EFFECTS OF ADULTERATED AND RE-USED COOKING FAT ON METABOLIC SYNDROME IN MALE SPRAGUE DAWLEY RATS (*Rattusnorvegicus*)

Thesis presented to the faculty of Science and Technology, Department of Biology in partial fulfillment for the award of the degree of Master of Science Applied Physiology and Cellular Biology.

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DEDICATION

To my family who have been with me, every step of the way

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Without you all, a lot of what was achieved could not be possible.

God bless you all!

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LIST OF ABBREVIATIONS

IDF. -International Diabetes Federation.

MFMER. -Mayo Foundation for Medical Education and Research.

SREBP-CAP. -Sterol regulatory element-binding protein Cleavage-activating protein.

EDTA. -Ethylenediaminetetraacetic acid

DBPC. -Dibromochloropropane

Mdn. -Median

HFD. -High fat diet.

RHFD. -Reused high fat diet

RHFD+T.O. -Reused high fat diet with transformer oil.

ND. -Normal diet.

RBC. -Red blood cells

PCBs -Polychlorinated biphenyls.

ESR. -Erythrocytes sedimentation rate

TGs. -Triglycerides.

HDL. -High density lipoproteins.

LDL - Low density lipoprotein.

VLDL - Very low density lipoprotein

ALT -Alanine aminotransferase.

AST - Aspartate aminotransferase.

FFA - Free fatty acid.

IDL - Intermediate density lipoprotein.

MetS - Metabolic syndrome

ATSDRs - Agency for Toxic Substances and Disease Registry

TAGs - Tiacyglycerol

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ABSTRACT

Globally, metabolic syndrome (MetS) has been on the rise and yet its risk factors are diverse in different populations. In most low income estates of major Kenyan cities and towns, roadside eating establishments reuse their both their cooking fats and oils in an attempt to minimize their businesses operating expenses. Some unscrupulous businessmen mix cooking fat with transformer oil to extend its cooking life: this over exposure of cooking fat to high temperatures and adulteration results in consumption of highly unstable oxidized and contaminated fats. The extended exposure to these abused fats poses a health risk and its physiological and toxic effects may have far reaching public health implications. The consumption of foods fried in these fats can possibly have an adverse effects to the body's immune system and subsequentlyexacerbatelong term conditions like HIV/AIDS, tuberculosis and cancers and other non-communicable diseases.

This study aimed to explore the effects of transformer oil-adulterated and reused cooking fats on the metabolic syndrome in male Sprague Dawley rats (*Rattusnorvegicus*).

Using a randomized experimental design, twenty four (24) weaned male Sprague Dawley rats were assigned to four (4) groups (n=6): ND, as control group I (fed a standard rat diet *ad libitum*), HFD, group II (fed 20% high fat diet *ad libitum*), RHFD, group III (fed 20% reused high fat diet) and group IV, RHFD + T.O, (fed a mixture of 20% reused high fat and transformer oil diet in the ratio of 3V:1V).To minimize on the expenses, measurementswere undertaken at the end of the study period but not monitored over time. The groupsmean body weights in grams were compared at the end of the study period as well as fastingblood sugar after 12 hours of fasting the experimental animals. After an intraperitoneal injection of Insulin,blood sugar was

established at T0, T30, T60 and T120 and their mean of area under curve (AUC) usedas a measure insulin tolerance. Venous blood was also collected at the end of the study period by a standard retro-orbital puncture for liver enzymes, lipid profile and osmotic fragility of the red blood cells assays. The study animals were then sacrificed and groups liver to body weight ratios determined. Finally the harvested liver samples were fixed in 10% neutral buffered formal saline (40% formaldehyde, 9% sodium chloride) and processed for Histopathology.

The experimental data were analyzed using One-way ANOVA followed by suitable post-hoc comparison performed using the statistical software Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA). The significance level was set at $p<0.05$.

As compared to other study groups, group IV (RHFD+T.O diet) results hada significantly elevated osmotic fragility of the red blood cells of 108.8 ± 16.7 % at 0.34%NaCl, ($p = 0.0043$) as compared to the ND group, liver weight to body weight ratio was highest between RHFD + T.O and HFD groups ($p = 0.0043$), elevatedliver enzyme ALT between RHFD + T.O and HFD groups ($p = 0.0043$), and a lipid disorder. Additionally, the same group had a significantly reduced body weight as compared to HFD, ($p = 0.0087$) with acute liver necrosis and significant portal inflammation of 2 according to theNon-alcoholic Steatohepatitis Clinical Research Network Histological grading system, indicating liver toxicity and bad health. Group III (20% RHFD) study animals were highly indicative of Non-alcoholic Steatohepatitis-inflammation of the liver concurrent with fat accumulation; the liver histology showed fat accumulation, inflammation, and fibrosis in different stages, this results were not well demonstrated in other study groups.Overall results further showed that all the groups on 20% high fat diet presented with differing levels of lipid disorders.

These findings demonstrate the hazardous effects of reused cooking fats and transformer oil mixtures and recommends for further studiesand surveys on commercial use of cooking fats and oils in Kenya.

CHAPTER 1.0: INTRODUCTION

1.1 General introduction

MetS is a collection of the most critical heart attack risk factors that include: diabetes and pre-diabetes, central obesity, hyperlipidemia and an elevated blood pressure(I.D.F.,2006).It's approximated that 20-25% of the world's adult population has the array of risk factors that may predispose them to MetS(Alberii et al., 2006).

The risk of having MetS is closely associated to obesity or abnormal weight gain that leads to a higher Basal Metabolic Index (BMI)(Cornier et al., 2008), other factors are; a sedentary life style, age, race, education status and gender.(Bankoski et al., 2011).However statistics of the syndrome in Kenya are not available, this may be due to consideration of the risk factors separately but not collectively as a syndrome.

A person is also determined as having the MetS if they have a central obesity plus two of the following four additional factors: elevated triglycerides (TGs), a depressed HDL-cholesterol, an elevated blood pressure or high than normal fasting plasma glucose level. Further studies have considered gender and ethnicity specific cut-off points for central obesity as determined by waist circumferences(International Diabetic Foundation, 2004).

The established guidelines of 2005 from the National Heart, Lung, and Blood Institute (America) and the American Heart Association consider any three of these traits in the same individual as the threshold for MetS: abdominal obesity established by a waist circumference of 102 cm (40 inches) or more in men and 88 cm (35 inches) or more in women, however for the Asian Americans, the cutoff values are ≥90 cm (35 in) in men or ≥80 cm (32 in) in women: a serum triglycerides of 3.9 mmol/L or above in both men and women: HDL cholesterol ≤1.04 mmol/ L in men and 1.3mmol/L or lower in women: blood pressure of 130/85 mmHg or more and a fasting blood glucose of 5.6 mmol/L and above.(Grundy et al., 2005).

It has been shown that subjectively healthy individuals may have biochemical abnormalities in keeping with the presence of MetS (Gami et al., 2007). In a study carried out in Kenya in 2017,a total of 528 participants found a prevalence of MetS was 25.6% (95% CI: 22.0%–29.5%). Among the surrogate markers of visceral adiposity, lipid accumulation product was the best predictor of MetS, while triglyceride was the best predictor among the lipid parameters for all participants. The optimal waist circumference cut-off for diagnosing MetS was 94 cm and 86 cm respectively for males and females. (Omuse et al., 2017). The study concluded that "The prevalence of MetS was high for a healthy population highlighting the fact that one can be physically healthy but have metabolic derangements indicative of an increased cardiovascular disease (CVD) risk. This is likely to result in an increase in the cases of CVD and type 2 diabetes in Kenya if interventions are not put in place to reverse this trend. We have also demonstrated the inappropriateness of the WC cut-off of 80 cm for black African women in Kenya when defining MetS and recommend adoption of 86 cm."(Omuse et al., 2017).

A stratified sampling among University students in Kenya established that "1.9% of the participants met the criteria for diagnosis of metabolic syndrome according to Harmonized Joint Scientific Statement (HJSS) criteria. Among the elements, there was statistical difference in gender body mass index (BMI) and waist circumference. 11.8% of subjects had two metabolic syndrome components while 3.1% had three components while none of the subjects had all six components. Elevated triglycerides was the most prevalent defining component for metabolic syndrome. There is a statistically significant relationship between sedentary lifestyle and dietary habits as risk factors to metabolic syndrome."(Mbugua et al., 2017)

In another cross-sectional study carried out at Riruta Health Centre (Kenya) in 2016, a population of 360 adults infected with HIVwere recruited and a questionnaire was employed to collect data on socio-demography. Their blood was analyzed for fasting glucose and lipid profile. The result indicated MetSpresent in 19.2% of the sampled population with prevalence higher among females participants than males. Obesity, lack of formal education, and family history of hypertension were associated with increased risk of metabolic syndrome while physical activity was associated with decreased risk. The study concluded that,"MetS is prevalent in this study population and the lack of formal education, obesity, physical inactivity and a family history of hypertension are associated with an elevated risk of MetS. Screening for risk factors, promotion of healthy lifestyle, and nutrition counselling should be offered routinely in HIV care and treatment clinics."(Kiama et al., 2018).

A comparative study on the demographic characteristics i.e.abdominal circumference, weight, height, blood pressure, lipid profileand blood glucose) in Eldoret referral hospital (Kenya) on prevalence and correlates of metabolic syndrome and its components in 300 adults with psychotic disorders and 300 controlsfound out that patients with psychosis had a higher mean random blood glucose [5.23 vs 4.79, p = 0.003],higher triglycerides [1.98 vs 1.56, p<0.001], higher body mass index [5.23 vs 4.79, p = 0.001], lower high density lipoprotein [1.22 vs 1.32, p<0.001] andlarger waist circumference [89.23 vs 86.39, p = 0.009] . The risk of developingMetS were elevated with age and were reduced with female gender among those who were never married and among the widowed/separated/ divorced. Over a half of patients in this study were not receiving treatment for the various components of MetS. The study concluded that"metabolic syndrome and its components were more prevalent among patients with psychotic disorders than in controls; and a clear treatment gap for these disorders was evident. There is a need for efforts to ensure adequate screening and treatment for these physical disorders."(Kwobah et al., 2021)

A cross-sectional baseline survey on gender variations in the pattern of socio-demographics relevant to MetS between Kenyan adults with central obesity at a Mission Hospital in Nairobi, Kenya, recorded a high occurrence of 87.2% MetS correlated with advanced age in males ($p < 0.001$) and females ($p = 0.002$). MetS was more likely among divorced/separated/widowed ($p = 0.021$) and high income males ($p = 0.002$) and females ($p = 0.017$). The unemployed males ($p = 0.008$) and the females with tertiary education ($p = 0.019$) unlikely to have MetS. Advanced age was more probable to lead to high blood pressure, fasting blood glucose and triglycerides ($p < 0.05$). Males were most probable ($p = 0.026$) to have raised triglycerides, while females

($p < 0.001$) had diminishedhigh density lipoproteins. The study did conclude that a high prevalence of MetS is highly associated with both social and gender differences among Kenyan adults with central obesity. The study finally recommended the need to look beyond behavioral and biological risks and focus with urgency on gender differences in dealing withMetS and cardiovascular diseases. (Okube et al., 2020).

It has also been indicated that these risk factors can also be an interplay of diets, genetics and gender. (Tian et al., 2017).The correlating data specifically for the Kenyan population is not available.

In Kenya, the prevalence rate of diabetes in adults is estimated at 2.0%(Guidelines-for-Screening-and-Management-of-Diabetic-Retinopathy-in-Kenya.Pdf.), however the World Health organization (WHO) has approximated the prevalence rate at 3.3% and projects a rise to 4.5% by 2025(Jones, 2013).This ballooning dilemma of diabetes in developing countries has been driven largely by a rise in obesity, according to WHO. In a 2012 WHO report, of the estimated 1.5 million global diabetes mortalities, greater than 80% occurred in low and middle income countries and W.H.O's global activity on diabetes attributed 1% of the total deaths in Kenya directly to diabetes in the same year. (Jones, 2013).

The Kenya Directorate of Promotive Health Services outlines processed food, declined physical activities, Tobacco and alcohol abuse as important risk factors in diabetes(Mwenda et al., 2018).This has prompted the Kenya Diabetes Management and information Centre to embark on offering educative trainings on diabetes management and prevention, diabetes awareness,

screening and monitoring: blood glucose, blood pressure, and Body Mass Index (BMI) among other activities in an attempt to scale down the prevalence of diabetes and its complications.

The Kenya Demographic and Health Survey (KDHS) estimated national prevalence of overweight and obesity for women of 15-49 years of age at 23% with a disproportionately higher rate in urban centers.(Mbochi et al., 2012)

The 2018 Health Sector Performance Review Report for 2016/2018 puts Hypertension as the leading Non Communicable Disease (NCDs) diagnosed during outpatient visits. The report also highlights the number of new cases of hypertension as three times the number of Diabetes as reported in outpatient department. These major NCDsare mostly brought about by lifestyle habits that include the type and quantity of cooking fats and oils used in preparation of meals.

Most solid fats are higher in saturated and/or trans-fats and lower in monounsaturated or polyunsaturated fats (PUFAs) than oils. Tran-fats and saturated fats are known to increase the low density lipoproteins (LDL) in the blood which eventually elevates the risk for heart disease. (Hunter, 2005).

In Kenya some of the most popular brands of cooking fats include; Fry mate (Pwani oil products Ltd), Mallo and Chipsy (Bidco oil Refineries), and Kasuku (Kapa oil Refeniries). (Best Cooking Oil and Fats in Kenya and Their Available Sizes - Info KE, 2021). This study used FryMate cooking fat due to its popularity by then as asserted by the fish vendor with whom i collaborated with in recycling of the cooking fat.

The high prevalence of diabetes in social and economically disadvantaged urban populations has been capturedby two studies in two major slums in Kenya (Korogocho and Viwandani).The studies indicated prevalence of diabetes in this impoverished population as moderately high.(Van de Vijver et al., 2013). The same study of this urban population revealed obesity as an important risk factor for raised blood glucose levels especially among women(Van de Vijver et al., 2013).

In another study in the same urban slum areas, it was found that the rates of treatment, awareness, and control were low and obesity was single most important risk factor for a raised blood glucose especially in women. (Oti et al., 2013). The study further noted that once individuals are aware of their hypertensive status, most will seek remedial measures. "Overall, there is urgent need to implement strategies that improve on prevention, detection, and affordable access to effective treatment in these populations."(Van de Vijver et al., 2013).

In a 2012 cross sectional study among some five urban social economic clusters (upper, lower upper, middle and lower) of Langata constituency in Nairobi, Kenya, the prevalence of MetS was found to be at around 34.6 %.(Kaduka et al., 2012), and the most distinctive characteristics of MetSwere: elevated blood pressure, a higher than normal waist circumference, and lower than normal HDL cholesterol. This study however noted that elevated fasting blood glucose and triacylglycerol (TAGs) were less frequent (Kaduka et al., 2012).The study further noted that the main risk factors linked to prevalence of MetS in these urban populations were increase in age, a

lower measure of their education level and income occupation: all of which inform their choice of diets (Kaduka et al., 2012)

In developing countries, there has been a rapid nutrition transition from a high fiber, high spices and whole grain diet to a highly processed caloric diet coinciding with increases in obesity, MetS, and type 2 DM(Misra et al., 2010). Available data shows an increase in supply and use of cooking fats that is consistent with high intake of these saturated fats in these developing countries (Misraet al., 2010). Most likely these modern diets together with a sedentary lifestyle have compounded the risk factors for MetS.

Mixtures of Transformer oil and standard cooking fat have been reported to be used in deep-frying foods in informal food kiosks in a number of countries including Kenya, Zambia, Zimbabwe, Tanzania, Malawi, and Bangladesh. Deep fried food stuffs are sold almost anywhere and most clients unknowingly buy these foodstuffs for consumption. (The Post Newspaper, Zambia. Monday 29 March 2010).In Kenya transformer oil siphoning for cooking among other uses was estimated to cost the Power firm Ksh 60 million a year. (The Daily Nation Newspaper. Thursday 22 November, 2012). In a 2007 report entitled: "challenges of vandalism on power equipment" by Engineer Joseph K. Njoroge, Managing Director, The Kenya Power and Lighting Company Limited, he notes that among many other uses of vandalized Transformer oil, it is mixed with vegetable cooking oil and sold as cooking oil(Njoroge, 2007). The main reason behind this dangerous business behavior is a longer frying period and maximization of profit.

Overall, the recommended remedies for MetS are lifestyle changes that include losing excess weight, eating a healthy diet, being physically active, stopping smoking in addition to medications for high blood pressure, cholesterol and elevated blood sugar.

Conclusively, prevention and any meaningful control strategies that target possible modifiable risk factors for diabetes and increase access to affordable treatment in any disadvantaged settings are urgently needed(Kaduka et al., 2012).

1.2 Rationale

This study hopes to contribute to the general understanding how reused cooking fats and transformer oil may be some of the precipitating factors in Metabolic Syndrome,

1.3 Objectives

1.3.1 General objectives

To investigate mixtures of reused cooking fat and transformer oil as risk factors in development of Metabolic Syndrome in male Sprague Dawley rats(*Rattusnorvegicus*).

1.3.2 Specific objectives

1. To compare lipid profiles of experimental animals fed different lipid dominated diets.

2. To compare levels of liver function enzymes in the study animals fed different lipid dominated diets.
3. To compare fasting and random blood glucose levels in the study animals fed different lipid dominated diets.
4. To compare liver to body weight ratios and overall body weights in study animals fed different lipid dominated diets.
5. To compare and assess the form and structure by liver histology of the different study animals.

1.4 Research questions

i. How do mixtures of reused cooking fat and transformer oil affect blood lipid profiles in male Sprague Dawley rats?
ii. How do mixtures of reused cooking fats and transformer oil affect critical liver enzyme levels in male Sprague Dawley rats?
iii. How do mixtures of reused cooking fats and transformer oil influence the body weight of the study animal'slivers?
iv. How do mixtures of cooking fats and transformer oil affect blood sugar levels in the study animals?
v. How do mixtures of cooking fats and transformer oil affect the form and structure of the study animals?

CHAPTER 2.0 LITERATURE REVIEW

2.1Saturated and unsaturated fats

Saturated fats are simply fat molecules that have no double bonds between their carbon molecules because they are completely filled with hydrogen molecules i.e. carbon is joined to its neighboring carbons by a single bondunlike unsaturated fats which contain one or more double bonds with a terminal carboxylic group (–COOH), (figure 1 compares the structures of both saturated and unsaturated fat molecule).Unlike unsaturated fats, saturated fatsare basically solid at room temperatureand hard to break down, a characteristic that gives it the ability to store and provide high energy than other nutrients.(Cymet et al., 2016)Saturated fats are a type of dietary fat found in high amounts in foods like red meat, butter, milk, coconut and palm oil (Boston et al 2014). In this study the solid saturated fats were used by fast melting at low temperatures.

Mice studies with dietary saturatedfat have revealed a stimulatory effect on weight gain and hepatic lipid accumulation than dietary unsaturated fat that eventually resulted in obesity and liver steatosis. (De Wit et al., 2012).Saturated dietary fat as an environmental factor have has been associated with human genotype interaction to affect the risk of obesity and metabolic syndrome. (Phillips et al., 2012).

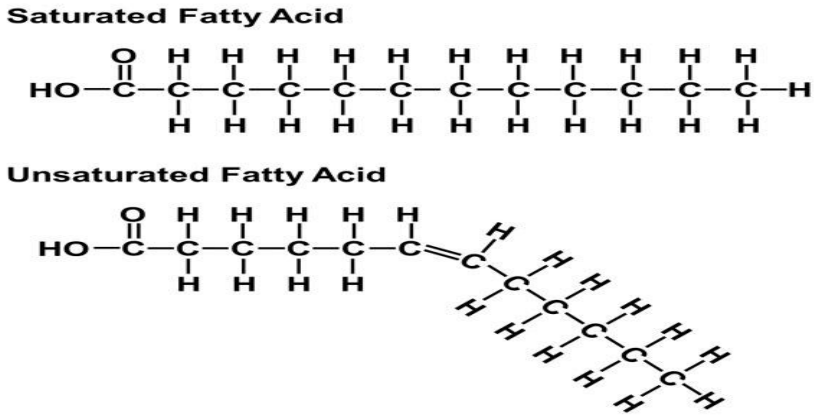


Figure1: A comparison of a saturated and unsaturated fat acid molecule.
Source, *(http://courses.washington.edu/conj/membrane/fattyacids.htm)*

Fresh saturated cooking fat contains mainly Triacylglycerol also called Triglyceride/ TAGs/Triacylglyceridein whichthe saturated fatty acid binds to the number 1 carbon atom of glycerol, (Lichtenstein, 2013).Triglyceride is the major storage form of lipid in the body in animals, (Allen, 1976).

