

**CONSUMPTION OF BOILED EGG CHOLESTEROL FORMULATED DIET
ON THE HISTOLOGY AND ANTIOXIDANT OF LIVER STATUS OF WHISTAR
RATS**

BY

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CERTIFICATION

This is to certify that this project research was carried out by MISS NJOKU BLESSING CHIDINMA with MAT NO. LSC2103771. from the department of Biochemistry. Faculty of Life Sciences, University of Benin, Edo State. This was done for the Partial Fulfillment for the Bachelor of Sciences (B.sc) degree of Biochemistry.



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DEDICATION

I wholeheartedly dedicate this project to God Almighty and my parents for their full support, guidance and encouragement. Thank you God for the strength so far despite having the feeling to give up. Thank you Dad and Mom for always encouraging your daughter.

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I sincerely want to acknowledge my supervisor, DR CYRIL UGBENI for his ever guidance, support and his professional insights in my project research. Thank you so much sir. God richly Bless you always. I also want to acknowledge the entire staff, lecturers, professors for the knowledge passed onto me in acquiring this degree. I'm forever grateful. In addition. I want to acknowledge my family, my parents Mr and Mrs Ben Njoku for always supporting me financially, morally and spiritually. For always encouraging me by saying, Greatness is not for The Lazy nor Weak but For the Strong. Be Strong for the End is Beautiful. These words impacted a lot of success in my work.

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ABSTRACT.

The liver plays a crucial role in metabolism and detoxification, making it highly susceptible to oxidative stress. This study dives into the effect of a boiled egg yolk-formulated diet on the antioxidant status and histological integrity of the liver of female Wistar rats. A total of 16 female Wistar rats were randomly assigned to four groups: group one(which was fed with boiled egg).group 2 (which was fed with synthetic cholesterol), group 3(which serve as positive control group) and group 4 (which serves as negative control group) for six weeks. After six weeks of feeding, the rats were sacrificed and the liver were taken for examination/analysis. After this was done, the various antioxidants enzymes were examined such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), along side with malondialdehyde (MDA) which serves as a marker of oxidative stress. The Liver tissues were also examined histologically to assess structural changes. The results showed a significant increase in antioxidant enzyme activities in the boiled egg yolk-fed group compared to the positive control group, suggesting enhanced antioxidant defense mechanisms. In addition,MDA levels were reduced, stating decreased lipid peroxidation and oxidative stress. The histological analysis revealed better-preserved liver architecture in the boiled egg yolk group, with minimal inflammatory changes compared to the cholesterol-rich diet group, which showcased signs of cellular degeneration. In conclusion, the consumption of a boiled egg yolk- formulated diet may contribute to improved liver antioxidant status and histological integrity. These findings highlight the nutritional benefits of egg yolk and its potential role in maintaining liver health.

CHAPTER ONE

1.1 Introduction

Dietary cholesterol plays an important role in nutrition and has many functions in both humans and animals. It is a key part of cell membranes, helping them stay flexible and work properly. Cholesterol is also needed to create important substances like steroid hormones (such as cortisol, estrogen, and testosterone), bile acids, and vitamin D. which are all crucial for metabolism, reproductive health, and strong bones (Kritchevsky, 2000).

In animals, cholesterol is essential for developing and keeping physiological systems like the nervous and immune systems healthy. For humans, eating cholesterol in moderation helps support these functions, but too much can lead to health problems like heart disease and metabolic issues (Lecerf & de Lorgeril, 2011).

Even with worries about how cholesterol affects blood levels, dietary cholesterol has a limited effect on most people's blood cholesterol because the liver helps regulate it (Fernandez, 2012). This highlights the importance of a balanced diet since cholesterol is necessary for keeping our cells and overall health in good shape.

Eggs are one of the best sources of dietary cholesterol, with the yolk containing about 200-250 milligrams per egg. While there are concerns about cholesterol, eggs are still seen as a valuable part of both human and animal diets because of their nutrition. They provide high-quality protein, including all nine essential amino acids needed for muscle growth, tissue repair, and overall body function (Haug & Lantsch, 1983).

In addition to protein. Eggs are packed with important nutrients besides protein. They contain various vitamins like A, D, E, B12, riboflavin, and folate, as well as minerals such as iron, phosphorus, and selenium. They also have healthy fats, including omega-3 fatty acids (Micha et al., 2017). Additionally, eggs are a great source of choline, which is vital for brain health, liver function, and cell stability (Zeisel et al., 1999). These nutrients help with overall health by supporting metabolism,

boosting the immune system, and enhancing brain performance. Eggs also provide antioxidants like lutein and zeaxanthin, which are good for eye health and can help prevent age-related vision problems (Rural et al., 2014). Even though eggs contain cholesterol, when eaten in moderation, they can fit well into a balanced diet without harming heart health for most people (Fernandez & Wolf, 2012).

Boiled eggs are a key source of cholesterol, but boiling them has health benefits and helps keep their nutrients intact. Unlike frying, which can lead to harmful changes in cholesterol and create unhealthy compounds like trans fats, boiling eggs reduces these risks. This makes boiled eggs a healthier choice for getting cholesterol without the negative effects linked to other cooking methods (Dunford et al., 2013). Cholesterol from boiled eggs is important for keeping our cells healthy since it is a major part of cell membranes and helps with making hormones, bile acids, and digesting fats (Lecerf & de Lorgeril, 2011). Boiling eggs doesn't change their cholesterol levels much, but it does help keep it stable and less likely to oxidize compared to raw eggs or those cooked at high temperatures (Liu et al., 2014).

Moreover, Boiling eggs helps keep important nutrients like protein, vitamins A, D, B12, and minerals intact. These nutrients provide various health benefits, including boosting the immune system and improving brain health (Zeisel et al., 1999). Because of this, boiled eggs are a healthy and safe way to add cholesterol to a balanced diet.

Dietary cholesterol is important for our health, especially for how it affects the liver. The liver is the main organ that processes cholesterol, and eating too much can cause problems like fat buildup, inflammation, and changes in liver structure, which can lead to issues like fatty liver disease or fibrosis (Siri-Tarino et al., 2010). These changes can make it harder for the liver to detoxify substances, causing more health problems.

Moreover, a high intake of cholesterol can increase oxidative stress, resulting in the creation of reactive oxygen species (ROS) that can harm liver cells. This damage can overwhelm the liver's

natural defenses, leading to a drop in important antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx), which are vital for keeping cells healthy (Grattagliano et al.. 2012). When there is too much ROS and not enough antioxidants, liver damage can worsen and lead to diseases such as non-alcoholic fatty liver disease (NAFLD) and cirrhosis.

Knowing about these effects is crucial for creating diet plans that can reduce the negative impact of cholesterol on liver health and help restore the balance of antioxidants, ensuring that the liver continues to function well.

The liver has an important and varied role in managing cholesterol and protecting against oxidative damage. It is the main organ that controls cholesterol levels by producing it, turning it into bile acids, and sending it out through bile. The liver carefully controls cholesterol production with enzymes like HMG-CoA reductase, which regulates the main step in making cholesterol (Brown & Goldstein. 1986). The liver also packages cholesterol into lipoproteins, such as low-density lipoproteins (LDL), which carry cholesterol to other parts of the body, and high-density lipoproteins (HDL), which help bring cholesterol back to the liver for disposal (Rader & Daugherty, 2008).

Besides managing cholesterol, the liver is also crucial for the body's defense against antioxidants. It creates important antioxidant substances like glutathione, which helps neutralize harmful reactive oxygen species (ROS) and protects liver cells from damage (Sies & Jones. 2020). The liver's antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, work together to lower oxidative stress, preventing liver damage and keeping metabolic balance (Kang & Park, 2017). If cholesterol management or antioxidant defenses are disrupted, it can lead to liver problems, oxidative stress, and diseases like fatty liver or cirrhosis.

1.2 Importance

Understanding how boiling affects a diet rich in egg cholesterol is significant because it can help us

learn more about the connection between dietary cholesterol, liver health, and antioxidant defenses. Cooking methods like boiling can change the chemical properties of cholesterol and other nutrients, which might affect how they influence liver structure and oxidative stress.

This research aims to explore the effects of boiled eggs on liver health by:

1. **Providing Insights into Liver Health:** It will show how boiling eggs impacts the structure of the liver, highlighting whether it helps prevent or worsen conditions like fatty liver or fibrosis.
2. **Enhancing Nutritional Guidelines:** The study will give evidence-based advice on how to safely eat eggs to support liver health.
3. **Clarifying Antioxidant Dynamics:** It will explain how the liver responds with antioxidants to the cholesterol found in boiled eggs, helping us understand how to manage oxidative stress.
4. **Addressing Public Health Concerns:** This research will offer strategies to reduce the risks linked to high cholesterol diets, especially for people at risk of liver diseases.
5. **Encouraging Safer Dietary Practices:** It will emphasize how cooking methods can change the health effects of dietary cholesterol, promoting safer eating habits.

1.3 Aims and Objectives

1. **Investigate the Impact on Liver Histology:** The study will look at how a diet high in boiled egg cholesterol affects the liver's structure, including cellular health, fat buildup, and possible damage.
2. **Assess Antioxidant Status:** It will check the levels of important antioxidant enzymes (like glutathione peroxidase, superoxide dismutase, and catalase) and other antioxidants in the liver.
3. **Determine Oxidative Stress Levels:** The research will measure oxidative stress markers, such as malondialdehyde (MDA), to understand how reactive oxygen species (ROS) and antioxidants balance each other.
4. **Compare Effects with Other Cholesterol Sources:** It will analyze how boiling eggs changes the effects of egg cholesterol on the liver compared to raw' eggs or other cholesterol sources.

5. Evaluate Protective Mechanisms of Boiling: The study will explore how cooking eggs might protect the liver from damage due to cholesterol, providing insights into safer eating choices.

CHAPTER TWO

2.1 Introduction to Dietary' Cholesterol

Dietary cholesterol refers to the cholesterol we get from food, particularly from animal products. It is essential for building cell membranes, producing hormones, and making bile acids. While our liver makes cholesterol, the amount we eat also affects the total cholesterol in our bodies. This cholesterol is a key type of steroid found in our tissues and plays an important role in many biological processes (Grundy, S.M., et al. (2018)).

2.1.1 Sources of Dietary Cholesterol

1. Egg Yoiks

- Egg yolks are among the highest sources of dietary cholesterol, with about 186 mg in each large egg. Even though they contain a lot of cholesterol, research indicates that eating eggs in moderation does not significantly raise the risk of heart disease for most people (Source: Shin et al., 2013).

2. Shellfish

- Shellfish, including shrimp, crab, and lobster, are high in cholesterol but have low levels of saturated fat. Studies show that when eaten as part of a healthy diet, shellfish have a minimal effect on blood cholesterol levels (Source: Grundy et al., 2018).

3. Organ Meats

- Organ meats like liver and kidneys are packed with nutrients and cholesterol. Although these foods are usually eaten in small amounts, they can significantly add to your dietary cholesterol intake.

2.1.2 THE ROLE OF CHOLESTEROL IN HUMAN AND ANIMAL NUTRITION

Cholesterol is important for both human and animal nutrition. Here are six main functions it serves:

1. Cell Membrane Structure: Cholesterol is a key part of cell membranes. It helps keep the membranes stable and flexible, which is vital for cells to work properly (Jiang et al.. 2016).

2. **Precursor to Hormones:** Cholesterol helps create steroid hormones such as cortisol, estrogen, testosterone, and progesterone. These hormones are essential for metabolism, reproduction, and the immune system (Toth et al., 2012).
3. **Bile Acid Synthesis:** The liver converts cholesterol into bile acids, which are necessary for digesting and absorbing dietary fats and fat-soluble vitamins (Puddu et al., 2009).
4. **Vitamin D Production:** When sunlight hits the skin, cholesterol helps produce vitamin D. This vitamin is important for calcium use and bone health (Meyer et al., 2011).
5. **Nervous System Function:** Cholesterol is a major part of myelin, the protective layer around nerve fibers. It is crucial for transmitting nerve signals and for brain function (Pfrieger, 2003).
6. **Immune Function:** Cholesterol helps the immune system work properly. It plays a role in forming lipid rafts in cell membranes, which help with cell signaling and immune responses (Liu et al., 2014).

2.1.3 HEALTH IMPLICATIONS OF EXCESSIVE CHOLESTEROL INTAKE.

Consuming too much cholesterol, especially from foods high in saturated and trans fats, can lead to several health problems:

1. **Atherosclerosis:** High cholesterol levels can cause plaque to build up in arteries, a condition known as atherosclerosis. This can narrow blood vessels, decrease blood flow, and raise the risk of heart disease, stroke, and peripheral artery disease (Libby. 2002).

2. Heart Disease: High levels of low-density lipoprotein (LDL) cholesterol, often called "bad cholesterol." can lead to plaque buildup in the coronary arteries. This can raise the chances of developing coronary artery disease, experiencing heart attacks, and suffering from angina (Stone et al., 2014).
3. Hypertension (High Blood Pressure): When cholesterol levels are high, they can make blood vessels stiff, which can cause more resistance to blood flow. Over time, this can raise blood pressure (Ginsberg et al., 2000).
4. Gallstones: Too much cholesterol in bile can result in gallstones, which can be painful, lead to infections, and sometimes require surgery (Korman et al., 2015).
5. Obesity: Diets that include a lot of foods high in cholesterol often also contain high calories and fats, which can lead to obesity. Being obese is a major risk factor for heart disease, type 2 diabetes, and some cancers (Lindstrom et al., 2003).
6. Liver Damage: Eating too much cholesterol can put extra stress on the liver, possibly causing fatty liver disease. If not treated, this condition can progress to cirrhosis or liver failure (Leung et al., 2015).
7. Increased Risk of Stroke: Cholesterol buildup in the arteries can also heighten the risk of stroke. If plaque blocks or reduces blood flow to the brain, it can cause an ischemic stroke (Warlow et al., 2008).

2.2. NUTRITIONAL COMPOSITION OF EGGS.

Eggs are very nutritious and have a complex mix of chemicals that provide important nutrients in a balanced way. They are a food packed with nutrition, offering a good mix of high-quality protein, healthy fats, vitamins, and minerals. The makeup of eggs differs slightly between the white and the yolk; the yolk contains most of the fats, vitamins, and minerals, while the egg white is mostly made up of water and protein.

