SEXUAL DIMORPHIC RESPONSE OF METABOLIC VARIABLES IN HIGH FAT DIET-INDUCED OBESITY IN WISTAR RATS ON LOW CALORIE DIET

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A Thesis in the Department of Physiology
Submitted to the Faculty of Basic Medical Sciences
In partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

FEBURARY, 2023

CERTIFICATION

I certify that this work titled **'Sexual dimorphic response of metabolic variables in high fat diet-induced obesity in wistar rats on low calorie diet'** was carried out by Ubong Edem, **DAVID** in the Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

To:

God Almighty, through Jesus Christ my Lord, in whom lie hidden all the treasures of wisdom and knowledge, and;

The blessed memory of my loving father; Dr E. E. David, who was so eager to see the completion of this work but passed on five years ago; your life and the uncompromising principles that guided it will forever be in my memory.

ACKNOWLEDGEMENTS

I give God all glory, honour, praise and adoration for taking me this far in my academic pursuit. If not for HIM, I will not be able to achieve anything in life and academically. Thank you, Lord, I will always worship and adore you while I have my breath.

I wish to appreciate my tolerant, amiable and indefatigable supervisor and mentor, Prof. A. A. Fasanmade who has been so accommodating and patient with me even when I am not too proactive in my work. I really appreciate your tutelage towards the triumphant conclusion of this thesis.

Special thanks to the down-to-earth HOD of Physiology Prof. S. B. Olaleye for his enormous support and backing during the period of my program. I am specially grateful for the encouragement and support of all other members of the academic staff especially Prof. A.R.A Alada, Prof. Y. Raji, Prof. E. O. Adewoye, Prof. F. S. Oluwole, Prof. S. A. Onasanwo and other Lecturers in the Department. Finally, I thank the late Mr Okon and all other non-teaching staff of the Department for the great technical support given to me in the course of my study.

Special thanks to Dr Abu, Dr Fada, Dr Tunde all from the Department of Animal Sciences for assisting me in the formulation of my diets. I truly appreciate very Physiology mentors of mine, for enlisting my passion in the knowledge of the standard physiology of the body; I say thank you for being a part of my career life make-up. Special thanks to Dr O. Owoeye (Department of Anatomy), Dr Aina (Veterinary Anatomy), Mr Otegbade and Mr Olaleye (Histopathology, UCH), Mr Adebowale (Laboratory in Ilorin) and Mrs A. O. Korede (Central laboratory, UI), Prof. Temidayo Olutayo Omobowale and Dr. Ademola Adetokunbo Oyagbemi (Veterinary Medicine) for their assistance in different aspects of my research work. I appreciate specially my colleagues in the Department for their great assistance during the course of this research. May God Almighty bless you all.

Also, my appreciation goes to Miss Patience Emmah and Miss Omoefe Loveth Godwin, your love and kindness towards me can never be forgotten.

My special thanks go to my ever-loving mother Mrs Imeh Edem David and my siblings Mr Idongesit Edem David and Mr Emem Edem David for their love and encouragement both morally and spiritually. The cord of love among us will not be broken.

ABSTRACT

Striking differences exist between men and women in lipid kinetics possibly due to sexual dimorphism in metabolism. However, the effect of prolonged intake of High Animal Fat Diets (HAFD), High Plant Fat Diets (HPFD) and Low Calorie Diets (LCD) in both sexes have not been fully investigated. This study was designed to evaluate the influence of sexual dimorphism on metabolic variables following prolonged intake of HPFD and HAFD in normal, and LCD-treated obese Wistar Rats (WR).

Gross energy, fat and fiber content of formulated feeds from proximate composition were HPFD (4.50 Kcal/g, 16.30, 3.60 %), HAFD (5.90 Kcal/g, 31.00, 3.10 %) and LCD (2.66 Kcal/g, 2.27, 20.64%). In experiment one (designed to evaluate sexual dimorphism in high fat diets induced obesity rats), 30 WR were divided into 15 males (mWR) and 15 females (fWR). Each sex group was sub-divided into 3 groups (n=5) and fed with Standard Chow (SC), HPFD and HAFD respectively, for 17 weeks. Serum was obtained from blood, samples of the liver, Small Intestine (SI) and heart were excised. Serum, liver, heart and SI lipid profile [High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Total Cholesterol (TC), triglyceride, Free Fatty Acids (FFA)] were measured by spectrophotometry. Serum luteinizing hormone (LH), Apolipoprotein A and B were analysed using ELISA while SI Saturated, Monounsaturated and Polyunsaturated Fatty Acids (SFA, MUFA and PUFA, respectively) were measured by gas chromatography using standard methods. Duodenal section of SI was examined for Cluster of Differentiation 36 (CD36) expression using immunohistochemistry. In experiment two, 30 WR were divided into two sexes (n=15). Each sex subgroup was divided into 3 groups (n=5). Subgroups 1 and 2 were fed on SC and HPFD for 22 weeks while subgroup 3 was fed HPFD for 17 weeks followed by LCD for 5 weeks. The same experimental analyses in study one were thereafter implemented. Data were analysed using descriptive statistics and ANOVA at $\alpha 0.05$.

In experiment one, the SC showed no significant difference in LDL, TC and triglyceride between fWR and mWR. In HPFD; serum FFA, heart LDL, SI SFA decreased significantly while heart HDL increased significantly in fWR compared with mWR (815±55.00 vs 1754±75.00µmol/L; 27±1.80 vs 38±2.90mg/dL; 41.03±2.07 vs 50.49±1.31%; 20±1.50 vs 14±1.40mg/dL, respectively). The HAFD significantly increased serum TC, LH, apolipoprotein B:A ratio, SI PUFA in fWR compared with mWR (251±17.00 vs 191±3.90mg/dL; 3.5±0.09 vs 4±0.20µlU/mL; 3.5±0.46 vs 2.8±0.07; 13.47±1.34 vs 8.5±0.75%, respectively). No significant difference was observed for MUFA between the sexes. Duodenal section of SI showed increased CD36 expression in fWR compared with mWR (5.83±0.26 vs 8.65±0.83%). In experiment two, serum TC and triglyceride significantly increased in subgroup 2, while in subgroup 3, liver LDL, TG and FFA significantly reduced in fWR compared with mWR (258±11.00 vs 217±9.80; 272±19.00 vs 202±6.90; 14±0.85 vs 31±1.60; 109±2.1 vs 182±5.8mg/dL; 834±72 vs 1409±74µmol/L respectively).

Sexual dimorphic response that was more pronounced in males on low calorie diet may be associated with sexually distinct lipid profile and hormonal modulations between the sexes.

Keywords: High fat diets, Low calorie diet, Sexual dimorphism, Lipid profile, Obesity

Word count: 493

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LIST OF ABBREVIATIONS

AC	–	Abdominal Circumference
AI	–	Adiposity Index
ApoA	–	Apolipoprotein A
ApoB	–	Apolipoprotein B
BAT	–	Brown Adipose Tissue
BL	–	Body Length
BMI	–	Body Mass Index
BW	–	Body Weight
CD36	–	Cluster of Differentiation 36
CRF	–	Cardiovascular Risk Factor
CVD	–	Cardiovascular Disease
EI	–	Energy Intake
FA	–	Fatty Acid
FATP4	–	Fatty Acid Transporter Protein 4
FFA	–	Free Fatty Acid
FE	–	Feed Efficiency
FFI	–	Final Feed Intake
FSH	–	Follicular Stimulating Hormone
GSH	–	Reduced Glutathione
HAFD	–	High Animal Fatty Diet
HDL-c	–	High Density Lipoprotein Cholesterol
HFD	–	High Fat Diet
HPFD	–	High Plant Fatty Diet
IFI	–	Initial Feed Intake
LCD	–	Low Calorie Diet
LCHFDs	–	Low-Carbohydrate, High-Fat Diets
LDL-c	–	Low Density Lipoprotein Cholesterol
LH	–	Luteinizing Hormone
LPL	–	Lipoprotein Lipase
MDA	–	Malondialdehyde
MUFA	–	Mono-Unsaturated Fatty Acid
PUFA	–	Poly-Unsaturated Fatty Acid
SAT	–	Subcutaneous Adipose Tissue
SC	–	Standard Chow
SFA	–	Saturated Fatty Acid
SOD	–	Superoxide Dismutase
TC	–	Total Cholesterol
TG	–	Triglyceride
ThC	–	Thoracic Circumference
T2DM	–	Type 2 Diabetes Mellitus
VAT	–	Visceral Adipose Tissue
WAT	–	White Adipose Tissue
WHO	–	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Excessive weight and obesity are well defined by the World Health Organization (WHO), 2017, as the increased buildup of adipose tissue that can affect the state of well-being. In line with WHO, no less than 2.8 million men and women die annually because of excessive weight and obesity (WHO, 2017). Obesity alters the endocrine and metabolic function and could exacerbate several metabolic abnormalities, like Diabetes Millitus Type 2 (T2DM), cardiovascular abnormalities, clothing of blood vessels, abnormal lipid accumulation and high blood pressure.

Currently, obesity has developed into a global health issue and in 2014, about 1.9 billion (39% of the population of the world) are overweight and 600 million (13% of the population of the world) are obese (WHO, 2017). In 2022, WHO further estimated that by 2025, approximately 167 million people (adult and children) will become less healthy because they are either overweight or obese (WHO, 2022). Dietary fats contain about double the amount of energy (9kcal/g) contributed by either protein or carbohydrate (4kcal/g) (Wang *et al.,* 2011). Fatty acids are the building block of dietary fats and are mainly in the saturated and unsaturated form. The key distinction between these fatty acids is the presence or absence of carbon-carbon bonds within the organic compound chain. The degree of saturation (saturated or unsaturated) or source (animal or plant) of fat has being the major determinant of dietary fat classification as good or bad.

Animal fats possess greater amount of saturated fats while plant fats possess greater amount of unsaturated fats. Excessive consumption of fatty diets has been greatly implicated in the exacerbation of arteriosclerosis and some cardiovascular risk factors (CRF) like high cholesterol level, high triacylglycerol level, lowering of high density lipoprotein cholesterol (HDL-c), higher blood pressure, T2DM and obesity (Wang *et al.,* 2011; Santos *et al.,* 2013). Great attention has been shown regarding the influence of

edible fats with varying levels of saturation on utilization or storage of energy in the body (Mercer and Trayhurn, 1984).

Saturated fats have been shown to elevate low-density lipoprotein cholesterol (LDL-c) thereby amplifying cardiovascular risk (Brouwer *et al.*, 2010), increasing oxidative stress and moderately increasing LDL-c (Han *et al.*, 2002). While unsaturated fats have been shown to reduce high cholesterol levels which results in the decreased prevalence rate of cardiovascular disease (CVD). In addition, unsaturated fats prevent triggering of nuclear factor κappa B and expression of Cyclooxygenase-2 (Lee *et al.*, 2013). Lastly, unsaturated fats positively impact the enhancement of autonomic function, improved irregular heartbeat, reduced platelet aggregation and blood pressure, enhanced endothelial activities, equilibration of atheromatous plaque and triglyceride levels (Al Rasadi *et al.*, 2016). Also, researches with mice, which consumed high plant fat diet had increased body generation of heat (Mercer and Trayhurn, 1984) and also exhibited lower body energy gain than mice, which consumed high animal fat diet (Mercer and Trayhurn, 1987).

Despite the advantages of unsaturated fat consumption, a research showed that oxidation is more rapid in polyunsaturated fatty acid (PUFA), an unsaturated fatty acid, compared with saturated fatty acids [SFA] (Li *et al.*, 2015). Furthermore, in 2010, a study showed no relationship amongst edible SFA and risk of CVD (Siri-Tarino *et al.*, 2010). But in a 12-year follow up cohort study, there was a relationship amongst consumed fatty diets (consisting of both saturated and unsaturated fatty acids) and a reduced threat of central adiposity in matured males (Holmberg and Thelin, 2013). A follow up study concluded that fatty diets and the resulting health issues are due both to the amount and type of fat consumed (Pavlisova *et al.*, 2016). An earlier research by Raclot and Groscolas, (1997), showed that the metabolic activities of the nutrient (dietary fats) and also the blood level of cholesterol. Also, dissemination in lipoproteins were greatly controlled by fatty acid chain length, level of saturation and double bonds arrangement (Raclot and Groscolas, 1997).

A calorie restriction diet or low calorie diet, which is the reduction in calorie intake without malnutrition, encourages weight lost, extends life span and prolongs the inception of sicknesses associated with old age (Speakman and Mitchell, 2011). It also

increases anti-inflammatory factors, immune responses and antioxidant enzyme activities (Speakman and Mitchell, 2011). However, inappropriate caloric restrictions could increase food intake, body weight, adiposity, encourage hepatic portal inflammation, fibrosis, bile stasis, focal necrosis and metabolic syndrome in numerous models (Swithers *et al.,* 2010).

A lot of dietary manipulations targeted at decreasing the occurrence of CVD and encourage reduction in weight after Dr. Atkins' Low-carbohydrate, high-fat diets (LCHFDs) (Atkins, 1972) have been developed. However, current reviews that appraised the properties of LCHFDs on cardiovascular disease risk factors so far have produced conflicting outcomes, with some findings suggesting that LCHFDs have promising outcomes on cardiovascular risk factors (Ruth *et al.,* 2013) while others reported that LCHFDs were related with higher all-cause and cardiovascular mortality post-myocardial infarction (Li *et al.,* 2014). Interestingly, LCHFDs composed with animal fat were observed to possess elevated chances of chronic diseases (Benetou *et al.,* 2008) compared to those from plant source, which improved low-density lipoprotein (LDL) levels when related to a high-carbohydrate diet (Jenkins *et al.,* 2009).

The source of the protein and fat, that is if it is of animal or plant origin determines its effect as a factor that exacerbates cardiovascular risk. A finding suggested that elevated weight loss observed in the usage of low-carbohydrate diets compared to low-fat diets (Shai *et al.,* 2008) while another researcher observed no notable similarities in the two diets (Foster *et al.,* 2010). Notable challenges with dietary trials consist of reduced adherence and elevated attrition rates (15–50%) (Nordmann *et al.,* 2006). There are three well established types of dietary intervention which are:

- High Protein 'ketogenic' diet (where the carbohydrate content was less than 40g).
- Low Carbohydrate diets (had carbohydrate containing portion of the diet below 60g).
- Low Fat (had energy derived from fat being about 30% or less).

Irrespective of the intervention, attentions are focused majorly on the safety, together with its influence on;

a.) development and lipid variables
b.) metabolic properties of low carbohydrate and fat intake
c.) state of elongated ketosis (Spear *et al.,* 2007).

At this point, it is necessary to differentiate between gender and sex. Sex variations are results from chromosomal peculiarities, like XY and XX in males and females respectively. These variations are as such feminine or masculine and continue to exist regardless of the regulative control of the different environmental factors and hormonal background. But, gender differences are a combination of sex hormone, epigenetic and sex chromosome communications with the environment and are characteristics of a given phase of existence. Sexual variations cannot be completely secluded from gender variations due to enormous confounding variables. Even though oestrogen and androgen are of little importance in metabolic research in healthy males, it is of great value mentioning that they are never limited only to the female gender.