Animal studies with this TAGshave indicated a strong positive correlation between the types of fats in consumed diets to the development of the MetS.(Damião et al., 2006). Since high consumption of these saturated fats is strongly associated to development of obesity and insulin

resistance, then the primary focus in alleviation of metabolic and cardiovascularabnormalities, and insulin resistance is diet composition and weight reduction.(Riccardi&Rivellese, 2000).

Synthesis of a triacylglycerol/Triglyceride molecule in animals occurs in many body organs andcell types, but the intestines,adipose tissue and the liver are most active. (Alvarez, 2016).

Three main pathways for triacylglycerol biosynthesis exist;dihydroxyacetone phosphate and sn-glycerol-3-phosphate pathways that are predominate in liver and adipose tissue, and lastly a mono-acyl-glycerol pathway which dominates in the intestines (Alvarez, 2016). More than 90% of liver triacylglycerol is synthesized by the sn-glycerol-3-phosphate/Kennedy pathway (figure 2 below) making it the most important pathway (Alvarez, 2016).

The first step in the Kennedy pathway as shown the figure 2 below involves the precursor *sn*-glycerol-3-phosphate, it's esterified by a fatty acid coenzyme A ester in a reaction catalyzed by a glycerol-3-phosphate acyltransferase (GPAT) to form lysophosphatidic acid, which in turn isacylated by an acyl-glycerophosphate acyltransferase (AGPAT) to form phosphatidic acid.(Alvarez, 2016)

Next, the phosphate group is removed by enzymes phosphatidic acid phosphohydrolases (PAPs). (Alvarez, 2016). In the final step in this pathway, the resultant 1, 2-diacyl-*sn*-glycerol is acylated by diacylglycerol acyltransferases (DGAT), in animals, DGAT1 is mostlylocated in endoplasmic reticulum and is normally expressed in skin, intestine and skeletal muscle. DGAT1 can utilize a

wider range of substrates, including mono-acyl-glycerols, long-chain alcohols and retinol. DGAT2 is the main form of the enzyme in hepatocytes and adipocytes. (Alvarez, 2016).

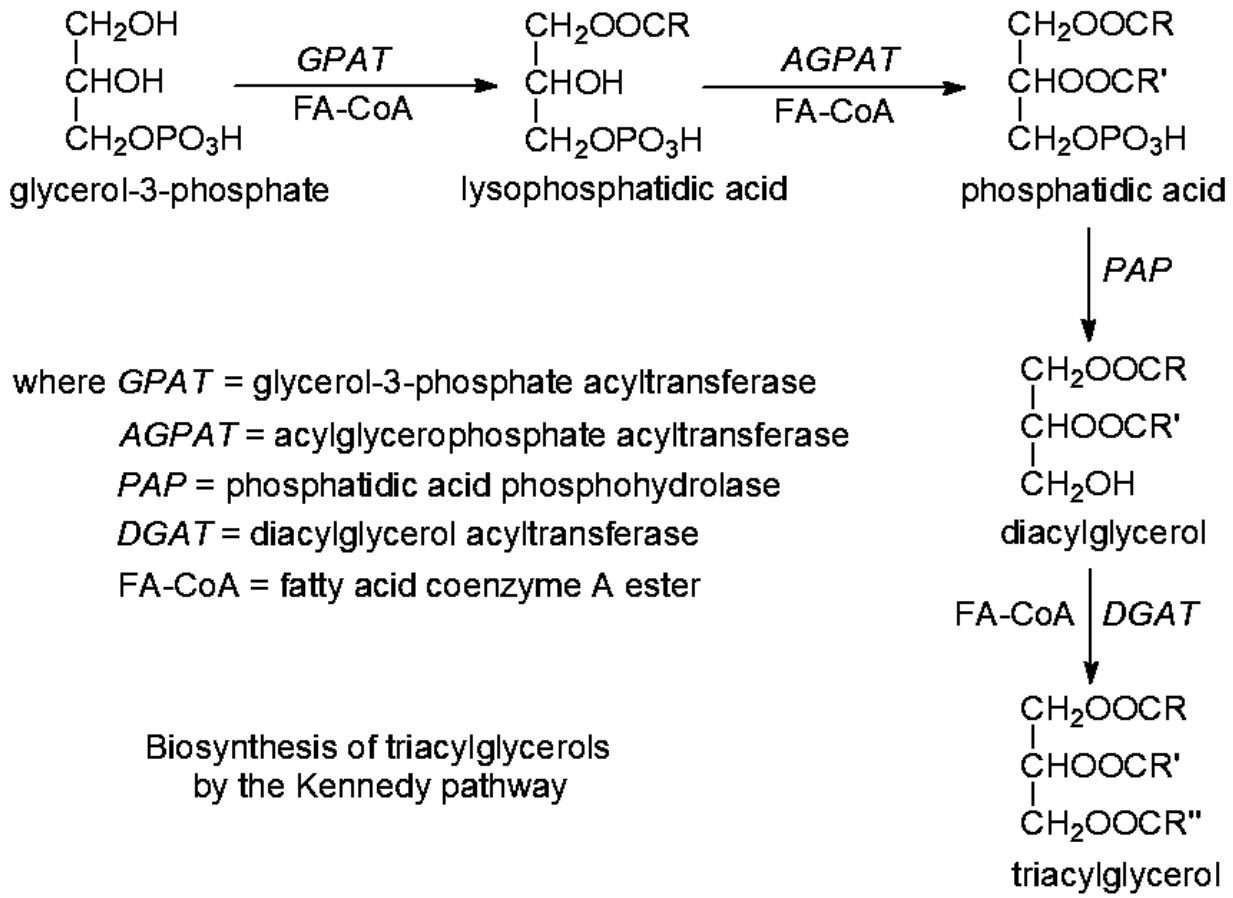


Figure 2. Synthesis of a triglyceride (Triacylglycerol) molecule by the Kennedy pathway.

Source: www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/tag2/Figure1.png

In a second pathway for triacylglycerol biosynthesis as shown in figure 3 below, dihydroxyacetone-phosphate in the endoplasmic reticulum can be acylated by a specific

acyltransferase to form 1-acyl dihydroxyacetone-phosphate, which is then reduced by dihydroxyacetone-phosphate oxido-reductase to lysophosphatidic acid, which then enters the pathway above to tri-acyl-glycerols(Alvarez, 2016). The forerunner dihydroxyacetone-phosphate is important as part of the biosynthetic channel to plasmalogens, which can be a significant components of cytoplasmic droplets in many mammalian cell types but not adipose tissue (Alvarez, 2016).

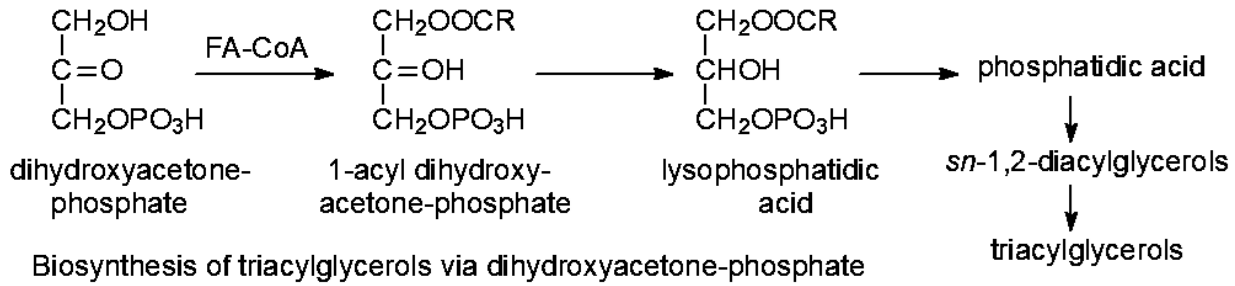


Figure 3. Biosynthesis of triacylglycerol molecule by dihydroxyacetone.
Adapted from:

https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/tag2/Figure2.png

After a meal, up to 75% of thetriacylglycerolsare formed through a monoacylglycerol pathway. 2-Monoacyl-*sn*-glycerols and free fatty acids are released from dietary tri-acyl-glycerols by the action of pancreatic lipase in the intestines and are taken up by the enterocytes. In the enterocytes mono-acylglycerols are initiallyacylated by an acyl coenzyme A: mono-acyl-glycerol acyltransferase with formation of *sn*-1, 2-diacylglycerols as the first intermediate in the process, some *sn*-2, and 3-diacylglycerolsare also generated.

1-Monoacylglycerols can also be synthesized by the acylation of glycerol as depicted in figure 4 below. Three isoforms of mono-acyl-glycerol acyltransferase exist in humans of which MGAT2 is the most active in the intestines and liver and MGAT1 in the adipose tissue. Finally, the acyl coenzyme A: diacylglycerol acyltransferase (DGAT1) reacts with the *sn*-1, 2-diacylglycerols only, to form triacylglycerol.

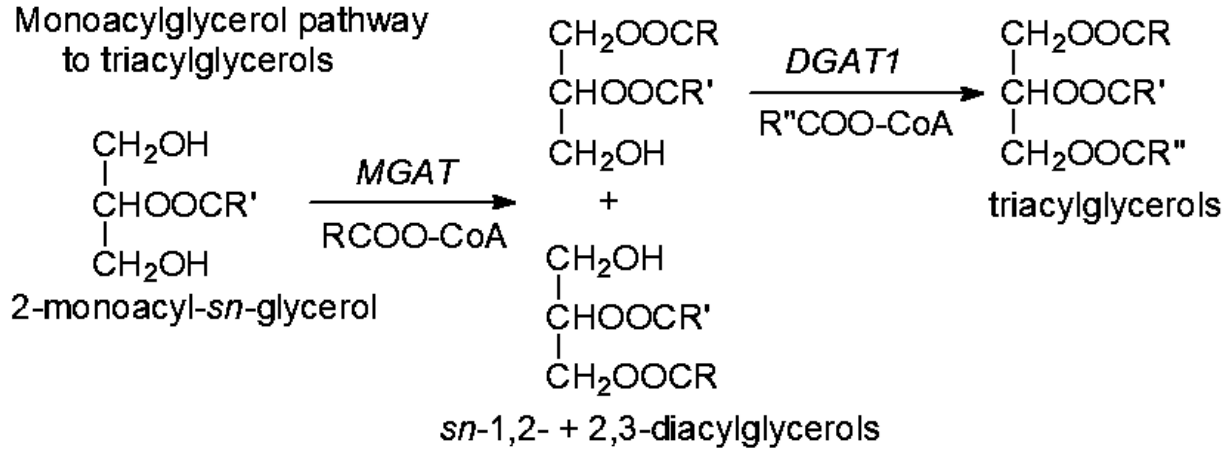
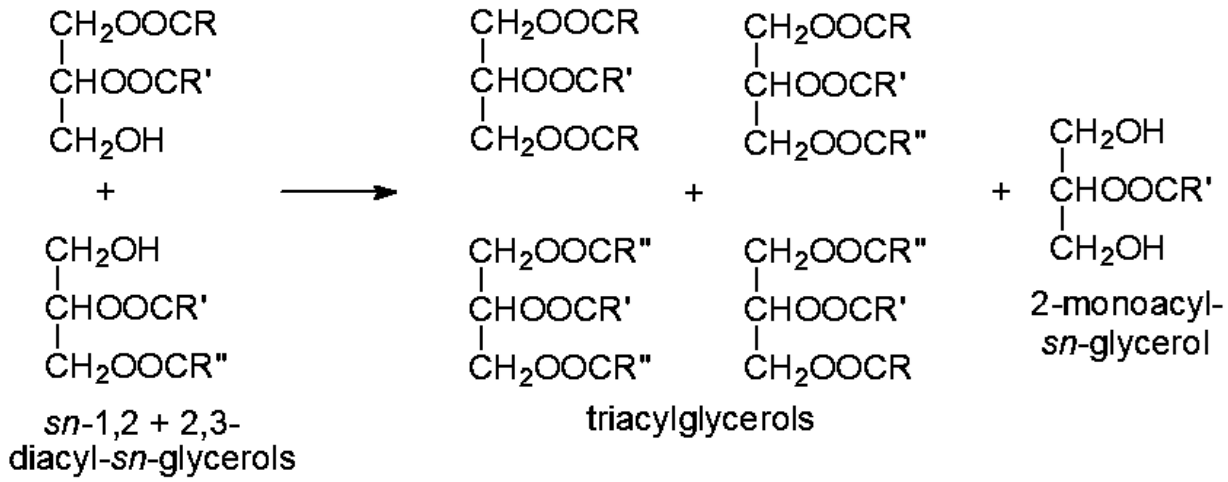


Figure 4. Synthesis of triacylglycerol molecule by the Monoacylglycerol**.**
Adapted from:

https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/tag2/Figure3.png

The fourth triacylglycerol biosynthetic pathway is by trans-acylation between two racemic diacylglycerols that is independent of acyl-CoA (figure 5 below). It occurs in the endoplasmic

reticulum of intestinal micro villus cells and is catalyzed by a diacylglyceroltransacylase. The twodiacylglycerol enantiomers participate in the transfer a fatty acyl group resulting in the generation of tri-acyl-glycerols and a 2-monoacyl-*sn*-glycerol.



Triacylglycerol biosynthesis via diacylglycerol transacylases

Figure 5: Synthesis of triacylglycerol molecule via the diacylglyceroltransacylases. Adapted from:https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/tag2/Figure4.png.

Figure 6 below depicts the chemical structure of a saturated triglyceride/triacylglycerol (De Weirdt, 2013). Triglycerides are lipid compounds composed of a glycerol esterified to 3 fatty acid chains of varying length and composition.

Structure of a saturated triacylglycerol/Triglyceride

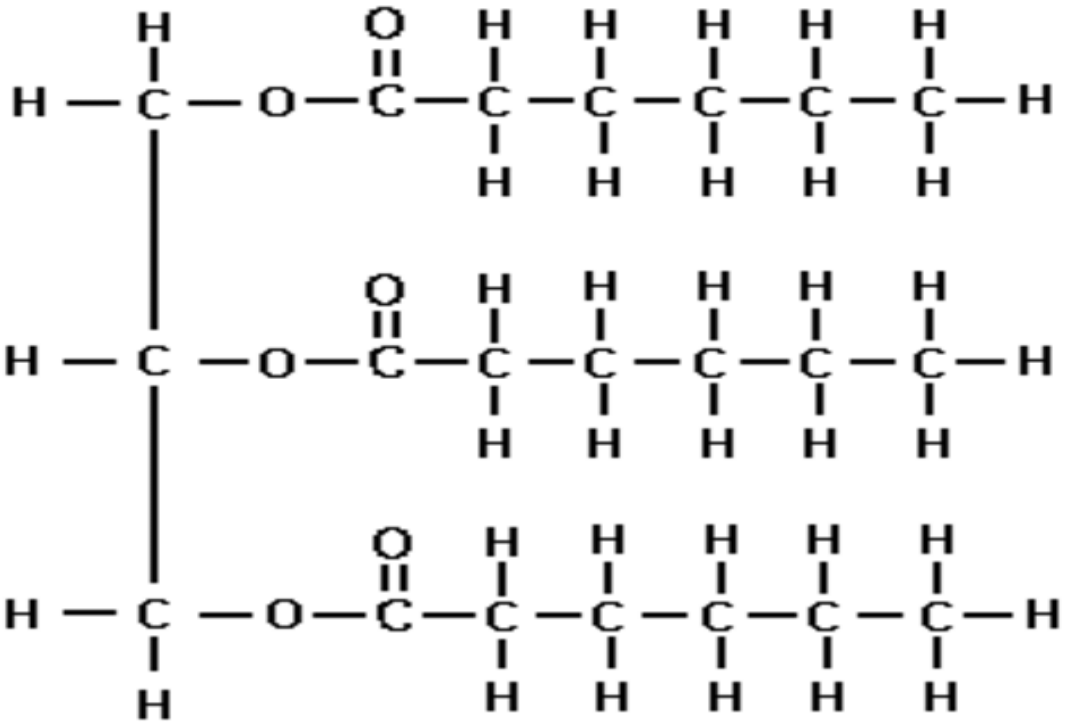


Figure 6: Chemical structure of a saturated triglyceride/triacylglycerol.(De Weirdt, 2013).

2.2 Reused cooking fats.

When saturated fats are excessively heated under high temperatures for a prolonged time, they undergo decomposition into hydro peroxides and eventually into polymers. (Frankel et al., 1984). Fats start breaking down producing free fatty acids, diacylglycerols, and mono-aclyglycerols that break down further to produce hydro peroxides and finally transformed into polymers that are of high molecular weight. (Frankel et al., 1984). Fats containing these products may also smoke easily at lower temperatures resulting into inhalation of volatile compounds like aldehydes, ketones, alcohols, hydrocarbons, lactones, and substituted furans. (Chow & Gupta, 1994). Ingestion of these decomposition products or unstable oxidized lipids causes several alterations in the lipid and fatty acid metabolism that might be of great physiological relevance(Crnjar et al., 1981).

Oxidized fats as constituents of over-heated and deep fried foods play a highly significant role in the pathogenesis of various diseases (Eder, 1999a). In oxidized fats are lipid peroxidation products that are known to affect metabolism in animals in various ways including increasing lipids in the plasma, heart and liver(Eder, 1999b). A report of Nov, 2018 by Euromonitor International titled “edible oils in Kenya” predicts an increased consumption especially in densely populated areas in the country.

The peroxide value and Refractive index are some of the analytical methods that can be used in determination and comparison of various physiochemical properties of cooking fats before and after frying. (Godswill et al., 2018). This analytical methods gives applicable information on the changes in the quality of cooking fat and oil which can be used in the determination of their range and rates of degradation. (Godswill et al., 2018). In thisway possible health complications

related to such degradation can be outlined.This methodshowever are expensive and too technical to be employed easily.

The roadside eateries relay on the physical characteristics of the used cooking fat to assess their quality.In most cases contamination of the cooking fat with food and water is the major determinant factor.

Refractive index is known to increase with an increase in the sum of conjugated fatty acids whilst peroxide values differ with the accumulation of primary oxidation products. But both methods are utilized in the detection of deterioration of rancidity(Godswill et al., 2018). Rancidity is normally noticeable as a foul smell in food and within the oil itself. Refractive index escalates with increase in oxidation same as peroxide value. Rancid oil will normally have a PV of 30mEq/kg to 40mEq/kg. (Godswill et al., 2018)

2.3 Transformer oil

Transformer oil is a transparent mineral oil. It contains organic compounds, paraffin's, naphthenes (cyclic aliphatic hydrocarbons e.g. cyclohexane) obtained from petroleum), aromatics, olefins and dissolved metals like copper and silver, corrosive Sulphur, oxidative inhibitor butylated hydroxytoluene (DBPC) among others(Kaplan et al., 2010). The mineral oils have the general formula – C_nH_{2n+2} and C_nH_{2n}.It's of low density and viscosity. These types of oils are for low-temperature and even though they oxidize more easily, the main product formed by this process i.e. sludge, is soluble. (Kaplan et al., 2010).

Studies have demonstrated metals like iron are capable of catalyzing aromatic compounds to form free radicals resulting in production of both reactive oxygen species (ROS) and reactive radicals

(Crichton, 2016). Thus, presence of un-complexed metals in biological systems can notably rise the level of oxidative stress(Pizzino et al., 2017).

Elevated oxidative stress (OS) in accumulated fat cells is a recorded pathogenic mechanism of obesity-associated MetS(Gluckman& Hanson, 2004).

Further the polychlorinated bi-phenyls (PCBs) in the transformer oil can cause nausea, irritation of the mucosal membrane, dehydration, weight loss, altered functions of the neuromuscular system, liver, and kidneys(Stein et al., 2002). It is estimated that PCBs can remain in human fatty fat cells up to 120 years with a possibility of altering the DNA structure and traces of it can be transferred to offspring(Stein et al., 2002). Ironically, ant oxidation stabilizers **(**phenol and naphtholic compounds) contained in the oil may prevent further oxidation of the cooking oil under high temperatures(Stein et al., 2002).

The insulating oil has the advantages of being stable under high temperatures and does not deteriorate as fast as standard cooking oil(Hering, 2020,). The oil reportedly looks just like regular cooking oil, but it lasts much longer. These properties have lured some unscrupulous business men to illegally siphon the transformer oil and latter mix with the standard cooking oil and sell as normal cooking oil for deep frying in roadside stalls(Iraki, 2014).