Here's a simplified breakdown of the important parts of eggs and their significance;

1. Proteins:

Eggs are a fantastic source of high-quality protein, especially found in the egg white (albumen). The

proteins in eggs have all the essential amino acids, making them a complete protein source.

-Albumen Proteins: The main proteins in egg white include ovalbumin (which makes up about 54% of the total protein), ovotransferrin, ovomucoid, and lysozyme. These proteins have various roles, such as protecting the embryo, supporting the immune system, and keeping the egg stable (Borkowski et al., 2021).

- Yolk Proteins: The yolk contains lipoproteins like vitellin and phosvitin, which store nutrients for the developing embryo. These proteins also help bind minerals like iron and add to the yolk's nutritional value.

2. Lipids:

Eggs are loaded with lipids, especially in the yolk, and have a good mix of saturated and unsaturated fats.

- Cholesterol: As mentioned before, eggs, especially the yolk, have a lot of cholesterol, which is important for making cell membranes, hormones, and bile acids. A large egg has about 186 mg of cholesterol (Micha et al., 2017).

- Phospholipids: The yolk is also full of phospholipids, especially lecithin. Lecithin helps mix different substances together and is crucial for how cells are built and work (Chang et al., 2016).

- Fatty Acids: Eggs have both saturated and unsaturated fatty acids. Unsaturated fatty acids include oleic acid (monounsaturated) and linoleic acid (polyunsaturated), which are good for your heart. The amounts of these fatty acids can change based on what the hen eats (Nestel et al., 2006).

3. Carbohydrates:

Eggs have a tiny amount of carbohydrates, mostly found in the egg white. These carbohydrates come mainly as glycoproteins and glucose, adding up to about 0.6 grams of carbs in a large egg. This amount is very small, so eggs are considered a low-carb food (Liu et al., 2013).

4. Vitamins:

Eggs are packed with many vitamins, especially those that dissolve in fat and water.

- Vitamin A: The yolk has a good amount of vitamin A, including retinol and carotenoids, which are

vital for vision, immune health, and skin care (DePew et al., 2013).

- Vitamin D: Eggs are one of the few natural food sources of vitamin D, especially in the yolk.

Vitamin D is important for helping the body absorb calcium and keeping bones strong.

- B-Vitamins: Eggs are a source of several B-vitamins, including B2 (riboflavin), B12 (cobalamin), B5 (pantothenic acid), and folate. These vitamins help with energy production, making red blood cells, and supporting the nervous system (Borkowski et al., 2021).

5. Minerals:

Eggs offer a variety of important minerals.

- Iron: The yolk is a good source of iron, especially heme iron, which the body absorbs more easily than iron from plants (Liu et al., 2013).
- Calcium: While eggs don't have a lot of calcium, they still provide a small amount of this mineral, which is important for strong bones and muscle function.
- Phosphorus: The yolk is high in phosphorus, which is crucial for bone health, energy production, and cell function (DePew et al., 2013).

6. Water:

About 74% of an egg's weight comes from water, mainly found in the egg white. Water is essential for keeping the egg's structure intact and helps with dissolving proteins and transporting nutrients.

7. Other Bioactive Compounds:

Eggs have several bioactive compounds, including carotenoids like lutein and zeaxanthin found in the yolk. These compounds act as antioxidants and help protect eye health by lowering the chances of macular degeneration (McNamara et al., 2016).

2.2.1. Biochemical Effect of Eggs as a Source of Dietary Cholesterol

Eggs, especially the yolk, are an important source of dietary cholesterol because of their unique biochemical makeup and the presence of cholesterol-rich lipoproteins. Here's how eggs provide dietary cholesterol:

1. Composition of Egg Yolk: The yolk contains a lot of lipids, including cholesterol. Cholesterol in the yolk is part of the lipid bilayers in lipoproteins, mainly low-density lipoproteins (LDL), which are the main carriers of cholesterol. In the yolk, cholesterol is combined with fatty acids to form cholesterol esters, and it is also found as free cholesterol (Micha et al., 2017).

2. Synthesis of Cholesterol in Eggs: The hen makes cholesterol during the egg formation process. It is created in the hen's liver from acetyl-CoA, a molecule produced when fats, carbohydrates, and proteins are broken down. The mevalonate pathway is used to synthesize cholesterol, involving several enzyme-driven steps that convert acetyl-CoA into cholesterol (Brown & Goldstein, 1986).

3. Transport of Cholesterol: After cholesterol is made, it is added to the yolk and carried in the form of lipoproteins, such as very-low-density lipoproteins (VLDL). These lipoproteins move from the hen's liver to the ovary, where the egg develops, and cholesterol gets deposited into the growing egg yolk (Shimomura et al., 1990).

4. Cholesterol in Egg Yolk: The cholesterol present in egg yolk exists mainly in two forms: free and esterified. This cholesterol helps keep the yolk's structure stable and maintains the integrity of its membrane. It also plays an important role in keeping cellular membranes flexible and fluid, which is essential for embryo development (Bjorn et al., 2012).

5. Dietary Impact: When you eat egg yolk, the cholesterol is absorbed in your intestines and enters your bloodstream. This cholesterol can affect the levels of LDL cholesterol, often called "bad cholesterol." However, how eating eggs impacts blood cholesterol levels can differ from person to person due to factors like genetics. Recent studies indicate that for most people, eating eggs in moderation does not significantly raise blood cholesterol levels (Hu, 2012).

2.2.2 Comparison of Egg Cholesterol with Other Dietary Sources

Cholesterol is a type of fat molecule that is important for many body functions, including making cell

membranes, producing hormones, and creating bile acids. However, the amount and type of cholesterol found in different foods, such as eggs compared to other items, can be quite different in their biochemical makeup. Below is a comparison of the cholesterol found in eggs and other common food sources:

1. Cholesterol in Eggs:

Eggs, especially the yolks, are one of the highest natural sources of dietary cholesterol. A large egg has about 186 mg of cholesterol, mostly located in the yolk, while the egg white has very little cholesterol (Micha et al., 2017). The cholesterol in eggs is part of lipoproteins, like phospholipids, with around 60-70% in the form of cholesterol esters and the rest as free cholesterol (Jiang et al., 2016).

- Bioavailability: After digestion, the body absorbs cholesterol from eggs in the intestines, which then enters the bloodstream through lipoproteins. However, for healthy individuals, the effect of egg cholesterol on blood cholesterol levels is quite small, as the body can manage cholesterol balance effectively.

2. Cholesterol in Meat:

Meat, such as beef, pork, lamb, and poultry, also has a good amount of cholesterol. For example, a 3-ounce portion of beef contains about 60-90 mg of cholesterol. Red meats like beef and lamb usually have higher levels of saturated fats, which can affect how the body processes cholesterol by raising LDL (low-density lipoprotein) cholesterol, also known as "bad" cholesterol (Liu et al., 2004).

- Saturated Fat: The cholesterol in meat often comes with saturated fat, which can boost the liver's cholesterol production. This may lead to higher blood cholesterol levels, especially LDL cholesterol, increasing the risk of heart disease and atherosclerosis.

3. Cholesterol in Dairy Products:

Dairy items, including milk, cheese, and butter, have different amounts of cholesterol. For instance, whole milk contains about 24 mg of cholesterol per cup, while cheddar cheese has around 30-40 mg per ounce. The cholesterol in dairy products is usually found with saturated fats, especially in full-fat

options (Haddad et al., 2012).

Bioavailability and Effects on Cholesterol Levels: The cholesterol found in dairy products, especially those rich in saturated fats like butter and cheese, tends to impact blood cholesterol levels more than the cholesterol in eggs. This often leads to an increase in both LDL cholesterol and total cholesterol. However, recent studies suggest that dairy fat might not be as bad for heart health as previously believed. Some research even indicates that fermented dairy products could help lower cholesterol levels (Praagman et al., 2016).

Cholesterol in Shellfish:

Shellfish, including shrimp, lobster, and crab, is known for having a relatively high cholesterol level. For instance, a 3-ounce serving of shrimp has about 150 mg of cholesterol (Kritchevsky, 2004). Despite this, shellfish are low in saturated fats, and the omega-3 fatty acids they contain may help balance out some of the negative effects of cholesterol.

- **Omega-3 Fatty Acids:** Shellfish provide omega-3 fatty acids, which have been shown to lower blood triglyceride levels and boost high-density lipoprotein (HDL) cholesterol, commonly known as "good cholesterol" (Patterson et al., 2015). This may lessen the impact of the cholesterol in shellfish on heart health.

Cholesterol in Processed Meats:

Processed meats such as sausages, hot dogs, and deli meats also contain cholesterol, along with high amounts of saturated and often trans fats. These meats can be particularly harmful to heart health due to the mix of cholesterol, trans fats, and high sodium. For example, processed meats can have 70-90 mg of cholesterol in a 3-ounce serving (Strazzullo et al., 2011).

- **Trans Fats:** The trans fats found in processed meats are especially dangerous because they not only raise LDL cholesterol but also lower HDL cholesterol, which significantly increases the risk of heart disease (Mozaffarian et al., 2006).

6. Comparing with Plant-Based Foods:

Plant-based foods like fruits, vegetables, grains, and legumes do not have any cholesterol because they

come from plants, not animals, which produce cholesterol. Plant sterols, such as sitosterol found in nuts, seeds, and vegetable oils, can help lower cholesterol absorption in the intestines, which in turn reduces blood cholesterol levels (Jones, 2009).

- Effect on Cholesterol Levels: While plant-based foods do not add cholesterol to your diet, they can help lower LDL cholesterol. This is due to their dietary fiber, plant sterols, and healthy unsaturated fats. On the other hand, animal-based foods often contain dietary cholesterol and can sometimes raise LDL cholesterol levels.

In summary, although eggs are a significant source of dietary cholesterol, they usually do not affect blood cholesterol levels as much as other animal sources like meat and dairy do. The saturated fat in these foods has a bigger impact on cholesterol levels than cholesterol itself. In contrast, cholesterol from shellfish and lean meats tends to influence blood cholesterol less, especially when eaten with omega-3 fatty acids. Plant-based foods, which have no cholesterol, can help control cholesterol levels, especially by lowering LDL cholesterol.

2.2.4 IMPACT OF COOKING ON EGGS

Cooking, or thermal processing, has a big effect on the nutrients in eggs. Different cooking methods like boiling, frying, and scrambling can change how easily our bodies can use the nutrients, as well as their makeup and stability. Here's a closer look at how cooking influences the main nutrients found in eggs:

1. Proteins:

Egg proteins are very responsive to heat, and cooking can cause them to change shape, a process known as denaturation. This change doesn't affect the amino acids in the proteins, but it does impact how well our bodies can digest them and how they work.

- Denaturation of Proteins: When eggs are cooked, the proteins in the egg white, such as ovalbumin, ovotransferrin, and lysozyme, change from their original shape. This change makes it easier for our bodies to digest these proteins because they become more accessible to digestive enzymes (Kramer et al., 2016).

- Biotin Binding: A concern with eating raw eggs is that they contain avidin, a protein in egg whites that attaches to biotin (a B-vitamin), making it hard for our bodies to absorb it. Cooking eggs denatures avidin, which decreases its ability to bind to biotin, allowing for better absorption of this important vitamin (Bragagnolo & Rodriguez-Amaya, 2002).

2. Lipids (Fats):

Egg yolks have a high amount of lipids, including cholesterol, phospholipids, and fatty acids.

Cooking can also change the structure and properties of these lipids.

- Cholesterol: Cooking eggs does not greatly change their cholesterol levels, but when eggs are fried at high temperatures for a long time, a process called lipid oxidation can happen. This may create oxidized cholesterol, which could be harmful to health, as it might help form plaques in arteries (Kanner et al., 2002).

- Phospholipids: The lecithin found in egg yolk can change when heated, affecting how well it can mix ingredients. While this does not remove the benefits of lecithin, it may reduce its ability to create emulsions, which is important in cooking and food preparation (Borek et al., 2011).

3. Vitamins:

Many vitamins in eggs are sensitive to heat and may break down during cooking, especially the water-soluble and fat-soluble vitamins.

- Vitamin A: Cooking can break down carotenoids (which turn into vitamin A) in egg yolks, but the form of vitamin A known as retinol stays stable during cooking. Moderate heat does not significantly lower vitamin A levels in eggs (Kerr et al., 2013).

- Vitamin D: The vitamin D in eggs is generally stable when heated and is not likely to be affected by regular cooking methods. However, cooking for too long or at very high temperatures might cause some loss (DePew et al., 2013).

- B-Vitamins: Riboflavin (B2) and B12 are mostly stable when heated. On the other hand, folate and pantothenic acid (B5) can break down with heat, especially if eggs are cooked at high temperatures for a long time (Gao et al., 2014).

4. Minerals:

Minerals like calcium, phosphorus, and iron in eggs usually stay stable during cooking. However, cooking can affect how well these minerals are absorbed by the body.

- Iron: The heme iron in egg yolks stays stable when cooked, but cooking can slightly lower the absorption of non-heme iron. However, when eggs are cooked with certain plant foods, the heat can help improve iron absorption by breaking down compounds like phytic acid, which makes it easier for the body to use the iron (Haug & Lantzsch. 1983).
- Phosphorus and Calcium: Cooking has little effect on these minerals. Still, the way calcium is absorbed from eggs can be affected by other food components, such as oxalates. This is more important when eggs are eaten with other foods instead of how they are cooked.

5. Antioxidants: Eggs have lutein and zeaxanthin, which are carotenoids that help protect the eyes. These carotenoids stay stable during cooking but can break down if they are exposed to heat for too long.

- Lutein and Zeaxanthin: Research shows that boiling or scrambling eggs keeps these carotenoids intact better than frying them, which can break them down due to the high heat (Wong et al., 2017). However, cooking eggs with fat, like when frying or scrambling, can make these carotenoids easier for the body to absorb (Golub et al., 2006).

6. Cholesterol Oxidation: As mentioned before, high heat from frying or cooking eggs for a long time can create oxidized cholesterol. This type of cholesterol is linked to heart problems and can be harmful to health.

- Oxidative Stress: Frying eggs at high temperatures or for too long can lead to harmful oxidized fats and cholesterol, which are worse for health than non-oxidized ones (Kanner et al., 2002). Cooking eggs for shorter times and at lower temperatures can help reduce this risk.

7. Water-Soluble Nutrients:

Water-soluble nutrients, particularly those in the egg white, are more prone to leaching into the cooking water, particularly if eggs are boiled or poached. These nutrients include riboflavin (B2) and

folate.