From the above explanations, gender will not be a perfect word to use when conversing on metabolic variations amongst females and males. For this research work to exhibit a focus, sex/gender dimorphism denotes the morphological/systematic variations amongst males and females further than those associated with basic reproductive functions (Cook, 1997; Robert, 2019). The acknowledgement of the importance of sex variation in the control of eating habits can be traced to the initial study of Holt *et al.*, (1936), who discovered that castration increased feed consumption in female rats but had only a minor depressing result in male rats. Ever since, focus of studies have been directed at determining the exact role of sex variations in eating pattern and the role of sex steroids in controlling this pattern. During diet-induced obesity, female mice were shown to be protected against insulin resistance and generally possess reduced reactions to high-fat diets in a research environment.

Many theories have emanated in a bid to understand what defends women from an equivalent metabolic impairment observed in men. Studies so far point to clear differences in the anatomical distribution of fatty tissues and the accumulation of these fats in specific areas in both sexes in mice. Also, research revealed that despite the intense monitoring of dietary intake and other ecological factors, male mice exhibited considerably bigger spreading out of whole body mass comprising the visceral adipose

tissue (VAT), subcutaneous adipose tissue (SAT) and liver compared with their feminine equivalents (Romanski *et al.,* 2000). Outcomes of these works have shown that it is very challenging making a flawless model due to the fact that male mice have a tendency to lose weight more slowly than females even when fed with HFD for the same time interval. Therefore, females usually exhibit lesser weight relative to males. Surprisingly, males possess less adipose tissue mass and altered glucose metabolism compared to females of similar body weight (Nickelson *et al.,* 2012). Also, males have lesser adipose tissue mass compared to females all through their lifetime (Smiderle *et al.,* 2014).

With regards to the distribution of fatty tissues, men are known to possess more in the 'android' or 'apple' phenotype (central or abdominal region), which shows a bigger danger indicator of metabolic abnormalities (Karastergiou and Fried, 2012). But in females, the distribution of fatty tissues is seen more in the lower body and known to exhibit a 'gynoid' or 'pear' phenotype (Votruba and Jensen, 2006), due to the fact that they possess more SAT and fewer VAT (Camhi *et al.,* 2011).

Also, although ladies do exhibit the upper-body obese phenotype, the decrease in the tendency of metabolic abnormalities seen in ladies could be as a result of their ability to accumulate adipose tissue in the SAT depot, especially in the gluteal-femoral region. This is welled observed in menopausal females, who possess a decreased in flowing estrogen concentrations (Lovejoy *et al.,* 2008) that results in a rise in visceral adiposity, due to a move in the direction of a central/android adiposity. Also, studies revealed an exhibition of a far larger increase in VAT volume in postmenopausal relative to premenopausal ladies (Lovejoy *et al.,* 2008). Furthermore, there has being a reduced abdominal circumference and VAT, in ladies especially in those who received hormone replacement therapy (HRT) in relation to subjects that did not receive therapy (Munoz *et al.,* 2002).

1.2 Statement of Problem

In the past, intake of SFA was well-thought-out as a cardiovascular disease risk issue. Therefore, substitution of nutritive SFA and trans-fat with MUFA and PUFA have been suggested for a long time to prevent cardiovascular disease (Kritchevsky, 1998;). However, a growing literature in obesity studies has generated more controversies with

studies either showing SFA as a positive or negative contributing factor that exacerbates cardiovascular disease (Currenti *et al.,* 2022). For example, a research work published in 2010 found out no relationship amongst consumed SFA and the threat of cardiovascular disease (Siri-Tarino *et al.,* 2010). Contrary to this, a 12-year follow up cohort study showed a relationship amongst increased consumption of fatty diets and a reduced danger of central obesity in matured males (Holmberg and Thelin, 2013). Even with the change in diet (which in majority of cases is cutting down on high fat diets and substituting it with low calorie diet) and activity patterns (such as increased exercise), the frequency of occurrence of obesity still progresses at a rapid rate of 12% as observed in a study in 2008 (Shinagawa *et al.,* 2015). Based on these findings, the American Heart Association (AHA) produced a dietary guideline for 2015-2020 which aims at healthy eating patterns with emphasis on quality and quantity of food eaten which still has not achieved its desired objectives. This brought about their current guideline which now emphases on 'dietary forms' rather than thinking of foods as good or bad (AHA, 2022)

1.3 Justification for the study

Most preclinical studies investigating Obesity and cardiovascular disease are usually carried out in male subject alone, creating a scarcity of evidence concerning the sexually dimorphic responses to obesity and cardiovascular disease in the literature (Sondergaard *et al.,* 2012; Kleisner *et al.,* 2021). This has led to numerous guidelines and therapies formulated with knowledge of researches done only in males but being executed in both genders (Hodson *et al.,* 2015; Kleisner *et al.,* 2021). This study aims at giving a sound physiological basis on the sexual dimorphic response of metabolic variables on Wistar rats that were made obese through prolonged consumption of either plant or animal fatty diets and on obese rats treated with a low calorie diet.

1.4 Aim of the Study

This study was designed to investigate sexual dimorphism on metabolic variables following prolonged intake of high plant fat diet (HPFD) and high animal fat diet (HAFD) in normal, and low calorie diet (LCD) treated obese Wistar Rats.

1.5 Objectives

The objectives of this study were to study:

a.) the obesogenic influence of high plant fat diet (HPFD) and high animal fat diet (HAFD) in Wistar rats on anthropometric and metabolic biomarkers.

b.) the outcomes of LCD intake on obese Wistar rats on anthropometric and metabolic biomarkers.

c.) the sexual dimorphic tendency associated with HPFD and HAFD intake in Wistar rats on anthropometric and metabolic biomarkers.

d.) the sexual dimorphic tendency associated with LCD intake in Wistar rats on anthropometric and metabolic biomarkers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Animal model validity

Animal model for metabolic research must fulfil a multifactorial groups of conditions of validity before it can be deliberated as a model usable in metabolic research. There are two types of validity (figure 2.1);

a.) Internal validity: this deals with how accurate the experimental design is such as: reproducibility, how reliable it is when observed by other researchers, indiscrimination, polycentric blueprint, blind investigation, design (test-control), blind and so forth.

b.) external validity: it is concern with the widespread examination of relevance of the result on a representative to the target inhabitants.

Currently, these validities criteria have been simplified into four namely;

a.) Predictive validity

b.) Face validity

c.) Construct validity

d.) Others

2.1.1 Predictive Validity

As stated by Willner (1984), predictive validity depends on 5 sub-conditions which are based on the model correctly identifying antidepressant treatments of pharmacologically multiple categories and not creating blunders of oversight or intentional. Also whether the effectiveness in the model relates with clinical effectiveness. As stated in this definition, this condition certainly depends on a pharmacological relationship which non-pharmacological diagnosis are not stated. It is obvious from the above instances that this condition does not put into consideration pathophysiology research in animals as it is just worried with the pharmacological properties but in a different article by the same researchers, additional condition was added to the existing ones which including other forms of treatments not exclusive to pharmacological approach (Willner *et al.,* 2002).

2.1.2 Face Validity

Face validity is evaluated by;

i.) determining if the antidepressant properties remain merely existing on, or remain heighten by chronic administration

ii.) if the prototype simulates depression in several ways

iii.) checking the most precise for depression,

iv.) which really cohabit in a precise sub-classification set of depressions

v.) the prototype that do not exhibit characteristics usually noticeable in clinical analysis. Willner (2002)

This means that face validity entails roughly diagnostic characteristics and symptomatic features together. Also, face validity relates to the level of affiliation amongst the prototype and the abnormality being studied (Willner *et al.,* 2002). In another word, face validity relates to an effort to imitate treatment conditions of the pathophysiological environment.

2.1.3 Construct Validity

Construct validity looks at;

1.) the conduct in the model

2.) the characteristics of insults that is prototyped

3.) models that could be precisely inferred and are specific

4.) closeness of the model to the in vivo subject

5.) theoretical relationship to the insult

Most models do not justify the construct validity condition, Willner (2002).

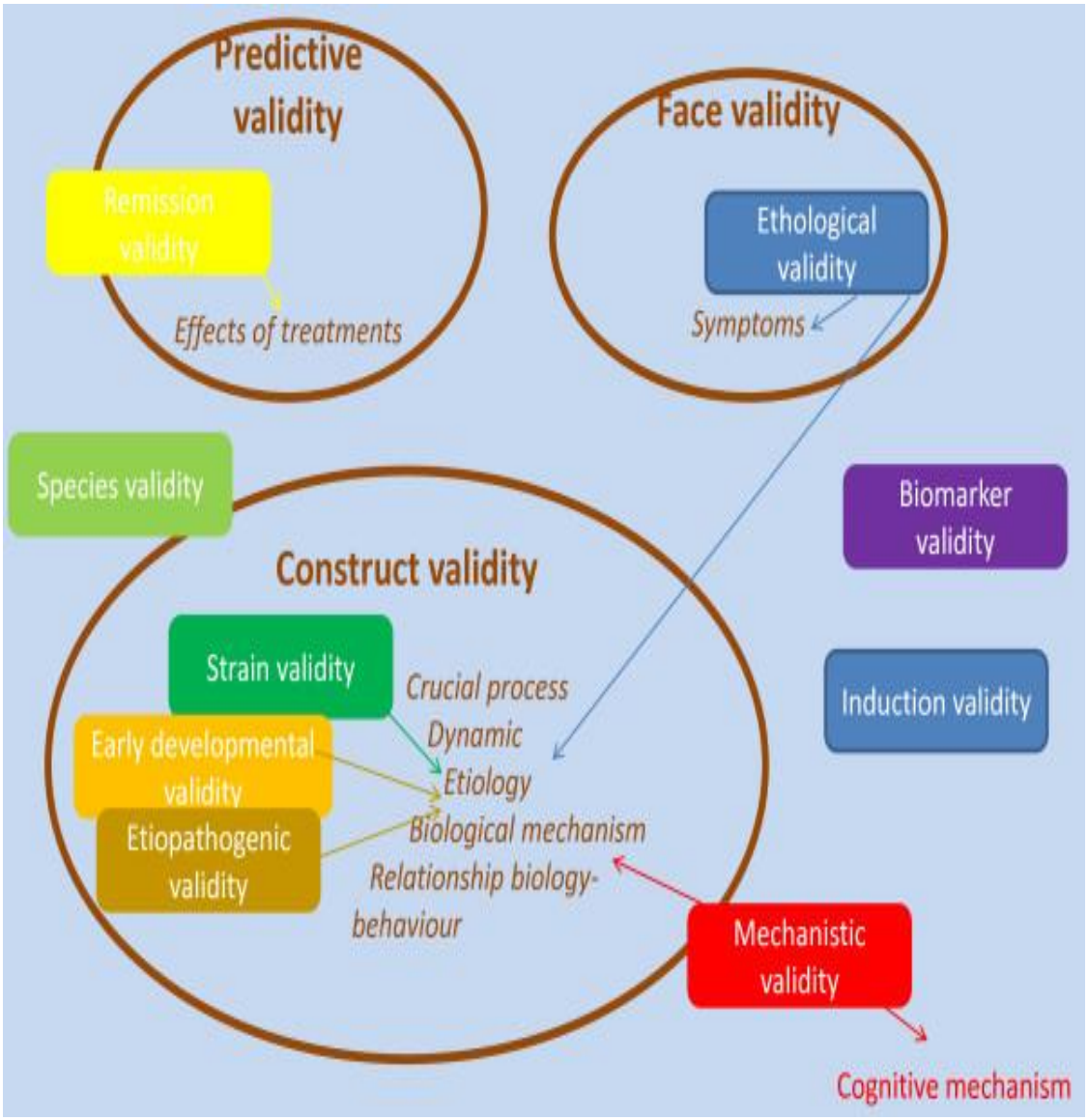


Figure 2.1: A schematic representation of Willner's proposed criteria with modifications by Belzung and colleagues. from Belzung et al. 2011

2.2 Animal Model

The induction of obesity can occur through different ways in animals such as genes modification, feeding or the neuroendocrine changes (Younossi *et al.,* 2016). The models used in inducing obesity depends on the aim of the study such as: Genetically modified models, also known as Monogenic or Transgenic Models (Mercer and Trayhurn, 1987); Diet-induced Models or Polygenic Models (Kritchevsky, 1998); Surgically and Chemically induced Models (Vaidya *et al.,* 2008); and Seasonal Models (Younossi *et al.,* 2016). Regardless of the model, diet is being used as the facilitator of these models.

Animal models have been very useful in studying metabolic insults such as obesity. With reference to the embryology, anatomy and physiology, rodents are closely related to humans and most research with these models has broadened greatly our knowledge of the fundamental factors that cause these metabolic abnormalities. Due to the availability, cost-effectiveness and easiness to manipulate for studies, rodents have numerous benefits in experimental studies in contrast to other mammals.

2.2.1 Diet Induced Obesity Model (DIO Model)

The diet induced obesity is any obesity animal prototype whose obesity was as an outcome of feeding of excessive fat or high energy yielding diet (Younossi *et al.,* 2016). Diets used in feeding experimental animals are in two classes;

a.) Conventional diets: Refer to grain or cereal-based which do not supply specific nutrients (Michael and Matthew, 2020).
b.) Purified diets: Refer to diets in which every ingredient supplies a specific nutrient (Michael and Matthew, 2020).

The main aim of DIO is not just to induce increased weight but also to simulate the pathogenic features commonly observed in humans (Younossi *et al.,* 2016). Numerous animals such as rats, mice, non-human primates or dogs have been utilized in these models to study in vivo obesity, obesity's comorbidities and other associated diseases (Michael and Matthew, 2020). Factors such as duration and type of diet, environmental and animal's age must be put into consideration while inducing this model. The reason for this is because the promotion of bodyweight, fat percentage or behaviors differ with these factors (Michael and Matthew, 2020). With obesity now assuming an epidermal

state no longer perculiar to the western world, DIO model has become a vital part of research in understanding the relationship between excessive dietary consumption on one hand and obesity and other metabolic abnormalities on the other hand (Michael and Matthew, 2020). (figure 2.2)

2.2.2 History of DIO Model

The DIO model was conceived in response to increasing worries over the ravaging health implications of obesity and also the increasing obesity rate worldwide, creating a controlled environment were obesity can be extensively studied. The was first created in 1948 and has been evolving ever since.

2.3 High-Fat Diets

2.3.1 High-Fat Diets and Metabolic Activities

Excessive intake of diets rich in fats are typically connected to a rise in plasma LDL cholesterol (LDL-c) and exacerbated heart abnormalities (Brouwer *et al.*, 2010). Further studies by numerous researchers went further to show that trans-fatty acids increase inflammatory markers in man and also slightly rises LDL-c levels (Han *et al.*, 2002), TC (Simons and Ikonen, 2000) which definitely has effects on the cardiovascular system. Also, studies have shown that SFA stimulate nuclear factor κB activation and cyclooxygenase-2 expression while PUFA has preventive effects (Lee *et al*., 2013). Although fatty acids are necessary for many functions in the body, replacement of SFA with MUFA in consumed feed is usually recommended in the controlling of excess blood cholesterol level and also minimizing the occurrence of CVD.

