

**EFFECT OF CONSUMPTION OF DIET FORMULATED WITH GROUNDNUT OIL  
FRIED EGG YOLK ON SOME LIPIDS AND HISTOLOGY OF THE HEART IN  
FEMALE WISTAR RAT**

**BY**

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**UNIVERSITY OF BENIN**

**EDO STATE**

**JANUARY, 2025.**

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**A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY IN PARTIAL  
FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF  
BACHELOR OF BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY.**

**JANUARY, 2025.**

## CERTIFICATION

This is to certify that this final year project research was carried out by **OSILAMA EMIKE (MAT. NO LSC2003042)** of the Department of Biochemistry, Life Sciences, University of Benin, Benin City, in partial fulfillment for the Bachelor of Science (B.Sc) degree in Biochemistry.

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(Head of Co-ordinator)

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Signature & Date

## **DEDICATION**

I wholeheartedly dedicate the successful completion of my project research to God Almighty. It was your grace and guidance that enabled me to navigate the challenges and obstacles along the way. Thank You for granting me the strength, wisdom, and grace to overcome every hurdle, and for providing the resources and opportunities that enriched my knowledge and experience throughout this journey. May this accomplishment stand as a testament to your faithfulness and love, inspiring others to trust in your unwavering support and guidance.

## ACKNOWLEDGEMENT

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I also extend my gratitude to the entire staff of the Department of Biochemistry, whose teachings on various concepts and courses in the field of biochemistry have equipped me with the knowledge and skills necessary to carry out this research successfully.

Additionally, I express my heartfelt appreciation to Almighty God for His constant guidance and protection during this journey. His grace and mercy have made it possible for me to achieve this milestone.

I am deeply grateful to my parents, **Mr. and Mrs. Osilama**, for their unwavering love, support, guidance, and prayers, which have been a pillar of strength and motivation throughout this process. Their encouragement has been vital to my success.

My friends also played a part in my education and I can't but say thanks to them... Cynthia, Gift, Akpewe, Abigail, Ernest, Mummy Zara, Joan and Hope.

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## ABSTRACT

Dietary cholesterol plays a crucial role in maintaining cardiovascular health. Consuming high amounts of cholesterol can lead to hyperlipidemia and cardiac dysfunction. This study aims to investigate the effect of frying of egg yolk with groundnut oil on the lipid profile and histological changes in the heart of female Wistar rats (*Rattus norvegicus*). A total of sixteen female Wistar rats were divided into four groups labelled I-IV. Group I was administered soybean, groundnut oil fried egg yolk, corn, premix, soybean oil, group II was administered synthetic cholesterol, corn, premix, soybean oil, soybean, group III was administered corn, premix, soybean oil, soybean and group IV was administered normal feed (grower mash) for 42 days. The rats had unrestricted access to food and water throughout the study. On the 43<sup>rd</sup> day, the animals were sacrificed and biochemical analyses of cholesterol and triglyceride were conducted. And histopathological examination was done on the heart tissue. The results showed that rats fed with formulated diet of groundnut oil fried egg yolk had significant decrease in triglyceride level ( $259.53 \pm 3.05$  mg/dL) compared to those fed synthetic cholesterol ( $307.63 \pm 7.74$  mg/dL) suggesting that frying egg yolk with groundnut oil may reduce the hypertriglyceridemia effect of egg yolk. While, there was an increase in cholesterol level in the group fed with formulated diet of groundnut oil fried egg yolk ( $589.19 \pm 4.03$  mg/dL) when compared to the group fed with synthetic cholesterol ( $577.09 \pm 9.53$  mg/dL) suggesting that frying may influence lipid metabolism. The experimental group fed groundnut oil fried egg yolk showed significant histopathological changes such as active congestion and vasodilation, while the group fed with synthetic cholesterol showed mild perivascular mobilization of fibroblasts. However, excessive consumption of dietary cholesterol, regardless of preparation method, remains a risk factor for hyperlipidemia and cardiovascular complications. Further research is needed to elucidate the mechanisms behind these effects and their implications for human dietary recommendations.

## CHAPTER ONE

### INTRODUCTION AND LITERATURE REVIEW

#### 1.1 BACKGROUND OF THE STUDY

The consumption of fried foods has become increasingly prevalent in modern diets, leading to concerns about their potential impact on cardiovascular health. Groundnut oil, commonly used for frying, has been the subject of research due to its potential modulation of blood lipid profiles and heart histology (Kusnandar *et al.*, 2022). This study aims to investigate the effect of frying with groundnut oil on the lipid profile and histological changes in the heart of female Wistar rats (*Rattus norvegicus*) fed an egg cholesterol-formulated diet.

Groundnut oil, commonly referred to as peanut oil, is a widely used cooking oil derived from peanuts (*Arachis hypogaea*). It is abundant in monounsaturated and polyunsaturated fatty acids, which research has indicated may have positive effects on heart health (Kris-Etherton *et al.*, 2017). Additionally, groundnut oil is rich in antioxidants, such as vitamin E and polyphenols, which aid in guarding against oxidative stress and inflammation (Wu *et al.*, 2020).

Recently, groundnut oil has attracted interest for its possible health advantages, such as lowering the likelihood of heart disease, enhancing blood lipid levels, and providing anti-inflammatory benefits (Liu *et al.*, 2019; Zhang *et al.*, 2020).

Cholesterol is an essential lipid molecule that is integral to various physiological functions, such as maintaining cell membrane structure, producing hormones, and synthesizing vitamin D (Qin *et al.*, 2020). However, elevated cholesterol levels in the bloodstream can contribute to the onset of cardiovascular disease (CVD), which continues to be a leading cause of illness and death worldwide (Benjamin *et al.*, 2019). As stated by the World Health Organization (WHO), CVD is

responsible for around 17.9 million fatalities globally each year, with high cholesterol being a significant risk factor (WHO, 2020). This discussion aims to give a comprehensive overview of cholesterol, its types, the factors that influence its levels, the health risks linked to elevated cholesterol, and recent developments in cholesterol management.

Cholesterol is an important molecule and both too much and too little of it can lead to illness. The majority of medical professionals are aware of its significance in maintaining the stability of cellular plasma membranes.

For years, dietary cholesterol was implicated in increasing blood cholesterol levels leading to elevated risk of cardiovascular disease (CVD). To date, extensive research did not show evidence to support a role of dietary cholesterol in the development of CVD. Additionally, intake of dietary cholesterol is usually associated with an increased intake of saturated fatty acids which is documented to increase LDL cholesterol and the risk of CVD. In fact, eggs are the only dietary source of cholesterol that is low in saturated fatty acid but is also nutrient-dense, economical and affordable.

## **1.2 AIM OF THE STUDY**

To investigate the effects of consuming high cholesterol obtained from fried egg yolk on lipid profiles (total cholesterol and triglycerides levels) as well as evaluating histological changes in the heart of Wistar rats.

## **1.3 OBJECTIVES OF THE STUDY**

1. To evaluate the effects of high cholesterol intake from fried egg yolk on total cholesterol and triglyceride levels in the serum of female Wistar rats.