- **Leaching into Water:** If eggs are boiled and the cooking water is discarded, some of the water-soluble nutrients are lost. To minimize nutrient loss, eggs can be boiled with the shell intact, as the membrane between the egg white and shell helps to preserve nutrients (Haug & Lantzsch. 1983).

In essence, thermal processing has both positive and negative effects on the nutrient profile of eggs. While cooking enhances the digestibility of proteins and reduces the effects of biotinbinding proteins like avidin, it can also cause the degradation of certain vitamins (especially folate and some carotenoids). Prolonged exposure to high heat, such as in frying, can lead to the oxidation of cholesterol and fats, potentially creating harmful compounds. To retain the most nutritional value, moderate cooking methods, such as boiling, poaching, or light scrambling, are ideal.

2.3 THERMAL PROCESSING AND ITS EFFECTS ON CHOLESTEROL

Thermal processing (cooking) can affect cholesterol in eggs and other foods in several ways, primarily through oxidation and the breakdown of lipids. The key effects include:

1. **Cholesterol Oxidation:** Prolonged exposure to high temperatures, such as in frying, can lead to the formation of oxidized cholesterol. This altered form of cholesterol is considered more harmful, as it can contribute to the development of atherosclerosis (plaque buildup in arteries) and other cardiovascular diseases (Kanner et al., 2002).

2. **Minimal Impact on Cholesterol Content:** While cooking can cause changes in the structure of lipids, it does not significantly reduce the total cholesterol content in eggs. However, the oxidation process at high temperatures can produce potentially harmful compounds (Kanner et al., 2002).

3. **Temperature and Cooking Time:** Boiling and poaching are gentler cooking methods that cause less cholesterol oxidation compared to frying or grilling, which involve higher temperatures and longer cooking times (Kerr et al., 2013).

In conclusion, while cholesterol levels in eggs remain largely unaffected by thermal processing, cooking at high temperatures or for long periods can lead to the formation of oxidized cholesterol, which is linked to negative health outcomes. To minimize these effects, it's advisable to use lower

temperatures and shorter cooking times.

2.3.1. EFFECT OF BOILING ON EGG CHOLESTEROL, STABILITY, AND BIOAVAILABILITY

Boiling is a popular way to cook eggs, and it affects cholesterol levels, stability, and how well our bodies can use the nutrients in eggs. The changes that happen when eggs are boiled mainly depend on the heat, how long they are boiled, and the egg's shell, which helps keep nutrients from being lost. Here's how boiling impacts the cholesterol in eggs:

1. Cholesterol Content:

Boiling eggs doesn't really change the overall cholesterol amount. Cholesterol is a type of fat mostly found in the egg yolk (about 186 mg in a large egg). When you boil eggs, there is very little loss of cholesterol because it doesn't dissolve in water and won't leak into the boiling water (Haug & Lantzsch, 1983). So, the cholesterol level mostly stays the same after boiling.

2. Cholesterol Stability:

- **Cholesterol Oxidation:** A big worry with cooking at high temperatures is that cholesterol can oxidize. But boiling is a gentler cooking method compared to frying or grilling, which means there is less oxidation of cholesterol. Oxidation happens when cholesterol reacts with free radicals created by high heat (Kanner et al., 2002). Since boiling usually occurs at a lower temperature (around 100°C) and in water, it is less likely to cause oxidation like frying does, where the egg is exposed to higher temperatures and air.

- **Protective Shell:** The eggshell helps protect the cholesterol from damage while boiling. This shell acts as a barrier, reducing the chances of harmful oxidized cholesterol forming, which could increase heart risks if eaten in large amounts (Kanner et al., 2002).

3. Cholesterol Bioavailability:

Boiling eggs doesn't change the amount of cholesterol they contain, but it can influence how well cholesterol is absorbed in the digestive system.

- **Better Digestion:** Boiling eggs makes both proteins and fats easier to digest. When you boil eggs,

the proteins in the egg white change shape, which helps digestive enzymes break them down more easily. While the cholesterol itself remains unchanged, this breakdown of proteins can help the body absorb fats, including cholesterol, more effectively (Kramer et al., 2016).

- **Cholesterol Absorption:** The way your body absorbs cholesterol from boiled eggs is similar to how it absorbs it from raw eggs. Some studies suggest that cooking eggs, including boiling, might actually help the body absorb fats better overall, as cooking can improve how well lipases and bile acids work to break down and absorb fats, including cholesterol (Wong et al., 2017). However, this benefit is not as strong with boiling as it is with cooking methods that use fat, like frying or scrambling with butter.

4. Effects on Other Nutrients:

- **Fat-Soluble Vitamins:** Boiling eggs has little impact on the loss of fat-soluble vitamins like vitamin A and vitamin D, which are present in the egg yolk along with cholesterol. These vitamins are fairly stable when exposed to heat, especially during boiling (DePew et al., 2013).

- **Water-Soluble Nutrients:** In contrast, some water-soluble vitamins such as B-vitamins (B2, B12, folate) can seep into the boiling water, especially if the eggs are boiled for a long time (Gao et al., 2014). However, the amount of these nutrients that you can absorb from boiled eggs remains relatively high as long as the eggs aren't overcooked and the cooking water isn't thrown away.

In conclusion, boiling eggs does not greatly change the cholesterol levels, but it helps keep cholesterol stable because of the lower temperatures and the egg's protective shell. Boiling reduces the oxidation of cholesterol, which helps keep the cholesterol molecule intact. Moreover, boiling makes cholesterol and other nutrients in eggs easier to digest and absorb, although this effect is stronger for proteins and fats in general. Boiling is a gentle cooking method that helps preserve the nutritional value of eggs, especially their cholesterol, while preventing the creation of possibly harmful oxidized cholesterol.

2.4 LIVER PHYSIOLOGY AND CHOLESTEROL METABOLISM

2.4.1. LIVER STRUCTURE AND FUNCTIONS

The liver is an important organ found in the upper right part of the abdomen, just below the

diaphragm. It is crucial for metabolism, detoxifying substances, aiding digestion, and storing nutrients. The liver is one of the largest organs in the body and has a complex structure made up of specialized cells and tissues that support its many functions.

The liver weighs around 1.5 kilograms (3.3 pounds) and sits on the right side of the abdomen, just below the diaphragm. It has two main parts: the larger right lobe and the smaller left lobe. The liver is made up of tiny functional units called lobules. Each lobule contains a central vein surrounded by liver cells called hepatocytes, which are arranged in a circular pattern. These lobules serve as the basic building blocks of the liver. Hepatocytes are the main type of cell in the liver and make up about 70-80% of its weight. They handle most of the liver's important jobs, like making proteins, detoxifying harmful substances, and producing bile. Between the hepatocytes are sinusoids, which are special tiny blood vessels that help exchange materials between the blood and liver cells. These vessels bring in nutrient-rich blood from the intestines and oxygen-rich blood from the heart. Hepatocytes release bile into small channels called bile canaliculi, which combine to form larger bile ducts. Bile is crucial for digestion, especially for breaking down and absorbing fats. Kupffer cells are special immune cells found in the liver sinusoids. They help protect the body by eating up germs, dead cells, and waste in the blood. The liver gets blood from two main sources: the hepatic artery, which brings oxygen-rich blood, and the portal vein, which carries nutrient-rich blood from the digestive system.

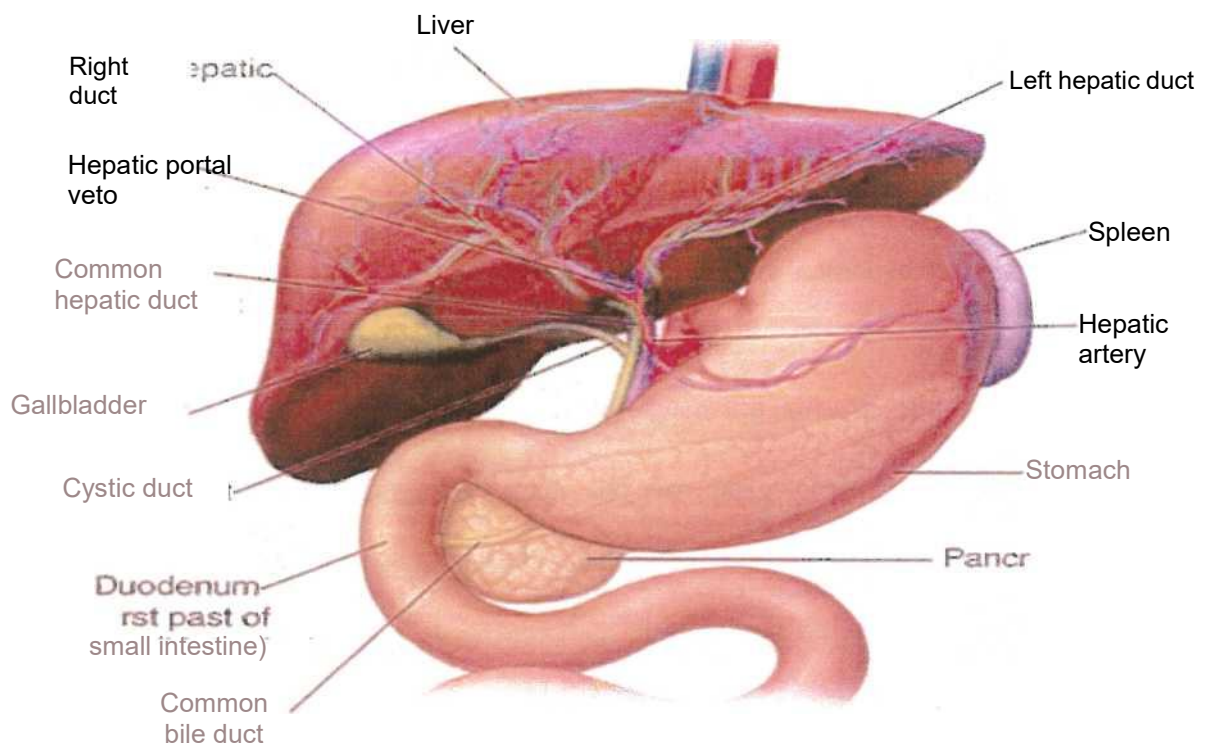


Figure 2.1: The liver

2.4.2. FUNCTIONS OF THE LIVER

The liver plays many important roles in metabolic processes that are essential for maintaining health:

a. Metabolism of Nutrients:

- **Carbohydrate Metabolism:** The liver helps control blood sugar levels by storing glucose as glycogen and releasing it when needed. It also creates glucose from non-carbohydrate sources like amino acids and glycerol through a process called gluconeogenesis.
- **Fat Metabolism:** The liver produces and breaks down fatty acids and lipoproteins. It changes extra carbohydrates and proteins into fat for storage and also makes cholesterol, which is important for creating hormones, bile acids, and cell membranes.
- **Protein Metabolism:** The liver creates many important plasma proteins, such as albumin, which helps maintain blood volume and pressure, and clotting factors that are essential for blood clotting. It also removes the amino groups from amino acids, turning them into forms that can be used for energy or other metabolic activities.

b. Detoxification:

- The liver cleanses harmful substances like drugs, alcohol, and waste products from metabolism, including ammonia. It changes these toxic substances into less harmful ones that are either excreted in bile or sent to the kidneys for removal in urine.
- The liver uses special enzymes called Cytochrome P450 to break down various toxins and medications. These enzymes help convert fat-soluble substances into water-soluble ones, making them easier to eliminate.

c. Bile Production:

- The liver produces bile, a digestive fluid that helps break down and absorb fats in the small intestine. Bile contains bile salts, bilirubin, cholesterol, and phospholipids. Bilirubin, which comes from the breakdown of red blood cells, gives bile its greenish-yellow color.

d. Storage:

- The liver stores glycogen, which is the main source of stored glucose in the body. It also keeps

fat-soluble vitamins (.A. D. E. K) and vitamin B12. releasing them into the blood when needed.

- Additionally, the liver holds iron as ferritin and copper, both of which are important minerals for the body.

e. Synthesis of Blood Proteins:

- The liver makes several plasma proteins, including albumin, which helps control blood volume and pressure, and clotting factors, which are crucial for blood coagulation. This process is vital for keeping blood healthy and stable.

f. Immune Function:

- The liver acts as a filter for the blood, removing harmful germs like bacteria and viruses with the help of Kupffer cells. These cells engulf and eliminate harmful particles from the bloodstream. The liver also produces acute-phase proteins during infections, which help with inflammation and the immune response.

g. Regulation of Hormones:

- The liver helps manage various hormones, such as insulin, glucagon, and thyroid hormones, through its metabolic activities. It also breaks down excess hormones to keep their levels balanced.

2.4.3 DISEASES ASSOCIATED WITH THE LIVER

The liver can be affected by many diseases that harm its structure and function, leading to broader health problems. Some common liver diseases include:

1. Hepatitis:

- Description: Hepatitis is the inflammation of the liver, usually caused by viral infections like Hepatitis A, B, C, D, and E. It can also occur due to alcohol consumption, autoimmune disorders, or certain medications.

- Symptoms: Signs include jaundice, tiredness, stomach pain, and increased liver enzymes.

- Biochemical Impact: Viral hepatitis affects liver cell function, leading to less protein production, issues with detoxification, and blockages in bile flow.

2. Non-Alcoholic Fatty Liver Disease (NAFLD):

- Description: This condition involves too much fat building up in the liver, and it isn't caused by drinking alcohol. It often happens with obesity, type 2 diabetes, and metabolic syndrome.
- Symptoms: Many people don't notice any symptoms, but it can develop into non-alcoholic steatohepatitis (NASH), fibrosis, or cirrhosis.
- Biochemical Impact: The liver stores more lipids, and oxidative stress along with inflammation can lead to liver damage.

3. Alcoholic Liver Disease (ALD):

- Description: This disease occurs when the liver gets damaged from drinking too much alcohol. It can range from fatty liver (steatosis) to alcoholic hepatitis, fibrosis, and cirrhosis.
- Symptoms: People may experience jaundice, fluid buildup in the abdomen (ascites), fatigue, and signs of liver failure in later stages.
- Biochemical Impact: When alcohol breaks down, it creates acetaldehyde, which is a harmful substance that causes oxidative stress, inflammation, and damages liver cells.

4. Cirrhosis:

- Description: This is a serious liver condition marked by permanent scarring (fibrosis) and a decline in liver function. It can be caused by chronic hepatitis, NAFLD, ALD, or genetic issues.
- Symptoms: Common signs include jaundice, fluid retention, bleeding from enlarged veins (varices), and confusion (hepatic encephalopathy).
- Biochemical Impact: Severe fibrosis changes the liver's structure, reducing blood flow and affecting how liver cells work.