A research work revealed PUFA to be instrumental in the improvement of neuronal activities, cardiovascular system, clotting and triacylglycerols levels (Al Rasadi *et al.*, 2016). Deficiency of fatty acids have been noticed to have outcome on the well-being of the body, for example, loss of cognitive abilities has been attributed to docosahexaenoic acid (DHA, 22:6 n-3) deficiency (McNamara *et al.*, 2010) and high SFA consumption (Grant *et al.*, 2002). Studies also show that PUFA acts by regulating neuro-immune responses and apoptotic pathways, altering membrane physiology and/or rivaling precursors of inflammatory mediators (Angelis-Pereira *et al.*, 2017).

Fatty Acid Metabolism: Fatty acid (FA) is being accumulated and released by the adipose tissue in reaction to variations in energy balance. After eating food rich in fat, a reasonable percentage of plasma chylomicron-triacylglycerol (TAG) FA is accumulated in the adipose tissue through lipoprotein lipase (LPL) activities. LPL achieves this by stimulating the hydrolysis of FA from its glycerol backbone, which enables them to be absorbed by the adipose tissue (Koutsari *et al.,* 2012). Not all TAG are absorbed through this pathway, some escape to the systemic circulation and their re-uptake is through another mechanism that does not depend on LPL pathway (Koutsari *et al.,* 2012). The FFA released through the LPL pathway is used to generate the energy required by body tissues for fat oxidation and the remaining FA is stored in the adipose tissue (Liu *et al.,* 2015). Liu *et al.* (2015) classified the LPL pathway as the direct FFA storage.

Once FA enters into the adipocyte, it is either transported via passive diffusion through FA transport proteins such as CD36 or via membrane protein facilitated diffusion (Koutsari *et al.,* 2012). A cell membrane glycoprotein, CD36, is activated or stimulated when extracellular FA concentrations are decreased and are important in the transportation of long-chain FA to the adipocyte membrane via FA binding (Koutsari *et al.,* 2012). But, current research shows that CD36 enables muscle and adipose tissue to take up FFA in situation where there is decreased in circulating FFA, but this uptake varies with the cell, for example, the take-up of FFA in the liver cells depends on the availability of CD36 while the take up of FFA in heart tissues only moderately dependents on CD36 availability (Liu *et al.,* 2015). Many pieces of research have explored alternative proteins that are associated with the accumulation of FA such as acyl-CoA synthetase (ACS) and diacyl-glycerol acyl-transferase (DGAT).

Fatty Acid is activated inside the cell to their CoA form via the catalytic action of ACS (Mashek *et al.,* 2007). Also, DGAT, the concluding stage of the accumulation of FA as TAG, is proposed to be a rate-regulating enzyme in FA storage (Yen *et al.,* 2008). Adipose triglyceride lipase, hormone-sensitive lipase (HSL), and monoglyceride lipase are 3 vital enzymes in the hydrolysis of triglyceride (Lass *et al.,* 2011). But, research has been done extensively on HSL and it has been shown to be associated with irregularities in lipolysis related to obesity (Saltiel *et al.,* 2017), and it achieves this by hydrolyzing a variety of molecules which includes TAG, diacylglycerol, and monoacylglycerol (Lass *et al.,* 2011).

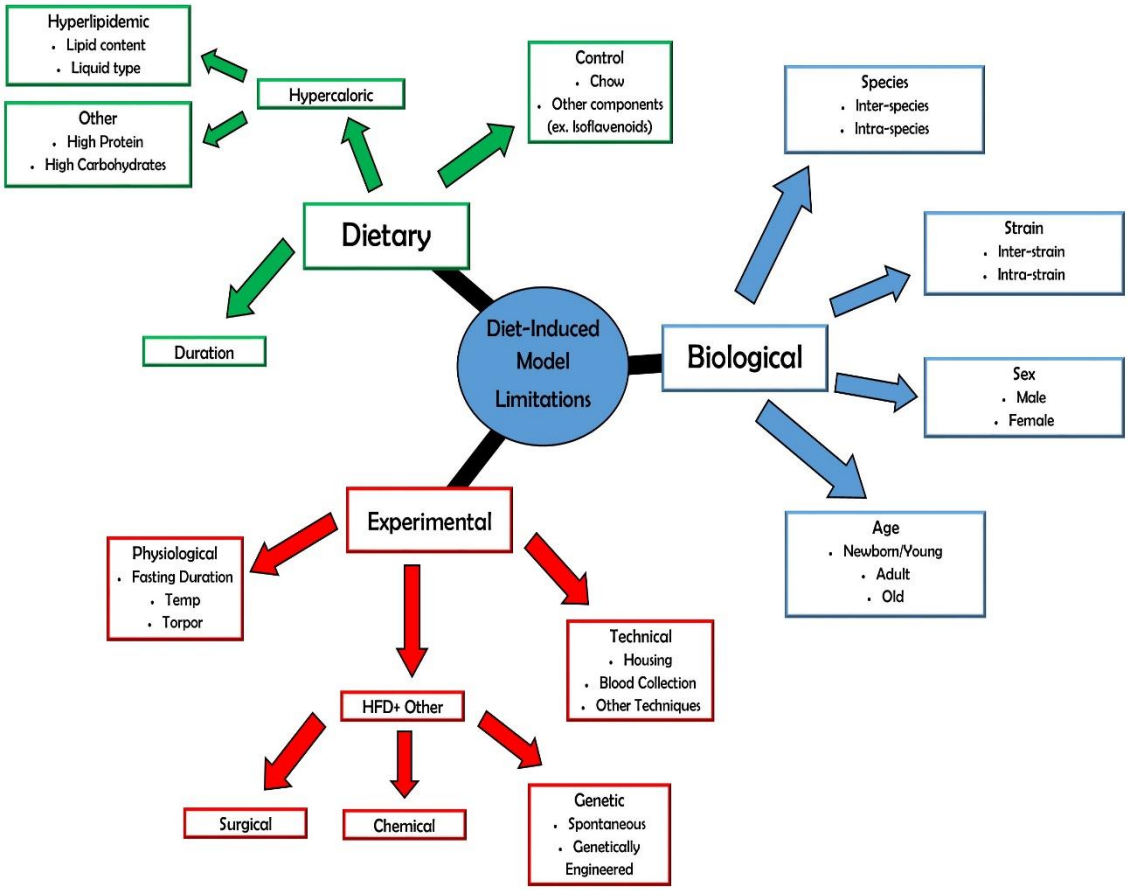


Figure 2.2: Different limitations of the diet-induced obesity model (Lai et al. 2015).

2.3.2 High Fat Diets and Digestive Activities

The powerful link between obesity and consumption of diets rich in fat has over the years being of great public health concern worldwide (Well, 2000) and has defiled numerous treatment such as lifestyle management (Santos *et al.,* 2013). Regulating hormones secreted from glands in the small intestine, such as glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK) and peptide YY (PYY) in respect to when there is availability of feed in the small intestine, has helped in moderating energy intake (Gregory *et al.,* 1989). Also, 12 carbon atoms chain length fatty acids have been reported as powerful inducers for the release of gastrointestinal hormone and the digestion of consumed diet in healthy humans (Cook *et al.,* 1997).

In contemporary times, studies have discovered the precise FA-sensing receptors found in the small intestine that are vital in facilitating the secretion of gastrointestinal hormones in reaction to consumption of food rich in fatty acids (Gregory *et al.,* 1989). One of such sensing receptors is CD36, which expression on enterocytes is mostly in the proximal small intestine (Votruba and Jensen, 2006) and has been shown to be vital in absorbing fats in the small intestine and also acts as a vehicle in the movement of FAs through the cell membrane (Shinagawa *et al.,* 2015). Further researches do show CD36 playing a vital role in facilitating the secretion of CCK when there is availability of fat in the small intestine (Shi and Clegg, 2009).

A recent study showed a decreased stimulation of pyloric motility and plasma CCK in reaction to elevated concentration of Oleic acid (C18:1) in the duodenum of obese in relations to lean subject (Wahlig *et al.,* 2012). The decreased GI stimulation was connected to decreased repression of the later energy intake by C18:1, and also, higher regular energy and fat intakes (Wahlig *et al.,* 2012). Researches with animal models show that these variations in GI nutrient responses are stimulated by the feeding of diets rich in fats (Bruce *et al.,* 2009). Rats made obese by feeding of diets rich in fats show a reduction in CCK, GPR40, GLP-1, GPR120 and PYY protein expressions and increased mRNA in the proximal small intestine (Bruce *et al.,* 2009). In summary, sensing and signaling of Fas in the small intestine can be compromised in obese situations and this could distort the regulation of energy intake.

2.3.3 High Fat Diet and Foetal Function

Research utilizing experimental animals do show that abnormal maternal nutrition, such as calorie restriction or excessive calorie intake, has an adverse implication on the offspring lifestyle (Yang and Huffman, 2013). The foetus sole source of nutrient for growth and wellbeing comes from the mother's nutritional supply, therefore any changes in the mother's metabolic status will subject the unborn child to a disturbed intrauterine environment, which can make the offspring susceptible to metabolic abnormalities in advanced lifespan (Muhlhausler and Ong, 2011).

Placenta: It acts as the connection between the mother and the foetal environment. Studies found out that mouse fed with 32% of calories from fat for 8 weeks before they were mated until breastfeeding was over had bigger foetal weight and placental SNAT2 mRNA and GLUT1 (Jones *et al.,* 2009).

Foetal Liver: Researches has documented the consumption of diets dense with energy to have adverse implication independent of the period in which the calorie was consumed in the liver (Ashino *et al.,* 2012, Volpato *et al.,* 2012). For instance, mouse that consumed 36% of calories from fat for 2 weeks before intercourse, throughout pregnancy and breastfeeding exhibited reduced foetal development and birth weight and high expression of liver lipogenic genes (Volpato *et al.,* 2012). Also, mouse that consumed 49% of calories from fat during gestation up till 3 months of after birth exhibited insulin resistance, high liver triglycerides, and hepatic steatosis (Volpato *et al.,* 2012). Furthermore, mouse fed 35% of calories from fat 1 week before intercourse, up till breastfeeding was over exhibited metabolic syndrome, hepatic lipid buildup, elevated hepatic JNK phosphorylation (Ashino *et al.,* 2012).

In another study, mouse fed 45% of calories from fat for 4 weeks before pregnancy up till breastfeeding was over exhibited higher hepatic lipogenesis, decreased mitochondrial function, changes the expression of inflammatory gene, higher oxidative stress (Bruce *et al.,* 2009). Also, mouse fed 49% of energies from fat throughout incubation and breast feeding stage had increased manifestation of liver lipogenic genes and Insulin resistance (Gregorio *et al.,* 2010). A different research observed that mouse fed 53% of calories from fat for six weeks before sexual intercourse up till pregnancy was over had higher protein levels in FA oxidation, higher birth weight, changed liver microRNA (Zhang *et al.,* 2009). Also, mouse fed 18% fat for six weeks before

pregnancy, up till breastfeeding ended had hypertension, liver steatosis (Elahi *et al.,* 2009).

While a mouse fed 20% (Polyunsaturated FA or Saturated FA) during pregnancy and breastfeeding exhibited decreased liver steatosis (Chechi *et al.,* 2010). Another study showed a mouse fed 45% of calories from fat for the period of pregnancy had higher natal being mass and glucose level, changed of hepatic histone alteration of PCK1 gene (Strakovsky *et al.,* 2011). Finally, a mouse fed 21% fat for two weeks before sexual intercourse up till breastfeeding was over exhibited CpG methylation of FADS2 promoter in the liver, aorta (Hoile *et al.,* 2012).

Related findings were seen in rats. For instance, rats fed 40% of calories from fat for 15 days before sexual intercourse up till breastfeeding exhibited reduced hepatic mtDNA copy figure and PGC1α mRNA manifestation (Burgueno *et al.,* 2013); rats fed 35% of calories from fat for six before sexual intercourse up till breastfeeding was over exhibited higher body weight, serum leptin, and changes in food-motivated behaviour (Mitra *et al.,* 2009). Finally, Non-Human Primate fed 32% of calories from fat for Up to four years before sexual intercourse had higher placental inflammatory cytokines, manifestation and methylation of hypothalamic gene, foetal metabolic profile, foetal thyroidal axis, reduced foetal HDAC activity, SIRT1 gene manifestation and activity, modulated serotogenic system, early reduced body weight and reduced lean mass, reduced foetal hepatic apoptosis, placental dysfunction, higher serum leptin, early-onset obesity, higher proinflammatory cytokine levels, higher hepatic expression of the gluconeogenic enzymes and transcription factor mRNA (Suter *et al.,* 2012).

Pancreas: studies in foetal pancreas shows that, mouse fed 60% of calories from fat for 8 weeks before sexual intercourse up till pregnancy is over expression of a decreased β-cell mass in second filial (F2) generation, diabetes type 2 in first filial (F1) and F2, increased β-cell mass in F1 (Galgani *et al.,* 2008). Also, rats fed 60% of calories from fat from postnatal day 24 up till incubation was over had elevated insulin secretion, glucose and insulin levels, and body weight (Sun *et al.,* 2012) while rats fed 40% of calories from fat throughout gestational and lactation periods had decreased pancreatic glucokinase mRNA and higher serum glucose (Cerf *et al.,* 2012).

Furthermore, rats fed 40% of calories from fat throughout gestational and lactation periods had higher pancreatic β-cell and serum leptin, higher serum glucose, hypertrophy and hyperplasia (Cerf *et al.,* 2012). Another study showed that rats fed 40% of calories from fat during the different period of gestation had impaired decreased PDX-1 and GK manifestation in β-cells, insulin release, changes in β-cell size and number, hyperglycemic, (Cerf *et al.,* 2009). Finally, rats fed 18% fat for two weeks before sexual intercourse up till weaning period elapsed exhibited scarcer huge islets on saturated fatty acids diet in the Pancreas which decreases natal born mass but increases with unsaturated fatty acids diet (Sun *et al.,* 2012).

Bone and Muscle: A research carried out on mouse fed 18% fat during pregnancy and breastfeeding affected the quantity and quality of bone (Malina, 1996). Also, rats that consumed 59% of energies from fat for four weeks prior to sexual intercourse and for the period of prenatal period exhibited variations in manifestation of insulin signaling proteins in skeletal muscle and higher serum insulin (Buckley *et al.,* 2005); rats fed 40% of calories from fat for ten weeks before sexual intercourse exhibited higher serum leptin, abdominal adiposity and insulin in skeletal muscle (Qing Wu *et al.,* 1998).