2. To examine the histological changes in the heart of Wistar rats fed with high cholesterol from fried egg yolk.

## **1.4 LITERATURE REVIEW**

### **1.4.1 DIETARY CHOLESTEROL AND IT'S ROLE ON HEART HEALTH**

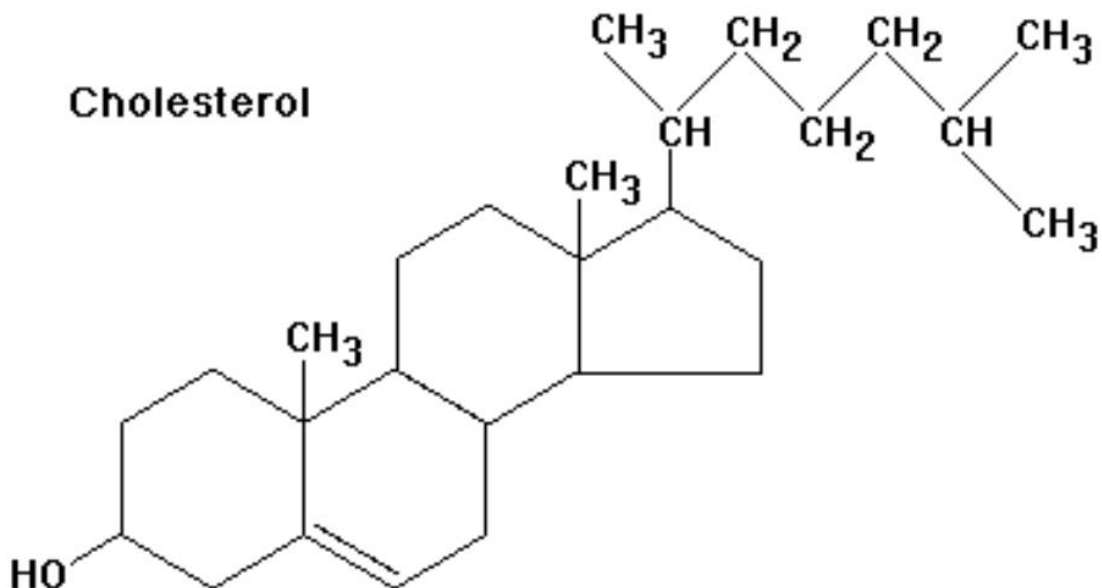
Dietary cholesterol is a type of fat found in animal-based foods, including meat, eggs, dairy products and seafood. It is essential for various bodily functions, such as hormone production and cell membrane integrity. While the body can synthesize cholesterol, dietary sources contribute to overall levels.

Studies have indicated that consuming dietary cholesterol can increase the levels of low-density lipoprotein (LDL) cholesterol, often referred to as "bad" cholesterol, in the bloodstream (Huang *et al.*, 2015). High LDL cholesterol is a significant risk factor for the onset of cardiovascular disease (CVD), which encompasses heart attacks, strokes, and peripheral artery disease (Benjamin *et al.*, 2019).

It is important to recognize that the relationship between dietary cholesterol and heart health is multifaceted, shaped by several factors including personal tolerance, the quality of the overall diet, and lifestyle practices (Krauss *et al.*, 2017). Certain research indicates that, for many healthy people, the impact of dietary cholesterol on blood cholesterol levels may be minimal (Hu *et al.*, 2019). It is worth noting that most foods that are rich in cholesterol are also high in saturated fatty acids and thus may increase the risk of CVD due to the saturated fatty acid content. The exceptions are eggs and shrimp (Soliman,2018).

The 2020 Dietary Guidelines for Americans recommend capping dietary cholesterol intake at 300 milligrams daily (USDA, 2020). Yet, certain experts contend that this limit could be overly

cautious, since the relationship between dietary cholesterol and heart disease may not be as definitive as once believed (Kritchevsky, 2018).



**Figure 1.1: Cholesterol Molecule**

Image: Google, Website: [www.toppr.com](http://www.toppr.com)

In conclusion, while dietary cholesterol may have some impact on heart health, the relationship is complex, and individual responses to dietary cholesterol vary widely. A balanced diet, regular physical activity, and a healthy lifestyle remain the best strategies for maintaining heart health.

#### **1.4.2 TYPES OF CHOLESTEROL**

Cholesterol is an essential lipid compound that acts as a fundamental component for cell membranes, hormones, and bile acids (Berg *et al.*, 2002). The liver generates about 80% of the

cholesterol found in the body, whereas the other 20% is obtained from the diet (Grundy *et al.*, 2004).

Cholesterol is transported in the bloodstream by lipoproteins, which are complex molecules composed of lipids and proteins (Goldstein *et al.*, 2009). These are the different types of cholesterol;

1. LDL, or low-density lipoprotein Cholesterol: Also referred to as "bad" cholesterol, low-density lipoprotein (LDL) transports cholesterol from the liver to the cells and can build up in the artery walls, increasing the risk of cardiovascular disease and causing plaque deposition (Goldstein *et al.*, 2009). One of the main risk factors for atherosclerotic cardiovascular disease (ASCVD) is elevated LDL levels (Grundy *et al.*, 2019).
2. High-Density Lipoprotein (HDL) Cholesterol: Often referred to as "good" cholesterol, HDL helps to eliminate excess cholesterol from the bloodstream and lower the risk of cardiovascular disease by transporting it from the cells to the liver for excretion. HDL also possesses antioxidant and anti-inflammatory qualities, which may also be responsible for its positive effects on cardiovascular health (Rye *et al.*, 2018).
3. Very-Low-Density Lipoprotein (VLDL) Cholesterol: VLDL is similar to LDL, but it carries more triglycerides and less cholesterol (Krauss *et al.*, 2017). VLDL can also contribute to plaque buildup in the arteries and increase the risk of cardiovascular disease.

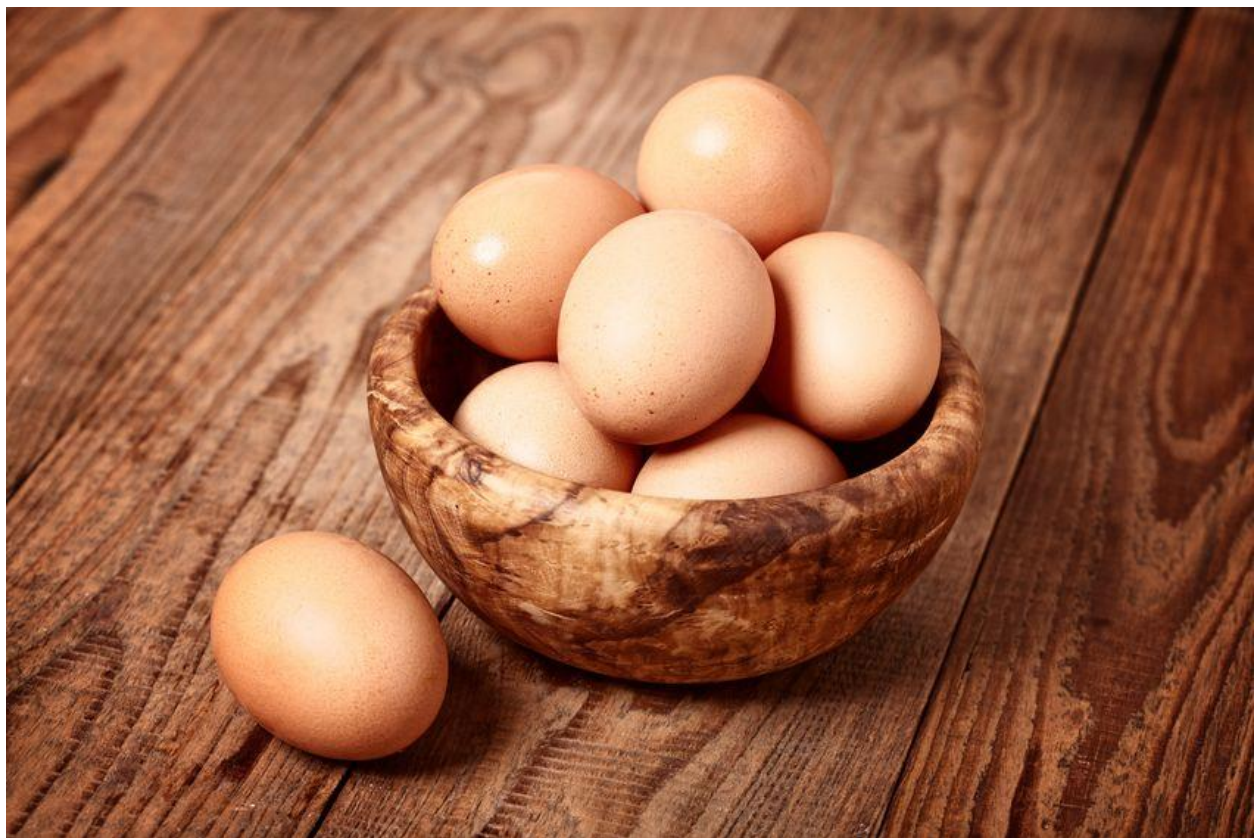
### **1.4.3 EGG YOLK AS A SOURCE OF DIETARY CHOLESTEROL**

Eggs are a significant source of dietary cholesterol, with a large egg containing approximately 186 to 275 mg of cholesterol, primarily found in the yolk (Man-Yun *et al.*, 2020). Despite their

high cholesterol content, research shows that moderate egg consumption does not significantly impact blood cholesterol levels for most individuals.