5. Liver Cancer:

- Description: This includes hepatocellular carcinoma (HCC), the most common liver cancer, which often develops alongside cirrhosis or chronic hepatitis B or C.
- Symptoms: People may notice weight loss, pain in the abdomen, jaundice, and a noticeable lump

in the liver.

- Biochemical Impact: Ongoing inflammation and genetic changes can lead to uncontrolled cell growth, resulting in tumors.

6. Hemochromatosis:

- Description: This genetic condition causes the body to absorb too much iron, which builds up in the liver and other organs.

- Symptoms: Patients may feel tired, have joint pain, and show signs of liver disease, such as cirrhosis in severe cases.

- Biochemical Impact: The extra iron creates oxidative stress and damages liver cells.

7. Wilson's Disease:

- Description: This rare genetic disorder leads to the accumulation of copper in the liver, brain, and other body tissues.

Symptoms: Jaundice, shaking, mental health issues, and Kayser-Fleischer rings in the eyes.

Biochemical Impact: The buildup of copper harms liver cells and disrupts liver function.

2.4.4. LIVER'S ROLE IN CHOLESTEROL SYNTHESIS, STORAGE, AND EXCRETION

The liver plays a key role in making, storing, and getting rid of cholesterol, which is important for keeping fats in balance. Cholesterol is vital for cell structure and various metabolic processes, and the liver manages its levels through specific biochemical processes.

1. Cholesterol Synthesis:

The liver is where cholesterol is mainly produced, taking place in the cytosol and smooth endoplasmic reticulum of liver cells. The process starts with acetyl-CoA, a small molecule made from carbohydrates, proteins, or fats.

- Mevalonate Pathway: Acetyl-CoA combines to create HMG-CoA (3-hydroxy-3-methylglutaryl-CoA), which an enzyme called HMG-CoA reductase changes into mevalonate. This step is crucial for cholesterol production and is controlled by the presence of cholesterol itself.
- The process continues as mevalonate turns into isoprenoid units, which then combine to form squalene. Squalene changes shape into lanosterol, which is then modified further to create cholesterol.

2. Cholesterol Storage:

After cholesterol is made, it is kept in liver cells mainly as cholesteryl esters.

- Esterification: The enzyme acyl-CoA:cholesterol acyltransferase (ACAT) helps create cholesteryl esters, which are less watery and better for storage or transport.
- These cholesteryl esters are then packed into VLDL (very-low-density lipoproteins) and released into the bloodstream to deliver cholesterol to other parts of the body.

3. Cholesterol Excretion:

The liver is the only organ that can get rid of cholesterol, which cannot be broken down into carbon dioxide or water (Dietschy & Turley, 2004).

Bile Acid Production: A large amount of cholesterol gets turned into bile acids. The enzyme known as cholesterol 7-alpha-hydroxylase (CYP7A1) starts this process by adding a hydroxyl group to

cholesterol, creating 7-alpha-hydroxycholesterol. This substance is then changed into cholic acid and chenodeoxycholic acid, which are the two main types of bile acids (Chiang. 2009).

Direct Release into Bile: The liver also releases free cholesterol straight into bile. This process involves transport proteins like ABCG5/ABCG8, found in the bile canaliculi (Dietschy & Turley. 2004).

Cholesterol and bile acids enter the intestine, where they help digest and absorb fats from food. Some bile acids get reabsorbed through the enterohepatic circulation, while the rest leave the body in stool (Goldstein & Brown, 2009).

Importance:

The liver plays a crucial role in controlling cholesterol creation, storage, and elimination, which helps maintain the right balance of cholesterol in the body. This balance is essential for:

1. Keeping cell membranes flexible (Nelson & Cox, 2017).
2. Making steroid hormones like cortisol, aldosterone, and sex hormones (Goldstein & Brown. 2009).
3. Producing bile acids, which are necessary for digesting and absorbing fats (Chiang, 2009).

If these processes do not work correctly, it can lead to issues like high cholesterol, which can contribute to diseases such as atherosclerosis and heart disease (Dietschy & Turley. 2004).

2.4.5 BIOCHEMICAL LINK BETWEEN DIETARY CHOLESTEROL AND LIVER FUNCTION.

The relationship between dietary cholesterol and liver function involves complicated biochemical processes that work to keep cholesterol levels stable in the body. Dietary cholesterol affects how the liver manages cholesterol, including how it makes, stores, transports, and eliminates it. Here's a simple explanation:

1. Absorption and Transport of Dietary Cholesterol

- Intestinal Absorption: Dietary cholesterol gets absorbed in the small intestine with the help of bile acids, which break down dietary fats. This absorption happens through a transporter called

Niemann-Pick C1-like 1 (NPC1L1) in the intestinal cells.

Chylomicron Formation: When cholesterol is absorbed, it gets changed into a form that can be transported by a special enzyme called acyl-CoA:cholesterol acyltransferase 2 (ACAT2) in intestinal cells. This modified cholesterol is then put into chylomicrons, which are tiny particles that carry dietary cholesterol through the lymphatic system and into the bloodstream (Nelson & Cox, 2017).

Uptake by the Liver: Chylomicron remnants, which are rich in dietary cholesterol, are taken in by the liver using specific receptors like the low-density lipoprotein receptor (LDLR) and the LDL receptor-related protein (LRP). The cholesterol from these remnants adds to the cholesterol stores in the liver (Goldstein & Brown, 2009).

Regulation of Hepatic Cholesterol Synthesis: When dietary cholesterol increases, the liver slows down the production of new cholesterol through a process called feedback inhibition that affects HMG-CoA reductase, the main enzyme in cholesterol creation. This process helps to prevent too much cholesterol from building up (Nelson & Cox, 2017). Additionally, high dietary cholesterol stops the activation of sterol regulatory element-binding proteins (SREBPs), which are important for controlling the genes that make cholesterol (Goldstein & Brown, 2009).

Hepatic Cholesterol Storage and Transport: The liver takes in extra dietary cholesterol and changes it into cholesteryl esters using an enzyme called ACAT1, which allows it to store it. It also packages cholesterol into very-low-density lipoproteins (VLDL) to send it to other parts of the body. Eating more cholesterol leads to increased production of VLDL, which can raise levels of LDL (bad cholesterol) in the blood (Chiang, 2009).

Excretion of Cholesterol: The liver takes some of the dietary cholesterol and turns it into bile acids using an enzyme called cholesterol 7-alpha-hydroxylase (CYP7A1). These bile acids are then released into bile to help digest fats and remove cholesterol. Additionally, any extra cholesterol is expelled into bile through special transporters known as ATP-binding cassette (ABC) transporters, such as ABCG5/ABCG8. Eating more cholesterol boosts this excretion process (Dietschy & Turley, 2004).

6. Effects of Too Much Dietary Cholesterol

- Dysregulation: When we eat too much cholesterol, it can throw off the liver's ability to manage cholesterol levels. This can lead to higher LDL cholesterol in the blood, which may cause atherosclerosis and heart disease.
- NAFLD: Too much cholesterol can build up in the liver, leading to non-alcoholic fatty liver disease (NAFLD). This condition is marked by fat buildup and inflammation in the liver (Schumacher et al., 2020).
- Cholesterol Gallstones: A high intake of dietary cholesterol can lead to a buildup in bile, increasing the chances of forming cholesterol gallstones (Dietschy & Turley, 2004).

Biochemical Importance: The liver plays a vital role in managing dietary cholesterol as part of overall fat metabolism. It helps keep cholesterol levels in check to support cell needs without causing harm. When these processes are disrupted, it can result in metabolic and heart-related diseases.

2.4.6 LIVER METABOLIC PROFILE

The liver's metabolic profile is essential for keeping the body balanced by managing carbohydrates, fats, proteins, and foreign substances. Here's a closer look at how the liver functions metabolically:

1. Carbohydrate Metabolism

The liver helps control blood sugar levels through various methods:

- Glycogenesis: When there is excess glucose, the liver stores it as glycogen, a process helped by the enzyme glycogen synthase (Nelson & Cox, 2017).
- Glycogenolysis: When we fast, the liver breaks down glycogen into glucose, thanks to the enzyme glycogen phosphorylase, and then releases it into the blood.
- Gluconeogenesis: During long periods without food, the liver creates glucose from non-sugar sources like lactate, glycerol, and amino acids. Important enzymes for this process include phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Goldstein & Brown, 2009).

- Regulation: Insulin and glucagon carefully manage these processes to keep blood sugar levels steady.

2. Lipid Metabolism

The liver also controls how fats are made, stored, transported, and broken down:

- Fatty Acid Oxidation: The liver oxidizes fatty acids in the mitochondria to produce acetyl-CoA and ATP through a process called beta-oxidation.

Ketogenesis: When you fast or don't eat many carbs, your liver changes acetyl-CoA into ketone bodies, like acetoacetate and beta-hydroxybutyrate. These ketone bodies provide energy to different parts of the body.

Lipogenesis: If there is too much glucose and acetyl-CoA, the liver turns them into fatty acids and triglycerides for storage. The enzyme called acetyl-CoA carboxylase (ACC) helps start the process of making fatty acids.

Cholesterol Metabolism: The liver makes, stores, and removes cholesterol. It also changes cholesterol into bile acids, which are important for breaking down and absorbing fats.

Protein Metabolism: The liver is very important for making proteins, breaking them down, and getting rid of ammonia:

- Albumin and Clotting Factors: The liver produces plasma proteins, such as albumin and fibrinogen, which help with blood clotting.

- Amino Acid Metabolism: The liver changes amino acids to create keto acids that can be used for energy or to make glucose. The enzyme aminotransferase plays a big role in this.

- Urea Cycle: The liver converts ammonia, a harmful waste from breaking down amino acids, into urea, which is then removed in urine. The urea cycle uses enzymes like carbamoyl acid phosphate synthetase I.

Detoxification and Xenobiotic Metabolism:

The liver cleans out harmful substances in two main steps:

- Phase I Reactions: This involves altering xenobiotics through oxidation, reduction, or hydrolysis

using cytochrome P450 enzymes.

- Phase II Reactions: In this phase, the liver makes metabolites water-soluble by combining them with glucuronic acid, sulfate, or glutathione, so they can be easily removed.
- Bilirubin Metabolism: The liver changes unconjugated bilirubin (which comes from breaking down heme) into conjugated bilirubin for removal in bile.

Storage Functions:

The liver keeps important nutrients for later use:

- Glycogen: This is the main form of stored carbohydrates.
- Vitamins: The liver holds onto fat-soluble vitamins (A, D, E, and K) and water-soluble vitamin B12.
- Iron and Copper: These minerals are stored in the liver, attached to ferritin and ceruloplasmin, for the body's needs.

6. Bile Production

The liver makes bile acids from cholesterol, which are important for:

- Breaking down fats and aiding digestion.
- Getting rid of cholesterol and bilirubin.

7. Hormonal Regulation

The liver also processes and controls hormones:

- Insulin: It breaks down insulin using an enzyme called insulinase.
- Thyroid Hormones: It changes T4 (thyroxine) into the more active T3 (triiodothyronine).

The liver's metabolic functions are crucial for keeping the body balanced, detoxifying harmful substances, and storing nutrients. When these processes are disrupted, it can lead to health issues like NAFLD, cirrhosis, and diabetes.

2.4.6. LIVER HISTOLOGY

Liver histology is the study of the liver's structure at a microscopic level. This includes looking at

the cells, blood vessels, and bile ducts that work together to keep the liver functioning. Understanding the liver's histological structure helps us learn more about its functions, diseases, and role in metabolism.

Studying liver tissue is essential for diagnosing diseases like hepatitis, cirrhosis, and cancer, as it reveals information about tissue damage, inflammation, and abnormal cells.

Key Components of Liver Histology:

1. Lobules: The liver is made up of hexagonal units called lobules. Each lobule contains:
 - Hepatocytes: The main cells that handle metabolism, detoxification, and bile production.
 - Sinusoids: Tiny blood vessel-like structures that allow blood to flow between hepatocytes.
 - Central Vein: Located in the center of each lobule, it collects blood from the sinusoids.
2. Portal Triad: Found at the corners of each lobule, it includes:
 - Hepatic artery: Delivers oxygen-rich blood to the liver.
 - Portal vein: Carries nutrient-filled blood from the digestive system.
 - Bile duct: Moves bile produced by hepatocytes.
3. Kupffer Cells: Special cells found in sinusoids that help defend the immune system and clean up debris.
4. Stellate Cells (Ito Cells): Found in the space of Disse, they store vitamin A and are involved in liver fibrosis when activated.

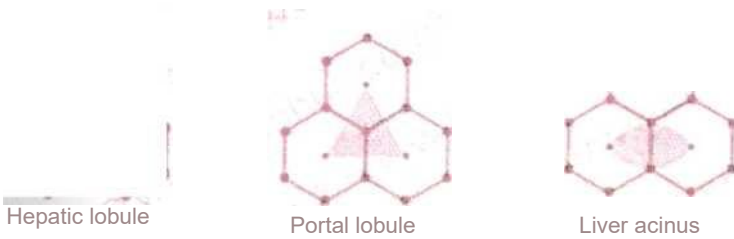
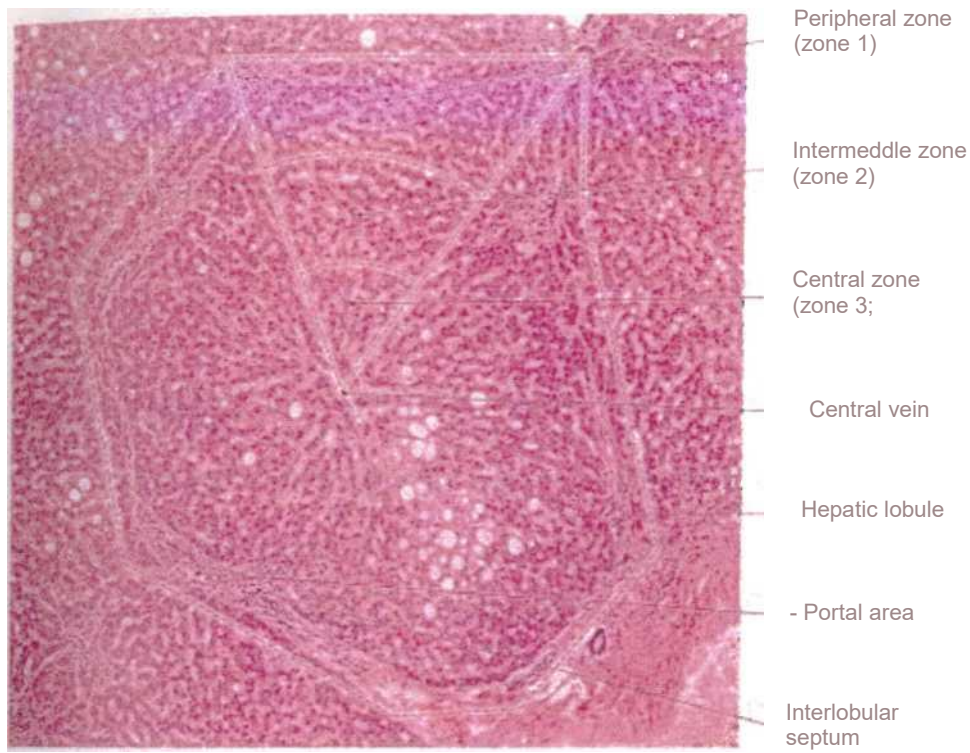


Fig 2.2: Histology of the liver

2.5. OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE IN THE LIVER

2.5.1 . THE LIVER'S ROLE IN HANDLING OXIDATIVE STRESS

The liver is essential in managing oxidative stress by neutralizing reactive oxygen species (ROS) and fixing oxidative damage to keep cells healthy. Oxidative stress happens when there is an imbalance between the production of ROS and the body's antioxidant defenses, which can harm cells and tissues.