Brain: studies show that, mouse fed 60% of energies from fat for three weeks prior to prenatal period up till the end of breastfeeding exhibited higher molecular changes and anxiety behaviour in the hippocampus (Pan *et al.,* 1994). Another study showed that mouse fed 20% fat (Polyunsaturated FA or Saturated FA) for the period of pregnancy and breastfeeding had Saturated FA raising the level of plasma TC and LDL-c (Chechi and Cheema, 2006). Also, mouse fed 60% of calories from fat for 12 weeks before copulation, up till the end of lactation had decreases in global methylation in brain (Vucetic *et al.,* 2010). Further studies showed that mouse fed 45% of calories from fat for 4 weeks before sexual intercourse up till breastfeeding elapsed exhibited distorted growth hormone axis, higher body length, distorted manifestation of paternally imprinted genes in F3 (Dunn and Bale, 2011).

Also, rats fed 40% of calories from fat for 6 weeks before sexual intercourse up till the end of weaning exhibited decreased hypothalamic leptin-dependent STAT3 phosphorylation, and higher insulin, body weight and serum leptin (Gupta *et al.,* 2009). Furthermore, rats fed 60% of calories from fat for 11 weeks before sexual intercourse

and throughout gestation exhibited higher serum insulin and leptin, and expression of hypothalamic appetite-regulating genes (Gupta *et al.,* 2009). Also, rats fed 50% of calories from fat starting from embryonic day 6 had high proliferation of neuronal precursor cells, and neuro-epithelial and precocious puberty (Vucetic *et al.,* 2010). Another study showed rats fed 29% of calories from fat for 8 weeks before sexual intercourse up till the end of weaning had increased blood glucose, bodyweight and leptin, decreased phospho- SOCS3 and STAT3 manifestation in the arcuate nucleus (Sun *et al.,* 2012). Finally, rats fed 60% of calories from fat from gestational day 2 had higher serum leptin and body weight, and decreased phosphorylation of STAT3 in the arcuate nucleus (Sun *et al.,* 2012).

Heart and vessels: studies show that, mouse fed 20% (Polyunsaturated FA or Saturated FA) for the period of pregnancy and breastfeeding had decreased heart DHA (Chechi *et al.,* 2010). While mouse fed 62% of calories from fat for 4 weeks before sexual intercourse up till the end of breastfeeding had higher blood pressure and body weight (Muhlhausler and Ailhaud, 2013). Furthermore, mouse fed 21% fat for 2 weeks before sexual intercourse up till the end of lactation had distorted vascular activities and CpG methylation of FADS2 promoter (Kelsall *et al.*, 2012).

Also, rats fed 21% fat from gestation to post-delivery day 10 had higher blood pressure, serum insulin, leptin, and left ventricular wall thickness (Parente *et al.,* 2008) and rats fed with 60% of calories from fat for 4 weeks before sexual intercourse up till the end of lactation had higher serum leptin and adiposity (Williams *et al.,* 2003). Another study showed rats fed with 33% fat for 10 days before sexual intercourse up till the end of lactation had reduced serum DHA (Zhao *et al.,* 2016). Finally, rats fed with 26% of calories from fat for ten days before sexual intercourse up till the end of lactation had insulin resistance, higher blood pressure, endothelial dysfunction, decreased heart rate, distorted aortic structure, renal function and vascular fatty acid content in the aaorta (Zhao *et al.,* 2016).

Adipose tissue: A study showed that mouse fed with 62% of calories from fat for 4 weeks before sexual intercourse up till the end of lactation had distorted histone modification of ADIPOQ promoter Adipose (Michaud *et al.,* 2012).

2.3.4 High Fat Diet and Adipose Tissue

Prolonged deposition of fats for a long time has been linked to severe co-morbidities and the way through which fat is distributed around the body is implicated to be a more improved indicator of health risk (Pischon *et al.,* 2008). Several pieces of research have worked on the relationship between the deposition of WAT in the body and metabolic risk in humans and attributed their findings was noted as an outcome of innate variations in the role of the adipose tissue (Amati *et al.,* 2012).

The 2 forms of WAT, VAT and SAT, are described through their locality (that is, where they are found in the body), and the pathways and developmental signals which explains the distinctive attributes regarding the way in which fats are located in the body. In man, VAT is normally seen as the omental deposition (in which the mesenteric fat is also part of) but in rodents by the perigonadal deposition (parametrial and epididymal fat pad); in man, SAT is normally seen as the abdominal, gluteal and femoral depots but in rodents as the inguinal fat pad. Researches show that central/abdominal (subcutaneous upper body and visceral) fat accumulation shows a relationship with higher predisposition for metabolic complications (Michaud *et al.,* 2012), but gluteal-femoral (lower body) fat accumulation is connected to a decreased metabolic risk and could be a defense shield in cases such as unfavourable health implications associated with excess body fat accumulation in both gender (Pinnick *et al.,* 2012).

2.4 Sexual Dimorphism in High Fat Diet Consumption

Sex hormones are endogenous regulators of adipocytes growth and activities and could impact also on the dispersal of SAT and VAT in the body (Shi and Clegg, 2009). In women of menopausal age do have higher visceral adipose tissue emanating from a swing from the subcutaneous adipose distribution to the direction of central/android body adipose distribution due to the decrease in the amount of estrogen in circulation (Piche *et al.,* 2008). A study showed that ta\he amount of VAT was greatly increased in females that have passed menopausal age when related to females who are yet to reach menopause (Shi and Clegg, 2009). Also, ladies that are placed on hormonal therapy were observed to exhibit a decreased waist circumferences and VAT compared to those without any therapy (Munoz *et al.,* 2002). Cooperatively, the above findings show that estrogen can alter fat dispersal by efficiently decreasing central adiposity in man.

Similarly, the accumulation of adipocytes in the gluteal-femoral SAT in females compared with the accumulation in the VAT in males could be linked to the increased level of estrogen in ladies who have not reached menopause in relation to men (Tchernof *et al.,* 2000). Male hormones could affect the distribution of fats in a way that is gender-specific. In male subject, the decrease in testosterone as a result of ageing and in females due to polycystic ovary syndrome (PCOS), are usually connected with an elevated VAT (Emmelot-Vonk *et al.,* 2008). There was a decrease in abdominal fat mass and elevated muscle mass in older male rats treated with testosterone (Emmelot-Vonk *et al.,* 2008). In the female, testosterone levels were directly related to meaningfully higher visceral fat (Haffner *et al.,* 1991). Furthermore, fat ladies who have passed menopause and had testosterone therapy had a reasonable elevated abdominal fat (Lovejoy *et al.,* 1996). A lot of researches have shown that both VAT and SAT in women and men exhibit sex hormone receptors, particularly the estrogen (ERα, ERβ and its modifications, and G protein-coupled ER) and androgen (AR) receptors but the gender with greater amount of ER has the greater influence on fat distributions (Lovejoy *et al.,* 1996).

ERβ1 expression was much decreased in VAT relative to SAT, while the manifestation of ERβ4 and ERβ5 mRNA levels were meaningfully increased in gluteal SAT relative to abdominal SAT of males and females, with elevated ERβ mRNA levels in females relative to males (Haffner *et al.,* 1991). Reports from Gavin *et al.* (2013) backs these findings, as their researches shows that abdominal SAT possesses additional ERα protein relative to gluteal. Also, gluteal SAT possesses additional ERβ protein in relation to abdominal SAT from weighty ladies that have not reached menopause and the research was done during the period of the follicular phase of the menstrual cycle (Gavin *et al.,* 2013).

Also, the waist-to-hip ratio adversely relates to the gluteal ERβ protein, signifying that ERs could have a function in regulating local fat dispersal. Furthermore, ERα and ERβ from researches are shown to facilitate depot-specific functions (Tchernof *et al.,* 2000). Circulating estrogens are responsible for the up-regulation of both ERα and ERβ mRNA expression in the fatty tissues of females, but in male, only ERα in the fatty tissues are expressed (Tchernof *et al.,* 2000).

Studies involving knockout models have produced fresh pieces of evidence concerning the precise function of estrogen, and also ERα and ERβ, in the regulation of WAT lipid and glucose homeostasis (Zirilli *et al.,* 2008). Therefore, the above researches have given indications to estrogen potential ability in regulating the storage of fat in a depot-specific fashion through the differences in ERs expression within the adipose tissue. With the storage of fat in obese subjects, ladies have been shown to have elevation of fatty cells but this was not noticed in males (Tchoukalova *et al.,* 2008).

Although the mass of fatty cells gets bigger in both gender, the frequency of fatty cell hypertrophy increases in male compared to females, especially in the lower-body fat. Cross-sectional evaluations of fatty cell morphology and adipogenesis in abdominal SAT and VAT depots before and after menopause in female showed that fatty cells hypertrophy in both depots but hyperplasia only in the abdominal SAT depot (Drolet *et al.,* 2008). Majority of this comparative studies were not only carried out in males, but in both sexes and such studies showed preadipocytes having a decreased adipogenic VAT capacity in relation to abdominal SAT of obese subjects of both sexes and with males having higher accumulation of visceral fat (Tchoukalova *et al.,* 2008) which has given an insight to the huge involvement of adipocyte hypertrophy in VAT enlargement in male compared to females.

The step rate-limiting in the absorbance of circulating TG-FFA by the fatty cell is the activities of lipoprotein lipase (LPL) and this determines the quantity of intracellular TGs that can be accumulated in adipose tissue-derived from FFAs. Numerous researches have evaluated the depot- and gender-specific modulation of LPL. In previous researches, LPL appearance and operations are revealed to be elevated in subcutaneous abdominal than in gluteal adipocytes in non-obese male (Hernandez *et al.,* 2011). In another view, the operations of LPL were raised in gluteal- femoral fatty cells of non-obese ladies in relations to the abdominal depot (Hernandez *et al.*, 2011).

Further researches observed that LPL operations are reduced in VAT compared to abdominal SAT-derived fatty cells secluded from ladies but increased in the VAT depot in obese males (de Bruin *et al.,* 1996). Similarly, LPL appearance and operations are increased in visceral than in gluteal fatty cells in non-fat or mildly fat males (de Bruin *et al.,* 1996). Further indication shows that testosterone was able to subdue LPL

operation in the femoral SAT of male, adding to the storage of abdominal fat (Rodriguez *et al.*, 2005), but some researchers are of the view that testosterone has no influence on LPL operations in the thigh (Marin *et al.,* 1995).

The above discrepancies could be attributed to gender-specific variances and the compound post-transcriptional modulation of LPL (Soeters *et al.,* 2007). However, there are possibilities that LPL appearance and operations may be very vital in sex-specific fatty cells depot growth and purpose. Deposition and gender-precise variances in the direct absorption of FFA in circulation have been well defined. The absorption of FFA as a result of immediate consumption of a meal has been observed to be elevated in upper body abdominal SAT when compared to lower-body SAT in both slim and fat males and females, with females possessing a greater amount in SAT compared to males (Koutsari *et al.,* 2012). Emphasis should be communicated that the rate of absorption of FFA in the research was analyzed per mass of the whole body fat. Consequently, the rate of absorption could be considered to be higher in SAT owing to its greater fatty mass but of no difference or lesser in VAT when considered per cell.

However, accumulation of fatty acids per gram of fat mass after consumption of meal rich in fats was significantly elevated especially in the gluteal-femoral SAT of females in relation to male (Votruba and Jensen, 2006). Furthermore, researches have observed that circulating FFA gotten from consumption of meal rich in fats are more effectively absorbed in the femoral SAT of ladies with lower-body obesity (Santosa *et al.,* 2008). These variations in the above-mentioned researches were not noticed in males and this in part could elucidate the gender-dependent distribution of adipose tissue, as classically seen in ladies having lower-body adiposity (Pouliot *et al.,* 1991).

On the other hand, new findings show that the absorption of FFA (gotten from the diet) in the VAT depot is bigger in slim males compared to females (Romanski *et al.,* 2000). There are also variances amongst the upper body depots, as FFA absorption (after consumption of a diet) was far bigger in the VAT when related to the abdominal SAT depot, which sequentially were bigger when related to the femoral SAT depot in obese women and men (Romanski *et al.,* 2000). Additionally, in males, testosterone stimulates FFA absorption (from consumed diet) preferably into abdominal SAT and reduces absorption in VAT (Marin *et al.,* 1995). Therefore, elderly males with reduced testosterone have the tendency of accumulating additional VAT. After the absorption of a meal, the absorption of FFA in the circulation into the femoral when related with the

abdominal SAT depot is much less proficient, whereas, in very fat subjects, absorption of FFA in the circulation is elevated precisely in the femoral depot of females (Shadid *et al.,* 2007).

Further researches back these findings, as Koutsari *et al.* (2012) showed that females had a bigger accumulation of FFA compared to males in both femoral and abdominal SAT after absorption of a meal. Remarkably, accumulation rates of adipose tissues were reasonably bigger in femoral when related to abdominal SAT in females, however, there were contradictory findings in males. In females, the femoral SAT depot of was observed to more successfully accumulate FFA in mobile conditions such as taking a walk, in relation to the abdominal SAT depot and each SAT depot in males (Koutsari *et al.,* 2012). As a group, these discoveries could imply that direct accumulation of FFA in fatty tissues is meaningfully bigger in females compared to males.

In totality, the upper-body abdominal adipocyte depot may be involved in the immediate day-by-day FFA absorption and management of circulating of consumed fatty acids (Frayn, 2002) but the lower-body gluteal-femoral depot seems to employ its defensive attributes in long-term adipocyte storage, mostly with females. Also, the absorption ability of gluteal-femoral WAT could have an effect on the level of abdominal/central fat deposition, as buttressed in research findings by Hernandez *et al*. (2011) which was portrayed in the form in which fat is rearranged after surgical removal of fat through suctioning (Hernandez *et al.,* 2011). Although various researches propose that the depot-variances in the researches thus far could have resulted from LPL actions (Nguyen *et al.,* 1996), further researches are needed to elucidate the fundamental principles that propel the absorption of FFA in upper- and lower-body depots of males and females.

As heretofore specified, a lot of the researches we have discussed were carried out to distinguish inflammatory variations when fatness as a result of high-fat consumption were performed in men. For example, female mice from researches are shielded from insulin resistance and in totality possess diminished reactions to high fat diets in experimental settings. Currently, numerous theoretical mode of action has been examined in an effort to comprehend what defends women from the same metabolic damages such as diabetics and hypertension which are more observed in men.

Studies on sexual dimorphism up till date indicates that male and female mice display intense variations in the structural fatty tissue arrangement and growth. Researches also indicate that even when regulating for consumed feeds and other ecological circumstances, male mice exhibit meaningfully bigger enlargement of total body mass including SAT, VAT, and liver when compared with their female counterpart (Nickelson *et al.,* 2012). These researches do encounter challenges in generating a flawless model due to the fact that female mice exposed to HFD for the same duration are likely to add more grams in a slower rate compared to male mice (Nickelson *et al.,* 2012). This is one of the proposed reason why females have lesser weight compared to males (Nickelson *et al.,* 2012). Remarkably, female do accumulate more fats but reduced cytokine gene expression and glucose impairment even with similar bodyweight, when related to males (Nickelson *et al.,* 2012).