Eggs serve as a rich source of nutrition, filled with bioactive nutrients and dietary elements essential for managing metabolic health, fat metabolism, immune response, and blood cell formation. Eggs contain a significant amount of minerals and vital trace elements. In particular, egg yolks are high in calcium, copper, iodine, iron, manganese, phosphorus, selenium, and zinc.

Studies suggest that consuming up to one egg per day is generally safe and may even be beneficial for heart health, particularly when considering the overall diet.



**Figure 1.2: Brown Egg**

Image: Brown eggs, Paul *et.al.*, (2024).

#### **1.4.4 GROUNDNUT OIL AND ITS EFFECT ON HEALTH**

Groundnut oil, commonly referred to as peanut oil, is a widely used cooking oil derived from groundnuts (peanuts). It is especially prevalent in Asian and African culinary traditions. Beyond being a tasty ingredient for numerous recipes, groundnut oil also provides various health advantages due to its distinctive nutritional profile.

The composition of groundnut oil includes mostly monounsaturated and polyunsaturated fats, which are known as beneficial fats. As reported by the United States Department of Agriculture (USDA), groundnut oil consists of about 46% monounsaturated fats, 32% polyunsaturated fats, and 20% saturated fats (USDA, 2020). Additionally, groundnut oil is high in antioxidants, especially vitamin E, which aids in shielding cells from damage and promotes skin health (Traber, 2007).

##### **Health Effects of Groundnut Oil:**

The intake of groundnut oil has been linked to various potential health advantages. The monounsaturated and polyunsaturated fats present in groundnut oil may contribute to lowering LDL (bad) cholesterol levels and raising HDL (good) cholesterol levels, thus minimizing the chances of cardiovascular diseases (Kris-Etherton *et al.*, 2002). Moreover, the antioxidants found in groundnut oil might assist in alleviating inflammation and enhancing overall health (Harris *et al.*, 2007).

Groundnut oil has also demonstrated anti-inflammatory effects, which could help decrease the likelihood of chronic illnesses such as cancer and neurodegenerative diseases (Baur *et al.*, 2006). Additionally, groundnut oil contains resveratrol, a compound identified to possess anti-cancer effects and may aid in lowering the risk of specific cancer types (Baur *et al.*, 2006).

On the other hand, groundnut oil does carry certain potential risks. A primary concern revolves around the potential for allergic reactions. Some people may have allergies to peanuts, which can lead to severe allergic responses (Sicherer *et al.*, 2010). Furthermore, groundnuts may become contaminated with aflatoxins, toxic substances produced by certain molds (Williams *et al.*, 2004). Another issue is the elevated levels of omega-6 fatty acids found in groundnut oil. Although certain omega-6 fatty acids are necessary, an overabundance can lead to inflammation and result in chronic health issues (Harris *et al.*, 2007). Hence, it is important to use groundnut oil in moderation and to pair it with other healthy fats.

In summary, groundnut oil can be a beneficial part of a well-rounded diet if used in moderation. Its distinctive blend of monounsaturated and polyunsaturated fats, along with antioxidants and compounds that reduce inflammation, makes it a nourishing option. However, those with peanut allergies or worries about aflatoxin contamination should be cautious. Furthermore, being aware of the omega-6 fatty acid levels and balancing them with other healthy fats is vital. By following these guidelines, we can enjoy the advantages of groundnut oil while reducing its potential risks.

#### **1.4.5 THE HEART OF THE WISTAR RAT**

The histology of the heart of Wistar rats is a crucial aspect of cardiovascular research. Studies have shown that Wistar rats are a suitable model for studying heart histology due to their similarities to humans in terms of cardiac anatomy and physiology.

The Wistar rat's heart is made up of four chambers: the right and left atria, along with the right and left ventricles (Greaves, 2012). Enclosing the heart is a pericardial sac that holds a small volume of pericardial fluid (Kumar *et al.*, 2018). Blood is delivered to the heart muscle via the coronary arteries, which originate from the aortic root (Tomanek *et al.*, 2017).

The Wistar rat's heart consists of three layers: the epicardium, myocardium, and endocardium (Greaves, 2012). The outermost layer, known as the epicardium, is made up of connective tissue and fibroblasts. The middle layer, called the myocardium, is composed of cardiac muscle cells (cardiomyocytes) that play a key role in contracting and pumping blood. The innermost layer, the endocardium, features endothelial cells that line the heart's chambers and valves.

#### **1.4.6 FUNCTIONS OF THE HEART**

1. **Circulation:** The heart circulates blood throughout the body, guaranteeing that tissues and organs receive oxygen and essential nutrients.
2. **Oxygen Transfer:** The heart takes in deoxygenated blood from the body and sends it to the lungs, where it absorbs oxygen and expels carbon dioxide.
3. **Nutrient Transportation:** The heart circulates blood enriched with nutrients, including glucose, amino acids, and fatty acids, to various tissues and organs.
4. **Excretion of Waste:** The heart pumps blood that transports waste products, such as urea and other harmful substances, to the kidneys and liver for elimination.
5. **Blood Pressure Regulation:** The heart plays a crucial role in maintaining blood pressure by adjusting the volume of blood pumped and the resistance within blood vessels.
6. **Heart Rhythm Control:** The heart produces electrical signals that govern the heartbeat, promoting a synchronized and effective pumping mechanism.

#### **1.4.7 HEART STRUCTURE AND FUNCTION**

The heart is made up of four Chambers:

1. **Right Atrium:** Accepts deoxygenated blood returning from the body.
2. **Right Ventricle:** Forces blood into the lungs for oxygenation.

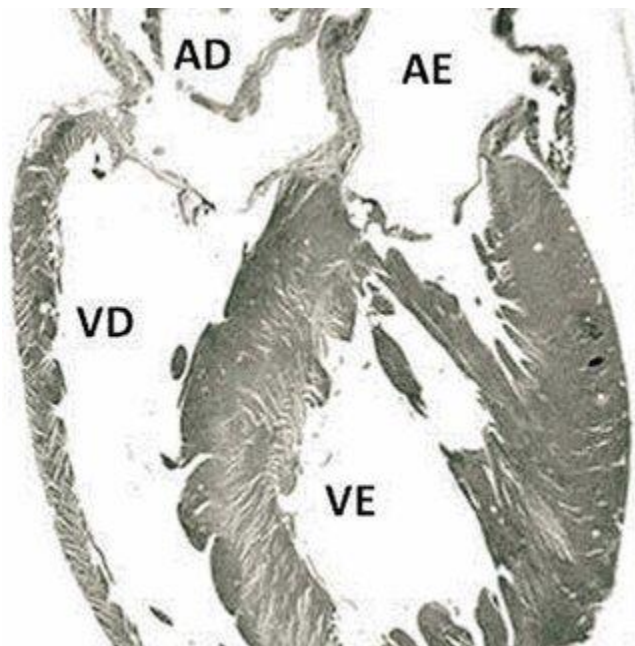
3. Left Atrium: Collects oxygen-rich blood coming from the lungs.

4. Left Ventricle: Distributes blood to the entire body.

### 1.4.8 HISTOLOGICAL STRUCTURE OF THE HEART

Histology is the study of the microscopic structure of tissues and organs. In Wistar rats, histological evaluation plays an essential role in several areas of research, such as toxicology, pharmacology, and disease modeling.

Wistar rats' hearts are made up of three layers: the epicardium, the myocardium, and the endocardium. The epicardium is the outermost layer, made up of connective tissue and fibroblasts. The myocardium is the central layer composed of heart muscle cells (cardiomyocytes) that contract and pump blood. The endocardium is the innermost layer, made up of endothelial cells that border the heart chambers and valves.