Coincidently the transformer oils also container substances like Ditertiary Butyl Para Cresol (DBPC) which are inhibitors that extend or delay the process of oxidization. DBPC as an antioxidant is used in oils and fats or in packaging materials for fat containing foods and feeds extending the life of the oil by three to four times of the actual period. Thin layer chromatography, infrared spectroscopy or gas chromatography are some of the methods used to detect the presence of DBPC in the oils or fats.

Rats fed high doses of DBPC have shown an increase in serum cholesterol. DBPChas also resulted in increased absolute liver weight and the ratio of liver to body weight. DBPC also increases the ratio of the left adrenal to body weight in male rats but with no consistent effect in female rats. DBPC when given to rats for 68-82 days caused a reduction in rate of increase in weight and fatty infiltration of their liver however no tumors occurred. When tested for teratogenic properties DBPC resulted inanophthalmia in the rat offspring, but not in mice. (National Center for Biotechnology Information (2021).

2.4 Digestion and Metabolism of fats.

Figure 7 below depicts a simplified fat digestion process in the stomach and intestines.

Digestion of fats

Fats *(bile and agitation)* **Emulsified Fats.**

Figure 7: A simplified flow diagram of fat digestion.

The digestion of fats comprises of the total sum of both physical and chemical changes that break down and synthesis fats in the living cells.The physical changes begins by breaking down fat in the mouth (mastication), using enzymes in saliva (lingual lipase), (Patricia &Dhamoon, 2021). Lingual lipase hydrolyzes ester bonds in triglycerides to resulting inmonoacylglycerols and diacylglycerols(Lai et al., 2019).

Mastication increases the surface area of foods, allowing the enzymes to break down food more effectively. Another important chemical that help with fat digestion in the mouth is phospholipids, which turn fats into small drops (Patricia &Dhamoon, 2021).

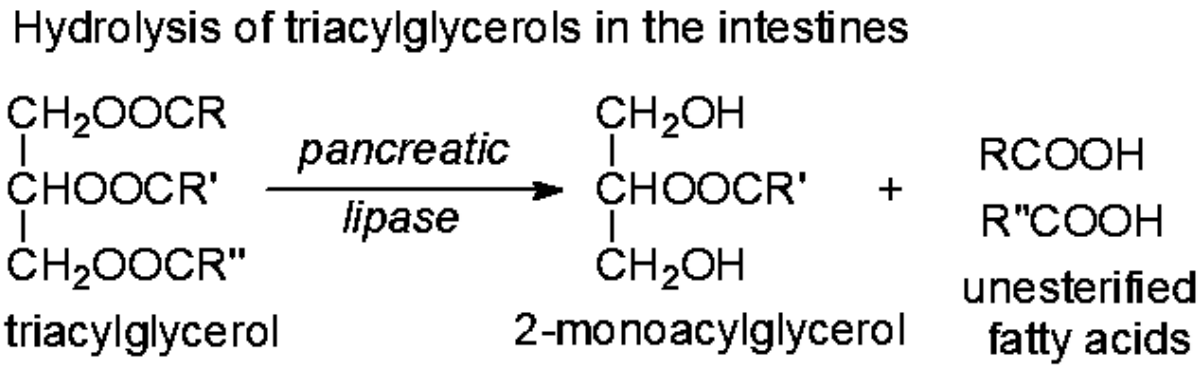


Figure8. Hydrolysis of Triacyglycerol by pancreatic lipase.
(https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/lipids/simple/tag2/Figure5.png)

The next phase in fat digestion occursin the stomach when gastric lipase further fragments fats. This phase in digestion is aided and intensified by rhythmical stomach contractions. The stomach digestion activity aloneconverts up to 30 percent of fats into fatty acids and diglycerides in about 2–4 hours after a meal. (Patricia &Dhamoon, 2021).

The stomach contents, including the di-glycerides and fatty acids, descend to the small intestine, specifically the duodenum, here the in addition to stomach motility, the liver releases bile containing lecithin and bile salts, (amphipathic molecules)that further assist in the break down fats.(Patricia &Dhamoon, 2021).Emulsifiers increases the fat's surface area, making them easier for the digestive enzymes to reacton. With an increased surface in form of fine droplets, the enzyme Lipase from the pancreas additionally digests fats into mono-glycerides and fatty acids as shown in figure 8 above. Bile further assist to move the fats to the tiny hair-like projections (microvilli) of the small intestines. The microvilli assist in shuttling the fats into the cells of the digestive system. (Patricia &Dhamoon, 2021).

Absorption of fats occurs by first regrouping of the broken down components into triacylglycerol. These triacylglycerol combine withcholesterol, phospholipids, and transport protein to form lipoproteins. Lipoproteins make way to the lymphatic system, and are then releasedinto the bloodstream. (Patricia &Dhamoon, 2021). Eventually the fats are stored in adipose tissue as a potential energy source or are broken down to produce required energy as demanded. Fats are also formed from excess dietary carbohydrates and amino-acids.

2.5 Exogenous Metabolism of lipoprotein

The Lipoprotein metabolism is understood to occur through the exogenous or endogenous pathway depending on the source of origin, dietary or hepatic. Around 95% of dietary lipids are triglycerides, while the remaining is a mixture of free fatty acids, phospholipids, fat-soluble vitaminsand cholesterol (Ros, 2000b). In a normal digestion process, Triglycerides from the diet are digested in the gastrointestinal tract resulting in monoglycerides and free fatty acids while Cholesterol esters from the diet undergo de-etherification to form free cholesterol. (Ros, 2000a)

These initial digestion products are soluble in bile acid micelles and can be absorbed from the chymus into the enterocytes. Further reassembly into triglycerides and the combination with cholesterol to form large chylomicron lipoproteins occurs in the enterocytes. Finally secretion of these particles into the lacteals is highly regulated by Apolipoprotein B-48; the chylomicrons then re-circulate through the lymphatic vessels and into the bloodstream. (Ros, 2000a)

Chylomicrons are the major carrier proteins (lipoproteins) of dietary triglycerides and cholesterol from the enterocytes and into the systemic circulation (through venous return). In the muscle and adipose tissue, the most of the triglycerides in the chylomicron are hydrolyzed to glycerol and fattyacids to provide energy. Upon depletion of energy, the remnantsof

chylomicrons that are basicallyrich in cholesterol, circulate back to the liver and are cleared from the body through a process mediated by Apoprotein-E. (Lambert & Parks, 2012)

2.6 Endogenous Metabolism of lipoprotein

Lipoproteins (chylomicron remnants) from the exogenous pathway are formed in the liver with endogenous triglycerides and cholesterol in the hepatic cells. (Feingold, 2000a) as shown in figure 9 below.

In this endogenous pathway, Apolipoprotein B-100 is known to synthesis these very low-density lipoproteins (VLDL) in the liver and releases them into the circulation. In the bloodstream, they encounter high-density lipoprotein (HDL) particles which donate apolipoprotein C-II and apolipoprotein E to the VLDL particles. (Feingold, 2000a).

The HDL particles that are initially free of cholesterol are formedin the enterocytes and the hepatocytes by acquiring cholesterol from lipoproteins and peripheral tissues for appropriate transportation. (Feingold, 2000a).

The VLDL particles are then transported in the bloodstream to the peripheral muscle and adipose tissues. Hydrolysis of triglycerides in these tissues provides fatty acids and glycerol to the cells as a source of energy. (Alves-Bezerra& Cohen, 2017).

Intermediate density lipoproteins (IDLs) are basically energy-depleted VLDL remnants; having a higher percentage of cholesterol, as the triglycerides have been depleted. (Burdge& Calder, 2015a). VLDL particles are circulated in the blood until they are absorbed in the liver with aid of a transporter protein- apolipoproetein E. (Burdge& Calder, 2015b). The IDLs are further hydrolyzed by hepatic lipase to release glycerol and fatty acids to form the low-density lipoproteins found abundantly in cholesterol. (Bauer, 2004).

LDL circulating in the blood can be absorbed by hepatocytes and peripheral tissues. The particles bind to the target tissue with the LDL receptor; a process that involves apolipoprotein B-100. The LDL particles are then absorbed by endocytosis and hydrolyzed to release lipids such as cholesterol. (Borén et al., 1994).

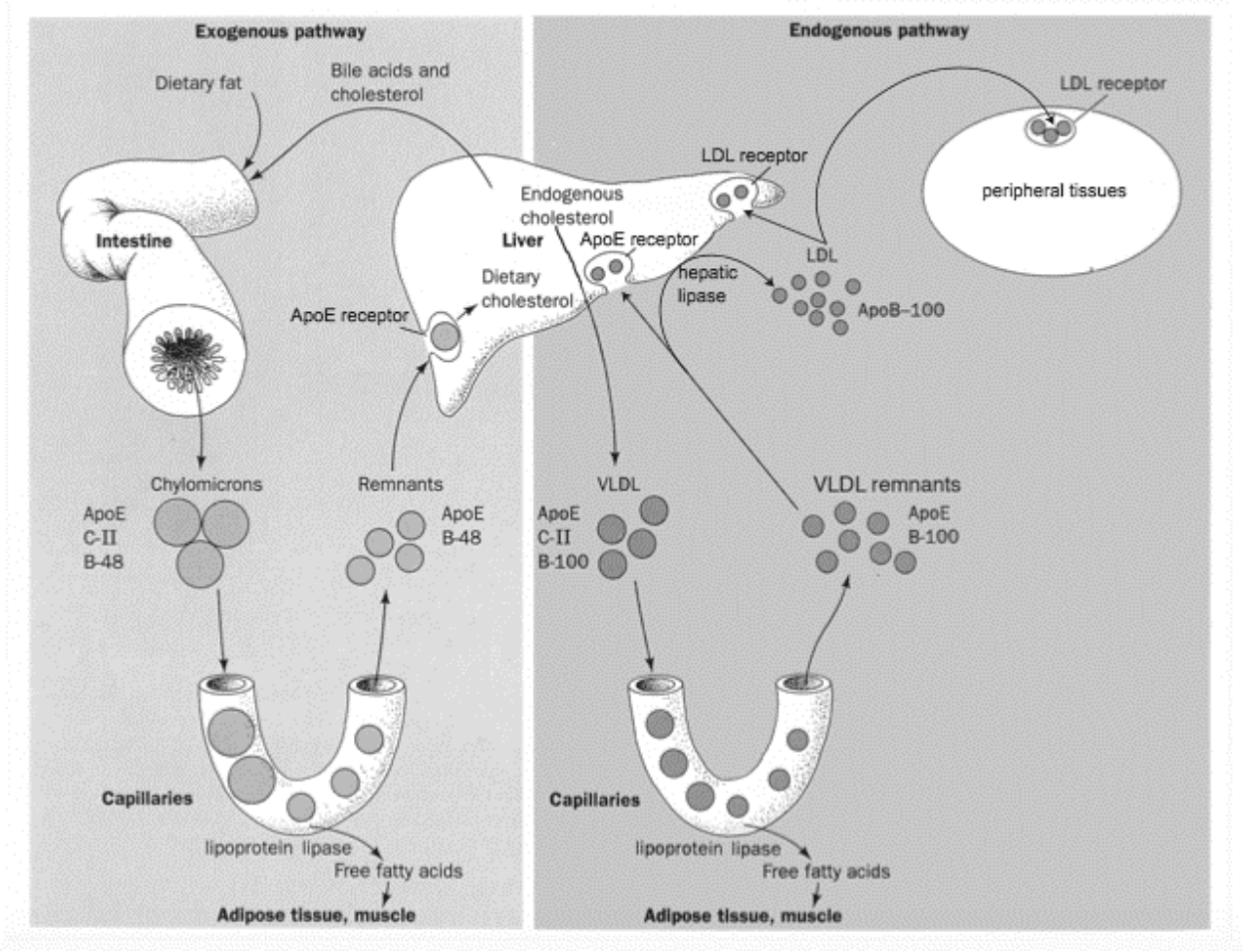


Figure 9. Exogenous and endogenous lipid transport pathways.Abbreviations. ApoE-Apolipoprotein E, apolipoprotein C-II, Apolipoprotein B-48, Apolipoprotein B-100

Source:https://o.quizlet.com/14fT.wehUMAEn8myrD8rUg_b.png

2.7 Compositions and types of lipoproteins in human plasma

Lipoprotein functions as ligands and cofactors for a number of receptors(Feingold, 2000a). the major lipoproteins includeVery low density lipoprotein (VLDL)-carry endogenous triglyceride,

Chylomicrons - carry dietary lipid, and some cholesterol, Intermediate density lipoprotein (IDL) - carry triglycerides and cholesterol esters, Low density lipoprotein (LDL) - that shuttle cholesterol esters(Feingold, 2000a). High density lipoprotein (HDL) is a small particle produced in hepatic and intestinal cells and is usually made of phospholipid and apolipoproteins; it functions to carry cholesterol esters(Feingold, 2000a).

Lipoproteins are basically the carrier vehicle in plasma for the water-insoluble lipids from their organs of formation to their sites of utilization. They are found in plasma as distinct entities, containing lipids and specific proteins. (Robert, 2009).

The most common alteration of lipoproteins in Type Diabetes is anelevation in VLDL, as reflected by either increased total triglyceride or VLDL triglyceride concentrations. Abnormalities in both production and clearance of VLDL triglyceride have been reported in NIDDM(Santen et al., 1972). Several studies have observed an overproduction of VLDL triglyceride which is more pronounced in diabetics with very high triglyceride values though it's also observed in people with modest elevations in plasma triglyceride.(Dunn et al., 1984).

Lipoprotein	Source	Diameter (nm)	Density (g/mL)	Composition		Main Lipid Components	Apolipoproteins
				Protein (%)	Lipid (%)		
Chylomicrons	Intestine	90-1000	<0.95	1-2	98-99	Triacylglycerol	A-I, A-II, A-IV,[a] B-48, C-I, C-II, C-III, E
Chylomicron remnants	Chylomicrons	45-150	<1.006	6-8	92-94	Triacylglycerol, phospholipids, cholesterol	B-48, E
VLDL	Liver (intestine)	30-90	0.95-1.006	7-10	90-93	Triacylglycerol	B-100, C-I, C-II, C-III
IDL	VLDL	25-35	1.006-1.019	11	89	Triacylglycerol, cholesterol	B-100, E
LDL	VLDL	20-25	1.019-1.063	21	79	Cholesterol	B-100
HDL	Liver, intestine, VLDL, chylomicrons					Phospholipids, cholesterol	A-I, A-II, A-IV, C-I, C-II, C-III, D,[b] E
HDL_1		20-25	1.019-1.063	32	68		
HDL_2		10-20	1.063-1.125	33	67		
HDL_3		5-10	1.125-1.210	57	43		
Preβ-HDL[c]		<5	>1.210				A-I
Albumin/free fatty acids	Adipose tissue		>1.281	99	1	Free fatty acids	

[a]Secreted with chylomicrons but transfers to HDL.
[b]Associated with HDL_2 and HDL_3 subfractions.
[c]Part of a minor fraction known as very high density lipoproteins (VHDL).
Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.

Table 1: Types and compositions of lipoproteins in human plasma.(Themes, 2017)

The Table 1above outlines the various types of lipoproteins, their sources, the major lipid components and their composition in human plasma among other characteristics. The major source is the intestine which reflects the diet and the liver which processes most of the proteins. Thus the conditionof this two major sources reflect on the physiological status of the individual.

2.8 Cholesterol metabolism

Lipids are basically cholesterol and triglyceride; are insoluble in plasma and thus are transported in lipoproteins particles. Their basic roles are energy utilization, steroid hormone production, bile acid production and lipid deposition (Cox &García-Palmieri, 1990).

Cholesterol is hydrophobic and thus cannot be transported as a free compound in blood circulation in plasma. It's combined with other compounds to form various lipoprotein particles. Among which are, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) contain the largest proportion of circulating cholesterol in plasma. Chylomicrons are also another type of lipoprotein formed by intestinal cells and primarily to shuttle cholesterol including dietary lipids. It's estimated that up to30 percent ofthe whole body cholesterol arise from dietary sources, indicating thatan increase in "exogenous" cholesterol levels are accompanied by low "endogenous" cholesterol synthesis. Cholesterol is found in the body in two forms, free and esterified. A number of earlier large clinical studies have suggested a positive alliance between total and LDL cholesterol levels (Gordon et al., 1977).

During the Exogenous pathway for lipid metabolism (figure 10 below): dietary cholesterol and fatty acids are absorbed while triglycerides are formed in the enterocytes from free fatty acids and glycerol as cholesterol is esterified.(Feingold, 2000a). Triglycerides and cholesterol combine to form chylomicrons which enter the blood circulation and travel to peripheral sites to release FFA. (Feingold, 2000a). The FFA are utilized as energy source, converted to triglyceride or stored in adipose tissue while the remnantsare used in the formation of HDL (Feingold, 2000a).

In Endogenous pathway as depicted in figure 10 below: very low density lipoproteins are formed in the liver cells from cholesterol esters and triglycerides, which are then hydrolyzed by lipoprotein lipase to form intermediate density lipoprotein or VLDL remnants. In this pathway, VLDL remnants are then removed from the circulation or integrated into LDL(Rosensen 2009).

The LDL particles have a basic of cholesterol esters and an amount of triglyceride. LDL is internalized by both hepatic and non-hepatic tissues. In hepatic tissues, LDL is made into bile acids and secretedthrough the portal circulation into the intestines (Rosensen, 2009).

In non-hepatic cells and tissues, LDL is used inthe synthesis cell membrane, hormone production, or stored. Excess LDL can be taken up by macrophages and other cells which may lead to excess accumulation and formation of foam cells: important in plaque formation. (Rosensen, 2009).

Disorders of cholesterol metabolism include; Familial hypercholesterolemia or more than normal total cholesterol, Familial together with hypercholesterolemia or more than normal total cholesterol and high triglycerides, Familial hyperapobetalipoproteinemia or high Apo lipoprotein B and polygenic hypercholesterolemia or high total cholesterol. (Barth et al., 1996).

Figure 10 below depicts an arbitrary diagrammatic representation of the two important pathways of cholesterol metabolism in humans.

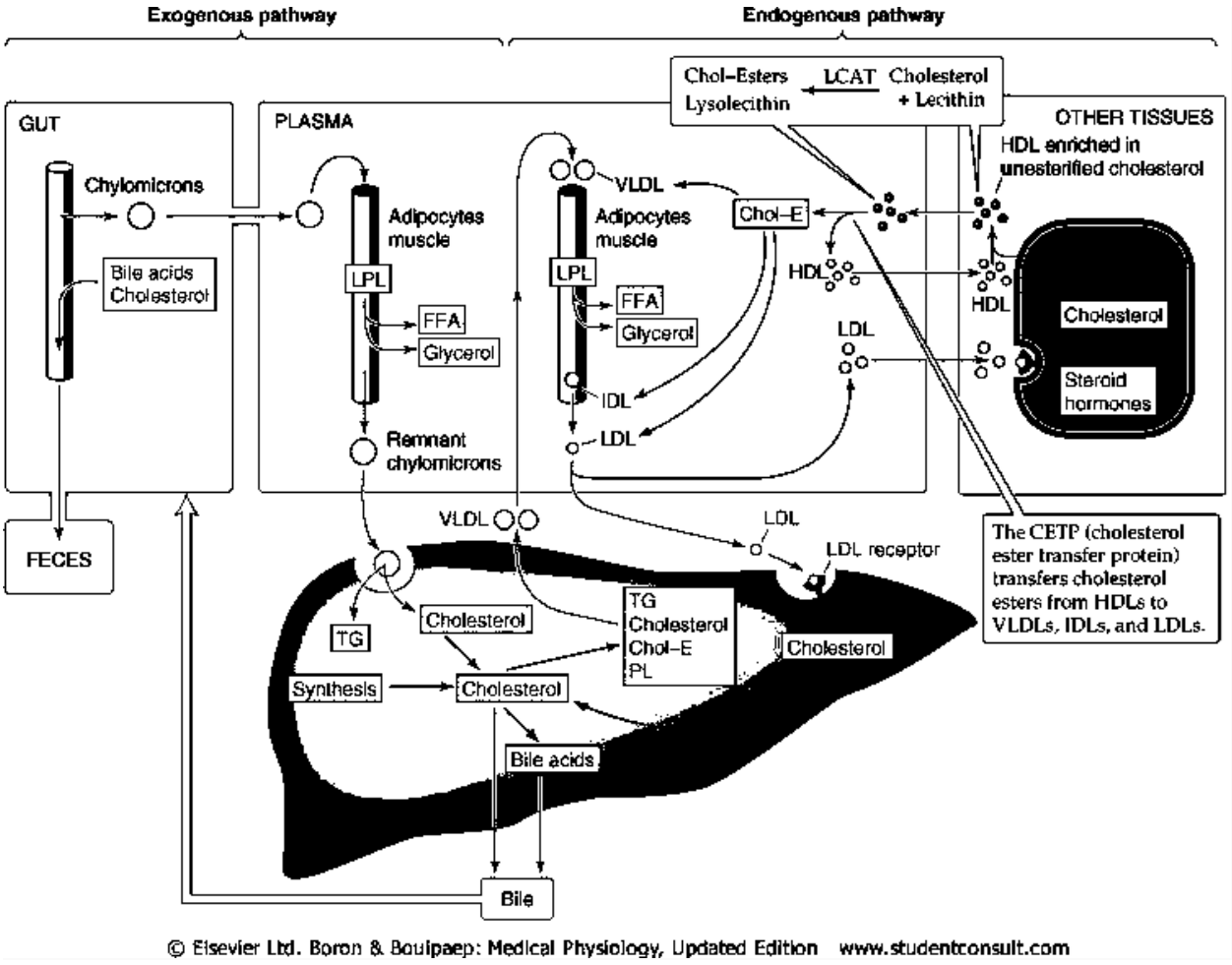


Figure 10. A diagrammatic representation of the two pathways of cholesterol metabolism. Abbreviations: LPL-Lipoprotein lipase; TG,triglyceride; CETP-cholesteryl ester transfer protein; Chol-E-cholesterylester;HDL, LCAT-lecithin-cholesterol acyltransferase.