2.4.1 Central Effects of Gender and High Fat Diets

There has been a general misunderstanding that variances in the adipose, intestine, liver and muscle lipid metabolism are exclusively accountable for gender variations in adipocytes dispersal, metabolic disease risk, energy expenditure, and substrate consumption. But, recent studies revealed that the CNS reacts to alterations in the nutritional and hormonal status, and provides a vital function of altering not only the entire body mass lipid metabolism but also eating pattern. Diet is a very potent force that stimulates both functional and psychological reactions and changes in eating patterns, in so doing defines the nutritional environment in the body (Shadid *et al.,* 2007). Key organs involved in nutritional metabolism react to alterations in nutrient status by discharging hormones that control eating pattern and nutritional metabolism (Frayn, 2002). There was a pronounced elevation in WAT depot size and weight ascribed to the reduced total body energy expenditure in mice fed standard chow and the lack of oestrogen receptor a (ERa) what implicated in both genders (Hernandez *et al.,* 2011).

2.4.2 Gender Variations in Diets Function and Lipid Absorption in the Gastrointestinal Tract

FATP4 and CD36 are transporters and apical protein channels that are controlled by adipocytes and facilitate the assimilation of fats in the small intestine (Jensen, 2008).

Researches, although limited, have shown that gender differences occurs in the transferring of molecules within the cells and this is mostly due to the knowledge that most biochemical analyses of lipid transfer within the cells are carried out in cell lines gotten from one gender. A research that was carried out with cells of the liver gotten from an early age teenage women and men rats observed a 65% higher rate flow of lipids in the cytoplasm of female cells of the liver, and this could be accredited to gender variances in FABP1 (Lu *et al.*, 1998).

Current research shows gender dimorphism in the utilization of lipids from sex-isolated embryonic rat, primary cortical neuron cells under conditions of diet withdrawal to stimulate autophagy (Jensen, 2008). In these cells, diet withdrawal instigates the buildup of autophagy markers and more rapid cell death in neuronal cells obtained from men. In disparity, women cells are much more resilient to autophagy and death and are distinguished by the buildup of TG, FFA, and neutral lipid droplets in the cytoplasm. Fascinatingly, pretreatment of cells with L-carnitine boosts survival in both men and women cells and eliminates the gender dimorphism as feedback to diet restriction (Lu *et al.*, 1998).

Sex differences in absorption of cholesterol: A big differentiation in gender-associated variances in cholesterol absorption has been described between various mouse model, showing that similar observation can be expected in men and women subjects. Consumption of diets rich in cholesterol by CD-1 mice had a higher absorption rate of cholesterol in women-like compared to men-like mice (Smiderle *et al.*, 2014). Estrogen treatment and age has been shown in researches to meaningfully raise the absorption of cholesterol in men-like and women-like AKR and C57 mice whose gonad had been surgically removed (Henderson *et al.*, 2007). In backing the above study, a research utilized humans with the age ranging from 17-80 years, there was no significant difference in the absorption rate of cholesterol of both women and men when cholesterol was analyzed using dual stable isotopes analysis (Smiderle *et al.*, 2014).

2.4.3 Sex Dimorphism in Size, Body Composition and Fat Distribution

Sexual dimorphism in the circulation of fat are been backed-up by numerous research findings and are related to the metabolic health of the entire human systems. On a broader view in the whole lifetime, females do have a relatively bigger fat deposition

compared to males (Lovejoy *et al.,* 2008). Nevertheless, males frequently exhibit greater fat depot circulated in the VAT (‘android’ or ‘apple’ phenotype), that portrays a bigger risk for metabolic disorders (Karastergiou and Fried, 2012).

But, in females, SAT is in great abundance compared to VAT (Camhi *et al.,* 2011), with special emphasis on the lower body (‘gynoid’ or ‘pear’ phenotype) (Karastergiou and Fried, 2012). This SAT/VAT distribution of fats is being distorted in females that have reached menopause, which is physiologically accompanied with a decrease in serum estrogen level in circulation were shown to possess a greater amount of VAT compared to SAT (Lovejoy *et al.,* 2008). Females who have passed menopause from studies also exhibited larger VAT volume when related to females who have not reached menopause (Camhi *et al.,* 2011).

The increase in SAT in postmenopausal female compared to premenopausal female could be credited to the predisposition to accumulated fat in the body in the SAT depot which is more in premenopausal female, especially in the gluteal-femoral likewise and this has a direct correlation with decreased metabolic rate especially in the premenopausal women. Also, ladies possessed reduced waist circumferences and VAT when given hormone therapy compared to those without treatment (Munoz *et al.,* 2002). In totality, these works put forward that estrogen could control white adipose tissue dissemination by successfully decreasing VAT in man. Androgens could likewise possess depot- and sex-specific properties on fat dissemination. For example, VAT is elevated in males as testosterone reduces with ageing (Camhi *et al.,* 2011) and in females with polycystic ovary syndrome, which are usually described by the excessive circulation of androgen (Munoz *et al.,* 2002). Also, treatment with Testosterone in elderly males reduces VAT mass and elevates lean muscle mass (Camhi *et al.,* 2011).

In females, testosterone concentration had a direct relationship with a meaningfully increased VAT (Haffner *et al.,* 1991). Furthermore, very fat females that had past menopause and were on testosterone therapy were found to possess meaningfully elevated VAT (Lovejoy *et al.,* 1996). Depot- and gender-specific variations in the absorption of plasma free fatty acid have also been described. In totality, females accumulated more SAT than males in studies which showed that absorption of FFA from

consumed meal was higher in upper-body abdominal SAT compared to lower-body SAT in both lean and very fat males and females (Koutsari *et al.,* 2012).

A very important observation is that the rate of serum FFA absorption in these studies were analyzed for each mass of the whole fat mass. Thus, absorption could be seen as bigger in SAT as a result of its big adipose tissue size and but alike or reduced in VAT when calculated per cell. However, absorption of FA per gram of fat mass after consumption of food rich in fats was hugely elevated especially in the gluteal–femoral SAT of females in relations to males (Votruba *et al.,* 2006). Furthermore, from research findings femoral SAT of females with lower-body adiposity do accumulate more circulating FFA from consumed feed (Santos *et al.,* 2013) and these local variations were not seen in males, hence, the above works could only in part describe gender-dependent adiposity, since females characteristically possess lower-body fat (Pouliot *et al.,* 1991). In another view, studies show that absorption of consumed FFA in the VAT depot is bigger in lean males than females (Romanski *et al.,* 2000).

Also, consumption of diet rich in fats from studies showed FFA absorption bigger in VAT compared to SAT depot in very fat male and female subjects (Jensen *et al.,* 2008). Moreover, testosterone stimulates the absorption of FFA majorly into abdominal SAT and reduces the absorption in VAT after consumption of a meal in males (Marin *et al.,* 1995). Therefore, elderly males with reduced testosterone are prone to have increased VAT. From the aforementioned studies, in lean subjects, the direct absorption of serum FFA is greatly pronounced in abdominal SAT depot not just in that of males but in very fat subjects.

Also, FFA absorption is higher in the femoral depot especially of that of females (Shadid *et al.,* 2007). Further works such as that of Koutsari *et al.* (2012) have given more backings to earlier researches which found out that females possessed more abilities to store FFA in both femoral and abdominal SAT in the post-absorptive state compared to males (Koutsari *et al.,* 2012). Remarkably, the rate at which FFA was stored were meaningfully bigger in the femoral when related to the abdominal SAT in females, while the revised was observed in males. And the femoral SAT depot of females had higher FFA storage rate especially during the period when the individual is taking a walk when related to the abdominal SAT depot and each SAT depot in males (Koutsari *et al.,* 2012).

Dimorphism in statures basically results at the period of postpartum development, with masculine neonates being just one percent lengthier than feminine neonates at birth (Rodriguez *et al.,* 2005). During adolescence, gender variation in physique configuration are utmost, with women inclining in the direction of an hourglass body shape while men incline in the direction of an inverted triangle with broad shoulders and a narrow waist (Wells, 2000) but with a rise in age the gender variations diminish, mainly as a result of variations in the figure of women (Wells, 2000). While the figure of men remains moderately constant across ages, in females, weight moves uphill from the thigh to the waist and bust.

At birth up till early infancy, males do have greater weight than females and this could be due to the elevated lean mass but not adipose tissue size which weight are alike between the sexes (de Bruin *et al.,* 1996). Comparably, women tend to have more fat tissues and a little greater mean skinfold thickness compared to men at birth (Rodriguez *et al.,* 2005). Both genders exhibit comparably reduction in adipose tissue mass from the age of 1-6 years; after that, females gradually gain more fat while the males gain more lean mass (Wells, 2000). These opposing development arrangements deviate from the period of teenage years, with females accumulating a reasonable sum of adipose tissue mass when compared to the lean mass, while males exhibit contrary arrangement (Hattori *et al.,* 2004).

Men who have reached early adulthood do possess bigger bone mass as a result of huge body size even though males bone density looks almost the same to that of women, just as it was observed in earlier-life growth periods (Maynard *et al.,* 1998). Current research shows that sexual variations in bone mass appear at about 15–17 years, with men having greater size and strength of bones (Schoenau *et al.,* 2001). At birth the mineral mass of both genders looks the same (Foster e*t al.,* 2010) but as they reach the adult stage, the skeletal mass of males become higher (4 kg) compared to the 2.8 kg of females probably due to hormonal variations (Malina, 1996). Gender variation in total body configuration is not static all through adult stage. For example, men do exhibit lean mass up to the fifth decade and then gradually starts decreasing in muscle weight (Pichard *et al.,* 2000) as an outcome of disparities in hormone levels and functions.

There is also a reduction in the lean mass of women, the only difference is that the fat mass increases more in females when related to males (Kyle *et al.,* 2003) despite both having a relatively balance weight (Zamboni *et al.,* 2003). Although these alterations linger into old age but could be highly subjective to environmental control (Visser *et al.,* 2003). It is well known that those from the industrialized countries are more predisposed to weight gain while those from non-industrialized nations are predisposed to malnourishment due to the energy contents of their meals (Kyle *et al.,* 2003). Since the decrease in lean weight is comparatively general, the gain in weight as ageing sets in both genders could be due to the fat mass (Pichard *et al.,* 2000).

The rise in lean mass of men could be as a result of excessive muscle mass males possess, which from MRI analysis of healthy adults, the proportion of muscle mass to lean mass was 0.53 in men and 0.47 in women (Wang *et al.,* 2001), with this gender variation reducing with advance age. There is rising appreciation about the differences between men and women by means of reverence to the primary function, physique arrangement and vulnerability to and development of a wide-ranging diversity of non-infectious diseases, in addition to responses to pharmacological interventions.

Accumulated adipose mass in the body is an important forecaster of metabolic disease risk and metabolic abnormalities basically affect individuals with upper-extremities fat deposition when related to individuals with lower-extremities fat deposition (Jensen, 2008). The association between upper body adiposity obesity and diabetes was first described by Jean Vague (Vague, 1947). Ever since then, many works have established the association amongst hypertension, central adiposity and diabetes risk, sleep apnea, cancer, cardiovascular disease risk and events and overall mortality rates (Wang *et al.,* 2001). Healthy, mildly active/inactive females with BMIs of 20–25 kg/m^2 often have 25–35% body fat, while males with similar BMI have 10–20% body fat while highly athletic females and males do possess lower body adiposity (Jeukendrup and Gleeson, 2010).

2.4.4 Sexual Dimorphism and the Brain

Lipids predominantly occupy fifty percent and above of the dry mass of the brain and it is the second tissue after adipose tissues with reference to the lipid content (Coppack *et al.,* 1999) and with the exclusion of essential FAs which must be present in the feed,

other FAs could be produced via de-novo lipogenesis in the cells of the brain (Coppack *et al.,* 1999). The blood-brain barrier is the passageway through which FAs are transported from the blood to the brain (Bazinet and Laye, 2014).

Polyunsaturated fatty acids especially those with double bonds at the omega-3 and omega-6 positions, such as arachidonic (AA; 20:4u6), docosahexaenoic (DHA; 22:6u3) and FAs are the most abundant lipids in the brain (Bazinet and Laye, 2014). Research have revealed that the declining intake of omega-3-PUFAs and saturated long-chain FAs from feeds and variations in lipid metabolism have implicated in neuropsychiatric diseases including neurodegenerative diseases such as cognitive degeneration, Alzheimer's disease and neuro-inflammation (Kennedy *et al.,* 2009). Regrettably, feeds eaten by industrialized nations are mostly rich in saturated long-chain FAs but low in polyunsaturated fatty acid intake of meals from industrialized nations are usually linked to the progression of obesity, cognitive dysfunction and also cancer due to the high energy and fatty contents. (Kennedy *et al.,* 2009).

2.4.5 Sexual Dimorphism in Macrophage in response to High-Fat Diet

Manlike and womanlike mice added some weight and fat mass when fed with a 60% lard-based HFD chow, although male mice added more bodyweight when related to female mice, females possessed a normal glucose tolerance and reduced insulin levels when related to men-like mice. To comprehend the role of fatty tissue in inducing inflammation, inflammatory cytokines have been expressed in HFD fed models and the females were observed to have a decreased expression when related to males (Azevedo *et al.,* 2001).

Gender has effects on oxidative stress. Studies have shown that females do have lesser oxidative stress compared to males (Kennedy *et al.,* 2009) especially H_2O_2 Plasma levels of oxidative stress markers were higher in males when related to females (Bazinet and Laye, 2014). A gender variation in oxidative stress could be associated with sexual dimorphism in antioxidant systems, since male rats from studies possess a lower expression of heart SOD (Barp *et al.,* 2002) and reduced catalase and SOD actions in macrophages when related to female (Azevedo *et al.,* 2001). Surprisingly, male rats were shown from studies to have elevated concentration of GPx in the heart when related to

female rats (Barp *et al.,* 2002), even though no variation were noticed in the action of GPx in rat macrophages (Azevedo *et al.,* 2001).

2.4.6 Sexual Dimorphism and Exercise

Females have been shown from research to utilized more of lipids compared to carbohydrates than men and this has been very useful especially during exercise where the demands for energy is higher (Henderson *et al.,* 2007). Numerous works have also pointed to the earlier findings with men having elevated lipid oxidation than women thereby sparing carbohydrate after exercise during the absorptive state (Henderson *et al.,* 2007). Afterwards, many works have also shown that in the stage after absorption, the elevation in lipid oxidation as a result of long duration exercise was very pronounced in males compared to females (Bazinet and Laye, 2014).

The study further showed that post-exercise diet inhibited the sexual dimorphism observations during the postprandial state (Bazinet and Laye, 2014). From these studies, it could be said that sexual dimorphism solely relies on the eating pattern. Gender variation in the course of workouts (with females depending less on carbohydrate) might hypothetically elucidate sexual dimorphism in dietary energy selections after workout in males and females via the actions of glycogen diminution on lipid oxidation. As a result, care should be given when elucidating a likely mechanism for the sexually dimorphic pattern of post-exercise lipid oxidation, since females have greater metabolic efficiency compared to males (Henderson *et al.,* 2007).