**Figure 1.3: Heart of a wistar rat**

#### **1.4.9 IMPACT OF HIGH DIETARY CHOLESTEROL ON HEART HEALTH**

Elevated dietary cholesterol can trigger various cardiac reactions, including:

1. **Increased LDL Cholesterol:** Consuming high levels of dietary cholesterol can raise the concentration of low-density lipoprotein (LDL) cholesterol, often referred to as "bad" cholesterol, in the bloodstream (Krauss *et al.*, 2017). Higher LDL levels can contribute to the onset of atherosclerosis, a disease marked by plaque accumulation in the arteries (Grundy *et al.*, 2019).
2. **Endothelial Dysfunction:** A high dietary cholesterol intake can also result in endothelial dysfunction, a state where the inner lining of blood vessels becomes impaired and unable to operate effectively (Reriani *et al.*, 2018). This dysfunction can diminish blood flow to the heart and elevate the risk of cardiovascular disease.
3. **High cholesterol intake can trigger inflammation in the body, which may elevate the chances of developing cardiovascular disease (Huang *et al.*, 2019).**  
This inflammation can harm the inner lining of blood vessels and promote plaque buildup.
4. **A high cholesterol diet can also induce cardiac remodeling, a condition where the heart undergoes structural and functional changes due to increased stress or injury (Lopaschuk *et al.*, 2018). Cardiac remodeling raises the likelihood of heart failure and various other cardiovascular conditions.**
5. **A diet rich in cholesterol can heighten the risk of cardiovascular diseases, such as heart attacks, strokes, and peripheral artery disease (Benjamin *et al.*, 2019).**

#### **1.4.9 IMPACT OF COOKING EGG YOLK WITH GROUNDNUT OIL**

Cooking egg yolks with groundnut oil can have both negative and positive impacts on the nutritional value and health effects of the dish.

One major issue with preparing egg yolks using groundnut oil is the possible reduction of omega-3 fatty acids. Omega-3 fatty acids are crucial for cardiovascular health, and egg yolks provide a significant amount of these beneficial acids (Li & Sinclair, 2002). However, heating egg yolks with groundnut oil, especially at high temperatures, can result in the depletion of these fatty acids (Chen *et al.*, 2018). Chen et al. (2018) state that "cooking egg yolks with groundnut oil at high temperatures can lead to the formation of cholesterol oxides and the loss of omega-3 fatty acids".

Another issue when cooking egg yolks in groundnut oil is the potential creation of cholesterol oxides. Cholesterol oxides are harmful compounds that may elevate the risk of heart disease (Kritchevsky, 2005). Kritchevsky (2005) notes that "the formation of cholesterol oxides during cooking can increase the risk of cardiovascular disease".

Despite these concerns, utilizing groundnut oil to cook egg yolks can also offer some advantages for nutritional content. For instance, the antioxidants found in groundnut oil can assist in safeguarding the egg yolks against oxidative damage and spoilage (Warner & Knowlton, 2017). Warner and Knowlton (2017) mention that "the antioxidants present in groundnut oil can help extend the shelf life of egg yolks".

Cooking egg yolks with groundnut oil presents both positive and negative implications for nutrition and health. While the antioxidants in groundnut oil can aid in protecting egg yolks from oxidative damage and spoilage, the risks of losing omega-3 fatty acids and producing cholesterol

oxides must be considered. Therefore, it is crucial to prepare egg yolks with groundnut oil at moderate temperatures and to consume them in moderation as part of a balanced diet.

## **CHAPTER TWO**

### **MATERIALS AND METHODS**

#### **2.1 APPARATUS AND EQUIPMENTS**

Plain bottle

Universal bottle

Beaker

Conical flask

Cuvettes

Centrifuge (Techmel & Techmel USA)

Wash bottle

Test tubes

Micro pipette

Syringes

Mortar and pestle

Cotton wool

Aluminium foil

Spectrophotometer (Techmel & Techmel USA)

Cages

Hand gloves

Paper tape

Polythene bag

Test tube rack

Scissor

Weighing balance

Water bath (Techmel & Techmel USA)

### **2.1.2 REAGENTS**

Distilled water

Saline

Chloroform

Cholesterol Kits

Triglyceride Kits

### **2.1.3 ANIMAL**

A total of 16 female Wistar rat were purchased from the animal house, Department of Biochemistry, Faculty of Life Science, University of Benin, Edo State. On average each rat weighed  $160 \pm 0.5\text{g}$  before sacrificing.

### **2.1.4 EXPERIMENTAL DESIGN**

A total of sixteen female Wistar rats were acquired and grouped into four categories. They were kept in individual cages at the Animal House of the Biochemistry Department at the University of Benin. During the entire experiment, the rats had constant access to both water and food. The animals were not allowed to acclimate and were given small amounts of the formulated diet.

### 2.1.5 FEED FORMULATION

A significant quantity of corn, soybean premix, and soybean oil was purchased from the market. Furthermore, one and a half crates of eggs and raw cholesterol were also obtained. And the animals were fed on the formulated pelleted feed and growers mash on a daily basis.

**Table 2.1: Feed Formulation**

<b>Groups/Diet</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
Normal Feed	-	-	-	-
Fried Egg Yolk with Groundnut oil	250g	-	-	-
Synthetic Cholesterol	-	2g	-	-
Corn	3.2kg	3.2kg	3.2kg	-
Soybean	750g	750g	750g	-
Premix	400g	400g	400g	-
Soybean oil	400g	400g	400g	-

### **2.1.6 ANIMAL SACRIFICE**

The rats were placed on overnight fast for 12 hours before sacrifice. At the end of the feeding period of 42 days, the animal (rats) were anesthetized in a chloroform chamber on the 43<sup>rd</sup> day. Organs were excised, placed in sterile sample bags and stored in a refrigerator for further biochemical assays such as cholesterol and triglycerides analysis. Blood was also collected from the veins of the Wistar rats and 2ml was placed in each of the EDTA bottles for biochemical analysis. The homogenate was prepared by homogenizing 1g of heart in 5ml of saline water. The homogenate was centrifuge at 3000rpm for 10min.

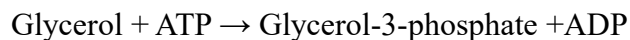
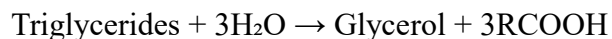
## **2.2 METHOD**

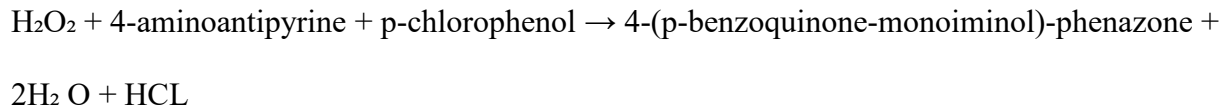
### **2.2.1 TRIGLYCERIDES**

#### **PRINCIPLE**

Lipase hydrolyzes the triglycerides to fatty acids and glycerol which in the presence of glycerolkinase and ATP, is phosphorylated to glycerol-3-phosphate and converted to glycerol-3-phosphate oxidase into Dihydroxyacetone phosphate and H<sub>2</sub>O<sub>2</sub>. The formed hydrogen peroxide reacts, in the presence of peroxidase, with 4-Chlorophenol and 4-Aminoantipyrin, giving a red colored compound, whose Absorbance, measured photometrically at 546nm, is proportional to the concentration of triglycerides.

Enzymatic colorimetric test:





## PROCEDURE

Plasma volume of 10 $\mu$ L was pipetted into 10 $\mu$ L standard distilled water and 1000 $\mu$ L of Reagent 1 (pipes buffer pH 7.8, 50mmol/l, p-Chlorophenol 2mmol/l, Lipoprotein Lipase 150000 U/I, Glycerolkinase 800 U/I, Glycerol-3-P-oxidase 400 U/I, Peroxidase 440 U/I, 4-Aminoantipyrine 0.7mmol/l, ATP 0.3mmol/l, Mg<sup>2+</sup> 40mmol/l, Na-cholate 0.20mmol/l, Potassium-Hexacyanoferrate(II) 1 $\mu$ mol/l. These were mixed, incubated for 5 minutes and absorbance was read at 546nm within 60 seconds.