Role of The Liver in Managing Oxidative Stress;

1. Production of Antioxidant Enzymes

- The liver creates important antioxidant enzymes, such as:
- Superoxide Dismutase (SOD): Changes superoxide radicals into hydrogen peroxide.
- Catalase: Breaks down hydrogen peroxide into water and oxygen.
- Glutathione Peroxidase (GPx): Reduces hydrogen peroxide and lipid peroxides using glutathione as a helper (Nelson & Cox, 2017).

2. Synthesis of Glutathione

- The liver makes glutathione (GSH), a strong antioxidant that helps neutralize ROS and repair damaged molecules. Glutathione is important for detoxifying the body and is recycled through the glutathione reductase system (Forman et al., 2009).

3. Detoxifying Xenobiotics

- The liver uses cytochrome P450 enzymes to break down xenobiotics, which can produce ROS as waste. These enzymes help lower oxidative stress by detoxifying harmful substances (Dietschy & Turley. 2004).

4. Regulating Metal Ions

- The liver stores and binds metal ions like iron and copper with proteins such as ferritin and

ceruloplasmin, preventing the formation of ROS caused by metals (Schumacher et al., 2020).

5. Repairing Oxidative Damage

- The liver has mechanisms to fix damaged DNA, lipids, and proteins caused by oxidative stress, which helps reduce the risk of injury to tissues and the development of diseases (Sies, 2017).

Clinical Importance

Oxidative stress is connected to liver diseases like non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease, and hepatitis. The liver's ability to fight against oxidative stress is vital in stopping these conditions from worsening into fibrosis, cirrhosis, or liver cancer (Schumacher et al., 2020).

2.5.1. EFFECTS OF DIETARY CHOLESTEROL ON OXIDATIVE STRESS AND ANTIOXIDANT STATUS

Dietary cholesterol impacts oxidative stress and the body's antioxidant levels by changing the balance of oxidation and reduction, increasing the creation of reactive oxygen species (ROS), and weakening the body's antioxidant defenses. These changes are especially notable in areas like the liver and blood vessel walls.

1. Effects on Oxidative Stress

- Increased ROS Production:

- Consuming too much cholesterol raises lipid peroxidation, mainly in low-density lipoproteins (LDL), which leads to oxidized LDL (ox-LDL). This ox-LDL produces ROS, making oxidative stress worse (Zhao et al., 2013).

- When there's too much cholesterol, it disrupts how mitochondria work, causing electrons to leak in

the electron transport chain and increasing ROS production (Nelson & Cox, 2017).

- Activation of Oxidative Enzymes:

- A high intake of cholesterol activates NADPH oxidase, an important enzyme that creates superoxide radicals, which increases oxidative stress in cells (Parthasarathy et al., 2010).

2. Effects on Antioxidant Status

- Depletion of Antioxidant Reserves:

- High ROS levels use up antioxidants like glutathione (GSH), vitamin E, and vitamin C as they try to neutralize free radicals (Forman et al., 2009).

- Inhibition of Antioxidant Enzyme Activity;

- Important antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase are less effective when oxidative stress is high due to too much dietary cholesterol (Zhao et al., 2013).

- Formation of Oxidized Derivatives:

- Products from cholesterol oxidation, known as oxysterols, can further weaken antioxidant defenses and lead to cell damage (Biasi et al., 2009).

3. Clinical Implications

- Long-term oxidative stress and weakened antioxidant defenses from high dietary cholesterol can lead to:

- Atherosclerosis: Caused by the oxidation of LDL and damage to blood vessels.

- Non-Alcoholic Fatty Liver Disease (NAFLD): Resulting from oxidative damage to liver cells.

- Inflammatory Diseases: Triggered by ROS activating pro-inflammatory pathways like NF- κ B (Schumacher et al., 2020).

Biochemical Strategies for Mitigation

- Eating more foods rich in antioxidants, like vitamins C and E and polyphenols, can help counteract reactive oxygen species (ROS) that come from high cholesterol levels.
- Boosting the body's antioxidant systems, such as making more glutathione, helps keep the balance of oxidative stress in check.

Effect of Boiling on Egg Cholesterol in Formulated Diets:

Cooking eggs by boiling can affect how cholesterol behaves in the body, mainly due to changes in its structure and how it interacts with oxygen. Cholesterol in eggs is sensitive to heat, which can change its stability and how well the body can use it. Boiling has specific effects on egg cholesterol:

1. Stability of Egg Cholesterol During Boiling

- Retention of Cholesterol Content: Boiling eggs usually does not greatly lower their total cholesterol levels. The cholesterol stays stable during cooking because it is protected within the fat of the egg yolk (Ahmad et al., 2020).
- Oxidation of Cholesterol: If eggs are boiled for a long time, it can lead to the creation of cholesterol oxidation products (COPs), like 7-ketocholesterol and 7 β -hydroxycholesterol. These harmful products form when cholesterol comes into contact with oxygen at high temperatures and can cause oxidative stress and inflammation if eaten in large amounts (Biasi et al., 2009).
- Minimal Lipid Peroxidation: Boiling eggs creates fewer COPs compared to frying or other high-heat cooking methods because there is less direct exposure to high oxygen levels or extra oils (Zhao et al., 2013).

2. Bioavailability of Cholesterol

- Boiling eggs can slightly change the structure of the lipoproteins in the egg yolk, which may make it easier for the body to absorb cholesterol during digestion. This means that the body can more effectively take in cholesterol from boiled eggs than from raw ones (Fernandez, 2001).
- The heat-induced denaturation of yolk proteins also makes it easier for digestive enzymes to access cholesterol, further increasing its bioavailability (Ahmad et al., 2020).

3. Implications for Formulated Diets

- Dietary Impact:

Formulated diets that include boiled eggs provide a stable source of cholesterol. However, excessive intake may contribute to oxidative stress, especially if other oxidative stressors are present in the diet.

- Health Considerations:

Including boiled eggs in diets should be balanced with antioxidants (e.g., vitamins C and E) to mitigate potential effects of COPs (Schumacher et al., 2020).

2.7. EFFECTS OF BOILING OF EGG CHOLESTEROL FORMULATED DIET ON THE HISTOLOGY STATUS OF THE LIVER OF WHISTAR RATS.

2.7.1. Effects of Boiled Egg Cholesterol Formulated Diet on Liver Histology in Wistar Rats

Feeding Wistar rats a diet formulated with boiled egg cholesterol can affect the liver's histological structure, primarily through cholesterol metabolism, oxidative stress, and lipid deposition. The biochemical and histological effects observed depend on the level of dietary cholesterol and its impact on hepatic functions.

1. Effects on Liver Histology

- Hepatic Lipid Accumulation (Steatosis):
- Diets rich in boiled egg cholesterol may lead to the accumulation of cholesterol and triglycerides in hepatocytes, resulting in fatty liver (steatosis). This occurs because excess cholesterol

overwhelms normal lipid metabolism pathways, leading to intracellular lipid droplet formation (Kritchevsky et al., 2000).

- Liver Inflammation:
- Oxidation of dietary cholesterol, even during boiling, can produce cholesterol oxidation products (COPs). When absorbed, these COPs contribute to oxidative stress and pro-inflammatory cytokine production, triggering infiltration of immune cells into liver tissue (Zhao et al., 2013).
- Histologically, this manifests as mononuclear inflammatory cell infiltration around the portal triads.

Hepatic Fibrosis:

- Eating a diet high in cholesterol for a long time can harm liver cells, activate certain liver cells called hepatic stellate cells, and cause more protein buildup in the liver. This can eventually lead to fibrosis (Biasi et al., 2009).

Disruption of Liver Structure:

- Too much cholesterol can lead to swelling in liver cells, which is seen as pale, foamy areas when looked at under a microscope. This swelling shows that there is fat buildup and damage to the cells (Schumacher et al., 2020).

2. Biochemical Processes Behind Liver Changes

- Problems with Fat Processing:
- When the liver processes too much dietary cholesterol, it makes more cholesterol esters and stores them in liver cells. This overloads the normal ways the liver exports fat, leading to a condition called steatosis (Nelson & Cox, 2017).

- Oxidative Damage:
- When cholesterol is heated or eaten, it produces substances that create reactive oxygen species (ROS) in the liver. These substances can harm liver cells, causing injury and inflammation (Zhao et al., 2013).

-Activation of Inflammatory Pathways:

-A diet high in cholesterol activates certain proteins, like NF-κB, that cause inflammation and tissue damage. These effects can be seen as changes in the liver structure under a microscope (Parthasarathy et al., 2010).

3. Findings in Wistar Rats

- Fatty Liver and Fibrosis:
- Studies of Wistar rats that were given high-cholesterol diets (including cholesterol from boiled eggs) showed more fat vacuoles in liver cells, disrupted blood flow spaces, and collagen buildup, which indicates fibrosis (Biasi et al., 2009).
- Activation of Kupffer Cells:
- High-cholesterol diets activate Kupffer cells, which are immune cells in the liver, and this leads to inflammation. Histological observations show an increase in the number of Kupffer cells and other immune cells in the liver (Zhao et al., 2013).

Antioxidants.

Antioxidants are molecules that prevent other molecules from oxidizing by neutralizing reactive oxygen species (ROS) and free radicals, which helps protect our cells from damage. Oxidation is a chemical process where electrons are transferred from one substance to an oxidizing agent, often creating free radicals as a result. These free radicals, like superoxide (O₂⁻) and hydroxyl radicals

(OH), are very reactive and can harm lipids, proteins, and DNA, causing oxidative stress (Sies, 1997).

Antioxidants work by stabilizing free radicals. They do this by donating electrons without changing themselves, which stops the harmful chain reactions caused by free radicals. Antioxidants can be either enzymatic, such as superoxide dismutase, catalase, and glutathione peroxidase, or non-enzymatic, like vitamin C, vitamin E, and glutathione (Halliwell & Gutteridge, 2015).

For example, vitamin E (tocopherol) helps eliminate lipid peroxyl radicals in cell membranes, while vitamin C can regenerate vitamin E and neutralize radicals in water. Glutathione, a small protein made up of three amino acids, is essential for converting hydrogen peroxide (H₂O₂) into water with the help of the enzyme glutathione peroxidase (Forman et al., 2009).

Antioxidants play an important role in protecting against oxidative damage, which is linked to aging, cancer, and heart diseases (Phaniendra et al., 2015). However, it's important to have the right balance, as too many antioxidant supplements can interfere with the normal functions of ROS in the body, such as cell signaling.

Effect of Boiling on Egg Cholesterol Diet and Antioxidant Status in the Liver of Wistar Rats.

The impact of boiling an egg-cholesterol diet on the antioxidant status of the liver in Wistar rats can be understood by looking at how dietary cholesterol, heat-related changes, and the antioxidant defense system interact on a biochemical level.

Key Biochemical Processes;

1. Lipid Oxidation from Boiling:

When eggs are boiled, the high heat can cause lipid oxidation, leading to the creation of oxidized

cholesterol compounds called oxysterols. These substances act as pro-oxidants, which can produce reactive oxygen species (ROS) in the liver after they are eaten (Savage et al., 2002). High levels of ROS can overwhelm the liver's ability to fight off these harmful molecules, resulting in oxidative stress.

2. Effects on Antioxidant Enzymes:

The liver has important antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) that help neutralize ROS. Research shows that a diet rich in oxidized cholesterol can lower the activity of these enzymes due to too much oxidative stress, making it harder for the liver to fight off free radicals (Osim et al., 1991).

3. Loss of Glutathione:

Glutathione (GSH) is an important non-enzymatic antioxidant in the liver that helps detoxify products formed by lipid peroxidation. Eating oxidized cholesterol from boiled eggs may increase the need for GSH to neutralize ROS, causing its levels to drop. This reduction can weaken the liver's antioxidant defenses and lead to more damage to liver cells (Shen et al., 2014).

4. Lipid Peroxidation in the Liver:

Diets high in oxidized cholesterol can increase the production of malondialdehyde (MDA), a sign of lipid peroxidation in the liver. Higher MDA levels show that there is more oxidative damage to liver cell membranes, which can harm their structure and function (Nwosu et al., 2017).

5. Boiling's Protective Effects:

However, boiling may lower the availability of some cholesterol in eggs by changing the structure of proteins and lipids, which could lessen its pro-oxidant effects. Therefore, the overall effect on the liver's antioxidant status depends on the balance between the harmful effects of oxidized cholesterol

and the protective effects of reduced cholesterol availability.

EFFECT OF BOILING ON EGG CHOLESTEROL FORMULATED DIET ON THE ANTIOXIDANT STATUS OF THE LIVER OF WHISTAR RATS:

Boiling an egg cholesterol diet can change how important antioxidant enzymes work in the livers of Wistar rats. The key enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). These enzymes are essential for fighting against harmful molecules called reactive oxygen species (ROS) and for keeping the liver healthy from oxidative stress.