2.4.7 Sexual Dimorphism in Lipolysis and Free Fatty Acid Mobilization

For lipids to be utilized by organs and tissues (such as adipose to muscle tissue), fatty acids in triglyceride must be freed by lipolysis. 3 FAs and 1 glycerol are discharged as a result of comprehensive lipolysis of a TG molecule. Nevertheless, the rate of appearance (Ra) of plasma FFA remains smaller than times 3 the Ra of glycerol (Coppack *et al.,* 1999). Consequently, FFA utilization is lower than the lipolytic rate, and this is thought to be a consequence of intracellular FA re-esterification in adipose tissue since this tissue know how to reprocess Fas nevertheless cannot exploit free glycerol for TG synthesis *in vivo* (Coppack et al., 1999). Glycerol Ra measurement is for lipolysis while FFA Ra characterizes the real utilization frequency of FFA for

dissemination amongst tissues through the use of stable isotope tracer (Hernandez *et al.,* 2011). Glycerol and FFA utilization are usually projected to go along the same way of variation in reaction to stimuli, while FFA utilization might be affected by a variation in the intracellular metabolism of FA after lipolytic stimulation. In reaction to food deprivation for longer period of days, lipolysis (Mittendorfer *et al.,* 2003) and FFA mobilization (Henderson *et al.,* 2007) are elevated. Lipolysis occurs over a period of 24 hours in situations where the individual does not eat any food (Mittendorfer *et al.,* 2003) and during exercises (Magkos and Mittendorfer, 2009).

However, it shows that lipolysis and related FFA utilization are quite reactive to the fuel availability and energy needs of the body. There was a reasonable increase in glycerol and FFA Ra for hours even after exercises in males compared to those without exercise (Henderson *et al.,* 2007). Further observations revealed that males could sustain these increase up to 24 hours after exercise (Magkos and Mittendorfer, 2009). But these rise in glycerol and FFA utilization after exercise were considerably higher in males compared to females even after carrying out the same exercise routine (Henderson *et al.,* 2007). The gender variation for inactive lipolysis after exercise was very remarkable in which males were seen possessing about 50% rise for hours after strenuous exercise, meanwhile females exhibited unequivocally no obvious rise in lipolysis rather they speedily re-established the resting lipolytic rate after exercise (Henderson *et al.,* 2007).

Couple of researches have revealed that plasma VLDL-TAG level is greatly elevated in males compared to females of the same age (Henderson *et al.,* 2007, Wang *et al.,* 2011). Elevated plasma TG levels are finer interpreters of cardiovascular disease risk compared to TC or LDL cholesterol in women due to their independent nature (Wang *et al.,* 2011), therefore in treating hypertriglyceridemia, more attention should be given to females compared to males. One of the limitations of those findings were excessive adipose mass compared to lean mass in females; therefore, it was difficult ascertaining the exact contribution of the excess fat to the lipolytic rate which could hugely affect the result (Wang *et al.,* 2011).

Interestedly, the reduced levels of triglyceride, LDL and VLDL elements in plasma in women in relation to men are shown to enhanced (instead of decreased) triglyceride, LDL and VLDL making; enriched plasma LDL, triglyceride and VLDL element

clearance returns for this and really leads to the total reduced steady-state levels (McLaughlin *et al.,* 2001). In divergence, the emission rate of VLDL apolipoprotein B-100 (VLDL particles) is reduced in females compared to males. Consequently, females produces smaller amount but (on average) triglyceride-richer VLDL compared to males (Magkos and Mittendorfer, 2009). The greater mean dimension of promising VLDL elements probably enables the elimination of triglyceride VLDL from the circulation by improving their vulnerability to hydrolysis by lipoprotein lipase (McLaughlin *et al.,* 2001).

The gender variation in HDL concentration is related with a higher HDL apolipoprotein A-I synthesis rate (minus variances in elimination rate) in females than in males and each with bigger HDL apolipoprotein A-II synthesis and plasma clearance rates (Magkos and Mittendorfer, 2009). A paucity of evidence exists with respect to the likely gender variances in cholesterol kinetics in the numerous lipoprotein segments in human subjects. Nevertheless, works with animal models give backing to the significant function of endogenous sex hormones in facilitating cholesterol metabolism in a gender variation manner (Santos *et al.,* 2013).

In situations where consumption of a diet has occurred, triglycerides move through apoB48-containing chylomicrons. In reaction to both mild and prolong consumption of diets rich in fats, females exhibit a greater clearance of diet-related triglycerides and elevated accumulation of those diets in subcutaneous gluteal adipose tissue, instead of abdominal adipose tissue, while accumulation is likely the same in abdominal versus subcutaneous adipose tissue in males (Santos *et al.,* 2013). The elevated triglycerides clearance by subcutaneous adipose tissue is further made noticeable by the adding of the carbohydrate-rich component to the feed in females (Santos *et al.,* 2013). Elevated serum triglycerides levels showed a relationship with an elevated risk of cardiovascular disease, a relationship which was greater in females in the Framingham Heart Study (Yost *et al.,* 1998).

Females usually exhibit a almost 10 years delay in foremost myocardial infarction relative to males, and this could hugely be as a result of hormonal properties of estrogens on cholesterol metabolism pre-menopausal period (Santos *et al.,* 2013). It was further observed that despite the reducing concentration of ovarian hormones with menopause,

females still exhibited reduced risk of cardiovascular disease in relation to males (Santos *et al.,* 2013). The occurrence of coronary heart disease with regards to 2011 – 2014 NHANES report is 19.7% in males and 11% in females 60 - 79 years old, and related drifts for elderly persons (Yost *et al.,* 1998). Rates of heart attack are a bit lower than half in the midst of females in this high-risk age group (Yost *et al.,* 1998).

Females before reaching menopause exhibit a reduced pro-atherogenic circulating lipid profiles compared to males (Magkos and Mittendorfer, 2009). Precisely, do possess higher HDL-c concentration (Magkos and Mittendorfer, 2009) and LDL-c, VLDL-c, circulating TG, and VLDL- triglyceride concentrations (Abbott *et al.,* 1983) during fasted and eating state together compare to males of the same age. Also, the concentration with the mean size of circulating VLDL are smaller (as a result of reduced levels of big and average size VLDL) (Magkos and Mittendorfer, 2009), whereas the concentration of LDL is smaller but the mean dimensions of LDL is larger (due to a swing in the direction of the bigger size at the detriment of the lesser LDL) (Magkos and Mittendorfer, 2009) in females related to males.

The level of flowing HDL is not dissimilar in males and females, but females exhibit a bigger HDL constituent part compared to males as a result of a swing to the direction of big and cholesterol-rich HDL (Vaidya *et al.,* 2008). These differences in plasma lipid concentrations and lipoprotein particle concentrations, subclass distributions, and sizes likely account for at least part of the cardio-protective effect of the female sex (Magkos and Mittendorfer, 2009).

Meal Fatty Acid Storage: After consumption of a meal and after absorption has taken place, there is no variation observed amongst males and females with regards to oxidation of FA but when exercise is going on, FA oxidation is greater in females (Votruba *et al.,* 2006). The means through which FA gotten from meals is accumulated in a way reflects the means through which adipose tissue is distributed in the body. Although, studies have shown no variation in the method through which FA gotten from the meal is accumulated in males and females as additional FAs derived from meal were accumulated (per gram lipid) in upper body SAT compared to the leg SAT in males and females (Votruba *et al.,* 2012).

But, after a diet rich in fats was eaten, females were observed to accumulate more quantity of fats derived from diet in lower body SAT while this was not observed in males (Votruba *et al.,* 2006). However, differences in local fat accumulations as a result of consumption of a meal were observed when FA accumulation as a result of consumption of a meal were related in males and females with unlike obesity phenotypes (Santos *et al.,* 2013). Females with lower-body obesity exhibited bigger accumulation of FA after meal in the gluteal than abdominal adiposity (Santos *et al.,* 2013), while accumulation was likely the same in the gluteal and abdominal adiposity in the upper body obese males and females. In addition, upper body obese males accumulated a smaller percentage of adipocytes as a result of consumed diets in subcutaneous adipocyte compared to either group of females (Santos *et al.,* 2013). From these findings, it could be said that FA accumulation due to the consumption of a diet could be very vital in influencing the gender variation in local fat storage.

FFA After a Meal: There is a remarkable reduction in plasma FFA level but the obtainability of plasma TG was elevated in the fed condition, demonstrating a comparative alteration in the obtainability of various shuttling patterns of FA according to Daskalopoulou *et al.* (2004). After consumption of a diet especially a diet rich in fats or even an assorted diet, there is an elevation of TG level in the blood for hours and this is referred to as postprandial lipemia. Also, as these events are on-going there is an elevation in plasma TG in the VLDL pool (liver TG release) furthermore to that in the chylomicron pool (small intestine TG release) (Park and Mun, 2013).

Fatty acids from a freshly consumed diet are quickly recycled immediately they enter into in chylomicrons in the VLDL units (Daskalopoulou *et al,* 2004). When TG is being transported after consumption of a meal, slight variations in TG level in the plasma could be metabolically suitable, and in situations of undue postprandial lipemia and could elevate CVD risks (Park and Mun, 2013); therefore, the control of the postprandial plasma TG excursion is very critical for wellbeing. If a little section of the exercise is done whether instantaneously before or even 24 hours before a diet can greatly give a negative reaction to postprandial lipemia (Daskalopoulou *et al,* 2004).

Moreover, postprandial lipemia is significantly reduced in females who have not reached menopause when compared to males (Henderson *et al.,* 2007), therefore, the essence of treating this area of metabolism is so insignificant in young, lean females compared to males. Studies have observed that VLDL-TG release rates are elevated in females when related to males (Magkos and Mittendorfer, 2009). On a common view, females are known to have a better regulating system compared to males. For instance, females have the ability to return back to euglycemia while males still stay in the condition of decreased blood glucose concentration for hours even after exercise (Henderson *et al.,* 2007).

The hepatic cells receive the FAs and bring them together into TGs, that is afterwards wrap up into TG-rich VLDL substance to be transported out of the hepatic cells. Fatness is linked to an elevated manufacturing of apoB-rich VLDL-TG substance by the hepatic cells to a larger extent in males compared to females (Mittendorfer *et al.,* 2003). Reduced VLDL-TG concentrations manufactured by the hepatic cells are somehow subordinate to the reduced FAs supplied to the hepatic cells as a result of improved FA clearance by muscle in females (Daskalopoulou *et al,* 2004).

It is worthy of noting that in reaction to FA supply to the hepatic cells that females release VLDL substance that is abundant in triglyceride (Hodson *et al.,* 2015), this could assist the hepatic cells transport triglycerides, thereby avoiding the hepatic cells storing too many fats which could result in fatness. Reacting to fatness, females exhibit elevated VLDL production mostly in situations where the fatness occurs in the abdomen region (Hodson *et al.,* 2015). In females, manufacturing of additional triglyceride-rich VLDL corresponded to the enhanced VLDL-TG clearance rates (Mittendorfer *et al.,* 2003), and in a long run could add to a lower hepatic cell fat and reduced plasma VLDL-TG concentrations in obese females.

It may be noted that only some studies propose a potent association amongst plasma FA concentrations and BMI and the most potent correlation amongst high FA concentrations and BMI occurs when there is elevation in BMI level. Females exhibit greater LPL actions compared to males, higher lipolysis in reaction to lipolytic inducement by fasting, however, there was a huge depression of lipolysis by insulin conditions were the subject has eaten (Hodson *et al.,* 2015). Mittendorfer *et al.* (2003), employed the use of

tracers for FFA flux, observed a greater similarity in the rate of appearance of FFA with greater fat accumulation in both men and women. Although elevated lipolysis connected with obesity, women do exhibit some form of resilient to FFA-induced insulin resistance.

In research models, infusion of lipid in the presence of heparin is a means of intensely elevating blood FA concentrations and known FA-mediated insulin resistance not depending on diet or mass variation amongst groups. Female rodents are resistant to FFA-induced insulin resistance and uphold regular insulin action in muscle and hepatic cells (Hevener *et al.,* 2002). Women in a way get sheltered from insulin resistance as a result of intramuscular lipid, and muscle is an essential facilitator of this sheltering (Frias *et al.,* 2001). But in males, this storage of intramuscular triglycerides is connected to insulin resistance and impaired glucose removal by muscle, while females moderately shielded from insulin resistance is connected with intramuscular triglyceride (Perreault *et al.,* 2010). Therefore, gender variation in FA management and intramuscular triglyceride metabolism correlates with gender variation in type-2 diabetes and risk for glucose tolerance.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Animals and Experimental Protocol

3.1.1 Study 1

The Animal Care and Use Research Ethics Committee of the University of Ibadan gave their express permission for this research work to be carried out UI-ACUREC/App/2015/070. Thirty male and thirty female Wistar rats of 28 days old weighing 40-50grams were utilized for this study. This was to enable the effective induction of obesity as observed from our pilot studies. In the course of this research work, rats were housed in a well-aerated cage and water and chow were given to them a *piacere.* The rats were acclimatized to the environment occurred for just 7days, this was closely followed by randomly distributing them into 3 identical groups, (each group having 10 rats), and administering chow for 17trgj weeks.

Group 1 (SC) were controlled males and females, which consumed normal rodent diet.
Group 2 (HPFD) were males and females, which consumed a diet rich in plant fats.
Group 3 (HAFD) were males and females, which consumed diet rich in animal fats.
The composition of each group's feed was as follow;

- SC group: Maize 50%, Soya bean 15%, Fish meal 5%, wheat offal 26%, Vitamin premix 0.5%, Oyster shell 1%, DCP 2% and Salt 0.5%.
- HPFD group: Maize 56%, Soya bean 15%, Fish meal 5%, Peanut oil 20%, Vitamin premix 0.5%, Oyster shell 1%, DCP 2% and Salt 0.5%.
- HAFD group: Corn Starch 30%, Powder milk 15%, Fish meal 5%, Sucrose 15%, wheat offal 5%, Lard 26%, Vitamin premix 0.5%, Oyster shell 1%, DCP 2% and Salt 0.5%.

Body Weight were observed and documented every seven days while feed intake was documented every 24 hours. The abdominal circumference (AC), thoracic circumference (ThC) and body length (BL) all got documented at the commencement

and conclusion of the bench-work. The rats were anaesthetized through the aid of sodium pentobarbital (50mg/kg body weight, within the peritoneal cavity), which enabled the measurement of variables such as ThC, BL and AC prior to dissection. This also facilitated the excavation and measuring/estimating brown adipose tissue mass (BAT), the white adipose tissue (WAT) mass, feed efficiency (FI) and energy intake (EI).

3.1.2 Study 2

After induction of obesity as described in study one. 20 obese (10 males and 10 females) were further separated into 2 equal groups and 10 control (5 male and 5 female) rats. These rats were randomly distributing into 3 identical groups, (each group having 10 rats) and consumed experimental diets for five (5) weeks.

Group 1 (SC) had controlled males and female which consumed normal rodent diet.
Group 2 (HFD) had male and female rats which consumed diets rich in plant fat.
Group 3 (LCD) had male and female rats which consumed low calorie diet, low in fat and high in fiber.
The composition each group's feed was as follow;

- SC group: Maize 50%, Soya bean 15%, Fish meal 5%, wheat offal 26%, Vitamin premix 0.5%, Oyster shell 1%, DCP 2% and Salt 0.5%.
- HFD group: Maize 56%, Soya bean 15%, Fish meal 5%, Peanut oil 20%, Vitamin premix 0.5%, Oyster shell 1%, DCP 2% and Salt 0.5%.
- LCD group: Maize 31%, Soya bean 15%, Fish meal 5%, Wheat offal 45%, Vitamin premix 0.5%, Oyster shell 1%, DCP 2% and Salt 0.5%.