The working procedure is as follow:

Wavelength: 546nm

Temperature: 37°C

Measurement: Against distilled water

Pipetted as follows:

Reagent 1: 1000 $\mu$ L

Sample, Std/Cal/H<sub>2</sub> O: 10 $\mu$ L

## CALCULATION

$$\text{Triglycerides conc.} = \frac{\Delta A \text{ sample}}{\Delta A \text{ stand}} \times \text{Stand conc.}$$

It was then mixed and incubated for 5 minutes and absorbance was read within 60 minutes.

### **2.2.2 CHOLESTEROL**

#### **PRINCIPLE**

Cholesterol is present in serum as cholesterol esters and free cholesterol. The cholesterol esters present in the serum are hydrolyzed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine present to form the red quinoneimine dye. The intensity of the dye formed is directly proportional to the level of cholesterol present in the sample.

#### **PROCEDURE**

10 $\mu$ L of DDH<sub>2</sub>O was added to blank with 1000 $\mu$ L of cholesterol reagent R1. 10 $\mu$ L of sample was added to 1000 $\mu$ L of cholesterol reagent R1. The content of each test tube was mixed and allowed to incubate for 5 minutes at 37°C, then the absorbance was read of the sample against the reagent blank at 500nm.

#### **CALCULATION**

Cholesterol conc. in sample =  $\frac{\Delta\text{abs Sample}}{\Delta\text{abs standard}} \times \text{conc of standard}$

### **2.2.3 HISTOPATHOLOGICAL EXAMINATION**

#### **PROCEDURE**

At the end of the experiment, the heart was collected and preserved in 10% neutral buffered formalin. Then it was later subjected to histological processing and dehydrated in a series of ethanol solutions for 1-2 hours. Then clear the dehydrated specimen using xylene or toluene for a

period of 1-2 hours. Soak the cleared specimen in molten paraffin wax. Position the infiltrated specimen within a paraffin block, ensuring correct orientation of the heart.

Cut the paraffin block into sections that are 5-7  $\mu\text{m}$  thick using a microtome. Place the sections onto glass slides that have been treated with a suitable adhesive (e.g., albumen or poly-L-lysine) and apply Hematoxylin and eosin (H&E) stain to the sections to visualize the morphology of cardiac tissue. Inspect the stained sections under a light microscope to assess the morphology of the cardiac tissue, then capture photomicrographs of representative regions for documentation and further examination.

#### **2.2.4 STATISTICAL ANALYSIS**

The data was presented as mean  $\pm$  SEM, Analysis of Variance (ANOVA) was used to test for significant differences between the mean.

SPSS version 25 was used as the statistical tool for analyzing the data.

## CHAPTER THREE

### RESULTS

#### 3.1 RESULTS

##### 3.1.1 MEASUREMENT OF THE BODY WEIGHT

The table below displays the average weight in grams of female Wistar rats measured prior to feed administration, as well as the average weight in grams after feed administration.

**Table 3.1.1: Weight of Female Wistar**

<b>Average Weight(g)</b>	<b>Groundnut oil Fried Egg Yolk</b>	<b>Synthetic Cholesterol</b>	<b>Positive</b>	<b>Negative</b>
Weight before Diet	37.89	39.34	45.42	
Administration				
Weight after Diet	110.74	128	126.4	
Administration (42 days)				
Percentage Increase %	192.27	225.37	179	

### 3.1.2 TRIGLYCERIDES AND CHOLESTEROL LEVEL IN THE HEART OF WISTAR

#### RATS

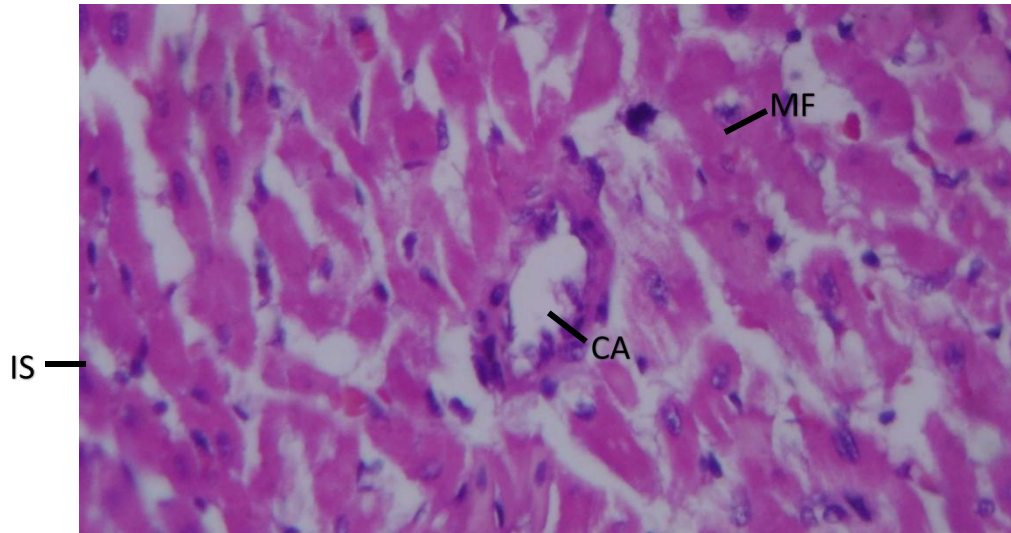
The cholesterol level for the Groundnut oil fried egg yolk ( $589.19 \pm 4.03$  mg/dL) shows a significant ( $p \leq 0.05$ ) increase when compared to the positive ( $569.46 \pm 5.50$  mg/dL) and negative ( $558.77 \pm 7.93$  mg/dL) control, while the raw cholesterol ( $577.09 \pm 9.53$  mg/dL) shows an intermediate level, slightly above the positive control but not significant as the groundnut oil fried egg yolk.

Groundnut oil fried egg yolk ( $259.53 \pm 3.05$  mg/dL) shows a significant decrease in triglycerides level, unlike the raw cholesterol group ( $307.63 \pm 7.74$  mg/dL) which shows a much more significant increase when compared to the negative control ( $277.09 \pm 9.16$  mg/dL).

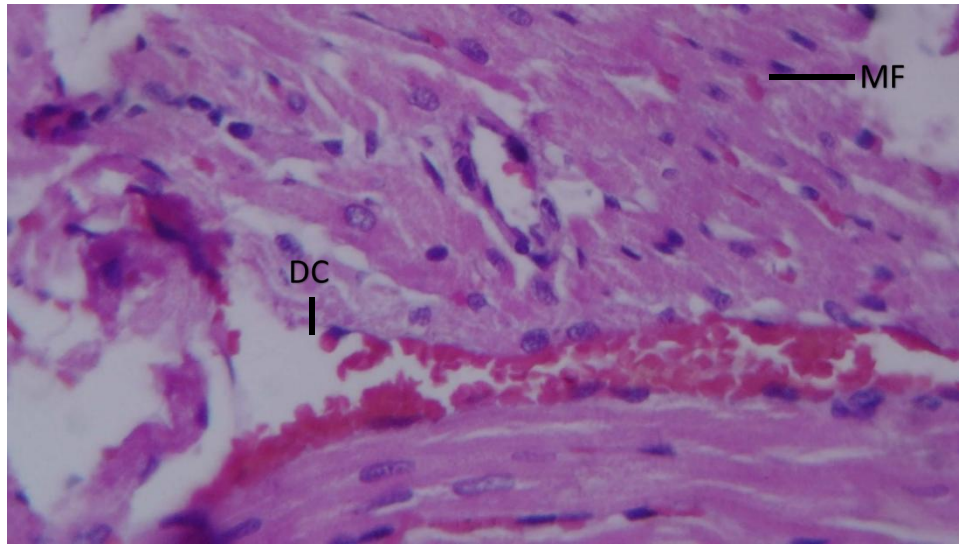
**Table 3.1.2: Triglycerides and Cholesterol levels in the heart of Wistar rats.**