2.9 Obesity and High fat diet

Epidemiological surveys have shown a significant positive link between a mean dietary fat intake and the occurrence of obesity and its associated complications and the risk factors. (Woods et al., 2003). Obesity is the central causative element in the progression ofMetS. Elevated oxidative stress in accumulated fat cells is an important mechanism in obesity-

associated Mets (Després&Lemieux, 2006). Hyperinsulinemia in high fat diet fed rats is a result of insulin resistance, a familiar feature of human obesity and one that is core to the progress of diabetes and cardiovascular disease, (Woods et al., 2003).

Increased dietary fat intake often has been cited as responsible for the increase in adipose tissue or adiposity(Hariri &Thibault, 2010). Human studies have indicated that high-fat diets of ≥ 30 % of energy from fat can very easily induce obesity (Hariri &Thibault, 2010). Epidemiological studies conducted in countries such as China, Canada and the USA have shown that, when the average amount of fat in the diet increases, the incidence of obesity also increases. This has led to an increased urgency worldwide to decrease the amount and type of fat in the human diets.

In animals too, diets rich in fat induce obesity(Hariri &Thibault, 2010). In both mice and rats a positive correlation has been established between the level of fat in the diet and body weight. Many Scientific experimentonanimals have shown that laboratory rats on diets containing high proportions of fat gain weight faster than those on diets containing less amounts of fats.

In 1949Obesity was for the first time induced in rats by *ad libitum* feeding of a semi-liquid diet(Ingle, 2016). In 1953, Fenton & Dowling employed a50 % high-fat dietin weaning mice and were able to induce obesity which they termed nutritional obesity but later the model was termed dietary obesity (Fenton & Dowling, 1953).

Epidemiological studies in humans has demonstrated a significant upward relationship has been established between the quantity of dietary fat and the percentage of the human population who

are obese(Lissner et al., 1987). Clinical studies between the amount of dietary fat and body-weight gain have demonstrated the link as well(Boozer et al., 1995). These positivealliancehave also been shown in a number of animal studies(Wade, 1982). This relationship in either humans or animal models shows that the fat content of any diet is an integral factor in energy balance. In general, human diets with more than 30 % of the total energy as fats results to the development of obesity.

Researchers have managed to induce obesity by diets of different percentages and sources of fats in laboratory rats and other study animals. It's noted thatthe individual characteristics of these study diets will always differbetween and within laboratories interms of the constituents of the macronutrient and energy properties. This major differences in the diets could have confounding effects on the study animals.

The great genomic similarity between the rodents and humans make animal models better indicator tools to study metabolic effects of high fat diet.(Panchal & Brown, 2010).

A lot of co-morbidity associated with obesity in both children and adolescents include diabetes mellitus, Dyslipidemia, hypertension, insulin resistance, and Liver diseases.(Pulgaron& Delamater, 2014).

2.10 Diabetes and high fat diet

Diabetes mellitus (DM) is a metabolic disorder characterized mainly by excess of glucose in the blood stream(Inzucchi, 2012a)It's a metabolic syndrome of impaired metabolism of substrates

including carbohydrates, fats and proteins and is caused by a deficiency of insulin secretion or decreased insulin sensitivity of tissues(Peters et al., 2004a).Lack of insulin prevents efficient blood glucose uptake and utilization by almost all animal cell types except the neurons which are insulin-independent in glucose uptake(Peters et al., 2004b).

Type 2 diabetes is strongly linked to obesity and insulin resistance(Skovsø, 2014) as well as faults in pancreatic β-cell function(Skovsø, 2014).These metabolic imperfections impede the core regulatory influence of insulin on lipid, protein metabolism and glucose, thus giving rise to a disease characterized by impairments in these basic physiological activities.(Kahn &Halban, 1997)

Majority of patients who develop type II diabetes often go through a state of obesity associated with reduced insulin sensitivity with an activated β-cell compensatory mechanism, such as enhanced basal insulin secretion and hyperproinsulinemia, as a part of their metabolic account.(Kahn &Halban, 1997).

In pre-diabetes, patients have either an impaired fasting glucose or an impaired glucose tolerance, or both, and is often linked with insulin resistance. In most healthy individuals, the adipose tissue acts as a safe store for lipids during a positive caloric balance (Frayn, 2002). Excess blood glucose is taken in by the liver cells and muscle tissue in the form of glycogen. For fully occupied glycogen stores, high glucose level might result in lipogenesis mainly in the liver and, to sometimes, in the adipose tissue (Donnelly et al., 2005). Lipogenesis essentially assist

maintain normal blood sugar levels by holding on excess glucose from the circulation. (Kahn &Halban, 1997).

Insulin resistance is observed in obesity and Type 2 diabetes mellitus, but it is also a common feature of in body fat changes that affect some people with HIV Aids, (lipodystrophy)(Frayn, 2002). Adipose tissue can play an important role in buffering the flux of fatty acids in the circulation after a meal. Adipose tissue provides its cushioning by diminishing the release of non-esterified fatty acids into the blood circulation and by elevating triacylglycerol clearance(Frayn, 2002).

The American Diabetes Association classifies diabetes mellitus into the following four types: Type 1 diabetes (as a result of β-cell destruction, resulting in an absolute deficiency of insulin), Type 2 diabetes (as a result of progressive loss of the capacity to secrete insulin on the background of tissue resistance to insulin), Gestational diabetes mellitus (a chronic state of hyperglycemia that is not clearly overt diabetes that is diagnosed in pregnancy during the 2^{nd} or 3^{rd}trimester), and diabetes secondary to other conditions affecting the exocrine pancreas like cystic fibrosis, monogenic diabetes syndromes like maturity-onset diabetes of the young, and chemical- and drug-induced diabetes(Chamberlain et al., 2016).

Type 2 diabetes is characterized by insulin resistance at early stages which increases with increasing hyperglycemia, and results in β-cell dysfunction that culminates in β-cell failure(Riccardi&Rivellese, 2000). Type 2 diabetes is preceded by an asymptomatic stage, termed pre-diabetes, that is characterized by mild elevation of blood glucose levels, relative

insulin resistance, and early decrements in the capacity of pancreatic β-cells to produce insulin(Inzucchi, 2012b).Complete β-cell failure involving both the loss of islet cell mass and decline of function of the β-cells is the trigger for transition from a state of insulin resistance and obesity to a full-blown type 2 diabetes (Muoio&Newgard, 2008).

Elevated levels of glucagon are seen in Type 2 DM and are implicated in development of hyperglycemia(Sandoval &D'Alessio, 2015a) and this is due to the lack of inhibition by insulin on the glucagon secretingα-cells after β-cell failure (Sandoval &D'Alessio, 2015b), however this may also be due to reduction of thehepato-incretin function on Glucago-like peptin 1(GLP-1) inhibitory effect on glucagon production(Ahrén, 2015).

High fat diet fed rat animal models as in Syndrome X (array of abnormalities resulting from high consumption of refined carbohydrates) do display excess amassing of muscle triglycerides coinciding with advancement of insulin resistance, this seems to occur in humans too and a number of studies indicate elevated muscle triglyceride levels in insulin resistant circumstances (Wong et al., 2016). Studies with animal models indicate that dietary changes and or exercises that decrease muscle lipid aggregation also improve insulin sensitivity(Wong et al., 2016).

It has been postulated that cytosolic aggregation of the long chain fatty acyl CoAs is majorly involved, resulting in changed enzyme activities like glycogen synthase or insulin signaling either directly or by activation of mediators.(Idris et al., 2001).

Physiologically diabetes is a MetS in which an individual has increased blood sugar that may be due to inadequate insulin, body cells failing to respond adequately to insulin or both(Gupta & Gupta, 2010). In type II diabetes, body cells are unable to respond adequately to insulin(Glaser et al., 1988). A high-fat diet (HFD), a decreased mitochondrial content and function have been closely associated with type II diabetes and obesity(Sparks et al., 2005).

A study to determine if HFDs down regulates the genes associated with mitochondrial oxidative phosphorylation in skeletal muscle did find out that HFDs in insulin-sensitive mice and humans correlated with reduction in the expression of the genes involved (e.g., the electron transport chain genes), nucleic genes that encode for mitochondrial proteins (e.g., mitochondrial carrier proteins), and genes involved in mitochondrial biogenesis (e.g., Peroxisome proliferator-activated receptor gamma co-activator 1-alpha- (PGC-1α)(Sparks et al., 2005). Seemingly, the studies supported the hypothesis that HFDs explain the reduction in oxidative phosphorylation genes seen in aging, a pre-diabetic state, and in advanced state (Sparks et al., 2005).

2.11 Blood Lipids, Cholesterol, Liver Enzymes and blood glucose levels.

A full cholesterol test is also termed a lipid panel or lipid profile, and is a determination ofTriglycerides, Total cholesterol, Low-density lipoprotein cholesterol, and High-density lipoprotein cholesterol. Obese and diabetic individuals' normally have high triglyceride levels(Castelli, 1986).

Lipids in circulating blood are either free or chemically bound to other molecules(Feingold, 2000a). They are mostly transported in a protein capsule (particle), and the density of the lipid particle determines its fate and the influence on metabolism(Blood Lipids | Environmentdata.Org, 2013).

The abundance of blood lipids depends on dietary intake, elimination, and uptake and production from functional cells(Feingold, 2000b). Blood lipids are mostly acids and cholesterol (Cox &García-Palmieri, 1990). Cholesterol transported as lipoproteins are water-soluble and carry both cholesterol and triglycerides(Feingold, 2000b).

Chylomicrons are re-synthesized monoglycerides and fatty acids in the intestinal epithelial cells. They enter the lymph and transport the lipids to the adipose tissue, cardiac muscle, skeletal muscle and the liver where they are hydrolyzed by lipoprotein lipase. (Hall et al., 2011).

When chylomicron particles reach the liver cells, they release triglycerides and cholesterol. Hepatocytes convert unutilized food metabolites into VLDL and then secrete them into plasma where they are converted to IDL, which thereafter are converted to both LDL particles and non-esterified fatty acids. Healthy individuals have relatively less but larger LDL particles. In contrast, large numbers of small LDL particles are actively associated with arteriosclerosis, a reason; LDL is referred to as "bad cholesterol" (Feingold, 2000).

HDL shuttle cholesterol back to the liver for excretion, thus implying that high numbers of large HDL particles is strongly linked to better health outcomes, and hence it’s commonly called

"good cholesterol". Hyperlipidemia is termed as presence of abnormally high levels of lipids/lipoproteins in the bloodcirculation which is a major risk factor for cardiovascular pathologies/disease(Daniels et al., 2005).

A 1987 report by the Adult Treatment Panels of the National Cholesterol Education Program (NCEP-USA), suggested total blood cholesterol levels should be: <200 mg/dl (5.17mmol/L) for normal blood cholesterol, 200–239 mg/dl (5.17-6.18mmol/L) as borderline-high, and >240 mg/dl(6.2mmol/L) as high cholesterol.In this report, serum levels of triglycerides, cholesterol, glucose, liver enzymes (ALT and AST) and body weights were used to evaluate both development and extent of MetSand the risk of cardiovascular diseases.

Determination of fasting blood glucose has been utilized as a screening test for detecting changes in glucose metabolism; normally the extent to which glucose drops following an insulin injection is symbolic of whole-body insulin activity(Danaei et al., 2011).

Liver weights and histology are symbolic of fat deposition in the liver (Altunkaynak, 2005).

CHAPTER 3.0: MATERIALS AND METHODS

3.1.0: Materials

3.1.1: Experimental animals

Twenty four (24) weaned male Sprague-Dawley rats were acquired from the School of Biological Sciences and housed in the animal house of the Department of medical physiology, University of Nairobi. At a density of 6 rats per cage(dimensions of 60cm by 40cm by 30cm) they were acclimatized for one week at room temperature (23^0C – 25^0C.). Plate 1 below shows some of the experimental animals before sampling.

Plate 1: Photograph of some of the experimental rats before the start of the study.

3.1.2 Neat cooking Fat

Thirty kilograms of fry mate brand cooking fat (plate 2below) was used. The brand was purchased and handed to a road side fish vendor in Kayole estate to use for two weeks. This was repeated until six (6) liters of filtered reused cooking fat was collected.

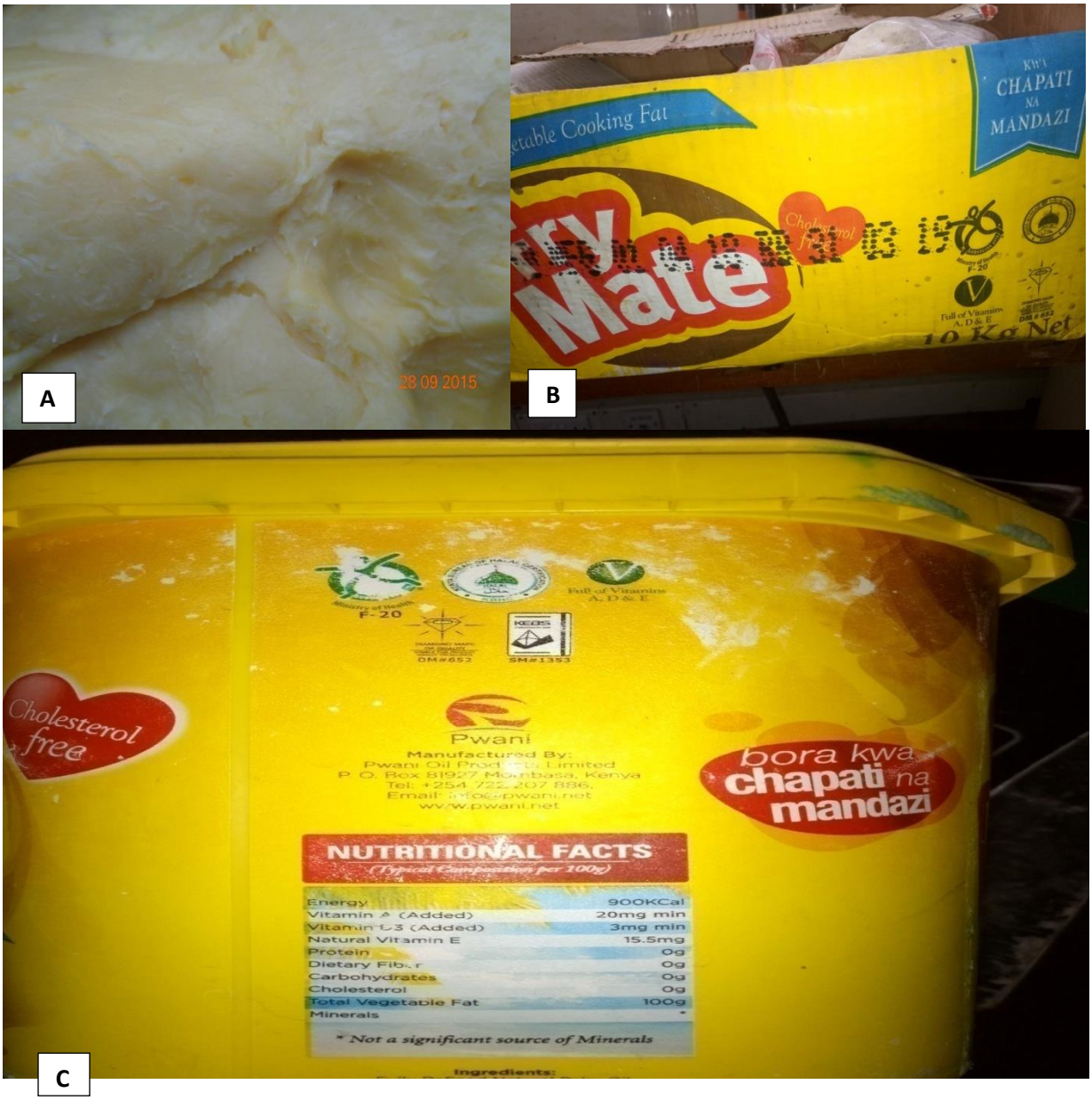


Plate 2: Photographsof Frymate™ cooking fat. A: neat solid cooking fat, B: Carton package of neat solid cooking, C: Nutritional facts of neat solid cooking.

3.1.3 Reused cooking fat.

Every two weeks, the reused cooking fat was collected, (plate 3), filtered to remove food particles and mixed with the previous collection. The fat was then stored at 4^0 C. Plate 4 shows the deep frying of fish in the pan and ready fish for consumption.

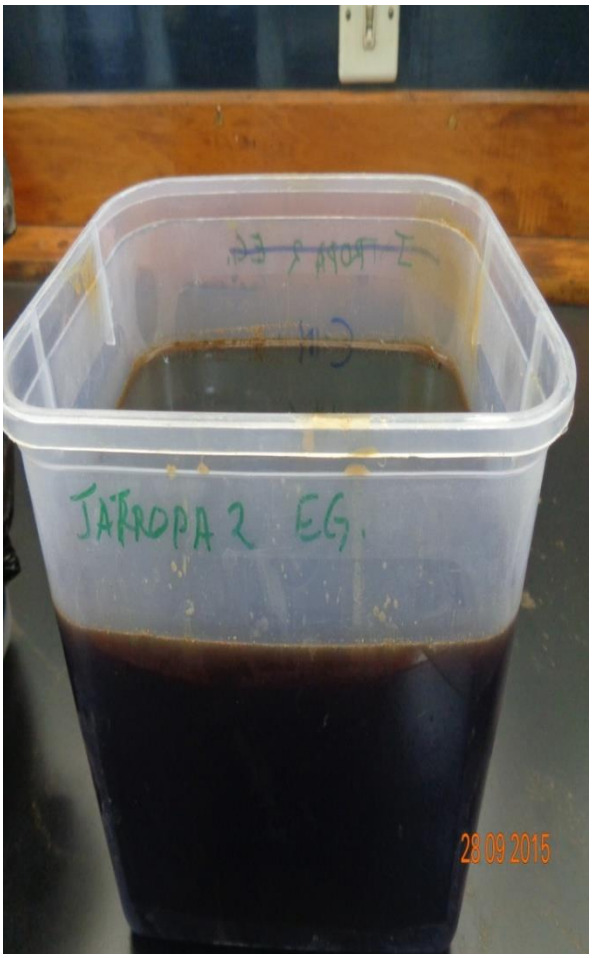


Plate 3**:** Photograph of the used cooking fat.Plate 4: A road side fish frying "kitchen."

3.1.4 Transformer oil

The transformer oil (Gulf Transformer oil)(plate 5) was sourced from Prof. Kabaru's Laboratory, School of Biological Sciences, Chiromo Campus, University of Nairobi. The oil was heated intermittently on a temperature controlled hot plate at 50 degrees centigrade for 6 hours to imitate the environment in the transformer machine. It was then cooled and stored at 4°C to be mixed latter with the reused cooking fat.

Plate 5: Photograph of a tin of the Gulf Transformer oil, UK.

3.1.5 Commercial rat pellets

The commercial rat pellets used in this study were sourced fromUnga Farm E.A limited, Nairobi. The maximum and minimum nutritional composition of rat pellets used in the studyare tabulated in table 2 below as provided by the manufacturer.