Body Weight were observed and documented every seven days while feed intake was documented every 24 hours. The abdominal circumference (AC), thoracic circumference (ThC) and body length (BL) all got documented at the commencement and conclusion of the bench-work. The rats were anaesthetized through the aid of sodium pentobarbital (50mg/kg body weight, within the peritoneal cavity), which enabled the measurement of variables such as ThC, BL and AC prior to dissection. This also facilitated the excavation and measuring/estimating brown adipose tissue mass (BAT), the white adipose tissue (WAT) mass, feed efficiency (FI) and energy intake (EI).

3.2 Anthropometric, Adiposity and Nutritional Variables

3.2.1 Proximate Analysis and Energy Evaluation

Proximate analysis and energy analysis were done on the feeds. The gross energy (G.E) of the diets and faecal energy were evaluated by means of a Gallenkamp® Ballistic Bomb Calorimeter (Gallenkamp & Co Ltd, London, United Kingdom). Digestible Energy was estimated by subtracting the gross energy from the faecal energy. Proximate analysis of the feeds was analyzed through a chemical method in accordance with the procedure stated by the Association of Official Analytical Chemists (A.O.A.C., 18^{TH} Edition, 2005). Every investigation procedure was done in a replica. The metabolizable energy of the diets was calculated through the well-established Atwater system.

3.2.2 Anthropometric Variables

The AC (directly frontal to the forefoot), TC (directly posterior to the foreleg), BL (nostril-to-anal measurement) were measured in each group at the commencement and the termination of the 17 weeks. These measurements were done using a non-elastic rope and a meter rule. The measured body length and weight at the termination of the research bench-work were also used in estimating;

1.) Body mass index (BMI) = Body weight (g)/Body length^2 (cm^2).
2.) Mean Cumulative body weight gain = Entire buildup of BW in seventeen weeks (grams).

3.2.3 Adiposity Index

The total fatty tissue mass was excavated and measured. WAT or whole adipose mass was quantified as the totality of the subsequent separate fat pad mass: epididymal fat + retroperitoneal fat + visceral fat. Brown adipose tissue was quantified as the sum of fats in grams found behind the neck area usually referred to as the interscapular area. The adiposity index (AI) was calculated as (total body fat/final BW) ×100 (Taylor and Phillips, 1996). AI is employed as a means of determining the adiposity due to the fact that the level of fat is observed to rise progressively with the rate of fatness (Leopoldo *et al.*, 2015).

3.2.4 Nutritional Analysis

Diet being eaten were evaluated daily, and BW was examined once every seven days. With respect to feeding and energy consumption, the following nutritional variables below were evaluated (Diniz *et al.*, 2005):

a.) Feed intake (g/day) = Average amount of feed eaten in the course of the seventeen weeks

b.) Energy intake (kJ/day) = Average amount of feed eaten multiplied by the metabolizable energy (Novelli *et al.*, 2007)

c.) Feed efficiency (FE; %) = (average body mass gain (g) x 100)/energy intake (kcal)

3.3 Biochemical Analysis

3.3.1 Preparation of Serum and Tissue for Biochemical Assays

Plan capillary tube were used to collect 2mls of blood through the sinuses around the eye into a plan serum bottle and immediately centrifuged at 3000rmp for ten minutes. After centrifugation, separation of blood cells from the serum occurred and then freeze for further analysis. This was followed by excision of one (1) gram of tissue and rapidly washing of the excised tissue in a very chilled 1.15 percent KCl. The next step in the tissue preparation was blotting, weighing and mincing with a sharp cutter into a plan bottle containing 5mls of chilled 0.1M phosphate buffer with a pH of 7.4. This was accompanied closely with homogenization in a chilled environment via a Tefflon homogenizer. The homogenized tissue was immediately separated through a cold separator at 10, 000rmp at a heat of four degrees Celsius for ten minutes. The supernatant was gathered and used for biochemical analysis.

Homogenizing buffer (0.1M Phosphate buffer, pH 7.4): 35.822 g of Na_2HPO_4 (BDH Chemical Limited, England) and 15.603 g of Na_2HPO_4 were dissolved in nine hundred millilitres of purified H_2O. The acid-base meter was attuned to 7.4 and the remaining added up to one litre with purified H_2O.

Potassium Chloride (1.15%): Potassium Chloride (1.15 g) (BDH Chemical Limited, England) was thawed in purified H_2O which was filled to complete 1000mL and kept at four degrees Celsius.

3.3.2 Measurement of Malondialdehyde (MDA)

MDA could be produced as a result of an oxidative mediator that has the ability to modify the lipid anatomy thereby giving rise to lipid peroxidation which can be evaluated by analyzed the thiobarbituric acid reactive substances (TBARS) which is a by-product of lipid peroxidation.

Material: Kit were purchased from G-bioscience, United Kingdom; Spectrophotometer microplate reader capable of reading wavelength of 532nm; 96 well microtiter plate and a micropipette of different sizes. Preparation of solutions was done as stated in the manual from the purchased kits

Procedure

The standard and sample of about 100µL were mixed with 25µL of MDA reagent D respectively and incubate for half an hour at sixty-degree centigrade and immediately accompanied by the addition of 100µL of MDA reagent D and 50µL of MDA reagent C to each vial and centrifuge at 10, 000rpm for 10 minutes. With great precaution, 200µL of supernatant were discarded to uncontaminated the vial and 100µL of the already prepared working solution were added and incubated for an hour at ninety-degree centigrade. This was followed by cooling of the vials with ice to stop the reaction. Finally, about 150µL was then dispense into the ninety-six well micro-plate and the OD at 532nm was evaluated.

3.3.3 Catalase measurement

Catalase is a catalytic enzyme found in the blood and tissues with anti-oxidative abilities. It enables the transformation of hydrogen peroxide into H_2O and O_2.

Material: Kit were purchased from Bioquochem, United Kingdom; Spectrophotometer micro-plate reader capable of reading wavelength of 540nm; 96 well micro-titer plate and a micropipette of different sizes. Preparation of solutions was done as stated in the manual from the purchased kits

Procedure

The following reagents; 100µL of Reagent B; 30µL of Reagent C and 20µL of reagent E solutions were dispensed to every well and allow it run for twenty minutes. After that, 30µL of the stop solution (Reagent F) was added to all wells. This was immediately accompanied with the accumulation of 30µL of the chromogen solution (Reagent G) and allowing the reaction run for ten minutes. Finally, 20µL of reagent H was added and then run for five minutes and read at 540nm.

3.3.4 Measurement of Superoxide Dismutase (SOD)

SOD is an important part of the cells defence system against oxidation and it catalyzes the dismutation of superoxide anion to O_2 and H_2O_2.

Material: Kit were purchased from Cayman Chemicals, United States of America; Spectrophotometer micro-plate reader capable of reading wavelength of 440-460nm; 96 well micro-titer plate and a micropipette of different sizes. Preparation of solutions was done as stated in the manual from the purchased kits

Procedure

Serum was diluted in a ratio of 1:5 with the sample buffer before analyzing for SOD actions. About 200µL of diluted radical detector and 10µL of standard and sample were added to each well respectively. This was followed by the addition of 20µL of diluted xanthine oxidase to all the wells. This with shaking the ninety-six well plate for some seconds to mix and cover with the plate cover and then incubate on a shaker for thirty minutes at room temperature. Read absorbance at 440-460nm.

3.3.5 Reduced Glutathione (GSH) Measurement

GSH is a key tissue antioxidant that helps to lessen catalytically lipid hydroperoxides to their equivalent alcohol and hydrogen peroxide to H_2O.

Material: Kit were purchased from Oxford Biomedical Research, United Kingdom; Spectrophotometer micro-plate reader capable of reading wavelength of 412 or 405nm with kinetic reading capabilities; 96 well micro-titer plate and a micropipette of different sizes. Preparation of solutions was done as stated in the manual from the purchased kits

Procedure

About 50µL of full blood was added to the lowermost part of a micro-centrifuge tube and the sample was freezed at a temperature of minus seventy degree Celsius for a maximum of thirty days. The next step was thawing of the GSH sample (fifty microliters) and mixing. This was followed by the addition of 350µL of freeze five percent MPA to the micro-centrifuge tube and shaking for a short time and then spinning to separate at 1000rmp for four degrees Celsius for ten minutes. 25µL of the supernatant was added to 1.5mL assay buffer in a clean micro-centrifuge tube and frozen until required. 50µL of standard, test substance, blank, DTNB and Reductase solutions were added to their matching wells on the microplate. The solution was properly missed by putting the plate on an orbital shaker and incubating at room weather for five minutes. This was followed by addition of 50µL of NADPH solution into respective well and placing the plate in a kinetic plate reader and recording the variation in absorbance at 412nm by taking recordings each minute for ten minutes

3.3.6 Lipid Measurements (Triglyceride [TG], Total Cholesterol [TC], High Density Lipoprotein [HDL], Low Density Lipoprotein [LDL])

Materials: Kit were purchased from Abcam, United Kingdom. In addition to microplate reader with the ability to detect absorbance at five hundred and seventy nm for TG, TC and HDL; ninety-six well. For TC, HDL and LDL only (Double distilled water) TC only (Micro-centrifuge; Pipettes and pipette tips; Orbital shaker; Vacuum drier).

Assay Procedure

Triglyceride

Triglyceride standard was dissolved while TG probe was defrosted and the enzyme mix and lipase were prepared. Triglyceride standard dilution was being prepared in a ratio of two to ten nmol per well, likewise the TG in optimal dilutions in other for it to properly adapt to the standard curve readings. Plates were prepared in duplicates for standard and samples in a measured volume of fifty microliters respectively. Two microliters of Lipase were then added to the standard and sample wells. This was followed by blending and gestating for twenty minutes at RT. A master mix was then prepared for TG Reaction Mix and fifty microliters of the reaction cocktail added to the standard and sample. The plate was then gestated at RT for half an hour and read at five hundred and seventy nm.

Total Cholesterol, High-density Lipoprotein and Low-density Lipoprotein Measurements

Standard dilutions of about 50µL were added into the first well (Standard well). Then, fifty microliters of TC/HDL/LDL were added into subsequent wells and assay buffer was used to regulate the measurements to fifty microliters per well. Reaction cocktail for each reaction was prepared and a master cocktail of the reaction cocktail was also prepared to guarantee uniformity. 50µL of total cholesterol reaction cocktail was added into Standard wells and total cholesterol sample well. This was followed by a proper mixing and incubation at thirty-seven degrees Celsius for an hour in a room shielded from light. The plate was then read at 570nm.

3.3.7 Apolipoprotein A and B and Lipoprotein Lipase Measurements

Materials: Kit were purchased from Abcam, United Kingdom. In addition to microplate reader capable of measuring absorbance at 450 or 600 nm; Deionized water; PBS, pipettes; Tubes; Plate shaker.

Assay procedure

The samples or standard were added to their respective wells at a volume of 50µL respectively. This was accompanied by putting 50µL of the antibody mix to respectively well. The plates were cover and gestated for sixty minutes at 21 ºC on a plate shaker apparatus to four hundred rpm. This was followed by rinsing of every well repeatedly with wash Buffer. At the end of the final wash, the plates were overturned and blotted with a clean paper towel to eliminate surplus liquid. About 100µL of TMB substrate were added to every well and gestated for ten minutes in the dim on a plate shaker apparatus to four hundred rpm. This was accompanied by putting 100µL of Stop Solution to every well and agitating the plate on a plate shaker for sixty seconds to blend and it was then read at four hundred and fifty nm.

3.4 Reproductive Hormones Measurements

3.4.1 Follicular Stimulating Hormone (FSH) and Luteinizing Hormone (LH) Assay

Materials: Kit were purchased from Calbiotech Inc. USA for 96 Test. In addition to Distilled H_2O; pipettes and tips; plate reader able to detect absorbance at four hundred and fifty nanometers; paper towel and graph.

Assay Procedure

All reagents and substances for the assay were brought to an assume temperature of twenty-one degrees and were gently mixed prior to beginning the procedure. The required amount of coated strips was added into the holder. 25µl of LH or 50µl of FSH were added to the normal and test sera into designated wells and was accompanied by putting 100µL of enzyme Conjugate into every well. The plates were properly mix by placing on a plate mover at six hundred for half a minute, after which the plates were covered and incubated for an hour at 20-25°C. All excess liquid was eliminated in every well before the wells were rinsed thrice with 300µL of rinse buffer and was accompanied by putting 100µL of TMB substrate into every well and gestating for fifteen minutes at twenty-one degrees. After which 50µL of stop solution were added into every well and the plate mildly agitated to combine the substance. The plate was then read at an absorbance of four hundred and fifty nanometers.

3.4.2 Testosterone and Estradiol Hormonal Assay

Materials: Kit were purchased from Abcam, UK, In addition to **a** micro-plate analyzer that detects absorbance at 450 nm or 620 nm; Incubator at thirty-seven degrees Celsius; Pipettes; Spinning processor; Distilled H_2O; Tubes and Timer.

Assay procedure

About 25µL of standard solution and the analyzing substance were added to the individual wells and was accompanied by putting 100µL of Testosterone-HRP or 200µL of seventeen beta Estradiol-HRP Conjugate into every well except an empty well that was kept as substrate blank. Every well was properly sealed with the foil provided in the kits and gestated for one or two hours at thirty-seven degrees Celsius. After gestation, the foil was eliminated and extraction of the substances of the wells occurred before every well was rinsed thrice for five seconds with three hundred microliters of wash

buffer. The leftover liquid was then eliminated through decanting at the end of the third wash. The plate was upturn and blot counter to an uncontaminated paper towel to eliminate surplus fluid. About 100µL TMB reagent was then added to every well and gestated for thirty minutes at twenty-one degrees Celsius in a dim environment and was accompanied by putting 100µL of stop reagent following a similar protocol as in TMB reagent and agitating the micro-plate mildly. A blue colouration at the period of gestation changes to yellow. The plate was then read at an absorption of four hundred and fifty nanometers.

3.5 Fatty Acids Measurements

3.5.1 Free Fatty Acid Measurements

Materials: Kit were purchased from Abcam, United Kingdom. In addition to microplate reader able of reading absorbance at five hundred and seventy nm; MilliQ water; Pipettes; Reagents and buffer solutions; Tubes; 96 well plate with transparent plane base.

Assay procedure

About 50µL of Standard dilutions were added into the Standard well and two to fifty microliter of FFA were added into the remaining wells and the volume adjusted to fifty microliters per well. This was followed by the addition of two microliters of ACS Reagent into all standard and FFA wells. The next step was mixing and then gestation of the reaction for half an hour at thirty-seven degrees Celsius. About 50 µL reaction cocktail was prepared for each reaction. Also prepared was a master cocktail of the reaction cocktail to guarantee uniformity and was accompanied by putting 50µL of reaction cocktail to every well. The reaction was then incubated at thirty-seven degrees Celsius for half an hour sheltered from the brightness. This was followed by quantification instantaneously on a micro-plate reader at five hundred and seventy nm.