1. Superoxide Dismutase (SOD)

SOD helps convert the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2), which lowers oxidative stress.

- Effect of Boiling:

Boiling eggs can create oxidized cholesterol compounds that raise ROS levels, including superoxide radicals, in the liver. If rats eat a boiled egg cholesterol diet for a long time, it may lead to too much superoxide being produced, which can overwhelm SOD. Extended oxidative stress can decrease SOD activity because ROS can inactivate the enzyme or lower the levels of important minerals like manganese (Mn) and copper (Cu) that SOD needs to work properly (Savage et al., 2002).

2. Catalase (CAT)

CAT breaks down H_2O_2 into water and oxygen, helping to protect cells from damage caused by H_2O_2 .

- Effect of Boiling:

Eating oxidized cholesterol from a boiled egg diet can raise H_2O_2 levels, increasing the need for CAT. However, if oxidative stress lasts too long, it can harm CAT activity by oxidizing the

enzyme or using up its necessary components like heme groups. If the production of ROS is too high, a boiled egg cholesterol diet might lead to lower CAT activity in the liver (Osime et al., 1991).

3. Glutathione Peroxidase (GPx);

GPx helps convert H₂O₂ and lipid hydroperoxides into water and alcohol, using reduced glutathione (GSH) as a cofactor.

Effect of Boiling:

When you eat a diet high in boiled eggs, the oxidized cholesterol can cause an increase in lipid peroxidation, which creates lipid hydroperoxides. These substances initially boost the activity of GPx, an enzyme that helps detoxify these peroxides. However, if oxidative stress continues for a long time, it reduces the levels of GSH, which is crucial for GPx to function properly. This ongoing lack of GSH makes GPx less effective at protecting against oxidative damage (Shen et al., 2014).

Glutathione Reductase (GR):

GR plays a key role in converting oxidized GSH (called GSSG) back into its active form, GSH, using NADPH. This process helps keep the redox balance in the liver.

Effect of Boiling:

A diet rich in boiled egg cholesterol leads to higher usage of GSH by GPx to remove reactive oxygen species (ROS). This raises the need for GR to refill GSH. However, if oxidative stress lasts for a long time, it can reduce GR activity by depleting NADPH, which is vital for GR to work. When GR activity decreases, it further lowers the GSH/GSSG ratio, making oxidative stress worse in the liver (Nwosu et al., 2017).

Summary of the Effects on Antioxidant Enzymes:

- SOD: Might decrease due to the inactivation of the enzyme by ROS.
- CAT: Likely decreases because of excess H₂O₂ and damage from ROS.

- GPx: Goes up at first but drops as GSH runs out.
- GR: Becomes less effective due to a lack of NADPH, which reduces GSH production.

In conclusion, a diet high in boiled eggs and cholesterol raises oxidative stress in the livers of Wistar rats. This happens because it increases ROS production, lipid peroxidation, and decreases GSH levels. As a result, the activity of important antioxidant enzymes (SOD, CAT, GPx. and GR) is negatively impacted, which could lead to oxidative damage if the oxidative stress surpasses what the liver can handle.

CHAPTER 3.

3.0 MATERIALS AND METHODS

3.1 Apparatus

EDTA BOTTLE

Test tube

Water bath(Techmel & Techmel USA).

Spectrophotometer (Vis Spectrometer).

Centrifuge(Searchtech instrument British Standard).

Cuvette

Microscope

pH meter

Microtome

Cover slips

Nose mask

Hand-gloves

Universal bottle

Micropipettes

Syringe 5ml

Aluminium foil

Cotton wool

Mortar and pestle

3.2 Chemical Reagents;

Pyrogallol

TCA-TBA-HCL

Phosphate buffer
Epinephrine
Hydrogen peroxide
Carbonate buffer
Sulphuric acid
Potassium permanganate
Distilled water
Chloroform
Saline
Formalin
Potassium Cyanide
Diethylene triaminepentacetic acid(DTPA).
Sodium carbonate
Thiobarbituric Acid(TBA)
Trichloroacetic Acid
Hydrochloric Acid
Phosphoric Acid
Hydrogen Peroxide
Potassium Permanganate
Reduced Glutathione
Nicotinamide Adenine Dinucleotide Phospate.

3.3 Experimental animals ;

Female Albina wistar rats weighing

and a total number of 17 rats were purchased from the animal house . Department of biochemistry,

Faculty of life sciences, University of Benin ,Benin city Edo state.

3.4 Experimental Design

Seventeen female Wistar rats were purchased and divided into six groups. They were housed in separate cages at the Animal House of the Biochemistry Department, University of Benin.

Throughout the experiment, the rats had continuous access to water and feed. The animals were not acclimatized and were fed small portions of the formulated diet.

3.5 Feed Formulation

Formulated Diet	Group 1	Group 2	Group 3	Group 4
corn.	3.2kg	3.2kg	3.2kg	3.2kg
Soybeans.	0.75kg	0.75kg	0.75kg	0.75kg
Egg Yolk.	0.25kg.	-	-	-
Corn oil	0.4kg	0.4kg	0.4kg	-
Vitamins.	0.4kg	0.4kg	0.4kg	-
Synthetic	■	2g		
Cholesterol	-	-	-	-

Table 3.1 : Feed Formulation for the animals

3.6 Measurements of the body weights

The average weight of female wistar rats in grammes measured before feed administration's and average weight in grammes of female wistar after feed administration is shown in the table below:

Average weight(g)	Group 1 (Boiled egg yolk)	Group 2 Cholesterol	Group 3 Positive control	Group 4 Negative control		
Weight before diet administration	38.50	39.34	45.32			
After administration (42days)	107.33	128	126.4			
Percentage increase %	178.8	225.37	179			

Table 3.2: Measurements of the body weights

3.7 Animal Sacrifice

At the end of the 42nd day, the animals were anesthetized in a chloroform chamber on the 43rd day. Organs were excised, placed in sterile sample bags, and stored in a refrigerator for further assays, including cholesterol, AST, ALP, lactate dehydrogenase, and albumin analysis. Blood samples were collected from the veins of the Wistar rats, with 2 ml transferred into EDTA bottles for biochemical analysis. While the organ was crushed with mortar and pestle and taken to the lab for histopathology analysis.

3.8 METHODS

3.8.1 DETERMINATION OF CATALASE (CAT) ACTIVITY

Catalase activity was estimated by the method described by Cohen *et al.*, (1970).

Principle: The estimation of catalase activity is on the measurement of the rate of decomposition of the hydrogen peroxide (H_2O_2) following the addition of the sample containing the enzyme. The quantity of hydrogen peroxide decomposed is directly proportional to the enzyme in the sample. The hydrogen peroxide produced is measured by reacting it with excess potassium permanganate ($KMnO_4$) and the absorbance was measured at 480nm and the amount of hydrogen peroxide hydrolysed was calculated for the catalase activity.

Procedure: The experiment was carried out by adding 2.5 ml of H_2O_2 to 0.25ml liver homogenate. The mixture was allowed to stand for 30mins. To terminate the reaction 0.6ml of phosphate buffer pH 7.4, 0.75ml of 6M of H_2SO_4 and 3.5ml $KMnO_4$ was added. Immediately, the mixture was inverted for proper mixture and transferred to a cuvette and placed in a spectrophotometer. The absorbance of the sample was measured against blank at 480nm for 30, 60 and 90 seconds

Calculation

Catalase activity = $\frac{\Delta OD \text{ of test sample}}{\text{min} \times V}$

M x V x L x Y

Where OD= absorbance

V= total reaction of the mixture

M=molar concentration

L=1 ight path

Y=mg of protein

V= volume of sample

3.8.2 DETERMINATION OF SUPEROXIDE DISMUTASE (SOD) ACTIVITY

The level of SOD activity was determined by the method of Misra and Fridovich (1972)

Principle: This assay is based on the ability of superoxide dismutase to inhibit the auto oxidation of epinephrine at Ph 10.2. The oxidation of the epinephrine to the adrenochrome was caused by the superoxide anion generated xanthine oxidase reaction.

Procedure: Liver supernatant sample measuring precisely 1 ml of the reading was diluted in 9 ml of water to achieve a dilution ration of 1 in 10. Subsequently, 0.2 ml of the resulting diluted enzyme preparation was added 2.5 ml of 0.05 M carbonate buffer Ph 10.2 to equilibrate within the spectrometer. The reaction commenced with the addition of 0.3 ml of freely prepared 0.3 Mm epinephrine to the mixture, followed by immediate inversion for thorough mixing. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Calculation:

% inhibition = $\frac{\text{change in absorbance of test sample}}{\text{Change in absorbance of blank}} \times 100$

Change in absorbance of blank

SOD (Units/mg protein) = $\frac{\% \text{ inhibition}}{\text{Mg protein} \times 50}$

NB: 1 Unit of SOD activity is the amount of SOD which caused 50% inhibition of the oxidation of epinephrine to adrenochrome less than 1 minute.

Where Y= mg of protein in the volume of sample used.

3.8.2 DETERMINATION OF GLUTATHIONE PEROXIDASE (Gpx)

This was determined according to Nyman (1959)

Principle: This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity resulting to a deep brown color disposition , read at 420nm.

Reagent preparation

Pyrogallol (20m): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

Procedure: To an aliquot of plasma (0.2ml) 2.5ml of phosphate buffer, 2.5ml hydrogen peroxide, 1.5ml of distilled water and 2.5ml of pyrogallol was added.

The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculation:

Activity = $\frac{\text{OD}/\text{min} \times V_i \times D_f}{\text{Mg protein} \times 50}$

OD= absorbance of the test

V_i= total volume of the reaction mixture

D_F= dilution factor

E=molar concentration

Vs= volume of sample

Y= mg of protein used.

3.8.3 DETERMINATION OF MALONDIALDEHYDE (MDA)

Malondialdehyde was determined using the thiobarbuturic acid assay(buege and aust 1978) **Principle:** malonaldehyde which is a product of lipid peroxidation react with thiobarbuturic acid (TBA) to give a red species.

Procedure: a volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL was mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling , the flocculent precipitate was removed by centrifuged at 1000g for 10min. the absorbance was determined using the formula;

MDA (mol/mg protein) = $A \times V \times 100$

$$M \times V \times Y$$

A= Absorbance

V= total volume of reaction mixture

M= molar extinction coefficient

V=volume of the sample

Y= mg protein

CHAPTER 4.

4.0 RESULT AND INTERPRETATION.

4.1 INTERPRETATION

SOD;

When group 1 (boiled egg) is compared with the group 2 (raw cholesterol), there's a significant increase ($P < 0.05$). While when compared to the positive and negative control, there tend to be a huge decrease .

CAT;

From the tabulated results, there tend to be a significant increase ($P < 0.05$) in the catalase value when group 1 (boiled egg) is being compared with group 2 (cholesterol). While when boiled egg is compared with the control (positive and negative control), there tend to be a decrease.

GPx;

There tend to be a an increase ($P < 0.05$) in the GPx value when boiled egg is compared with cholesterol according to our tabulated results. While when compared with the controls , there tend to be a decrease.

MAL;

From the tabulated results, there tend to be a huge decrease ($P < 0.05$) in the MAL values when boiled egg is compared with cholesterol.

While when boiled egg is compared with the control; there tend to be a decrease.

RESULTS.

Antioxidants;

GROUPS	SOD	CAT	GPx	MAL
Boiled egg yolk	0.40±0.18 a	0.42±0.05 a	2.87±0.33 a	3.63±0.34b
cholesterol	0.43±0.02 a	0.43±0.02 a	3.03±0.43 b	1.85±0.26a
positive control	0.27±0.09 b	0.32±0.12b	2.43±0.27 a	2.85±0.18 ab
Negative control	0.33±0.03 b	0.38±0.00b	2.80±0.06 a	1.37±0.09a

KEY;

CATALASE, CAT

Malondialdehyde, MAL;

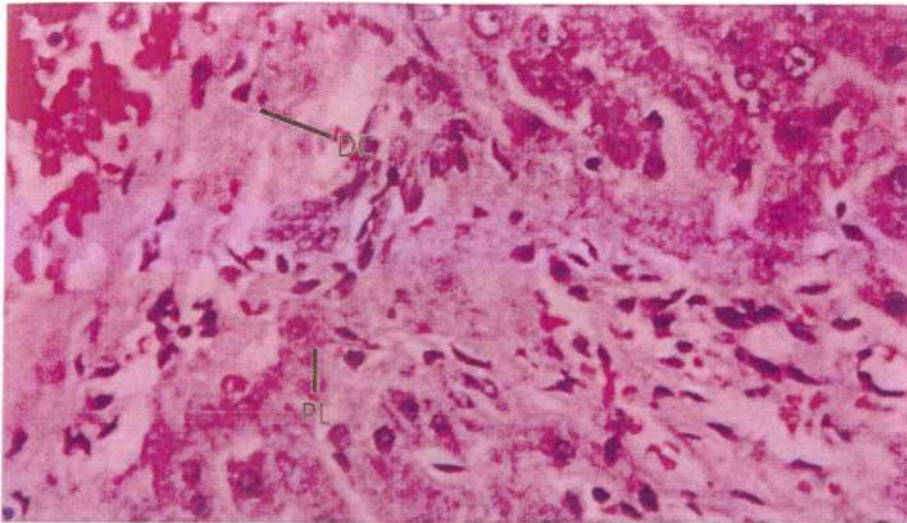
Gluthathione peroxidase, GPx;

Superioxide dismutase SOD.

Values are expressed as Mean ± SEM

HISTOLOGY.

Group 1.



----- NH

Plate 1. Rat liver given boiled egg yolk compounded diet show: active vascular congestion and dilatation (DC), periportal mobilization of lymphocytes (PL), normal hepatocytes (NH): H&E 400 X.

Group 2.