Nutrition information of rat pellets	
Parameters	%
Moisture	Max 12
Crude protein	Min 17.7
Fat	Min 2.2
Ash	Min 5.1
Crude Fibre	Max 6.0
Calcium	0.85
Phosphorous	0.79

Table 2. The nutritional information of rat pellets.

3.1.6 Study design

The study design in figure 11 below was observed, the fixation, sectioning and histology of the tissues was accomplished in two weeks.

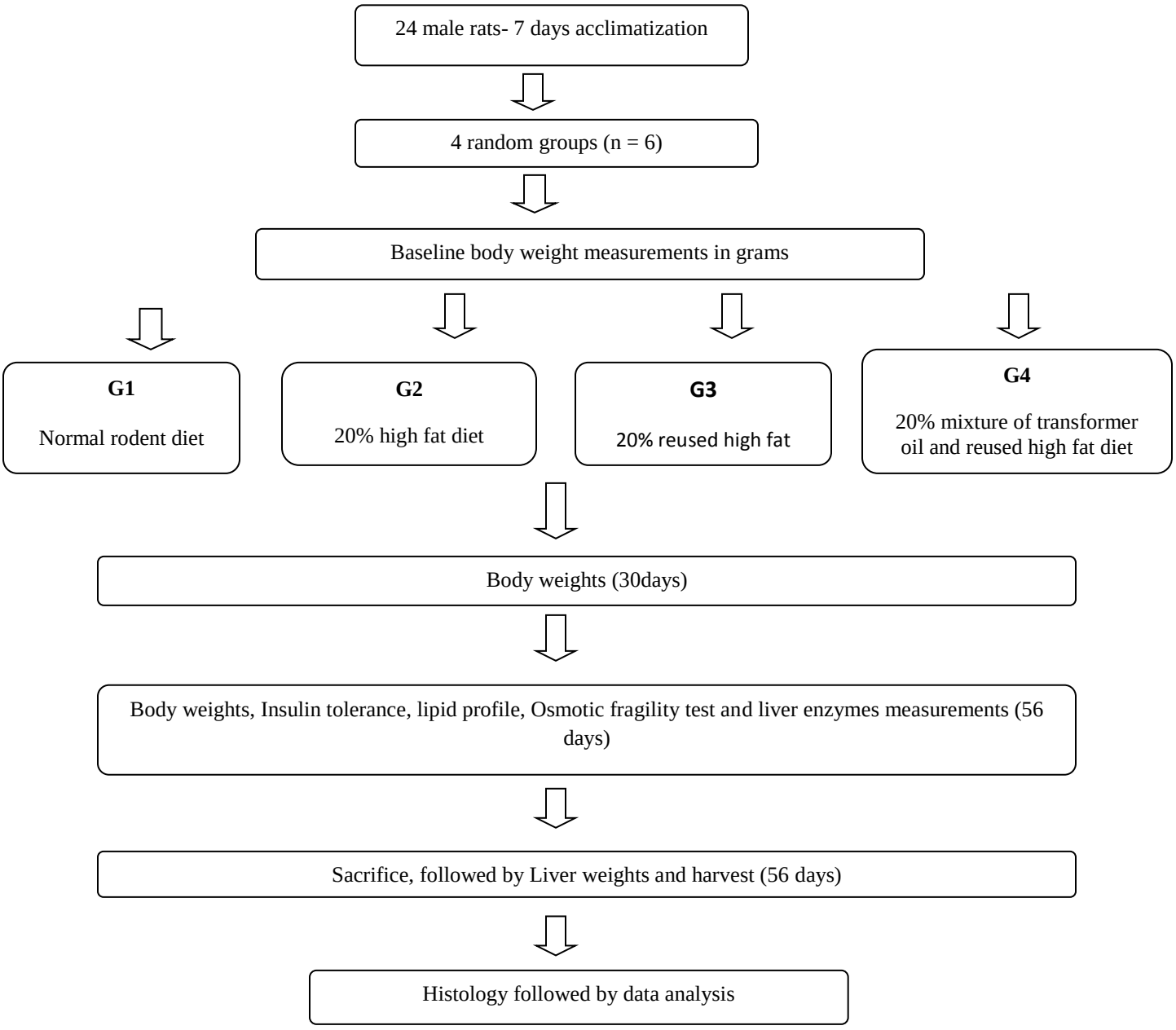


Figure 11: Study design for the study

3.2.0 Methods

3.2.1 Experimental design and diets

After seven (7) days of acclimatization and normal rat pellets feeding, the 24 study animals were randomly assigned four groups of six animals and allowed to freely access water by nipple bottles and fed appropriate rat pellets (Unga Farm E.A Ltd, Nairobi.Apendices1.0) *ad libitum*.

Group I: Normal rat pellets diets (ND) from Unga Farm E.A Ltd, Nairobi.

Group II: High Fat diet (HFD).

Group III: Reused high fat diet (RHFD).

Group IV: Reused high fat and Transformer oil diet (RHFD + T.O).

After eight weeks, insulin tolerance test was performed, venous blood drawn from the orbital sinus by the standard procedure of retro-orbital puncture (Sharma et al., 2014) into EDTA-coated vacutainer blood collection tubes on all the study animals. Plasma was prepared by centrifuging whole blood at 162.2g using an MSE centrifuge (Measuring and Scientific Equipment (UK) Ltd) for ten min and stored at -20˚C for further analysis. The animals were then sacrificed after an overnight fast.

3.2.2 Preparation of diets.

The 20% high fat and reused high fat diet was prepared by adding 200 g of either neat or reused cooking fat to 800g of normal rodent pellets. The mixture was then gently heated over low heat for 15 minutes, allowed to cool and stored in air tight polythene bags for future use.

Heated and cooled transformer oil was mixed with melted reused cooking fat in the ratio of 1V:3V; 200g of the transformer oil-cooking fat mixture was then added to 800g of normal rodent pellets and gently heated over a hot plate for 15 minutes. (The 1:3 ratio was arbitrary). The mixture was cooled and stored in air tightpolythene bags for use as reused high fat diet plus Transformer oil (RHFD+T.O). The study animals were fed on these 4 diets for 56 days of study after which the following measurements were done.

3.2.3 Body weights

Body weights per study group were taken at the end of the experimental period using OHAUS triple beam mechanical weighing balance. The mean body weights per group were calculated and then compared using the Graph-pad prism statistical software for significance.

3.2.4 Fasting blood sugar

Blood plasma glucose levels were determined using the Gluco Plus glucometer and test strips after a 12hours of fasting. (Food was withdrawn at 7:00 pm and blood sugar taken at 7:00 am the next day. Only water was provided during this period). The tip end of the rat's tail was

disinfectedwith cotton wool soaked in 70% alcohol and nipped with sterile dissection scissors. The first drop of blood was wiped off and a drop of subsequent blood drawn into the side of the test strip that has been inserted into the strip port of the glucometer. The tabulated results were expressed in mmol/L.

3.2.5 Insulin tolerance test

Insulin Tolerance test (ITT) is a measure of insulin sensitivity which characterizes Type 2 Diabetes Mellitus (DM). The glucose clamp technique is usually considered the gold standard for assessment of insulin sensitivity(Akinmokun et al., 1992). However in routine screening outside a clinical setting, the insulin tolerance test (ITT) is the most suitable substitute(Akinmokun et al., 1992). Food was withdrawn for 5-6 hours at 7:00 am. Tail blood for blood glucose determination was sampled at T0 minutes (for baseline glucose) and then T30, T60 and T120 minutes after an intra-peritoneal injection of 1.5U/kg body weight of regular insulin (ACTRAPID® Neutral insulin 100 IU/m, Novo Nordisk A/S, Denmark.) in 1.0 mL saline. Blood glucose concentration was determined with a hand held glucometer. (GlucoPlus, Inc. Quebec, Canada).

3.2.6. Liver enzymes

Liver transaminases, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined at the University of Nairobi, Department of Clinical Chemistry laboratory using (ELITech Group Puteaux.France). The stored serum was initially thawed, mixed with a reagent and poured in a cuvette for measurement. Two reagents, R1 and R2 are mixed in the

ratio of 4:1 to prepare a working solution. The working solution is then mixed with sample in the ratio of 10:1 and aspirated in the flow cell of the machine. The basic principle involves transmittance of light through a colored substance and measured/recorded as the absorbance at a selected wavelength of 340nm. The levels were expressed in IU/L.

3.2.7. Lipid profile

A full lipid profile was determined after 2 months. Food was initially withdrawn from the animals for 5-6 hours. Venous blood was drawn from the orbital sinus by the standard procedure of retro-orbital puncture (Sharma et al., 2014) into EDTA-coated vacutainer blood collection tubes.

Plasma was prepared by centrifuging blood using the MSE centrifuge (Measuring and Scientific Equipment (UK) Ltd) at 1409 g force for 10 min. The plasma was then mixed with a blocking reagent in the ratio of 100:1 and incubated for 10 minutes at 37^0 C. the mixture was then aspirated into a flow cell and absorbance measured/ recorded at 600nm using Selectra Pro S. Spectrophotometer (Eli Tech Group Puteaux.France) at the Department of Clinical Chemistry, University of Nairobi. The machine measures, Total cholesterol, Triglycerides and High density lipoproteins (HDL).

Very low density lipoprotein (VLDL) is calculated by dividing the amount triglyceride in a sample with five (5) while Low density lipoprotein is calculated by subtracting the amount of VLDL from HDL in the given sample (Warnick et al., 1990).

3.2.8.Percentages of liver weights to body weights

The livers from the sacrificed study animals excised and weight against the animal remains using ATY224 Unibloc Analytical Balance (Shimadzu). The ratios were determined and percentages established.

3.2.9Erythrocytes Osmotic fragility test.

Osmotic fragility is the easiness/fragileness or toughness with which RBCs (red blood cell) hemolysis when kept in a hypotonic solution(Fernández-Alberti& Fink, 2000). When RBCS are mixed with a hypotonic salinesolution;normally water is drawn in the cells by higher intracellular osmotic pressure due to its salts(Hendry, 1954).The cells then become spherical and the cell membrane tears releasing hemoglobin into its surrounding(Hendry, 1954). The resistance or toughness which the red blood cells redder to the hemolytic forces of the hypotonic saline solution is used as an indicator of the weakness or fragility of RBC's (Fernández-Alberti& Fink, 2000).

Among different salt concentrations, there's a range over which hemolysis occurs and therefore a range of toughness or fragileness of the red cell membrane. In various pathological conditions this range is therefore indicative of the defectiveness of the cell membrane.

The osmotic fragility of erythrocytes test for each study group was determined according O' Dell protocol: 20 microliters (μl) of freshly drawn blood was added to 10-mL of 0.125M hypotonic sodium chloride solutions of varying concentrations: 0.0%, 0.29%, 0.34%, 0.38%, 0.42%, 0.47%, 0.51% and 0.58%.(The varying concentrations were achieved by dilutions in eight (8) tubes of the 0.125M stock solution, with tube one containing 8.0 mL 0.125M sodium chloride diluted with 2.0mL distilled water and tube 8 containing 0.0 mL of 0.125M sodium chloride and 10.0mL of distilled water). The suspensions were then incubated for 15min at room ambient temperature and centrifuged at1409 g force for 5 min using an MSE centrifuge (Measuring and Scientific Equipment (UK) Ltd).

The absorbance of the decanted supernatants was determined using Selectra Pro S. (Eli Tech Group) Spectrophotometer at 540nm as a measure of hemoglobin concentration from hemolysedRBCs during incubation and used to calculate the percentages of RBCs hemolysis. The percentage hemolysis was calculated by a simple proportion since the readings in the last tube (had pure distilled water) gives the value of complete (100%) hemolysis. The reading of each tube was divided by the reading of tube eight and multiplied by 100. Distilled waterwas used as the blank solution in this procedure.

3.2.10 Liver histology

Livers from the sacrificed study animals were fixed in a 10% neutral buffered formalin solution, embedded in paraffin wax in the automatic tissue processor, (Leica TP 1020,Gemany) then, sliced with a microtome machine, mounted on microscope slides and stained according to the standard hematoxylin-eosin procedure, (Avwioro, 2011).

Histopathological evaluation of the stained slides was done according to Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN) Histological grading system and the grades scored for each rat. Mean liver weight in grams was presented as a percentage of total body weight.

3.3 Statistical methods

All Group data differences were analyzed using one way ANOVA by Graph-pad prism Version 6.0 for Windows (GraphPad Software, La Jolla, California, USA) statistical software. Data was then expressed as mean (SD)(Jaykaran, 2010). The significance level was considered at $P<0.05$ and the differences between the groups was determined using Mann Whitney post hoc test.

CHAPTER 4.0 RESULTS

4.1 Effects of the diet on mean body weight.

Figure 12 below compares the mean body weights in grams among the four treatment groups in the present study after 60 days of the experimental diets, (n = 6). The differences were statistically significant ; [186.5 ± 46.5 g HFD vs. 56.2 ± 36.8 g RHFD vs. 40.7 ±32.2 g RHFD+T.O vs. 152.4 ± 7.5 g ND (negative control): $P<0.05$]. Post-hoc analysis indicated the greatest differences between the HFD and RHFD+T.O groups (p = 0.0087).

The mean body weights per study group after 60 days.

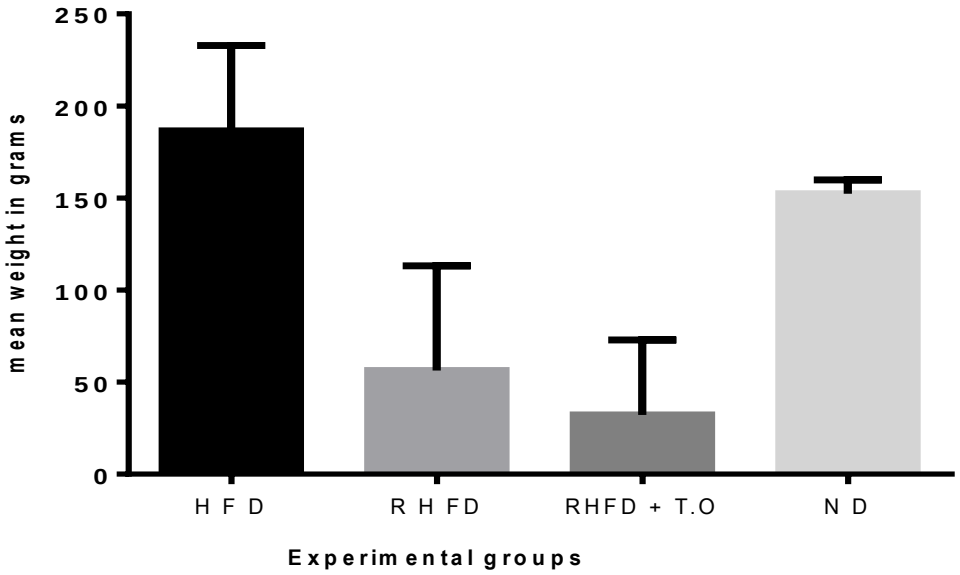


Figure 12: comparison of the mean body weightafter 60 days in grams for the four treatment groups under study. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.2 Fasting blood sugar.

Figure 13 below shows fasting blood sugar levels of experimental animals in the present study. The differences were statistically significant among the four groups. (n = 6).[3.4 ± 1.1mmol/L HFD vs. 3.1 ± 0.6 mmol/L RHFD vs. 2.8 ± 0.3 mmol/L RHFD+T.O vs. 2.0 ± 0.4mmol/L ND: $P<0.05$]. Post-hoc analysis indicated the greatest difference between the HFD and ND groups (p = 0.0087).

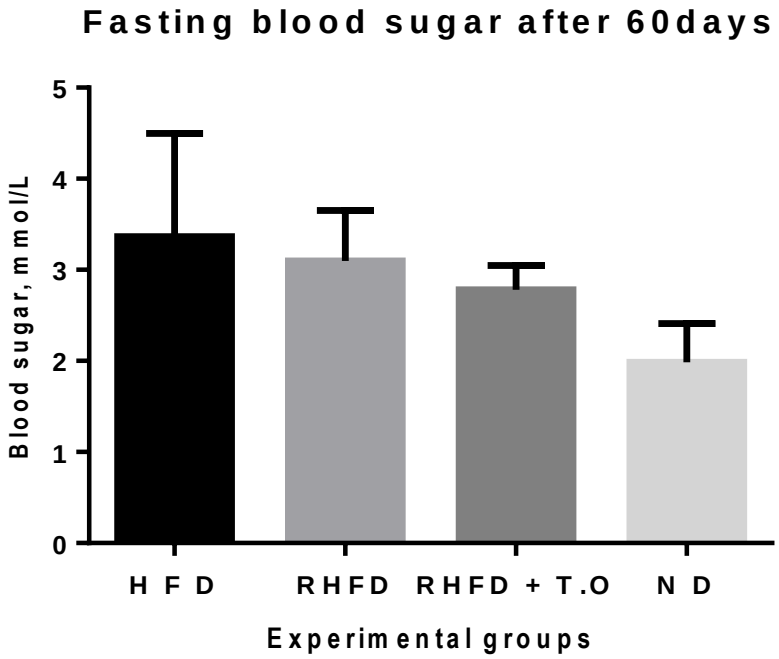


Figure 13: Fasting blood sugar levels in the experimental animals' under the currentstudy among the four groups.Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.3 Insulin tolerance test

Figure 14 below gives the mean measurement of blood plasma glucose per study group as an area under curve for the insulin tolerance test from T0 to T120 for each study. There were significant differences between the groups in the Area Under Curve (AUC) values [301.3 ± 4.8 mmol/l HFD vs. 302.8 ± 8.9 mmol/l RHFD vs. 289.5 ± 10.2 mmol/l RHFD + T.O vs. 245.0 ±

18.7 mmol/l ND: $p<0.05$]. The tukeys post hoc test indicated the greatest difference were between RHFD and ND groups followed by HFD and ND groups.

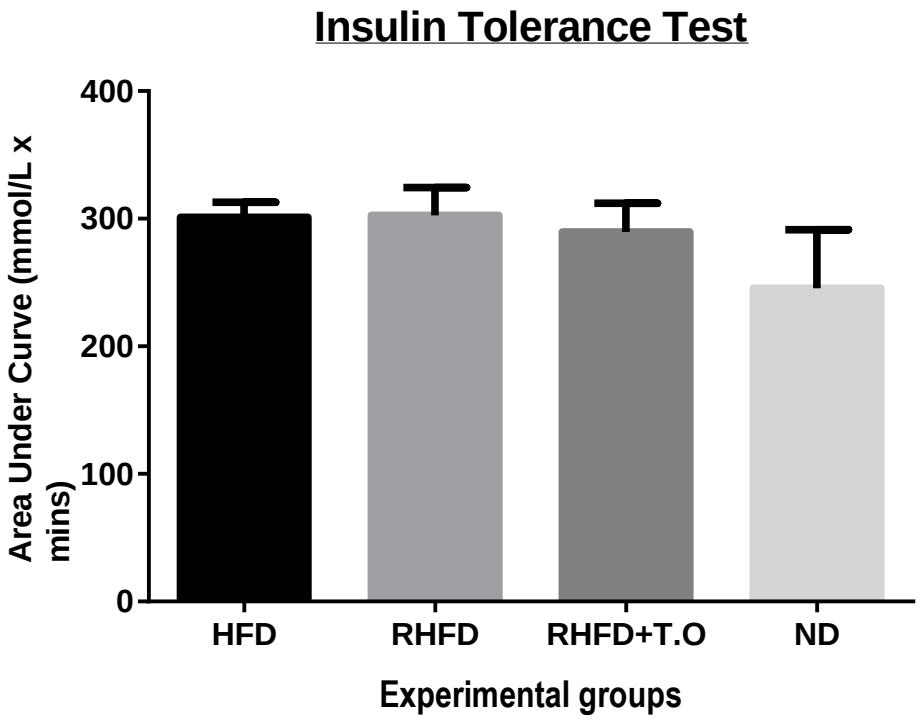


Figure14: Bar graphs showing the glucose response during Insulin Tolerance Test among the four study groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.4 Liver enzyme levels

4.4.1 Serum Aspartate transaminases (AST) levels.

Figure 15below depicts serum AST levels among the study animals. There were no statistically significant differences observed in AST levels among the four groups [157.3 ± 47.6 I.U, HFD vs. 164.2 ± 34.0 I.U, RHFD vs. 219.0 ± 74.5 I.U, RHFD + T.O vs. 156 ± 19.9 I.U ND: $p \geq 0.05$].