GROUP	TC(mg/dL)	TG(mg/dL)
GROUNDNUT OIL FRIED EGG YOLK	$589.19 \pm 4.03^a$	$259.53 \pm 3.05$
RAW CHOLESTEROL	$577.09 \pm 9.53$	$307.63 \pm 7.74^a$
POSITIVE	$569.46 \pm 5.50$	$259.53 \pm 4.03$
NEGATIVE	$558.77 \pm 7.93$	$277.09 \pm 9.16$

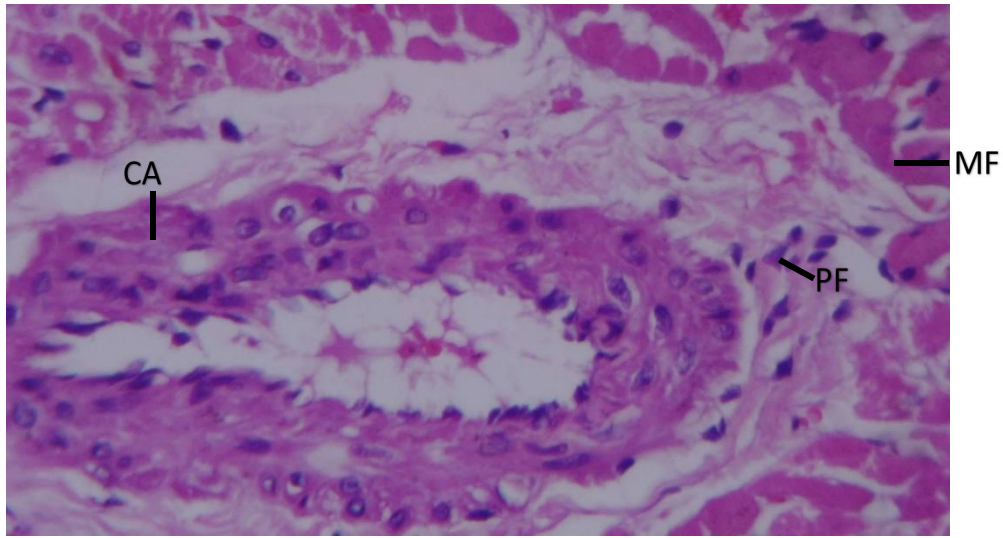
### 3.3.3 Histological Result of the rats from different groups.



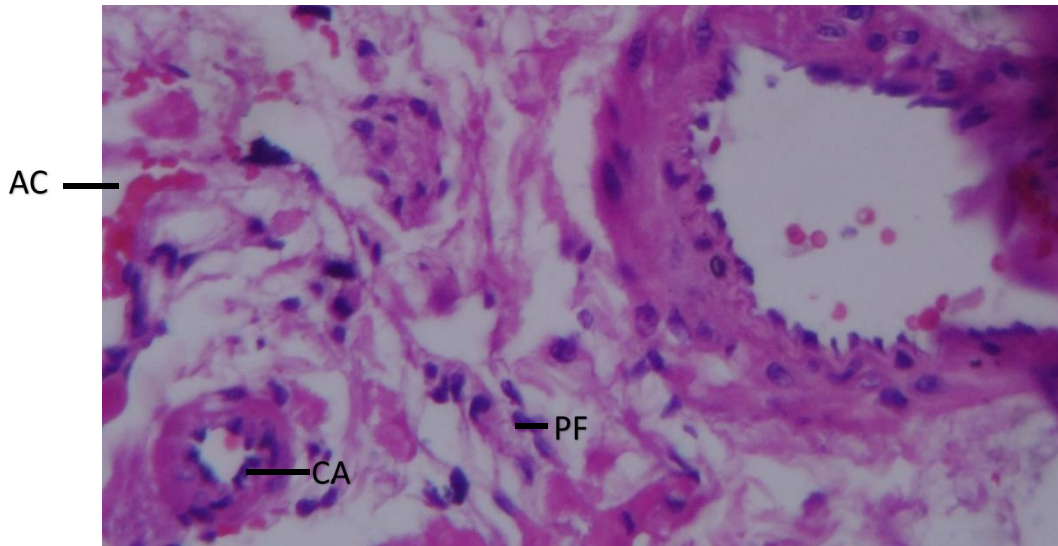
**Plate 1: Rat heart normal control, show normal bundles of myocardial fibres (MF), interstitial space (IS) and coronary artery (CA): H&E 400 X**



**Plate 2: Rat heart given fried egg yolk + groundnut oil show: normal bundles of cardiomyocytes (MF), vasodilatation and active congestion (DC):H&E 400 X**



**Plate 3: Rat heart given cholesterol only show: normal bundles of cardiomyocytes (MF), coronary artery (CA) and mild perivascular mobilization of fibroblasts (PF): H&E 400 X**



**Plate 4. Rat heart given Soya oil show: active interstitial congestion (AC), normal vascular architecture (CA) and perivascular mobilization of fibroblasts (PF): H&E 400 X**

## CHAPTER FOUR

### DISCUSSION AND CONCLUSION

#### 4.1 DISCUSSION

This study investigated the effects of frying with groundnut oil on egg cholesterol and its impact on the histology of the Wistar rats. The findings provide an insight in the significant increase and decrease in the triglyceride and cholesterol levels in the different groups with its varying formulated diet.

When cholesterol levels increase, it can result in atherosclerosis, a condition marked by the buildup of fats, inflammatory cells, and fibrous materials in the large arteries (Libby *et al.*, 2019). This can hinder blood circulation to essential organs such as the heart. Elevated cholesterol level can lead to heart related issues or even death if left untreated (Grundy *et al.*, 2019).

According to the American Heart Association (2022), elevated triglyceride levels can contribute to the development of small, dense low-density lipoprotein (LDL) particles, which are prone to oxidation and can more easily infiltrate the arterial wall, resulting in inflammation and atherosclerosis. Research published in the Journal of the American College of Cardiology indicated that high triglyceride levels were linked to an increased risk of cardiovascular incidents, such as heart attacks and strokes (Miller *et al.*, 2019).

Rats fed a standard diet with unrestricted access to water exhibited typical heart structure, characterized by clearly defined bundles of myocardial fibers (heart muscle cells), interstitial spaces and cardiac blood vessels, including branches of the coronary artery and cardiac veins' tributaries.

Those on a fried egg yolk with groundnut oil diet showed normal myocardial fiber bundles along with signs of increased blood flow and vasodilation, indicating beneficial effects of improved circulation and vessel expansion. Enhanced blood circulation and the expansion of blood vessels signal a robust cardiovascular system. The process of vasodilation, which refers to the enlargement of blood vessels, facilitates a greater blood supply to the heart muscle, supplying it with essential oxygen and nutrients (Brady *et al.*, 2018). This occurs through the relaxation of smooth muscle cells located in the walls of blood vessels, resulting in increased blood flow (Opie *et al.*, 2018).

Lastly, sections from rats on a cholesterol-rich diet revealed normal myocardial fiber bundles and coronary arteries, although there was a reactive mobilization of fibroblasts surrounding the blood vessels. The coronary artery (CA) is observed to be normal, indicating that the administration of cholesterol did not lead to major atherosclerotic alterations in these arteries (Grundy *et al.*, 2019). The coronary arteries are essential for delivering oxygen and nutrients to the heart muscle, and any impairment to these vessels can result in cardiac dysfunction.

The slight mobilization of perivascular fibroblasts (PF) suggests there is a mild inflammatory reaction occurring within the heart tissue (Libby *et al.*, 2019). Fibroblasts are crucial cells involved in the synthesis of extracellular matrix and collagen, and their mobilization may signal a response to tissue damage or inflammation.

The detection of mild perivascular fibrosis indicates that cholesterol administration may have resulted in some degree of vascular injury or inflammatory response, which could contribute to the onset of atherosclerosis (Libby *et al.*, 2019). Nevertheless, the observation that both the cardiomyocytes and coronary arteries remain normal implies that significant cardiac damage did not result from the cholesterol administration.

## **4.2 CONCLUSION**

The study demonstrated that dietary interventions involving Groundnut oil fried egg yolk and raw cholesterol significantly influenced the histological and biochemical status of the heart in Wistar rats.

These findings point out the importance of dietary choices in preventing cardiovascular diseases and maintaining the heart health. Future studies are needed to fully understand the effects of this diet on cardiovascular health.