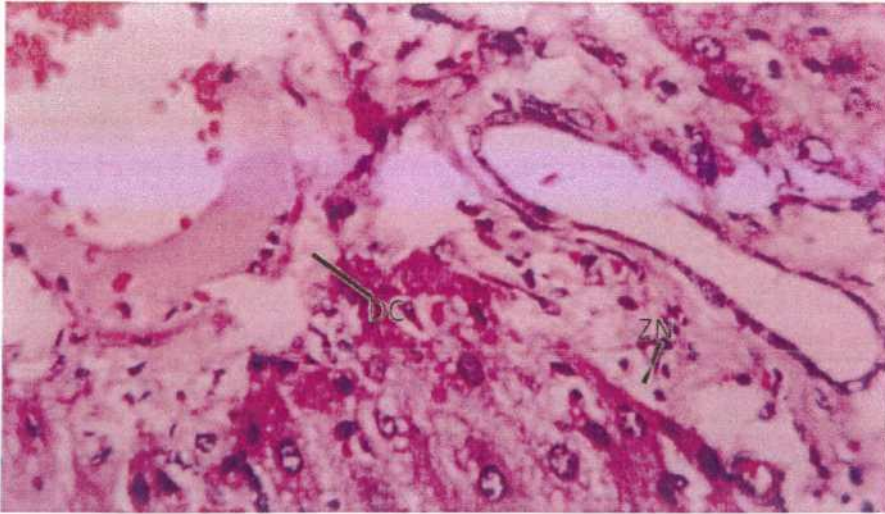


Plate 2. Rat liver given Cholesterol compounded show: vasodilatation and congestion (DC) and zonal hepatocyte necrosis (ZN): H&E 400 X.

Group 3.

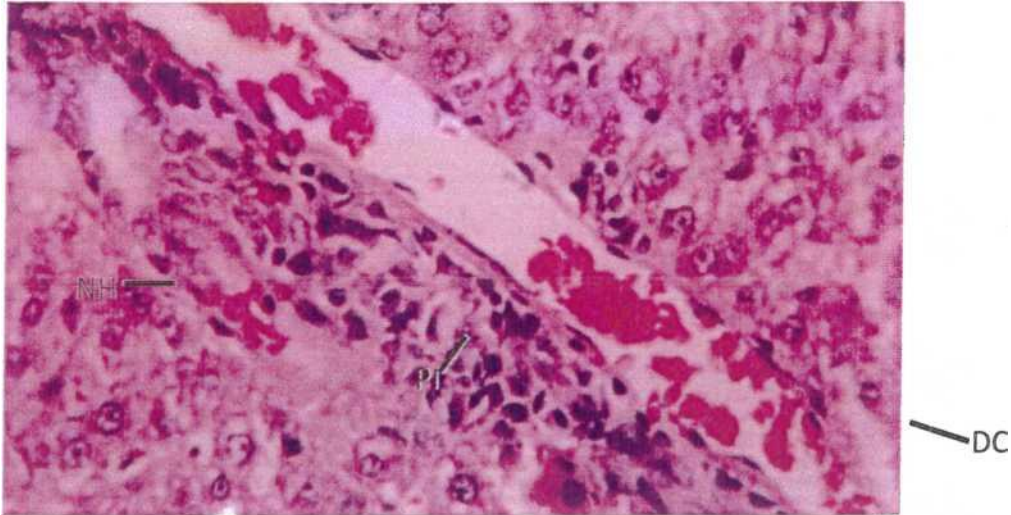


Plate 3. Rat liver given Soya oil compounded show: vasodilatation and congestion (DC), normal hepatocytes (NH) and periportal infiltrates of inflammatory cells (PI): H&E 400 X.

Group 4.

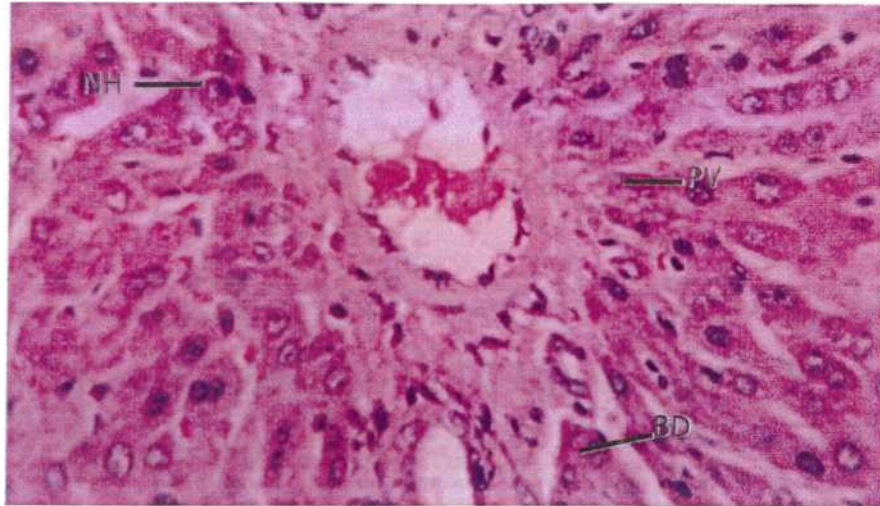


Plate 4. Rat liver, normal control show: normal hepatocytes (NH), sinusoids (SI), portal vein (PV) and bile ducts (BD): H&E 400 X.

CHAPTER 5

DISCUSSION AND CONCLUSION.

5.0 DISCUSSION.

Antioxidants.

SOD:

Superoxide dismutase (SOD) is a critical antioxidant enzyme that protects cells from oxidative damage by catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide. Elevated SOD activity in the liver indicates an adaptive response to increased oxidative stress.

An increase in SOD activity would imply that boiled egg consumption induces oxidative stress, prompting the liver to enhance its antioxidant response. This response is crucial for maintaining cellular integrity and function in the face of dietary-induced oxidative challenges (Fridovich, 1995).

A decrease in superoxide dismutase (SOD) activity when boiled egg is compared with positive and negative controls in the liver status of Wistar rats indicates a diminished antioxidant response, which has significant biochemical implications.

CAT;

A decrease in catalase suggests that the liver's overall antioxidant capacity is compromised. This may indicate that the boiled egg diet does not provide sufficient nutrients (e.g., vitamins or polyphenols) to support the antioxidant defense system, unlike a cholesterol-rich diet that may trigger a compensatory antioxidant response (Nikki 2009).

An increase in catalase activity, indicates that boiled eggs may induce oxidative stress, although potentially less severe than the cholesterol diet. The liver might still be capable of mounting a sufficient antioxidant response.

GPx;

- An increase in GPx activity suggests that the liver is adapting to oxidative stress caused by the consumption of boiled eggs. The oxidative stress may result from lipid peroxidation or the generation of reactive oxygen species (ROS) during the metabolism of dietary lipids and cholesterol (Halliwell & Gutteridge, 1999).

- A decrease in GPx activity indicates an impaired ability of the liver to neutralize hydrogen peroxide and lipid hydroperoxide. This can lead to the accumulation of reactive oxygen species (ROS), causing oxidative stress and damage to proteins, lipids, and DNA (Halliwell & Gutteridge, 1999).

MAL;

A decrease in MDA levels suggests that the boiled egg diet generates less oxidative stress and lipid damage in the liver compared to the cholesterol-rich diet. This indicates a lower production of ROS or a more effective antioxidant response in the boiled egg group (Halliwell & Gutteridge, 1999).

Histology.

LIVER

Sections of the liver taken from rats given baseline feed and water freely show normal microscopic architecture with well-defined hepatocytes, sinusoids, portal vein and bile ducts.

Sections taken from rats given the compounded diet mixtures show normal architecture with well-defined hepatocytes, sinusoids and portal triad (portal vein, hepatic artery and bile ducts). There was also increased blood circulation (active congestion) and stimulation of the local immune system of the liver through mobilization of lymphocytes and the sinusoidal Kupffer cells. However, in the group containing compounded cholesterol diet there was zonal hepatocyte necrosis, which signifies destruction of hepatocytes around the portal region. Moreover, the group given compounded soya oil induced mild inflammation around the portal region of the liver.

In conclusion, the compounded lipid containing diets caused beneficial reactions from the blood vessels and increased blood circulation, as well as stimulating the local immune system of the selected organs. However, in isolated cases there was vascular compromise and hepatocyte necrosis as observed in the diet containing cholesterol as well as mild inflammation in the portal region of the liver in rats given compounded soya oil.

CONCLUSION.

In conclusion .the findings from this study suggest that the consumption of boiled egg yolk positively influences the antioxidant defense mechanisms in the liver, as evidenced by enhanced activity of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). alongside a reduction in markers of oxidative stress, such as malondialdehyde (MDA). These results indicate that boiled egg yolk may help mitigate oxidative damage in hepatic tissues.

In Overall, the findings of this study provide compelling evidence that the boiled egg yolk-formulated diet supports antioxidant defense in the liver and maintains liver histology, thereby promoting liver health.

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SOD

Descriptives

VAR00001

95% Confidence Interval for

	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1.00	3	.4013	.03232	.01866	.3211	.4816	.38	.44
2.00	3	.3233	.20937	.12088	-.1968	.8434	.09	.49
3.00	3	.3070	.07794	.04500	.1134	.5006	.26	.40
4.00	3	.4343	.04922	.02842	.3121	.5566	.39	.49
5.00	3	.2773	.16397	.09467	-.1300	.6847	.09	.37
6.00	3	.3320	.15588	nannn	-.0552	.7192	.15	.42
Total	18	.3459	.12428	.02929	.2841	.4077	.09	.49

Mean Std. Deviation Std. Error Lower Bound Upper Bound Minimum Maximum
Mean

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.053	5	.011	.613	.692
Within Groups	.209	12	.017		
Total	.263	17			

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
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Between Groups	.053	5	.011	.613	.692
Within Groups	.209	12	.017		
Total	.263	17			

Multiple Comparisons

Dependent Variable: VAR00001

Mean Difference		95% Confidence Interval					
	(1) VAR00002 (J) VAR00002	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound	
Tukey HSD	1.00	2.00	.07800	.10779	.975	-.2841	.4401
		3.00	.09433	.10779	.946	-.2677	.4564
		4.00	-.03300	.10779	1.000	-.3951	.3291
		5.00	.12400	.10779	.851	-.2381	.4861
		6.00	.06933	.10779	.985	-.2927	.4314
		2.00	1.00	-.07800	.10779	.975	-.4401
	3.00	.01633	.10779	1.000	-.3457	.3784	
	4.00	-.11100	.10779	.899	-.4731	.2511	
	5.00	.04600	.10779	.998	-.3161	.4081	
	6.00	-.00867	.10779	1.000	-.3707	.3534	
	3.00	1.00	-.09433	.10779	.946	-.4564	.2677
		2.00	-.01633	.10779	1.000	-.3784	.3457
		4.00	-.12733	.10779	.837	-.4894	.2347
		5.00	.02967	.10779	1.000	-.3324	.3917
		6.00	-.02500	.10779	1.000	-.3871	.3371
	4.00	1.00	.03300	.10779	1.000	-.3291	.3951
		2.00	.11100	.10779	.899	-.2511	.4731

LSD

	3.00	.12733	.10779	.837	-.2347	.4894
	5.00	.15700	.10779	.695	-.2051	.5191
	6.00	.10233	.10779	.925	-.2597	.4644
5.00	1.00	-.12400	.10779	.851	-.4861	.2381
	2.00	-.04600	.10779	.998	-.4081	.3161
	3.00	-.02967	.10779	1.000	-.3917	.3324
	4.00	-.15700	.10779	.695	-.5191	.2051
	6.00	-.05467	.10779	.995	-.4167	.3074
6.00	1.00	-.06933	.10779	.985	-.4314	.2927
	2.00	.00867	.10779	1.000	-.3534	.3707
	3.00	.02500	.10779	1.000	-.3371	.3871
	4.00	-.10233	.10779	.925	-.4644	.2597
	5.00	.05467	.10779	.995	-.3074	.4167
1.00	2.00	.07800	.10779	.483	-.1568	.3128
	3.00	.09433	.10779	.399	-.1405	.3292
	4.00	-.03300	.10779	.765	-.2678	.2018
	5.00	.12400	.10779	.272	-.1108	.3588
	6.00	.06933	.10779	.532	-.1655	.3042
2.00	1.00	-.07800	.10779	.483	-.3128	.1568
	3.00	.01633	.10779	.882	-.2185	.2512
	4.00	-.11100	.10779	.323	-.3458	.1238
	5.00	.04600	.10779	.677	-.1888	.2808
	6.00	-.00867	.10779	.937	-.2435	.2262
3.00	1.00	-.09433	.10779	.399	-.3292	.1405
	2.00	-.01633	.10779	.882	-.2512	.2185
	4.00	-.12733	.10779	.260	-.3622	.1075
	5.00	.02967	.10779	.788	-.2052	.2645

4.00	6.00	-.02500	.10779	.820	-.2598	.2098
	1.00	.03300	.10779	.765	-.2018	.2678
	2.00	.11100	.10779	.323	-.1238	.3458
	3.00	.12733	.10779	.260	-.1075	.3622
	5.00	.15700	.10779	.171	-.0778	.3918
	6.00	.10233	.10779	.361	-.1325	.3372
5.00	1.00	-.12400	.10779	.272	-.3588	.1108
	2.00	-.04600	.10779	.677	-.2808	.1888
	3.00	-.02967	.10779	.788	-.2645	.2052
	4.00	-.15700	.10779	.171	-.3918	.0778
	6.00	-.05467	.10779	.621	-.2895	.1802
	6.00	1.00	-.06933	.10779	.532	-.3042
2.00		.00867	.10779	.937	-.2262	.2435
3.00		.02500	.10779	.820	-.2098	.2598
4.00		-.10233	.10779	.361	-.3372	.1325
5.00		.05467	.10779	.621	-.1802	.2895

GPX

Descriptives

VAROOOOI

		95% Confidence Interval for Mean						
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1.00	3	2.8667	.05774	.03333	2.7232	3.0101	2.80	2.90
2.00	3	2.9667	.37859	.21858	2.0262	3.9071	2.70	3.40
3.00	3	2.6333	.23094	.13333	2.0596	3.2070	2.50	2.90
4.00	3	3.0333	.75719	.43716	1.1524	4.9143	2.50	3.90

5.00	3	2.4333	.47258	.27285	1.2594	3.6073	1.90	2.80
6.00	3	2.8000	.10000	.05774	2.5516	3.0484	2.70	2.90
Total	18	2.7889	.40277	.09493	2.5886	2.9892	1.90	3.90

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.744	5	.149	.887	.519
Within Groups	2.013	12	.168		
Total	2.758	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(1) VAR00002	(J) VAR00002	Mean Difference		Sig.	95% Confidence Interval	
			(I-J)	Std. Error		Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	-.10000	.33444	1.000	-1.2234	1.0234
		3.00	.23333	.33444	.979	-.8900	1.3567
		4.00	-.16667	.33444	.995	-1.2900	.9567
		5.00	.43333	.33444	.782	-.6900	1.5567
		6.00	.06667	.33444	1.000	-1.0567	1.1900
2.00	1.00	1.00	.10000	.33444	1.000	-1.0234	1.2234
		3.00	.33333	.33444	.910	-.7900	1.4567
		4.00	-.06667	.33444	1.000	-1.1900	1.0567
		5.00	.53333	.33444	.616	-.5900	1.6567