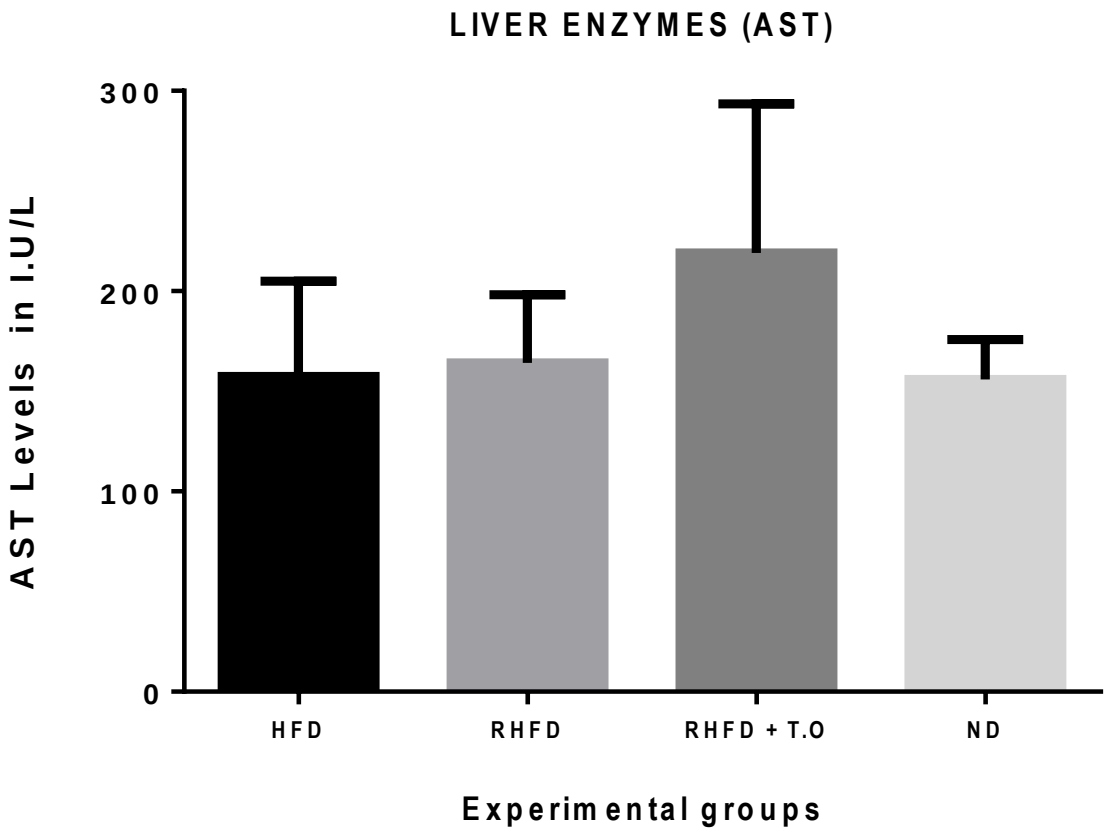


Figure 15: comparison of serum AST in I.U/l between the four groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.4.2 Serum Alanine transaminases (ALT) levels.

Figure 16 indicates the serum ALT levels among the four animal study groups. There were statistically significant differences in serum ALT concentrations among the different four groups [129.5 ± 9.4 IU HFD vs. 166.5 ± 70.0 IU RHFD vs. 169.0 ± 9.9 IU RHFD + T.O vs. 142.8 ± 13.5 IU ND: $P<0.05$]. The post hoc test indicated the greatest differences between the RHFD + T.O and HFD groups (p = 0.0043).

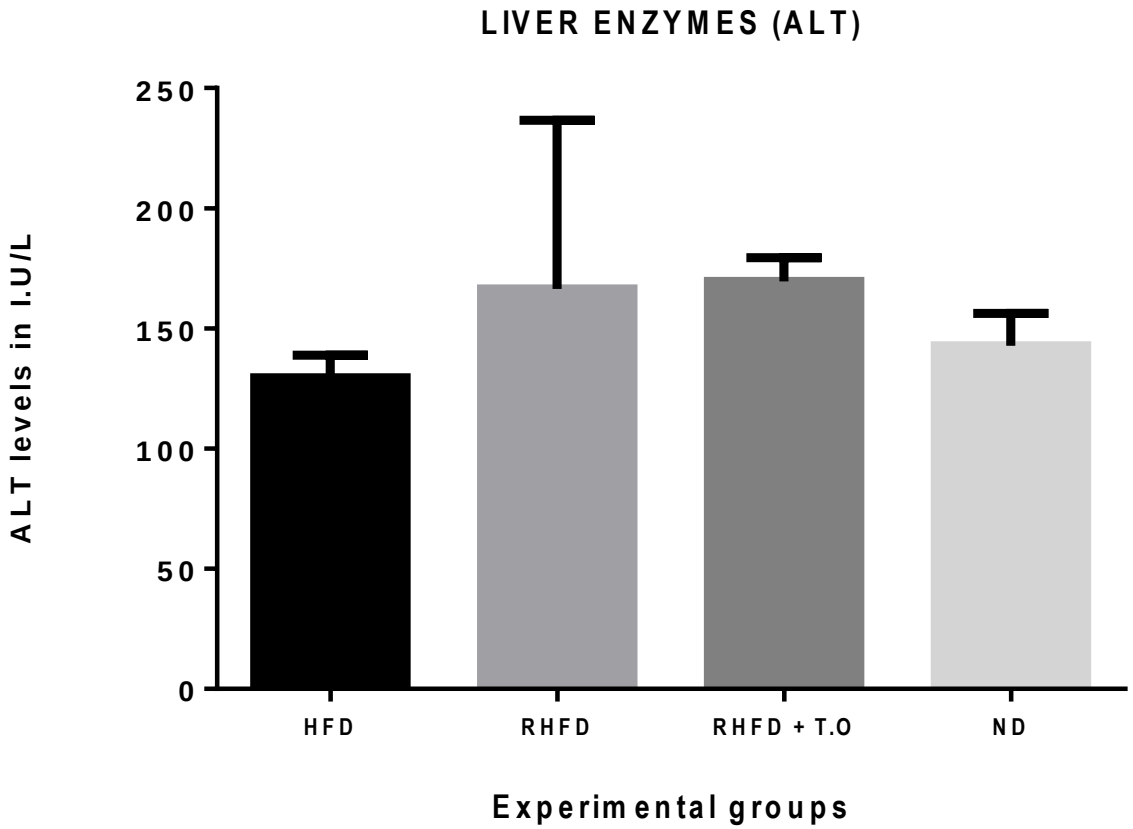


Figure 16: Comparisons of serum ALT levels between the four groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.5 lipid profile

4.5.1 Serum total cholesterol

Figure 17 depicts the total cholesterol among the four animal study groups. There were statistically significant differences in the total serum cholesterol levels among the four groups. [1.95 ± 0.1 mmol/L HFD vs. 1.62 ± 0.3 mmol/L RHFD vs. 1.93 ± 0.1 mmol/L RHFD + T.O vs. 1.40 ± 0.1 mmol/L ND: $P<0.05$]. The post hoc test indicated the greatest differences between the RHFD+T.O and ND groups (p = 0.0022.

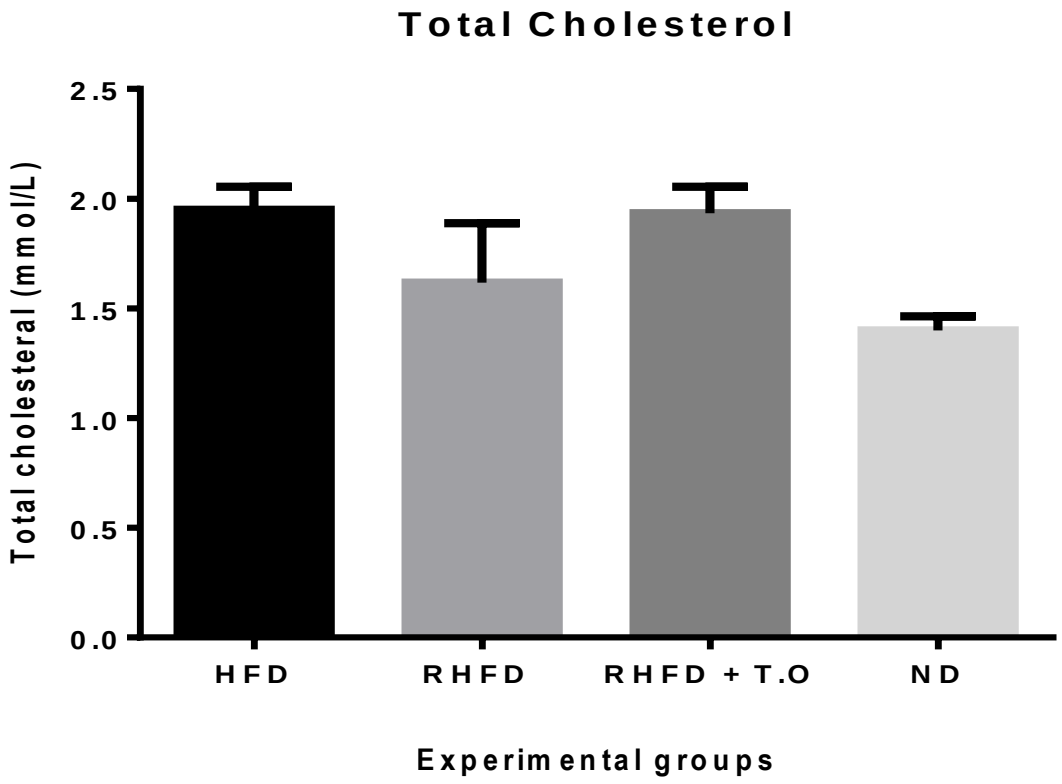


Figure 17. Comparisons of the serum total cholesterol between the four groups.Abbreviations:HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.5.2 Serum High density lipoproteins (HDL) levels

Figure 18 depicts the high density lipoproteins levels among the four study groups. There were statistically significant differences in the HDL serum levels between the groups [1.233 ± 0.24 mmol /L. HFD vs. 1.383 ± 0.194 mmol /L. RHFD vs. 0.900 ± 0.089 mmol /L. RHFD+T.O vs. 1.083 ± 0.0753 mmol /L ND.: $P<0.05$]. The Post-hoc analysis indicated that the greatest difference were between the RHFD + T. O and RHFD groups (p = 0.0130).

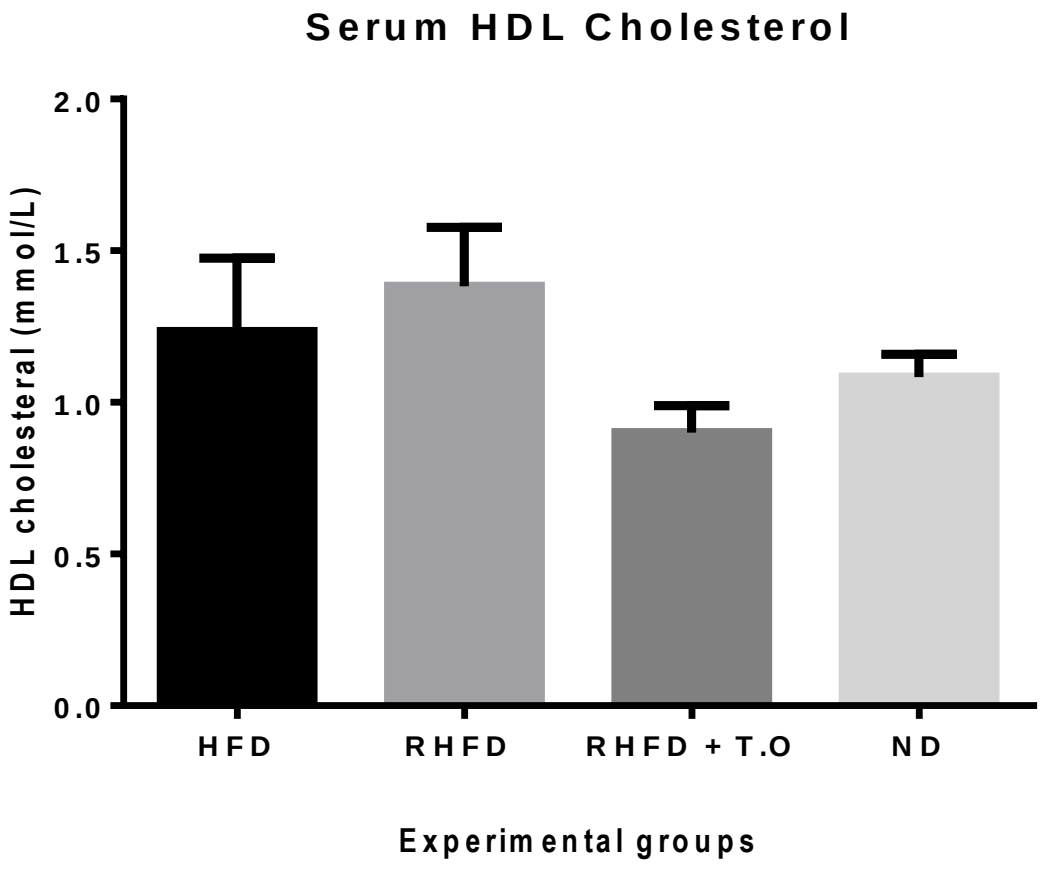


Figure18: Comparisons of serum HDL cholesterol between the four groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.5.3 Serum Triglycerides (TRIG) levels

Figure 19 shows the mean serum triglycerides levels among the four treatment groups in the present study. The differences were statistically significant between the groups [1.4 ± 0.52 mmol /L. HFD vs. 0.533 ± 0.16 mmol /L. RHFD vs. 0.567 ± 0.16 mmol /L. RHFD+T.O vs. 0.617 ± 0.098 mmol /L ND: $P<0.05$]. The post-hoc analysis showed that the greatest difference were between RHFD + T.O and HFD groups (p = 0.00430.

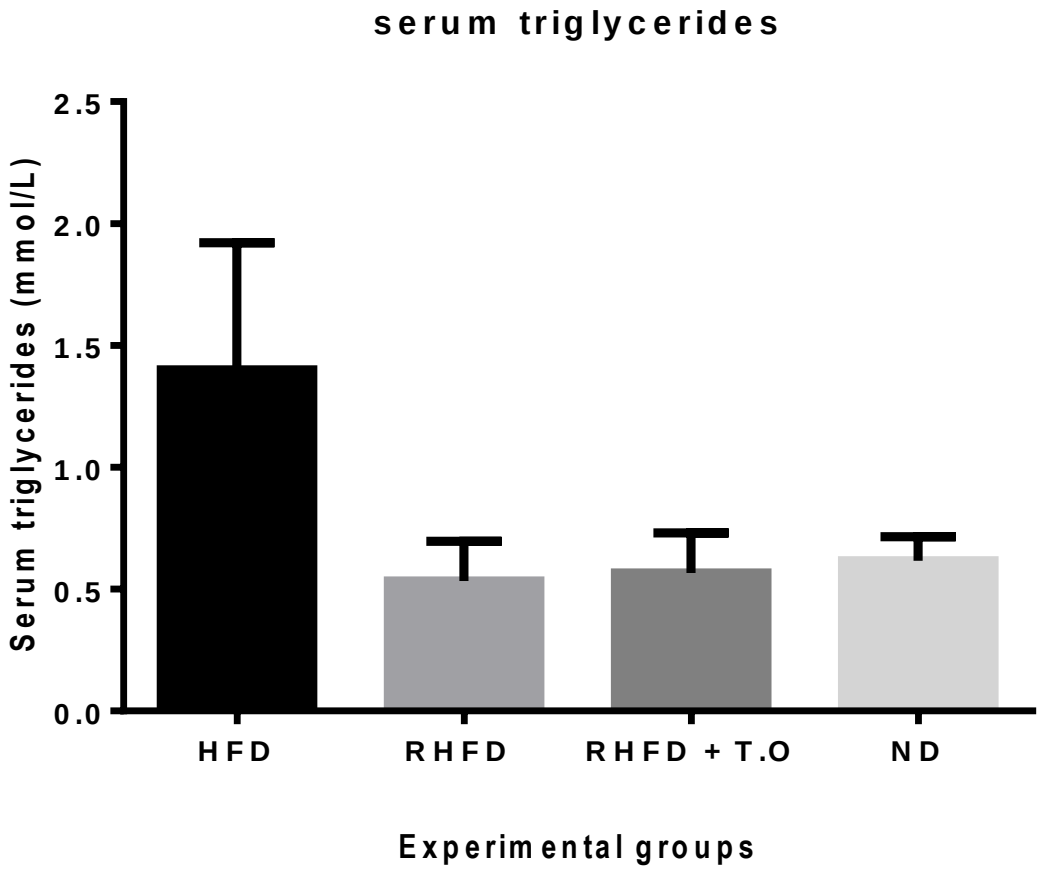


Figure19: Comparisons of serum triglycerides between the four groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.5.4 Serum low density lipoproteins (LDL) levels.

Figure 20 depicts the mean serum low density lipoproteins levels among the four treatment groups in the present study. The differences were statistically significantly between the four groups [1.12 ± 0..50 mmol /L.HFD vs. 1.45 ± 0.12 mmol /L. RHFD vs. 1.72 ± 0.17 mmol /L. RHFD+T.O vs. 1.083 ± 0.098 mmol /L. ND: $P<0.05$]. Post-hoc analysis showed the greatest difference were between the RHFD + T.O and ND (p = 0.0022).

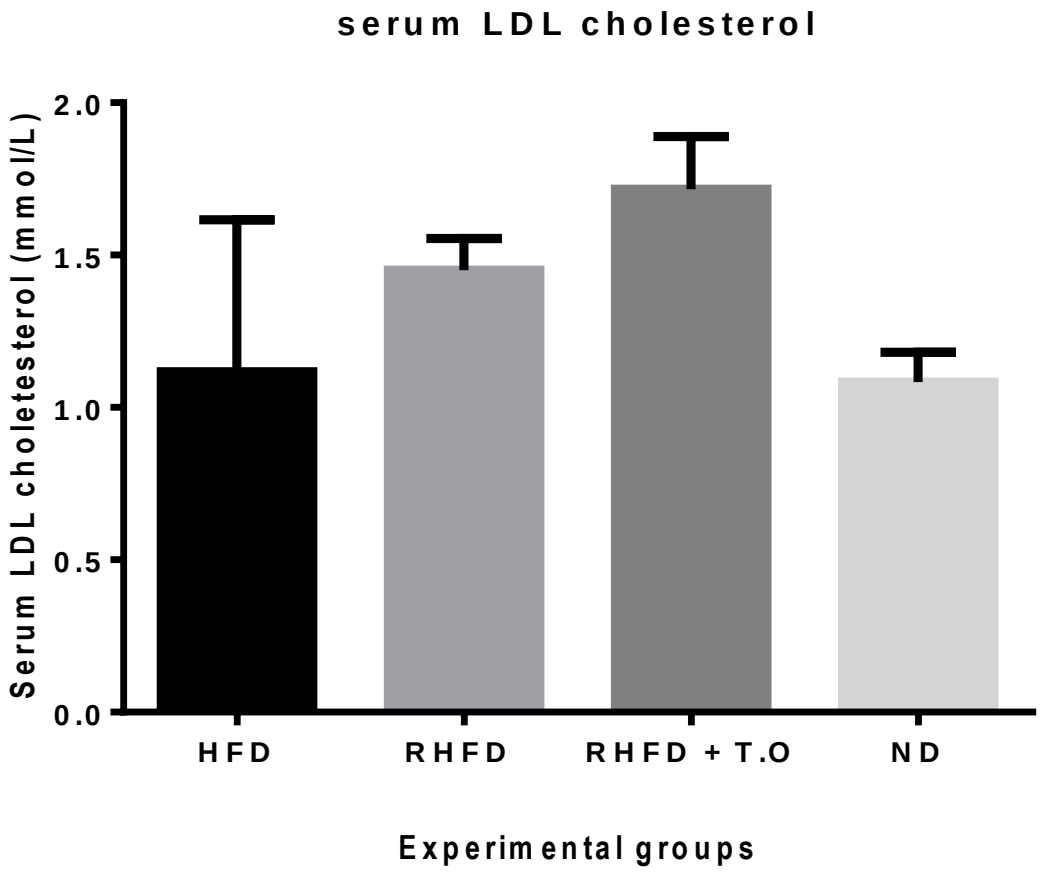


Figure 20: Comparisons of serum LDL cholesterol between the four groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.6 Percentages of liver weights to body weights.

