhydrous sodium sulfate. The solution was filtered to remove the drying agent, and the solvent was removed under reduced pressure in a rotary film evaporator. The extract was then stored in an air -tight container in a desiccator at 4$^{o}$C until when needed. The quail egg yolk lipid extract and cod liver oil were profiled for the fatty acids present using GC-MS equipments.

### Gas Chromatography and Mass Spectrophotometry

The content was concentrated to 1ml for gas chromatography analysis and 1µl was injected into the injection port of GC. The GC equipment used was HP 6890 powered with HP chemstation Rev. A09.01 (1206) software. The split ratio was 20:1, the carrier gas was nitrogen at inlet temperature of 250$^{0}$C with a column type of HP INNOWax and column dimensions of 30m x 0.25mm x 0.25µm. the oven program parameters include initial temperature at 60$^{0}$C, first ramping at 12$^{0}$C/minutes for 20minutes, maintain for 2minutes and second ramping at 15$^{0}$C/min for 3minutes, maintained for 8minutes. The detector used was FID at 320$^{0}$C at hydrogen pressure 22psi and compressed air of 35psi.

### Experimental Design

Male albino rats were used according to the standard guidelines of the Care and Use of Experimental Animal Resources. The rats were housed 5 per cage under constant environmental conditions (20–24 °C; 12 h light/dark cycle), and were given ad libitum access to standard pelleted food and water. 15 male albino rats weighing 200 ± 10 g obtained from standard animal house, were used to access the ability of the quail egg yolk lipid extract as anti-dyslipidemic agent with comparison with Cod liver oil for a duration of 4 weeks.

Group 1: normal chow *ad libitum* and served as control.
Group 2: quail egg yolk lipid (630µl/kg body weight).
Group 3: cod liver oil (630µl/kg body weight).

### Sample Preparation

The rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac punctures into plain bottles. Serum was prepared by aspiration of the clear yellowish liquid after clotting and centrifuged for 10 minutes at 3000g in a bench centrifuge. The clear supernatant was used for the estimation of serum lipids. The animals were quickly dissected and the heart and liver were removed and rinsed with ice cold 1.15% potassium chloride. The tissues were then homogenized in ice-cold 0.1M phosphate buffer solution using a Teflon homogenizer, and centrifuged for 10 minutes at 3000g in a bench centrifuge to collect the aliquot for biochemical evaluations.

### A -Lipid Profile

Enzymaticaly with commercial test kits from Randox Company, the following fractions of lipids were assayed:
-Total cholesterol (TCh) [11].
-HDL-Cholesterol (HDL-C [12].
-Triglycerides (TG) [13].

-Low Density Lipoprotein cholesterol (LDL-C) and Very Low Density Lipoprotein (VLDL-C) was calculated using the Friedwald equation [14], as follow: LDL-C = TCh - HDL-C - TG / 5.0
VLDL = TG / 5.0

**B-The atherogenic indices was calculated as follow:**

-Coronary Risk Index (CRI) = TCh / HDL-C [15].
-Atherogenic Coefficient (AC) = (TCh – HDL-C)/ HDL-C [16].
-Atherogenic Index of Plasma (AIP) = log (TG / HDL-C) [17].

**Statistical analysis**

Data presented were the means ± and standard deviations; student-t-test was used to compare the significance of the difference in the mean values of any two groups. (P≤0.05) was considered statistically significant.

**RESULTS**

Table 1: Lipid modulation effect on the atherogenic dyslipidemia

| | Atherogenic coefficient | Coronary risk index | Atherogenic index | HDL-C/LDL-C | TAG/HDL-C |
|---|---|---|---|---|---|
| Quail egg yolk oil | 2.308±0.217 | 3.308±0.261 | 0.922±0.111 | 1.571±0.111 | 8.361±1.012 |
| Cod liver | 2.0038±0.011 | 3.0038±0.171 | 0.931±0.221 | 3.373±0.212 | 8.537±0.926 |
| Control | 4.607±0.225 | 5.607±0.321 | 1.309±0.177 | 1.884±0.262 | 20.380±1.221 |