## REFERENCES

- Baur, J. A., Pearson, K. J., Price, N. L., Jamieson, H. A., Lerin, C., Kalra, A., ... & Sinclair, D. A. (2006). Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*, 444(7117), 337-342.
- Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., & Virani, S. S. (2019). Heart disease and stroke statistics—2019 update: A report from the American Heart Association. *Circulation*, 139(10), e56-e528.
- Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., ... & Virani, S. S. (2019). Heart disease and stroke statistics—2019 update: *A report from the American Heart Association*. *Circulation*, 139(10), e56-e528.
- Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *Biochemistry* (5th ed.). New York: W.H. Freeman and Company.
- Brady, A. J., Rockman, H. A., & Ross, R. S. (2018). Cardiac structure and function in the rat. *Journal of Molecular and Cellular Cardiology*, 114, 241-253.
- Chen, F., Zhang, J., & Chen, Y. (2018). Effects of cooking methods on the nutritional quality of egg yolks. *Journal of Food Science*, 83(5), S1448-S1456.
- Goldstein, J. L., Brown, M. S., & Anderson, R. G. (2009). Receptor-mediated endocytosis of low-density lipoprotein. *Annual Review of Cell Biology*, 25, 257-275.
- Greaves, P. (2012). *Histopathology of preclinical toxicity studies*. Academic Press.

Grundy, S. M., Cleeman, J. I., Merz, C. N., Brewer, H. B., Clark, L. T., Hunninghake, D. B., ... & Stone, N. J. (2004). Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Journal of the American College of Cardiology*, 44(3), 720-732.

Grundy, S. M., Stone, N. J., Bailey, A. L., Beam, C., Birtcher, K. K., Blumenthal, R. S., & Braun, L. T. (2019). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journal of the American College of Cardiology*, 73(24), e285-e350.

Grundy, S. M., Stone, N. J., Bailey, A. L., Beam, C., Birtcher, K. K., Blumenthal, R. S., & Braun, L. T. (2019). 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol. *Circulation*, 139(25), e1046-e1081.

Harris, W. S., Bulchandani, D., & Goodnight, S. H. (2007). Omega-3 fatty acids and cardiovascular disease. *Journal of Cardiovascular Medicine*, 8(1), 64-71.

Hu, F. B., Stampfer, M. J., Rimm, E. B., Manson, J. E., Ascherio, A., Colditz, G. A., ... & Willett, W. C. (2019). Dietary fat intake and risk of cardiovascular disease in women. *New England Journal of Medicine*, 381(25), 2459-2468.

- Huang, T., Yang, B., Zheng, J., Li, G., Wahlqvist, M. L., & Li, D. (2015). Cardiovascular disease mortality and cancer incidence in vegetarians: A meta-analysis and systematic review. *Annals of Nutrition and Metabolism*, 66(2), 113-122.
- Huang, T., Yang, B., Zheng, J., Li, G., Wahlqvist, M. L., & Li, D. (2019). Cardiovascular disease mortality and cancer incidence in vegetarians: A meta-analysis and systematic review. *Annals of Nutrition and Metabolism*, 74(2), 131-138.
- Krauss, R. M., Blanche, P. J., & Rawlings, R. S. (2017). Effects of dietary cholesterol on plasma lipoproteins and cardiovascular disease risk. *Journal of Lipid Research*, 58(3), 551-564.
- Krauss, R. M., Blanche, P. J., & Rawlings, R. S. (2017). Effects of dietary cholesterol on plasma lipoproteins and cardiovascular disease risk. *Journal of Lipid Research*, 58(3), 551-564.
- Kris-Etherton, P. M., Petersen, B. L., & Kritchevsky, D. (2017). Effects of peanut oil on cardiovascular disease risk factors. *Journal of Nutrition*, 147(12), 2241-2248.
- Kris-Etherton, P. M., Zhao, G., Binkoski, A. E., Coval, S. M., & Etherton, T. D. (2002). The effects of nuts on coronary heart disease risk. *Nutrition in Clinical Practice*, 17(2), 133-141.
- Kritchevsky, D. (2005). Dietary lipid requirements for optimal health. *Journal of Nutrition*, 135(11), 2795-2801.
- Kritchevsky, D. (2018). History of recommendations to restrict dietary cholesterol. *Nutrition Today*, 53(4), 164-170.

- Kumar, V., Kumar, N., & Sharma, P. (2018). Cardiovascular disease in Wistar rats: A review. *Journal of Cardiovascular Pharmacology*, 71(3), 151-162.
- Kusnandar, F., Kharisma, T., Yuliana, N.D., Safrida, S., Budijanto, S. (2022). The Hypocholesterolemic Effect of Analogue Rice with the Addition of Rice Bran. *Current Research in Nutrition and Food Science Journal*, 10(1):183-194
- Li, D., & Sinclair, A. (2002). Macronutrient and fatty acid profiles of eggs from different production systems. *Journal of Food Science*, 67(6), 2211-2216.
- Libby, P., Buring, J. E., Badimon, L., Hansson, G. K., Deanfield, J., Bittencourt, M. S., & Lüscher, T. F. (2019). Atherosclerosis: A tale of two diseases. *Journal of the American College of Cardiology*, 73(11), 1339-1348.
- Liu, X., Liu, Y., & Huang, Y. (2019). Peanut oil and cardiovascular health: A systematic review and meta-analysis. *Nutrients*, 11(11), 2531.
- Lopaschuk, G. D., Ussher, J. R., & Jaswal, J. S. (2018). Lipid metabolism and cardiac function. *Journal of Molecular and Cellular Cardiology*, 123, 111-118.
- Man-Yu, Y., Siu-Wai, L., & Ka-Hay, W. (2020). Egg consumption and risk of cardiovascular disease and mortality: A systematic review and meta-analysis. *Nutrients*, 12(11), 3221.
- Miller, M., Stone, N. J., Ballantyne, C., Bittner, V., Criqui, M. H., Ginsberg, H. N., & Jacobson, M. S. (2019). Triglycerides and cardiovascular disease: A scientific statement from the American Heart Association. *Journal of the American College of Cardiology*, 73(11), 1339-1348.

- Opie, L. H., Hasenfuss, G., & Pieske, B. (2018). *Heart physiology: From cell to circulation*. Oxford University Press.
- Paul, S. R. and Glenn W.F. (2024). Encyclopedia Britannica. <https://www.britannica.com/topic/egg-food#/media/1/180194/16098>.
- Qin, X., Deng, Y., & Huang, Y. (2020). Cholesterol and cardiovascular disease. *Journal of Clinical Lipidology*, 14(3), 251-258.
- Reriani, M. K., Lavi, S., & Lerman, A. (2018). Endothelial dysfunction in patients with cardiovascular disease. *Journal of Cardiovascular Medicine*, 19(12), 641-648.
- Rye, K. A., Bursill, C. A., & Barter, P. J. (2018). HDL and atherosclerosis: Insights from genetic studies. *Annual Review of Nutrition*, 38, 287-307.
- Sicherer, S. H., Sampson, H. A., & Leung, D. Y. (2010). Peanut allergy. *Journal of Allergy and Clinical Immunology*, 125(3), S85-S91.
- Soliman, G.A. (2018). Dietary Cholesterol and the lack of Evidence on Cardiac Vascular Disease. *Nutrients*, 10(6) 780.
- Tomanek, R. J., Torry, R. J., & Zheng, W. (2017). Cardiac hypertrophy and failure in Wistar rats: A model for human heart disease. *American Journal of Physiology-Heart and Circulatory Physiology*, 313(3), H533-H542.
- Traber, M. G. (2007). Vitamin E regulatory mechanisms. *Annual Review of Nutrition*, 27, 347-362.

USDA (United States Department of Agriculture). (2020). *Dietary Guidelines for Americans 2020*.

USDA (United States Department of Agriculture). (2020). Peanut oil. Retrieved from (link unavailable)

Warner, K., & Knowlton, S. (2017). Frying oils and oxidative stability. *Journal of Food Science*, 82(5), S1478-S1485.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *American Journal of Clinical Nutrition*, 80(5), 1106-1122.