Figure 21 indicates the mean percentages of the liver weights to body weights among the four experimental study groups. The differences were statistically significant between the four groups [3.47 ± 0.29 HFD vs. 3.733 ± 0.51 RHFD vs. 5.8 ± 0.51 RHFD+T.O vs. 4.00 ± 0.28 ND: $P<0.05$]. The Post-hoc analysis showed the greatest differences were between the RHFD + T.O and HFD groups (p = 0.0043).

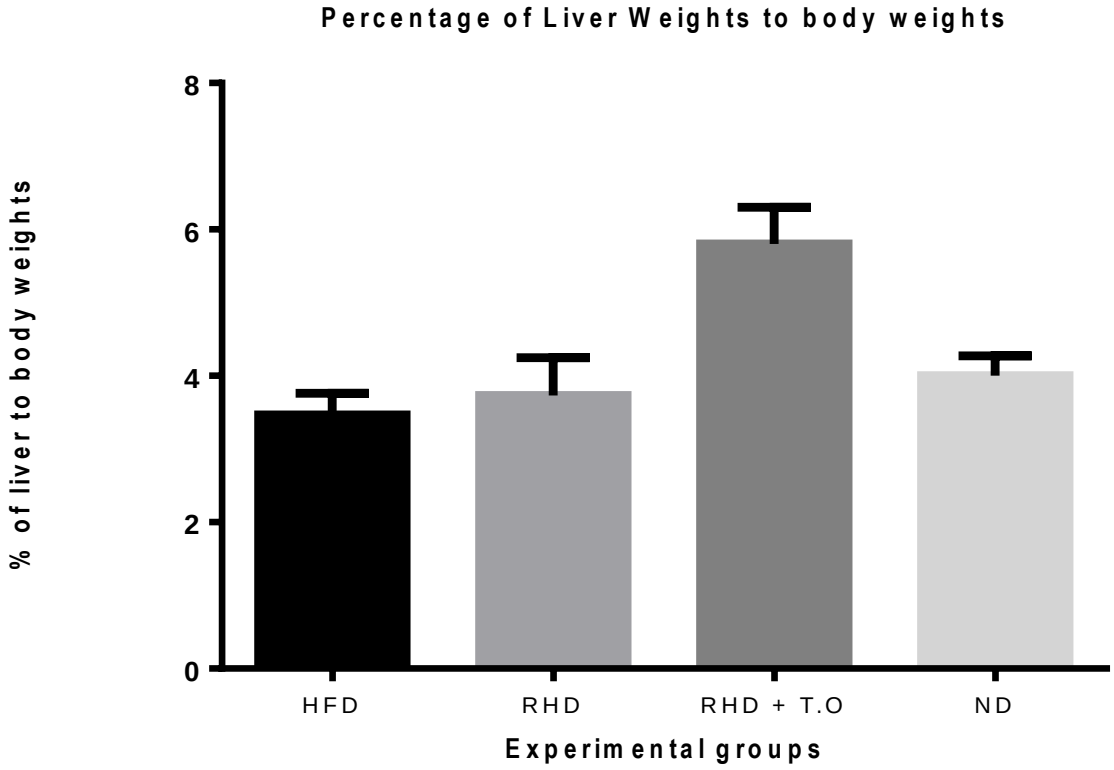


Figure 21: Comparison of liver weight/ body weight ratio between the four groups.

Abbreviations:HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.7 Osmotic fragility of erythrocytes

Figure 22 depicts the mean osmotic fragility of erythrocytes among the four study groups. The differences were statistically significant at 0.51% Nacl between the groups [44.3 ± 23.3 % HFD vs. 62.6 ± 21.1 % RHFD vs. 83.6 ± 3.7 % RHFD+T.O vs. 44.7 ± 24.4 % ND: $P<0.05$]. The post hoc test indicated that the greatest difference were between RHFD + T.O and ND groups (p = 0.0043). However, there were no statistically significant differences observed in osmotic fragility of erythrocytes among the four groups at Nacl concentrations of 0.34% (p value = 0.83), 0.38% (p value = 0.41), 0.42% (p value = 0.43), and at 0.47% (p value = 0.075). The highest percentage of haemolysis was observed in the RHFD + T.O group (108.8 ± 16.7 % at 0.34%), RHFD group (101.6 ±5.32 % at 0.38%), RHFD group (101.1±3.4 % at 0.42%), and RHFD + T.O group (96.7±5.7 at 0.47%).

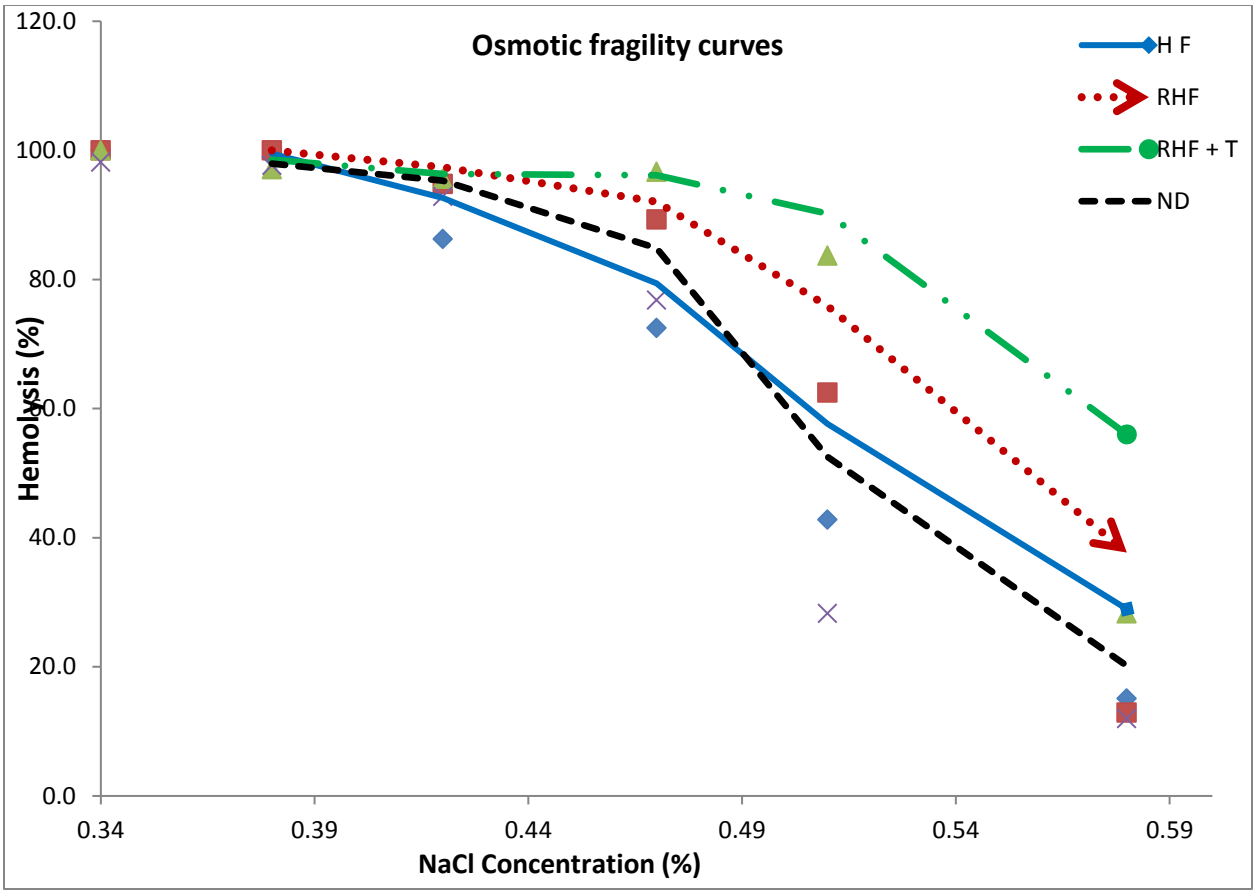
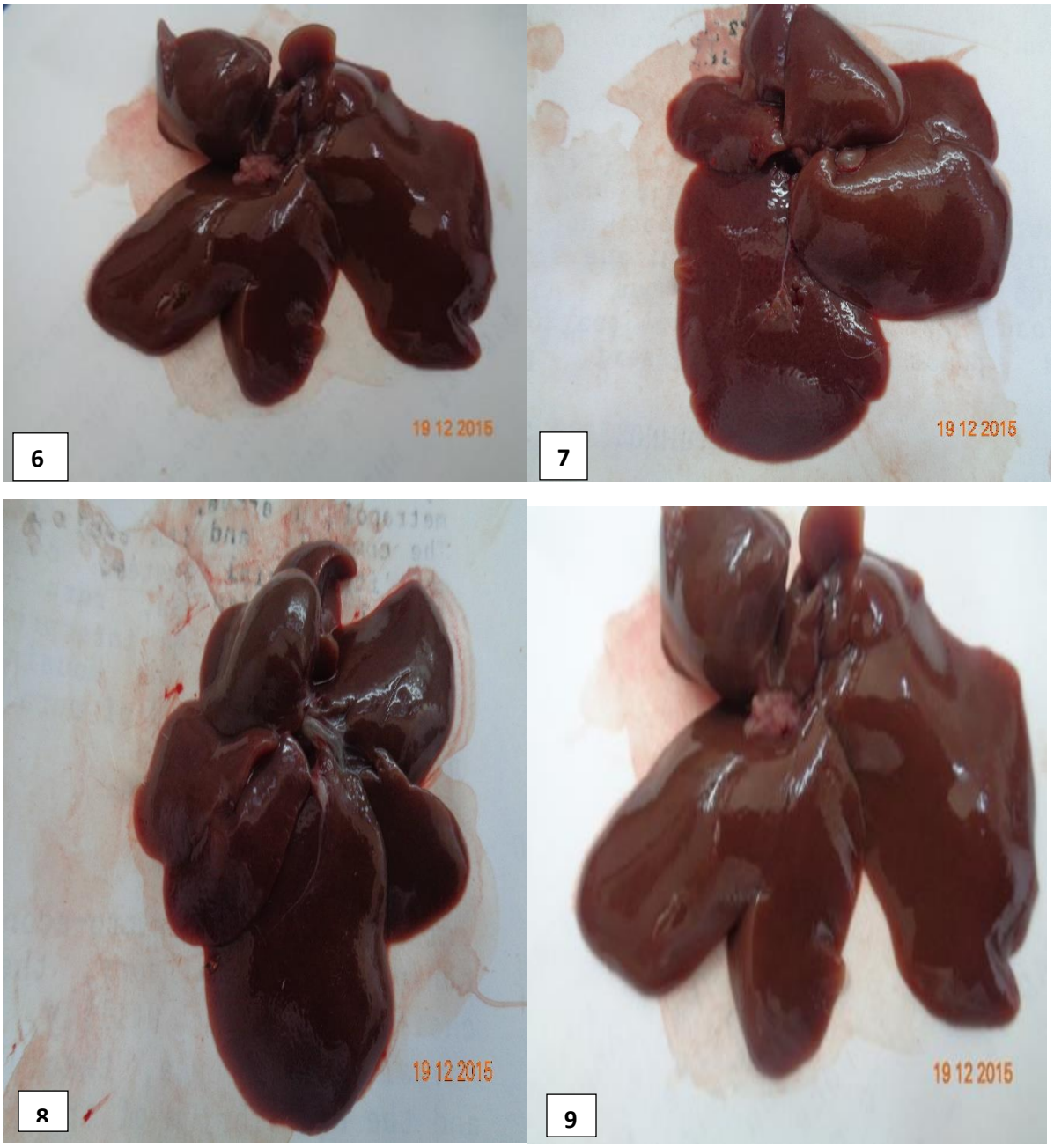


Figure 22: comparisons of the osmotic fragility curvesof erythrocytes between the four groups. Abbreviations: HFD, High fat diet; RHFD, Reused high fat diet; RHFD+T.O, Reused high fat with transform oil; ND, Normal diet (control).

4.8 Histopathological evaluation of the stained liver cells

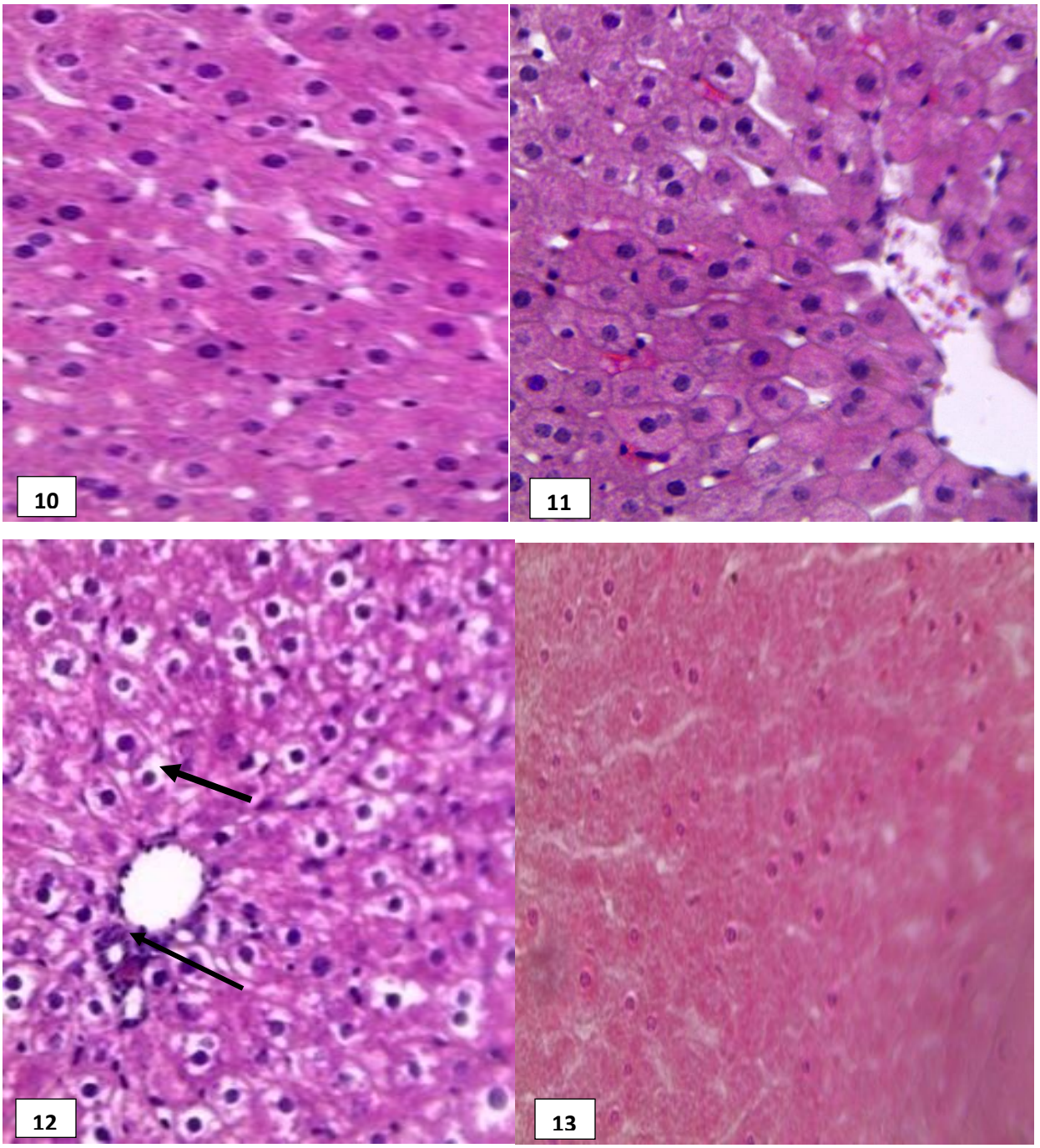
4.8.1 Physical appearances of the whole liver samples of the study

Plates 6 to 9 are the photographs of the different rat livers after sacrificing and excision.

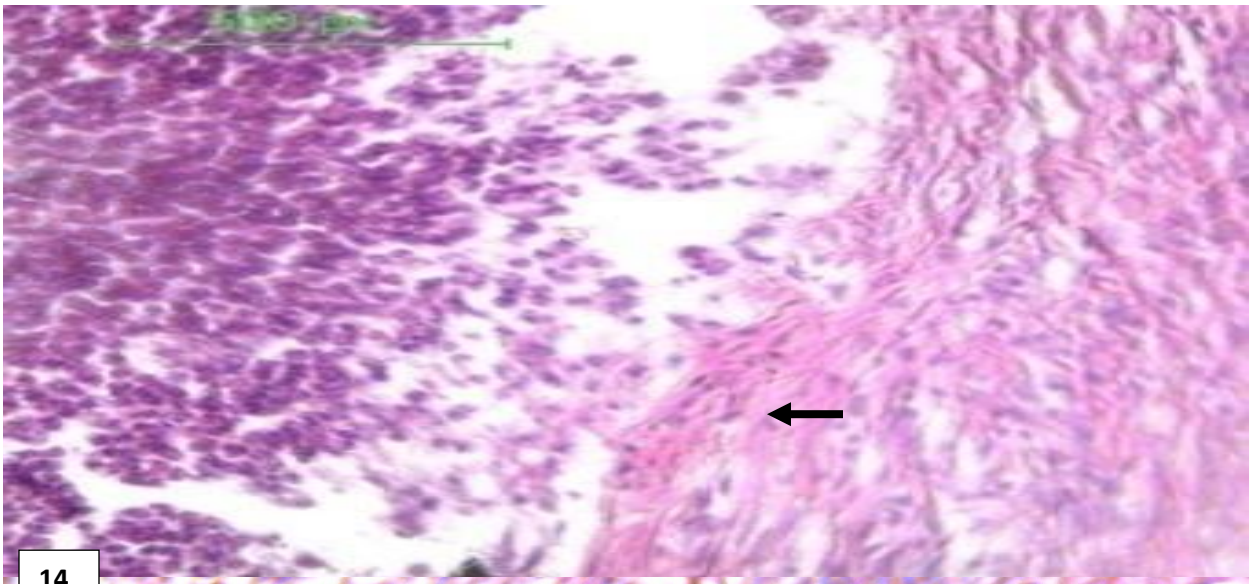


Whole liver samplesshowing different degrees of paleness. Plate 6: Normal diet groupI, showing a smooth dark brown well-formed liver. Plate 7: High fat group, Plate 8: Reused high fat group, Plate 9: Reused high fat and transformer oil group showing a pale brown smooth liver sample.

4.8.2 H & E Histological appearances of the study liver samples



Liver histology of the samples, H&E X4. Plate 10: Normal diet group I depicting centrally placed nucleus, Plate 11: Neat high fat group II, Plate 12: Reused high fat diet group III depicting features of NASH (Foci of inflammation & hepatocellular ballooning). Plate 13: Liver histology of Reused high fat and transformer oil group IV showing degenerated cell outlines.