Values are expressed in mean ± standard deviation. N=5

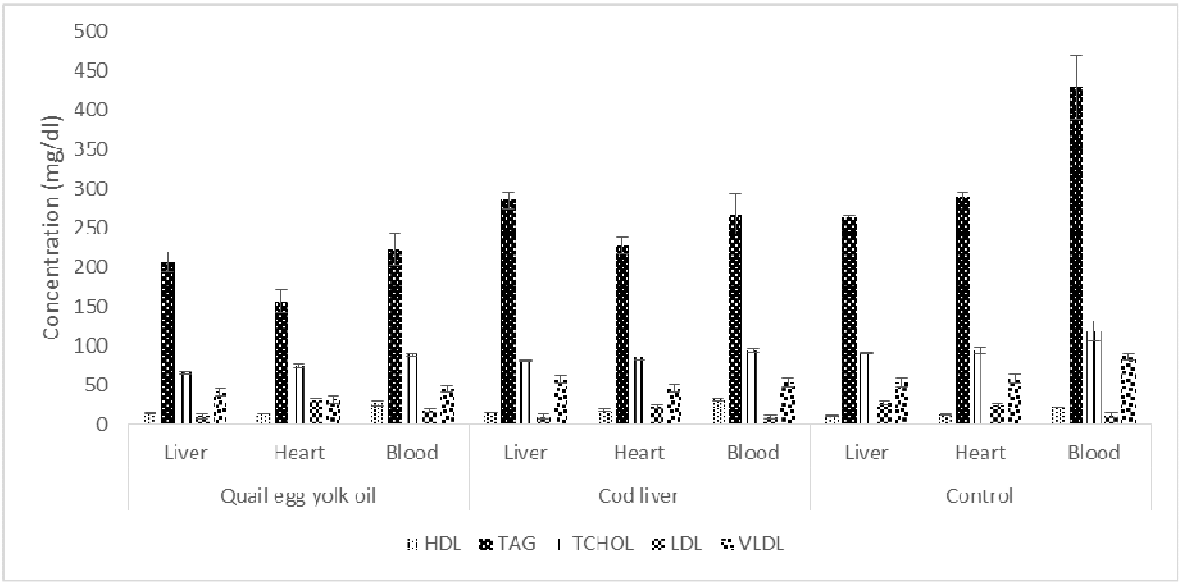


Figure 1: Lipid profile modulation by quail egg yolk hexane extract and cod liver oil in liver, heart and blood.

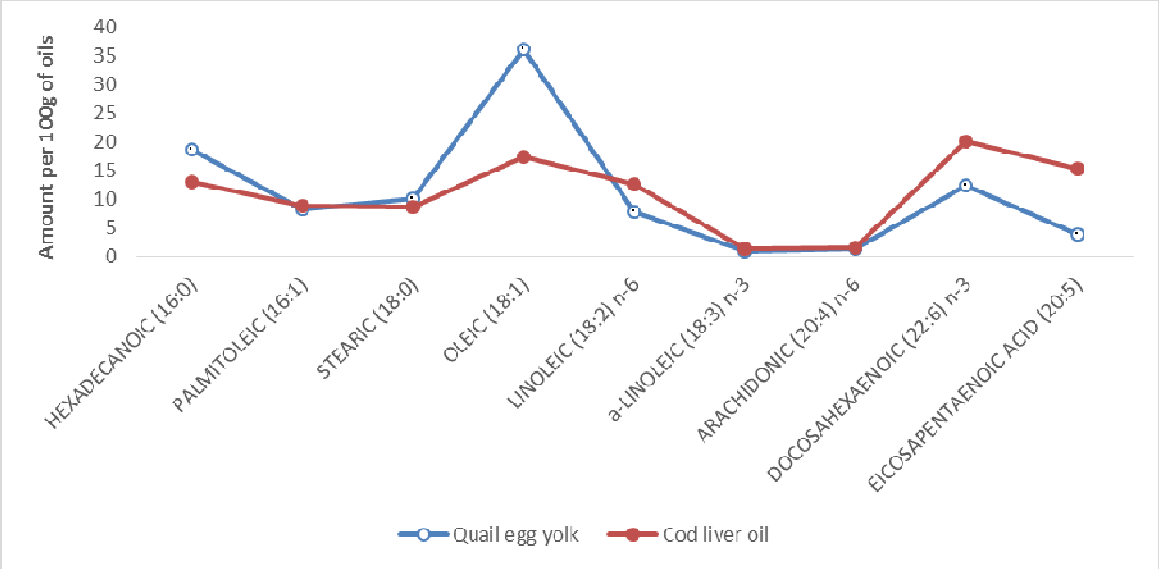


Figure 2: GC-MS lipid profiles of quail egg yolk hexane extract and cod liver oil.