	6.00	.16667	.33444	.995	-.9567	1.2900
3.00	1.00	-.23333	.33444	.979	-1.3567	.8900
	2.00	-.33333	.33444	.910	-1.4567	.7900
	4.00	-.40000	.33444	.831	-1.5234	.7234
	5.00	.20000	.33444	.989	-.9234	1.3234
	6.00	-.16667	.33444	.995	-1.2900	.9567
4.00	1.00	.16667	.33444	.995	-.9567	1.2900
	2.00	.06667	.33444	1.000	-1.0567	1.1900
	3.00	.40000	.33444	.831	-.7234	1.5234
	5.00	.60000	.33444	.503	-.5234	1.7234
	6.00	.23333	.33444	.979	-.8900	1.3567
5.00	1.00	-.43333	.33444	.782	-1.5567	.6900
	2.00	-.53333	.33444	.616	-1.6567	.5900
	3.00	-.20000	.33444	.989	-1.3234	.9234
	4.00	-.60000	.33444	.503	-1.7234	.5234
	6.00	-.36667	.33444	.874	-1.4900	.7567
6.00	1.00	-.06667	.33444	1.000	-1.1900	1.0567
	2.00	-.16667	.33444	.995	-1.2900	.9567
	3.00	.16667	.33444	.995	-.9567	1.2900
	4.00	-.23333	.33444	.979	-1.3567	.8900
	5.00	.36667	.33444	.874	-.7567	1.4900
LSD	1.00	-.10000	.33444	.770	-.8287	.6287
	3.00	.23333	.33444	.499	-.4954	.9620
	4.00	-.16667	.33444	.627	-.8954	.5620
	5.00	.43333	.33444	.219	-.2954	1.1620
	6.00	.06667	.33444	.845	-.6620	.7954
2.00	1.00	.10000	.33444	.770	-.6287	.8287

	3.00	.33333	.33444	.339	-.3954	1.0620
	4.00	-.06667	.33444	.845	-.7954	.6620
	5.00	.53333	.33444	.137	-.1954	1.2620
	6.00	.16667	.33444	.627	-.5620	.8954
3.00	1.00	-.23333	.33444	.499	-.9620	.4954
	2.00	~ 1.33333	.33444	.339	-1.0620	.3954
	4.00	-.40000	.33444	.255	-1.1287	.3287
	5.00	.20000	.33444	.561	-.5287	.9287
	6.00	-.16667	.33444	.627	-.8954	.5620
4.00	1.00	.16667	.33444	.627	-.5620	.8954
	2.00	.06667	.33444	.845	-.6620	.7954
	3.00	.40000	.33444	.255	-.3287	1.1287
	5.00	.60000	.33444	.098	-.1287	1.3287
	6.00	.23333	.33444	.499	-.4954	.9620
5.00	1.00	-.43333	.33444	.219	-1.1620	.2954
	2.00	-.53333	.33444	.137	-1.2620	.1954
	3.00	-.20000	.33444	.561	-.9287	.5287
	4.00	-.60000	.33444	.098	-1.3287	.1287
	6.00	-.36667	.33444	.294	-1.0954	.3620
6.00	1.00	-.06667	.33444	.845	-.7954	.6620
	2.00	-.16667	.33444	.627	-.8954	.5620
	3.00	.16667	.33444	.627	-.5620	.8954
	4.00	1'23333	.33444	.499	-.9620	.4954
	5.00	' .36667	.33444	.294	-.3620	1.0954

VAR00001

		Subset for alpha = 0.05	
		1	
VAR00002 N			
Tukey HSD ^a	5.00	3	2.4333
		3.00	...3 2.6333
		6.00	3 2.8000
		1.00	3 2.8667
		2.00	3 2.9667
		4.00	3 3.0333
		Sig.	.503

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

CAT

Descriptives

VAR00001

95% Confidence Interval for Mean

	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1.00	3	.4237	.09074	.05239	.1982	.6491	.35	.53
2.00	3	.3900	.07621	.04400	.2007	.5793	.30	.43
3.00	3	.4337	.06243	.03604	.2786	.5887	.37	.49
4.00	3	.4270	.03105	.01793	.3499	.5041	.40	.46
5.00	3	.3187	.21507	.12417	-.2156	.8529	.07	.47
6.00	3	.3800	.00693	.00400	.3628	.3972	.38	.39

Total 18 .3955 .09661 .02277 .3475 .4435 .07 .53

AN OVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.028	5	.006	.520	.757
Within Groups	.130	12	.011		
Total	.159	17			

Multiple Comparisons

Dependent Variable: VAR00001

95% Confidence Interval Mean Difference

	(1) VAR00002	(J) VAR00002	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	.03367	.08512	.998	-.2522	.3196
		3.00	-.01000	.08512	1.000	-.2959	.2759
		4.00	-.00333	.08512	1.000	-.2892	.2826
		5.00	.10500	.08512	.813	-.1809	.3909
		6.00	.04367	.08512	.995	-.2422	.3296
		2.00	1.00	-.03367	.08512	.998	-.3196
	3.00	-.04367	.08512	.995	-.3296	.2422	
	4.00	-.03700	.08512	.998	-.3229	.2489	
	5.00	.07133	.08512	.954	-.2146	.3572	
	6.00	.01000	.08512	1.000	-.2759	.2959	
3.00	1.00	.01000	.08512	1.000	-.2759	.2959	
	2.00	.04367	.08512	.995	-.2422	.3296	

	4.00	.00667	.08512	1.000	-.2792	.2926
	5.00	.11500	.08512	.753	-.1709	.4009
	6.00	.05367	.08512	.986	-.2322	.3396
4.00	1.00	.00333	.08512	1.000	-.2826	.2892
	2.00	.03700	.08512	.998	-.2489	.3229
	3.00	-.00667	.08512	1.000	-.2926	.2792
	5.00	.10833	.08512	.794	-.1776	.3942
	6.00	.04700	.08512	.992	-.2389	.3329
5.00	1.00	-.10500	.08512	.813	-.3909	.1809
	2.00	-.07133	.08512	.954	-.3572	.2146
	3.00	-.11500	.08512	.753	-.4009	.1709
	4.00	-.10833	.08512	.794	-.3942	.1776
	6.00	-.06133	.08512	.976	-.3472	.2246
6.00	1.00	-.04367	.08512	.995	-.3296	.2422
	2.00	-.01000	.08512	1.000	-.2959	.2759
	3.00	-.05367	.08512	.986	-.3396	.2322
	4.00	-.04700	.08512	.992	-.3329	.2389
	5.00	.06133	.08512	.976	-.2246	.3472
1.00	2.00	.03367	.08512	.699	-.1518	.2191
	3.00	-.01000	.08512	.908	-.1955	.1755
	4.00	-.00333	.08512	.969	-.1888	.1821
	5.00	.10500	.08512	.241	-.0805	.2905
	6.00	.04367	.08512	.617	-.1418	.2291
2.00	1.00	-.03367	.08512	.699	-.2191	.1518
	3.00	-.04367	.08512	.617	-.2291	.1418
	4.00	-.03700	.08512	.672	-.2225	.1485
	5.00	.07133	.08512	.418	-.1141	.2568

3.00	6.00	.01000	.08512	.908	-.1755	.1955
	1.00	.01000	.08512	.908	-.1755	.1955
	2.00	.04367	.08512	.617	-.1418	.2291
	4.00	.00667	.08512	.939	-.1788	.1921
	5.00	.11500	.08512	.202	-.0705	.3005
	6.00	.05367	.08512	.540	-.1318	.2391
4.00	1.00	.00333	.08512	.969	-.1821	.1888
	2.00	.03700	.08512	.672	-.1485	.2225
	3.00	-.00667	.08512	.939	-.1921	.1788
	5.00	.10833	.08512	.227	-.0771	.2938
	6.00	.04700	.08512	.591	-.1385	.2325
	5.00	1.00	-.10500	.08512	.241	-.2905
2.00		-.07133	.08512	.418	-.2568	.1141
3.00		-.11500	.08512	.202	-.3005	.0705
4.00		-.10833	.08512	.227	-.2938	.0771
6.00		-.06133	.08512	.485	-.2468	.1241
6.00		1.00	-.04367	.08512	.617	-.2291
	2.00	-.01000	.08512	.908	-.1955	.1755
	3.00	-.05367	.08512	.540	-.2391	.1318
	4.00	-.04700	.08512	.591	-.2325	.1385
	5.00	.06133	.08512	.485	-.1241	.2468

VAR00001

			Subset for alpha = 0.05
			1
	VAR00002 N		
Tukey HSD ^a	5.00	3	.3187

6.00	3	.3800
2.00	3	.3900
1.00	3	.4237
4.00	3	.4270
3.00	3	.4337
Sig.		.753

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

MDA

Descriptives

VAR00001

95% Confidence Interval for Mean

	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
1.00	3	3.6333	.58312	.33667	2.1848	5.0819	2.96	3.97
2.00	3	2.4633	.49075	.28333	1.2442	3.6824	2.18	3.03
3.00	3	3.2600	.56000	.32332	1.8689	4.6511	2.70	3.82
4.00	3	1.8500	.46000	.26558	.7073	2.9927	1.39	2.31
5.00	3	2.8567	.32501	.18765	2.0493	3.6640	2.53	3.18
6.00	3	1.3700	.15000	.08660	.9974	1.7426	1.22	1.52
Total	18	2.5722	.88930	.20961	2.1300	3.0145	1.22	3.97

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.976	5	2.195	10.672	.000
Within Groups	2.468	12	.206		
Total	13.445	17			

Multiple Comparisons

Dependent Variable: VAR00001

95% Confidence Interval Mean Difference

	(1) VAR00002	(J) VAR00002	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	1.00	2.00	1.17000	.37032	.070	-.0739	2.4139
		3.00	.37333	.37032	.907	-.8705	1.6172
		4.00	1.78333'	.37032	.004	.5395	3.0272
		5.00	.77667	.37032	.349	-.4672	2.0205
		6.00	2.26333'	.37032	.001	1.0195	3.5072
		2.00	1.00	-1.17000	.37032	.070	-2.4139
	3.00	-.79667	.37032	.325	-2.0405	.4472	
	4.00	.61333	.37032	.581	-.6305	1.8572	
	5.00	-.39333	.37032	.887	-1.6372	.8505	
	6.00	1.09333	.37032	.098	-.1505	2.3372	
3.00	1.00	-3.7333	.37032	.907	-1.6172	.8705	
	2.00	.79667	.37032	.325	-.4472	2.0405	
	4.00	1.41000'	.37032	.023	.1661	2.6539	
	5.00	.40333	.37032	.877	-.8405	1.6472	
	6.00	1.89000'	.37032	.003	.6461	3.1339	
	4.00	1.00	-1.78333'	.37032	.004	-3.0272	-.5395

LSD

	2.00	- .61333	.37032	.581	-1.8572	.6305
	3.00	-1.41000'	.37032	.023	-2.6539	-.1661
	5.00	-1.00667	.37032	.142	-2.2505	.2372
	6.00	.48000	.37032	.782	-.7639	1.7239
5.00	1.00	-.77667	.37032	.349	-2.0205	.4672
	2.00	.39333	.37032	.887	-.8505	1.6372
	3.00	-.40333	.37032	.877	-1.6472	.8405
	4.00	1.00667	.37032	.142	-.2372	2.2505
	6.00	1.48667*	.37032	.017	.2428	2.7305
6.00	1.00	-2.26333'	.37032	.001	-3.5072	-1.0195
	2.00	-1.09333	.37032	.098	-2.3372	.1505
	3.00	-1.89000*	.37032	.003	-3.1339	-.6461
	4.00	-.48000	.37032	.782	-1.7239	.7639
	5.00	-1.48667*	.37032	.017	-2.7305	-.2428
1.00	2.00	1.17000*	.37032	.008	.3632	1.9768
	3.00	.37333	.37032	.333	-.4335	1.1802
	4.00	1.78333*	.37032	.000	.9765	2.5902
	5.00	.77667	.37032	.058	-.0302	1.5835
	6.00	2.26333'	.37032	.000	1.4565	3.0702
2.00	1.00	-1.17000*	.37032	.008	-1.9768	-.3632
	3.00	-.79667	.37032	.053	-1.6035	.0102
	4.00	.61333	.37032	.124	-.1935	1.4202
	5.00	-.39333	.37032	.309	-1.2002	.4135
	6.00	1.09333'	.37032	.012	.2865	1.9002
3.00	1.00	-.37333	.37032	.333	-1.1802	.4335
	2.00	.79667	.37032	.053	-.0102	1.6035
	4.00	1.41000'	.37032	.002	.6032	2.2168

	5.00	.40333	.37032	.297	-.4035	1.2102
	6.00	1.89000'	.37032	.000	1.0832	2.6968
4.00	1.00	-1.78333'	.37032	.000	-2.5902	-.9765
	2.00	-.61333	.37032	.124	-1.4202	.1935
	3.00	-1.41000'	.37032	.002	-2.2168	-.6032
	5.00	-1.00667'	.37032	.019	-1.8135	-.1998
	6.00	.48000	.37032	.219	-.3268	1.2868
5.00	1.00	-.77667	.37032	.058	-1.5835	.0302
	2.00	.39333	.37032	.309	-.4135	1.2002
	3.00	-.40333	.37032	.297	-1.2102	.4035
	4.00	1.00667*	.37032	.019	.1998	1.8135
	6.00 ~~~	1.48667*	.37032	.002	.6798	2.2935
6.00	1.00	-2.26333*	.37032	.000	-3.0702	-1.4565
	ZOO	£"72865™				
	3.00	-1.89000*	.37032	.000	-2.6968	-1.0832
	4.00	-.48000	.37032	.219	-1.2868	.3268
	5.00	-1.48667*	.37032	.002	-2.2935	-.6798

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

VAR00001

	VAR00002	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD ^a	6.00	3	1.3700		
	4.00	3	1.8500	1.8500	
	2.00	3	2.4633	2.4633	2.4633
	5.00	3		2.8567	2.8567

3.00	3	3.2600
1.00	3	3.6333
Sig.	070

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.