World Health Organization. (2020). Cardiovascular diseases. Retrieved from (link unavailable)

Wu, J., Zhang, Y., & Wang, X. (2020). Antioxidant activity of peanut oil and its components. *Journal of Agricultural and Food Chemistry*, 68(2), 533-541.

Zhang, Y., Liu, X., & Huang, Y. (2020). Effects of peanut oil on blood lipid profiles: A systematic review and meta-analysis. *Lipids in Health and Disease*, 19(1), 1-11.

**APPENDIX**  
**TRIGLYCERIDES**

**Descriptives**

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	583.1967	6.99607	4.03918	565.8175	600.5759	577.09	590.83
2.00	3	577.0900	16.51342	9.53403	536.0684	618.1116	558.77	590.83
3.00	3	569.4567	9.53403	5.50447	545.7728	593.1405	558.77	577.09
4.00	3	558.7700	13.74000	7.93279	524.6379	592.9021	545.03	572.51
Total	12	572.1283	14.14462	4.08320	563.1413	581.1154	545.03	590.83

## ANOVA

VAR00001

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	998.127	3	332.709	2.213	.164
Within Groups	1202.647	8	150.331		
Total	2200.774	11			

## Multiple Comparisons

Dependent Variable: VAR00001

			Mean		Sig.	95% Confidence Interval		
			(I) VAR00002	(J) VAR00002		Difference (I-J)	Std. Error	Lower Bound
Tukey HSD	1.00	2.00		6.10667	10.01102	.926	-25.9522	38.1655
		3.00		13.74000	10.01102	.548	-18.3188	45.7988
		4.00		24.42667	10.01102	.146	-7.6322	56.4855
	2.00	1.00		-6.10667	10.01102	.926	-38.1655	25.9522
		3.00		7.63333	10.01102	.869	-24.4255	39.6922
		4.00		18.32000	10.01102	.327	-13.7388	50.3788
	3.00	1.00		-13.74000	10.01102	.548	-45.7988	18.3188
		2.00		-7.63333	10.01102	.869	-39.6922	24.4255
		4.00		10.68667	10.01102	.717	-21.3722	42.7455
	4.00	1.00		-24.42667	10.01102	.146	-56.4855	7.6322
		2.00		-18.32000	10.01102	.327	-50.3788	13.7388
		3.00		-10.68667	10.01102	.717	-42.7455	21.3722
LSD	1.00	2.00		6.10667	10.01102	.559	-16.9788	29.1921

	3.00	13.74000	10.01102	.207	-9.3455	36.8255
	4.00	24.42667*	10.01102	.041	1.3412	47.5121
2.00	1.00	-6.10667	10.01102	.559	-29.1921	16.9788
	3.00	7.63333	10.01102	.468	-15.4521	30.7188
	4.00	18.32000	10.01102	.105	-4.7655	41.4055
3.00	1.00	-13.74000	10.01102	.207	-36.8255	9.3455
	2.00	-7.63333	10.01102	.468	-30.7188	15.4521
	4.00	10.68667	10.01102	.317	-12.3988	33.7721
4.00	1.00	-24.42667*	10.01102	.041	-47.5121	-1.3412
	2.00	-18.32000	10.01102	.105	-41.4055	4.7655
	3.00	-10.68667	10.01102	.317	-33.7721	12.3988

\*. The mean difference is significant at the 0.05 level.

**VAR00001**

		Subset	for
		alpha = 0.05	
VAR00002 N		1	
Tukey HSD <sup>a</sup>	4.00	3	558.7700
	3.00	3	569.4567
	2.00	3	577.0900
	1.00	3	583.1967
	Sig.		.146

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

## CHOLESTEROL

escriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for		Minimum	Maximum
					Mean			
					Lower Bound	Upper Bound		
1.00	3	259.5333	5.28853	3.05333	246.3959	272.6708	256.48	265.64
2.00	3	307.6300	13.42187	7.74912	274.2882	340.9718	297.70	322.90
3.00	3	259.5333	6.99607	4.03918	242.1541	276.9125	251.90	265.64
4.00	3	277.0900	9.16000	5.28853	254.3353	299.8447	267.93	286.25
<b>Total</b>	<b>12</b>	<b>275.9467</b>	<b>21.97879</b>	<b>6.34473</b>	<b>261.9820</b>	<b>289.9113</b>	<b>251.90</b>	<b>322.90</b>

**ANOVA**

**VAR00001**

	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	<b>4631.808</b>	<b>3</b>	<b>1543.936</b>	<b>18.113</b>	<b>.001</b>
<b>Within Groups</b>	<b>681.932</b>	<b>8</b>	<b>85.241</b>		
<b>Total</b>	<b>5313.739</b>	<b>11</b>			

## Multiple Comparisons

Dependent Variable: VAR00001

		Mean			95% Confidence Interval		
		Difference (I-					
	(I) VAR00002 (J) VAR00002 J)		Std. Error	Sig.	Lower Bound	Upper Bound	
Tukey HSD	1.00	2.00	-48.09667*	7.53841	.001	-72.2373	-23.9560
		3.00	.00000	7.53841	1.000	-24.1406	24.1406
		4.00	-17.55667	7.53841	.170	-41.6973	6.5840
	2.00	1.00	48.09667*	7.53841	.001	23.9560	72.2373
		3.00	48.09667*	7.53841	.001	23.9560	72.2373
		4.00	30.54000*	7.53841	.016	6.3994	54.6806
	3.00	1.00	.00000	7.53841	1.000	-24.1406	24.1406
		2.00	-48.09667*	7.53841	.001	-72.2373	-23.9560
		4.00	-17.55667	7.53841	.170	-41.6973	6.5840
	4.00	1.00	17.55667	7.53841	.170	-6.5840	41.6973
		2.00	-30.54000*	7.53841	.016	-54.6806	-6.3994
		3.00	17.55667	7.53841	.170	-6.5840	41.6973
LSD	1.00	2.00	-48.09667*	7.53841	.000	-65.4803	-30.7131

	<b>3.00</b>	<b>.00000</b>	<b>7.53841</b>	<b>1.000</b>	<b>-17.3836</b>	<b>17.3836</b>
	<b>4.00</b>	<b>-17.55667*</b>	<b>7.53841</b>	<b>.048</b>	<b>-34.9403</b>	<b>-.1731</b>
<b>2.00</b>	<b>1.00</b>	<b>48.09667*</b>	<b>7.53841</b>	<b>.000</b>	<b>30.7131</b>	<b>65.4803</b>
	<b>3.00</b>	<b>48.09667*</b>	<b>7.53841</b>	<b>.000</b>	<b>30.7131</b>	<b>65.4803</b>
	<b>4.00</b>	<b>30.54000*</b>	<b>7.53841</b>	<b>.004</b>	<b>13.1564</b>	<b>47.9236</b>
<b>3.00</b>	<b>1.00</b>	<b>.00000</b>	<b>7.53841</b>	<b>1.000</b>	<b>-17.3836</b>	<b>17.3836</b>
	<b>2.00</b>	<b>-48.09667*</b>	<b>7.53841</b>	<b>.000</b>	<b>-65.4803</b>	<b>-30.7131</b>
	<b>4.00</b>	<b>-17.55667*</b>	<b>7.53841</b>	<b>.048</b>	<b>-34.9403</b>	<b>-.1731</b>
<b>4.00</b>	<b>1.00</b>	<b>17.55667*</b>	<b>7.53841</b>	<b>.048</b>	<b>.1731</b>	<b>34.9403</b>
	<b>2.00</b>	<b>-30.54000*</b>	<b>7.53841</b>	<b>.004</b>	<b>-47.9236</b>	<b>-13.1564</b>
	<b>3.00</b>	<b>17.55667*</b>	<b>7.53841</b>	<b>.048</b>	<b>.1731</b>	<b>34.9403</b>

\*. The mean difference is significant at the 0.05 level.

**VAR00001**

		Subset for alpha =	
		0.05	
VAR00002 N		1	2
Tukey HSD <sup>a</sup>	3.00	3	259.5333
	1.00	3	259.5333
	4.00	3	277.0900
	2.00	3	307.6300
	Sig.	.170	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.