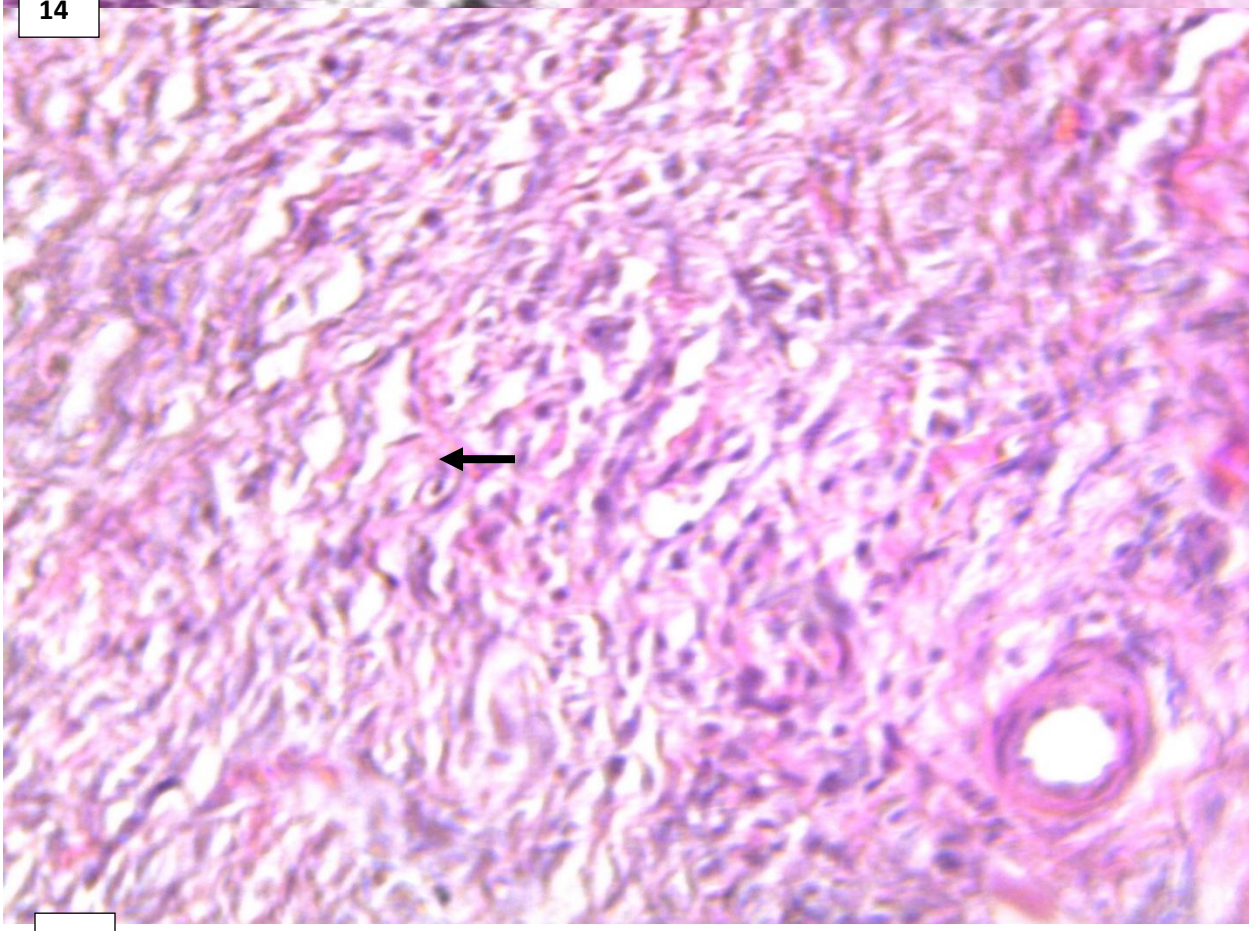


Plate 14 and 15:Further slides of Liver histology of group III study animals, reused high fat diet (RHFD) showing microscopic cirrhosis surrounded by fibrous tissue, H & E X40**.**

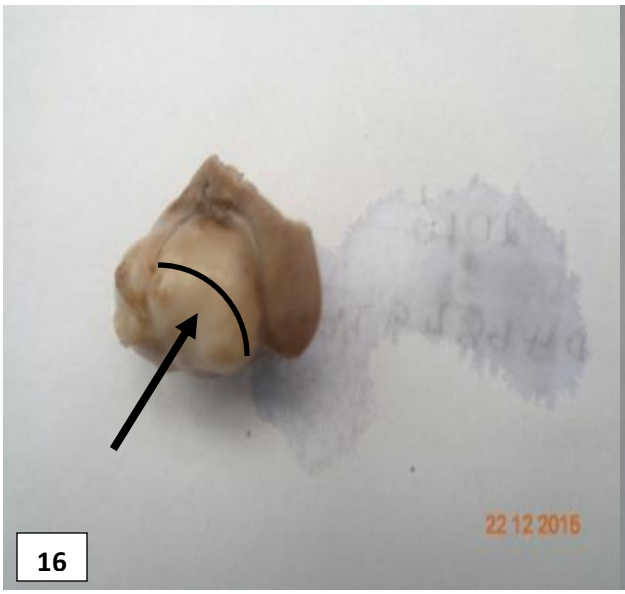


Plate 16: Macro-nodular cirrhosis of reused high fat diet (RHFD) group III study animals

Plate 17: Micro nodular cirrhosis of reused high fat diet (RHFD) group III study animals.

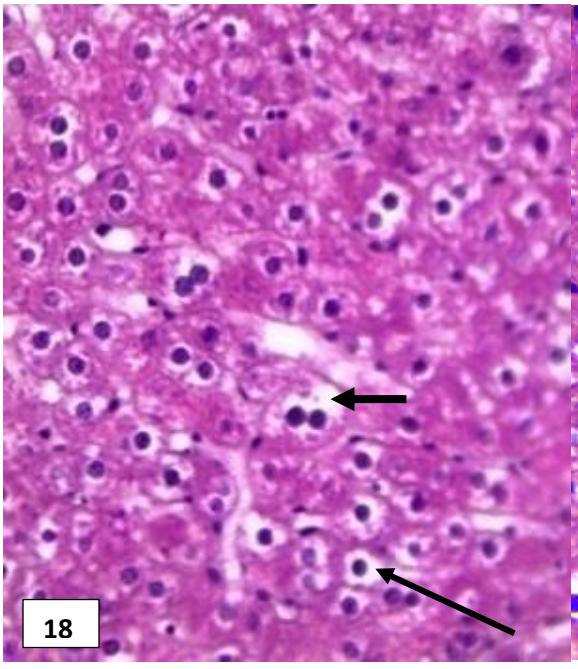


Plate 18: Liver histology of grp III study animals depicting features of NASH; ballooning. H&E X40)

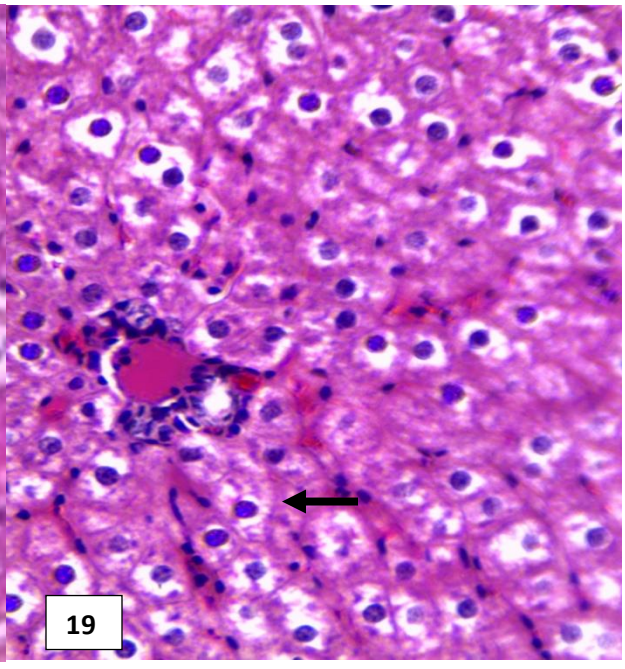


Plate 19: Liver histology of grp III study Animals showing more NASH features (micro vesicular steatosis, H&E X40.

Table 3: below shows the Histopathological evaluation of stained liver cells from the current study using the Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN) Histological grading system

HISTOLOGICAL FEATURE	Group I- (ND)	Group II- (HFD)	Group III- (HFD)	Group IV- (RHFD + T.O)
Macro-vesicular steatosis	**–**	**–**	**–**	**–**
Micro-vesicular steatosis	**–**	**–**	**2**	**–**
Hepatocellular ballooning	**–**	**–**	**2**	**–**
Lobular inflammation	**–**	**2**	**3**	**–**
Portal inflammation	**–**	**2**	**3**	**2**
Fibrosis	**–**	**–**	**3**	**–**

Table 3. Histopathological evaluation of stained liver cells under study. Steatosis 2(33-66%), Hepatocellular ballooning 2 (many cells), Lobular/Portal inflammation 3(≥4 foci/200X), Fibrosis 3(Bridging Cirrhosis).

CHAPTER 5.0: DISCUSSION

5.1 Changes in body weight

The HFD group results showed the greatest weight gain of 186.5 ± 46.5 g compared to other groups; a finding that reflected findings in other previous studies (Woods et al. 2003).

The RHFD + T.O showed the least weight gain of 40.7g ±32.2 g, an expected finding since transformer oil is a toxic mineral oil known to contain heavy metals and polychlorinated biphenyl (PCBs)(Petrosino et al., 2018). The heavy metals and PCBs have been shown to negatively affect the body weights and cause general toxicity in experimental rats(Bernhoft et al., 1994). The fact that the RHFD group showed less weight gain than the ND group but more weight gain than the RHFD + T.O reflects other previous study findings that have shown the negative effects of oxidized cooking fats on experimental rats (Eder 1999) . The RHFD + T.O experimental group that showed the least body weight gain of 40.7 ±32.2 g was probably due to the combined negative effects of the recycled fats and the toxic transformer oil.

Recycling cooking fats leads to oxidization; several studies have established that intake of oxidized oils in laboratory animals generally leads to a markedly lower growth rate than the control groups(Eder, 1999). The nutritional status of the diet may also have been compromised by the presence of toxic transformer oil. The molecular mechanisms underlying the reduced body weight are not yet elucidated but can be attributed to both liver and general toxicity.

5.2 Percentage of liver weight to body weight

The RHFD + T.O showed the highest liver weight gain of 5.8 ± 0.51g, probably due to the accumulation of toxic compounds of the transformer oil that are known to increase liver weights in laboratory rats(Wilber & Gilchrist, 1965). It has been previously established that at a normal test dose of 0.1percent Butylatedhydroxytoluene (BHT) produces a significant increase in the weight of the liver in relation to the body weight and the mean absolute weight(Brown et al., 1959).

It has also been shown in other studies that an increase in smooth endoplasmic reticulum (SER) surface area in the liver cells which are specialized in detoxification of toxic compounds produced by metabolic processes(Villeneuve, 2004) can result in liver weight gain.

5.3 Osmotic fragility

The RHFD + T.O experimental group had the highest osmotic fragility of 108.8 ± 16.7 % at 0.34%) as compared to the ND group. The absorption of exogenous organic solvents, metals and other possible toxins in the transformer oil could have been the source of exogenous oxidant stress that influenced lipid peroxidation of the erythrocytes(Clemens & Waller, 1987) resulting in the increased fragility. The uptake of these oxidative compounds could have resulted in the formation of Heinz-body anemia (aggregated and denatured Hb). Further oxidation may have involved Sulf-hydryl (-SH) groups in the RBCs membrane resulting in its damage and membrane loss (Slappendel 1998). Though so far established in cats alone, red blood cell hemolysis is also known to occur following hypo-phosphatemia in cats withhepatic lipidosis or diabetes mellitus (Slappendel, 1998).

5.4 Fasting blood glucose

The fasting blood sugar levels in all the high fat diets (3.4 ± 1.1mmol/L (HFD), 3.1 ± 0.6 mmol/L (RHFD), and 2.8 ± 0.3 mmol/L (RHFD+T.O), indicated slightly impaired fasting glucose or pre-diabetes compared to the ND (2.0 ± 0.4mmol/L) experimental group. This may have been due to the presence of the reused high-fats in the diet which are known to predispose experimental animals to a higher fasting blood glucose, as observed in the HFD group which had the highest levels and the RHFD group(Huang et al., 2004).

5.5 Lipid profile

The RHFD + T.O experimental group showed the highest LDL cholesterol, lowest HDL cholesterol, lower than ND Serum Triglycerides and the highest Total cholesterol. This indicated atherogenic dyslipidemia-AD which has been established to consist of a cluster of lipoprotein abnormalities including elevatedapolipoprotein B (apoB) and serum triglyceride,a reduced level of HDL cholesterol (HDL-C) and an increased small LDL particles. (Grundy et al., 2005).

Hepatic lipid homeostasis involves highly regulated and extremely complex interrelated signaling pathways in the liver, normally, lipid input is equal to hepatic lipid output, however perturbation of either the input or output pathways result in dysregulation of lipid metabolism(Zhou & Liu, 2014a). The transformer oil and the recycled cooking fat were the likely extrinsic source of oxidants that led to induction of Endoplasmic reticulum stress (ER

stress). Prolonged ER stress is known to induce dysregulation of hepatic metabolism(Zhou & Liu, 2014b).

5.6 Liver enzymes

There were markedly elevated levels of liver enzymes ALT in the RHFD + T.O (169.0 ± 9.9 IU) experimental group as compared to the ND (142.8 ± 13.5 IU) group. This was followed by high levels in the RHFD (166.5 ± 70.0 IU) experimental group, indicating a substantial injury in both groups. Elevated ALT is a maker of liver damage(Vutukuru et al., 2007) and it's more specific in hepatocellular injury. Elevated AST is significant in the later stages of fibrosis and non-specific as it is released on damage of other organs (Wang et al., 2012). Liver ALT is solely localized in the cellular cytoplasm whereas AST is both cytosolic and mitochondrial (Rej 1989), this indicates that the hepatic injury resulted in the destruction of the hepatocytes leading to elevated ALT levels.

Even though the mechanisms involved in liver damage may vary, generally many chemicals damage the mitochondria causing release of excess oxidants that injure hepatic cells. The injury may also cause activation of some enzymes in the cytochrome P-450 system that may lead to oxidative stress(Jaeschke et al., 2002). Injured hepatocytes and bile ducts cells lead to further accumulation of bile acids inside the liver promoting further liver damage(T et al., 1998).

5.7Histopathological examination

In this study, the Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN) histological scoring system was used. It's a grading system based on three parameters of

steatosis, ballooning and lobular inflammation. Other grading systems are modified Kleiner, the international association for study of the liver, Batts-Ludwig and Metavir.

The gross examination of the liver sample from the Normal diet group I showed a normal dark brown, smooth liver (plate 5). On histological examination (plate 9), the hepatocytes showed polygonal epithelial cell with one or more centrally located round nucleus. There were no morphological changes: normal lobules, lack of pigmentation, absence of congestion, steatosis nor necrosis indicating no hepatic injury.

Gross examination of the RHFD+T.O (group IV) liver sample (plate 8) showed a smooth pale brown liver but with no scaring.However histology of the same (plate 12) sample revealed morphologically degenerated liver cells with no clear cell boundaries. Two of the characteristic responses of the liver cells to exogenous hepatotoxic agents are the development of necrosis (cell death by factors external to the cell) and the accumulation of fats(Rees, 2008).

There was no pigmentation, steatosisnor necrosis in the hepatocytes in these experimental group indicating no fatty changes in the livers. The liver cell deaths may have been solely causedby chemical driven hepatotoxicity effects of the array of minerals and chemical substances present in the transformer oil. This is supported by the presence of portal inflammations (Brunt et al., 2009) and the elevated levels of the aminotransferases AST and ALT in the blood in this experimental group.

Studies indicate that liver injury is a constant and prominent finding in animals exposed to PCBs, particularly rats and monkey(ATSDR's Toxicological Profiles, 2002). The liver effects are similar in nature and characteristically include initially fat deposition then fibrosis, and hepatic microsomal enzyme induction (an external molecule initiating expression of an enzyme), increased serum levels of liver related enzymes indicative of hepatocellular damages, liver enlargement, and Necrosis (*ATSDR's Toxicological Profiles*, 2002). Other liver-related effects of PCBs include altered lipid and porphyrin metabolism. Increased serum levels of total lipids, triglycerides, and/or cholesterol are characteristic effects of short- and long-term oral exposures to PCBs that are well-documented in rats and monkeys ((*ATSDR's Toxicological Profiles*, 2002).

Gross observation of the RHFD study liver sample (plate 15 and plate 16) showed macro-nodular and micro-nodular liver cirrhosis respectively in two of the experimental rats in the RHFD group. On histological examination (plate 11,17 and 18) the stained study liver cells in this group exhibited micro-vesicular steatosis-clear appearances of lipid vacuoles in H & E (fatty change) indicating an abnormal accumulation of lipids.

Lipids accumulate when lipoproteins transport is disrupted and/or when fatty acids accumulate due to hepatotoxin accumulation.(Bangru, S., &Kalsotra, A. (2020).This strongly reflected an impairment of the normal process in the synthesis and elimination of triglycerides within the liver cells. Hepatotoxinsare known to interfere with mitochondrial and microsomal functions in hepatocytes. Though the biological mechanism still poorly understood, the results were indicative of Non-alcoholic Steatohepatitis-inflammation of the live concurrent with fat

accumulation (NASH). In NASH, the Pathophysiology involves fat accumulation, inflammation, and fibrosis(Ishii, H., &Okuyama, K. (1995).

It was further observed that the RHFD exhibited Hepatocellular ballooning, lobular and portal inflammation. Hepatocellular ballooning is a key indicator in NASH(Caldwell et al., 2010).

Liver cell polyploidy was also observed in some of the sample in RHFD (plate17) study group.Poly-ploidization of liver cells is in known to occur physiologically in adult animals. It has also been hypothesized to occur due to high level spontaneous ploidy chromosome aberrations, (cell not acting properly). The Poly-ploidization of liver cells thus ensures protection against these delirious consequences of the aberrant mitosis(Uryvaeva, 1981). The Reused high fat diet in group 2 may have been the initiator of aberrations that were not observed in other groups.

Peroxidized fatty acids, mostly cyclic fatty acid monomers are generated by heating of standard cooking oils and trigger Hepatic inflammation(Frankel et al., 1984). They can initiate free radical generation and oxidant stress that promote membrane injury(Udilova et al., 2003). It also has been shown that biopsied livers of animals treated with heated corn oil expresses higher levels of inflammation related genes that includes interleukin 1b, tumor necrosis alpha and cyclo-oxygenase 2. This has been linked to CD68 positive macrophage infiltration(Pais et al., 2013).

5.8Insulin Tolerance Test

Insulin Tolerance Test a measure of insulin sensitivity that characterizes Type 2 Diabetes. Statistically significant results were observed in RHFD and HFD experimental groups indicating Type 2 Diabetes that may have been caused by impaired insulin resistance and an increase in

insulin resistance. Since Insulin is the primary regulator of carbohydrate, fat, and protein metabolism, an oversupply of exogenous highfat may have resulted in high hepatic triglyceride.

In Type 2 Diabetes mellitus, in addition to other abnormalities, there's increased hepatic triglyceride production and storage that's driven by substrate availability(Vatner et al., 2015). High level of hepatic triglycerides was observed in all the high fat diets except the normal diet group. Furth more, hepatic triglyceride pile up leads to steatosis and an increased ratio of liver to body weight as demonstrated in this study.

CHAPTER 6.0 CONCLUSION.

The analysis of this study suggest that reused high fat adulterated with transformer oil is not only highly toxic to the liver but can accelerate development of metabolic syndrome in male Sprague Dawley rats.Absorption of transformer oils by biological systems can significantly increase the level of oxidative stress which when accumulated fat cells becomes an important physiological mechanism of obesity-associated metabolic syndrome. The implications of consumption of this fats and oil is not limited to metabolic syndrome, but may include Nonalcoholic fatty liver disease, liver cirrhosis, poor health and lipid disorders.

The fatty changes in the livers under study reflected an impairment of the normal synthesis and elimination of the lipids from the affected hepatocytes, further implications of these changes are failure of the liver to synthesis and secrete major plasma proteins.

Rats fed on a high neat cooking fats gain weights.

Elevated liver enzymeAlanine aminotransferase (ALT) was highly indicative of liver damage to cellscaused by the toxic mineral oil.

High fat diet resulted in high triglyceride in blood and high blood glucose.

A comprehensive evaluation of all the body organs, metabolic and growth hormones would have been very informative in the understanding of the general pathogenesis and determination of the effects of the reused cooking fat and transformer oil on the overall health of the experimental animals, however, consumption of both reused cooking oil and transformer oil is a health risk. Additionally initial basic measurements of glucose, lipids and weights and their subsequent

weekly monitoring of the same would have provided better understanding of the physiological mechanism underlying the changes.

6.1 Recommendations

MetS has surfaced as one of the major public health challenges worldwide(Daniels et al., 2005).It's estimated that 20 to 25 percent of the world's adult population possess the risk factors that is MetS(Alkerwi et al., 2012) and other related cardiovascular risk factors such ashypertension, visceral obesity, hyperglycemia anddyslipidemia.(Luecken& Gallo, 2008). Economically emerging nations are rapidly undergoing nutrition transition coinciding with related cardiovascular risk factors. An increased intake of both saturated and adulterated saturated fats may pose a major risk in these developing countries

In view of the findings in this present study, I would suggest the following,

1.The Kenya Power Lighting Company adopts use of Ester-based dielectric oilse.g. MIDEL eN 1204(rapeseed/canola) and MIDEL eN 1215 (soya bean) in their transformer machines to discourage siphoning of mineral based transformer oils.

2. A country wide surveyof used cooking fats and fried foods in both informal road side eateries and established fast food restaurants be undertaken to establish the magnitude offood contamination and the related health dangers be out lined and made available to the public.

3. The policy makers should consider emulating other countries inbanning use of cooking fats but instead advocate for cooking oils and other alternatives.

4. Government should adopt strict regulations (similar to those in Europe) regarding minimum acceptable cookingoil quality, publish the regulation and enforce them.

5. Greatresponsibility is to the individual to exercise caution and change the culture of consuming roadside friesshould be advocated.

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