Table 2: Summary of lipid profiles of extract

| | **Quail egg yolk** | **Cod liver oil** |
|---|---|---|
| **n-6 PUFAs** | **8.93** | **14.15** |
| **n-3 PUFAs** | **17.01** | **36.66** |
| **n-6:n-3** | **0.524985** | **0.385979** |

**DISCUSSIONS**

The roles of PUFAs become more important as they are not synthesized in human organism and have to be delivered with food [18]. The GC-MS result (Fig. 2) of the samples revealed saturated and unsaturated fatty acids presence in the samples. The sum of the amount of omega-3 fatty acids per 100g of samples are 17.01 and 36.66 for quail egg yolk hexane extracts and cod liver oil respectively. The ratio of n-6:n-3 are 0.525 and 0.386 approximately for quail egg yolk hexane extracts and cod liver oil respectively. These results justify the basis for the higher antiadyslipidemic activities of the cod liver oil over quail egg yolk hexane extracts.

The result (Fig. 2) revealed stearic acid of 10.04g and 8.72g per 100g of quail egg yolk lipid and cod liver oil samples respectively. Stearic or octadecanoic acid, classified as saturated fatty acid (SFA) had been shown to have recommendable effects on blood total and low density lipoprotein (LDL) cholesterol levels [19]. The concentration of stearic acid is considerably high to enhance stimulation of pericardial muscles as a result of the TChol and LDL-C reduction (hypocholesterolemic activity), thus depleting atherogenicity that could result from dyslipidemia. The effect of stearic acid is not peculiar with attenuating atherogenic dyslipidemia alone but could be responsible for attenuating dyslipidemia in other organs of the system including liver. Palmitoleic acid is a monounsaturated fatty acid resembling saturated fatty acids in its ability to lower LDL-C [20,21].

The implication of Elevated total cholesterol concentrations in dyslipidemic conditions and the progression to diseases affecting the organ systems has opened insight to attenuating recommendation that would reduce the TChol, LDL-C and TG and promote HDL-C distribution. An increased level of triglyceride (TG) is both independent and synergistic risk factor for CVD [22] and severe dyslipidemic conditions. TG/HDL-C was shown to be a more accurate predictor to atherogenic dyslipidemia. The logarithmatically transformed ratio of plasma TG/HDL-C could serve as an indicator of the atherogenic lipoprotein phenotype. The Atherogenic Index of Plasma (AIP) defined as log TG/HDL-C, has recently been proposed as a marker of plasma atherogenicity because it is increased in people at high risk for CVD and it is inversely correlated with LDL-C particles [23]. AIP predetermines the direction of the cholesterol transport in an intravascular pool i.e. the flux of newly produced cholystreyl esters by lecithin cholesterol acyl transferase (LCAT) towards atherogenic LDLs or beneficial HDLs [24].

Studies also showed that AIP predicts cardiovascular risk and that it is an easily available cardiovascular risk marker and a useful measure of the response to treatment [25]. The quail egg yolk extract and cod liver oil showed atherogenic condition attenuating activities. However cod liver oil showed higher antiatherogenic activity compared to quail egg yolk hexane extract. Cod liver oil gave a reduced atherogenic coefficient and coronary risk index while no significant difference occurred between the samples with logarithmic atherogenic index. TG/HDL-C result showed no significant difference between the samples, while

the activities of the samples for HDL-C/LDL-C ratio showed a higher value for cod liver oil and lower value for quail egg yolk extract. Elevated HDL/LDL cholesterol ratio was associated with low risk of diseased events [18].

Cod liver oil has been confirmed in this research as having antidyslipidemic and hypocholesterolemic activities. These activities of cod liver oil are competitive with the activities quail egg yolk hexane extract. The frequent occurrence of atherogenic and hepatic dyslipidemic conditions have led to death of many with complications resulting from genetic defect (as with hypertrophic cardiomyopathy and other genetical atherogenic disorders with the feasibility of treatment from the perspective of dyslipidemic modulation) or diet (with hypercholesterolemic diet). This study is not a cognitive activity between the samples but an elaborate antidyslipidemic activities of the samples and thus a recommendation for quail egg yolk hexane extract as dyslipidemic attenuating agent.

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