3.5.2 Fatty Acid Quantification

Tissue: Folch method is the most utilized tissue extraction method for fatty acid (*Folch et al., 1957*).

Fatty Acid Methyl Esters Preparation

The extracted sample (about 1 drop) of oil was drop into a screw-capped tube and an addition of 1ml of 0.5M methanolic potassium hydroxide was followed. The tube was cover tight with its cap and heated for 5 minutes in a water bath at 80 degrees, after which the tube was slightly cooled. The next step was the addition of 1ml BF_3 (14% in methanol) and then covering the tube tightly again and warming for ten minutes in a H_2O bath at 80 degrees. The next step was cooling of the tube slightly and then addition of 1ml of water followed by the addition of 1ml hexane. The cap of the tube was then tightly covered and vortex vigorously for 30 seconds and the tube contents allowed to settle to form layers, (centrifuge if necessary). The next step was remover of the hexane (top) layer into a tube containing a little quantity of sodium sulfate and swirl tube to eliminate any water from the solvent. Then hexane was transferred to GC vial for analysis.

Analysis

The FA profile of the lipid we are investigating was estimated via gas chromatograph Varian® (model 3800) equipped and the documentation of FAs was carried out with the aid of standard FAs in which the holding period of the FAs of the sample is associated with the holding period of the standards.

3.6 Structural Analysis

3.6.1 Histopathology

Tissue Processing: The tissues were observed and cut into little particles of nothing less than four mm dense into a pre-labelled molecule. This was followed by submerging into ten percent formalin saline for one day to fix through automatic tissue processor. This was followed with the tissues undergoing emersion with different reagents of a varying percentage of formalin and alcohol with the aim of dehydrating them. The tissues were further immersed in xylene of varying percentage with the aim of clearing, this was closely followed with the movement of the tissues into 3 wax baths for infiltration/impregnation.

Embedding, Microtomy, Floating, Drying and Staining: Every processed tissue was given a solid support medium (paraffin wax). The molten paraffin wax was dole out into a metal mould and the tissue was submerged and adapted to the already marked container

and later relocated to an ice plate to solidify. This was followed by disconnection of mould from the new tissue block. The staining technique used was Haematoxylin and Eosin technique.

Results

The nuclei will have a blue colouration while the cytoplasms will have a pink colouration (Avwioro, 2010)

3.6.2 Immunohistochemistry Technique

Reagent Preparation

Endogenous peroxidase blocking solution: nine millilitres of methanol and one millilitre of 30% H_2O_2

Phosphate Buffer (0.1 M pH 7.40): 17.418g of K_2HPO_4 and 13.601g of KH_2PO_4 were thawed in 900ml of purified H_2O, and pH fine-tuned to 7.4, and distilled H_2O was used to complete the remaining to one litre.

Preparation of DAB tablet: 1 tab of DAB was dissolved in 10 ml of PBS plus 1 ml of 30% H_2O_2

Preparation of wash buffer (0.1 M pH 7.4): 1 litre of PBS plus 0.5 ml Tween 20

Citrate buffer (0.01 M; pH 6.0): 2.1 g of citric acid monohydrate was dissolved in 10ml purified distilled H_2O to make solution A and 14.7 g of trisodium citrate dehydrate in 50 ml distilled water to make solution B. 9 ml of citric acid monohydrate solution (Solution B) was taken to make 50 ml. The mixture was then added to 430 ml of distilled H_2O and the pH fine-tuned to 6.0 and distilled H_2O used to makeup to 500 ml.

Preparation of Tissue for reading on the Microscope

The slides were labelled with Hb pencil and this was followed by dewaxing by submerging the slides twice in xylene for one hundred and twenty seconds both. The dewaxed slides were rehydrated with absolute ethanol, 90% ethanol and 80% ethanol for one hundred and twenty seconds each, followed by immersion in distilled H_2O for 300 seconds. The tissues were then incubated through an endogenous peroxidase blocking solution in the humidifying chamber at room temperature for ten minutes. Once

gestation was over, the slides were washed and positioned in a wash buffer tank for five minutes and washed again through with distilled H_2O. This was immediately followed with antigen retrieval in citrate buffer tank (boil) at a pH of 6.0 and later be left to calm down. The next step was washing of the slides again and placing them in a distilled H_2O tank for two minutes.

The serum applied on the slides was that of goat, followed by gestation in a dampening cavity for fifteen minutes. Then shaking was done to remove excess goat serum (please do not rinse). This was followed by incubation with the chief antibody of concern all-night in dampening cavity at room temperature. The slides were then rinsed using wash buffer and position in wash buffer tank for five minutes. This was followed by warming the dampening chamber to 21 degrees and then adding biotynated antibody to the slides and incubating in dampening chamber for half an hour. After which rinsing occured, followed by placing slides in a wash buffer tank for five minutes. Shaking of the slides followed, then incubation with Streptavidin-HRP for half an hour and then rinsing and placing slides in PBST tank for 5 minutes. This was followed by shaking of the slides and adding DAB for three minutes (look out for colour changes). Distilled water was then used to stop the reaction and then immersion of the slides in haematoxylin for 3 seconds and rinsing again with distilled water. The slides were then placed in 80% ethanol, 90% ethanol and absolute (100%) ethanol respectively for two minutes each and then in absolute xylene twice for two minutes each time. Finally, the moutant were placed on the slides and slides coverd and view under the microscope and snap.

3.7 Statistical Analysis

Values were expressed as mean ± SEM. The significance of the results for dietary groups was evaluated using analysis of variance (ANOVA) and the means were compared using Tukey-Kramer Multiple Comparison Test. $p<0.05$ was considered as statistically significant.

CHAPTER FOUR

RESULTS

STUDY ONE

4.1 Proximate Analysis of the 3 Feeds, Gross Energy and Metabolisable Energy of the 3 Feeds, Fecal Energy and Disgestable Energy of Wistar Rats in the 3 Diet Groups

No significant variation in the crude protein and fecal energy amongst the 3 formulated feed. Significant reduction in $p<0.05$ of crude fiber but a significant rise in $p<0.05$ of crude fat, gross, metabolizable and digestible energies in HPFD and HAFD when compared with SC. Finally, significant reduction in $p<0.05$ of moisture content, ASH and N.F.E only in the HAFD compared with SC. (table 4.1)

Table 4.1: Proximate analysis of the 3 feeds, Gross Energy and Metabolisable Energy of the 3 feeds, Fecal Energy and Disgestable Energy of Male and Female Rats in the 3 Diet Groups

Parameter	Groups		
	SC	HPFD	HAFD
Moisture (%)	9.28	9.39	5.03*
Crude Protein (%)	22.69	22.27	25.89
Crude Fat (%)	3.63	16.27*	30.96*
Crude Fiber (%)	8.64	3.64*	3.09*
ASH (%)	6.21	5.16	4.26*
NFE (%)	49.55	42.76	30.77*
Gross Energy (Kcal/g)	3.30	4.46*	5.90*
Metabolizable Energy (Kcal/g)	2.84	3.66*	4.62*
Fecal Energy Male (Female) Kcal/g	1.20(1.19)	1.20(1.20)	1.21(1.21)
Degestible Energy Male (Female) Kcal/g	2.10(2.10)	3.26(3.26) *	4.70(4.70) *

Values expressed as mean ± S.E.M of 2 samples where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. NFE = Nitrogen–free Extract or Carbohydrate portion. * = $p<0.05$ when HPFD and HAFD differ significantly compare with SC.

4.2 Effect of Sexual Dimorphism on Anthropometric Variables of Rats Fed with High Fat Diets

Significant decrease in $p<0.05$ of body length in HPFD of female group when compared to the HPFD of Males. (Table 2)

A significant rise in $p<0.05$ of thoracic circumference in HPFD and HAFD when compared to SC groups of both genders respectively. Also, significant rise in $p<0.05$ HPFD when related to HAFD groups in female rats. Finally, a significant reduction in $p<0.05$ Females when related with their males respectively. (Table 2)

Significant rise in $p<0.05$ of abdominal circumference in HPFD and HAFD when compared to SC respectively. Also, significant rise in $p<0.05$ HPFD when related to HAFD groups in both genders. Finally, there was a significant decrease in $p<0.05$ females when compared with their males in all groups respectively. (Table 2)

Finally, a significant increase in $p<0.05$ of final body weight in HPFD groups when compared with SC and HAFD groups respectively. There was a significant decrease in $p<0.05$ HPFD but a significant increase in $p<0.05$ HAFD of female rats when compared with that of their male counterpart respectively. (Table 2)

Table 4.2: Effects of Sexual Dimorphism on Anthropometric Variables of Rats Fed with High-Fat Diets

Group / Variable	SC	HPFD	HAFD
Body Length (cm)			
Male	21.0 ± 0.94	24.0 ± 0.3	22.0 ± 0.65
Female	21.0 ± 0.48	21.0 ± 0.12[a]	20.0 ± 0.34
Thoracic Circumference (cm)			
Male	5.7 ± 0.12	7.3 ± 0.29*	6.8 ± 0.21*
Female	4.3 ± 0.16*	5.8 ± 0.19[#, a]	4.9 ± 0.14[#, b, c]
Abdominal Circumference (cm)			
Male	7.0 ± 0.10	9.3 ± 0.22*	8.3 ± 0.09*[, a]
Female	6.4 ± 0.13*	7.9 ± 0.17[#, a]	6.6 ± 0.07 [#, b, c]
Initial Body Weight (cm)			
Male	44.0 ± 1.30	47.0 ± 1.00	58.0 ± 0.75
Female	43.0 ± 1.70	53.0 ± 1.40	42.0 ± 0.73
Final Body Weight (cm)			
Male	204.0 ± 3.90	266.0 ± 7.30*	185.0 ± 1.10 [a]
Female	202.0 ± 2.90	237.0 ± 1.20[#, a]	215.0 ± 1.70 [b, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); [a] $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); [b] $p<0.05$ Female (HAFD) compared with Female (HPFD); [c] $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.3 Effect of Sexual Dimorphism on Obesity Anthropometric indices of Rats Fed with High Fat Diets

A significant rise increase in $p<0.05$ of body mass index in HAFD and HPFD related with SC in both genders respectively. (Table 4.3)

A significant rise in $p<0.05$ of adiposity index in HAFD and HPFD related with SC in both genders respectively. A significant decrease in $p<0.05$ in HPFD when related to HAFD in males. Significant rise in $p<0.05$ of female (HAFD and HPFD) related with their male counterparts. (Table 4.3)

A significant rise in $p<0.05$ white adipose tissue in HAFD and HPFD related with SC in both genders respectively. A significant rise in $p<0.05$ HPFD when related to HAFD in female rats. A significant increase in $p<0.05$ female HPFD when compared to their male counterpart. (Table 4.3)

A significant rise in $p<0.05$ of female (HAFD and HPFD) and in male HAFD related with SC respectively. A significant reduction in $p<0.05$ HPFD when related to HAFD respectively. A significant increase in $p<0.05$ female HPFD when compared to Male HPFD but a reduction in $p<0.05$ Female HAFD when related to male HAFD respectively. (Table 4.3)

Table 4.3: Effect of Sexual Dimorphism on Obesity Anthropometric indices of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
Body Mass Index (g/cm^2)			
Male	8.6 ± 0.22	12 .0± 0.42*	11.0 ± 0.39*
Female	8.5 ± 0.16	11.0 ± 0.37$^{\#}$	11.0 ± 0.35$^{\#}$
Adiposity Index (%)			
Male	1.7 ± 0.13	3.2 ± 0.12*	3.8 ± 0.14*$^{, a}$
Female	1.7 ± 0.1	4.9 ± 0.14$^{\#, a}$	5.3 ± 0.18$^{\#, c}$
White Adipose Tissue (g)			
Male	2.9 ± 0.12	8.9 ± 0.67*	9.5 ± 0.56*
Female	3.3 ± 0.26	14.0 ± 0.75$^{\#, a}$	9.7 ± 0.72$^{\#, b}$
Brown Adipose Tissue (g)			
Male	0.6 ± 0.02	0.6 ± 0.05	1.4±0.025*$^{, a}$
Female	0.6 ± 0.06	0.8 ± 0.04$^{\#, a}$	1.2 ± 0.03$^{\#, b, c}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.4 Effect of Sexual Dimorphism on Feed indices of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ initial feed intake in male HPFD but a reduction in $p<0.05$ in female HAFD when related with their SC counterpart respectively. A significant rise in $p<0.05$ HPFD compared to HAFD in both genders respectively. Finally, a significant reduction in $p<0.05$ SC, HPFD and HAFD of female rats related with SC, HPFD and HAFD of female rats respectively. (table 4.4)

A significant rise in $p<0.05$ final feed intake in female HPFD but a reduction in $p<0.05$ male HPFD when related with their SC counterpart. Also, a significant reduction in $p<0.05$ HAFD when compared with SC in both genders. A significant rise in $p<0.05$ HPFD related with HAFD in both genders. Finally, a significant reduction in $p<0.05$ SC, HPFD and HAFD of females related with SC, HPFD and HAFD of males respectively. (table 4.4)

A significant increase in $p<0.05$ Energy intake in HPFD and HAFD related with SC in both genders respectively. Finally, a significant reduction in $p<0.05$ SC, HPFD and HAFD of females related with SC, HPFD and HAFD of male respectively. (table 4.4)

A significant decrease in $p<0.05$ Feed efficiency of HAFD when compared with SC and HPFD of male rats respectively. Also, a significant rise in $p<0.05$ SC and HAFD of female rats related with SC and HAFD of male rats respectively. (table 4.4)

Table 4.4: Effect of Sexual Dimorphism on Feed indices of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
Initial Feed Intake			
Male	49.0 ± 0.80	68.0 ± 0.70*	53.0 ± 0.72 [a]
Female	41.0 ± 0.75*	42.0 ± 0.40 [a]	31.0 ± 1.1[#, b, c]
Final Feed Intake			
Male	102.0 ± 0.59	96.0 ± 0.65*	74.0 ± 0.93*[, a]
Female	69.0 ± 0.71*	75.0 ± 0.23[#, a]	53.0 ± 0.4[#, b, c]
Energy intake			
Male	1006.0 ± 41	1409.0 ± 45.00*	1447.0 ± 38.00*
Female	816.0 ± 31*	1068.0 ± 42.00[#, a]	972.0 ± 33.00[#, c]
Feed Efficiency			
Male	31.0 ± 4.20	33.0 ± 4.20	21.0 ± 2.60*[, a]
Female	41.0 ± 5.90*	36.0 ± 4.70	38.0 ± 5.20[c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); [a] $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); [b] $p<0.05$ Female (HAFD) compared with Female (HPFD); [c] $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.5 Effect of Sexual Dimorphism on the abdominal region of Rats Fed with High Fat Diets

A significant rise in the deposition of abdominal fat in the HAFD and HPFD rats in comparison with SC of both male and female respectively. (plate 4.1)

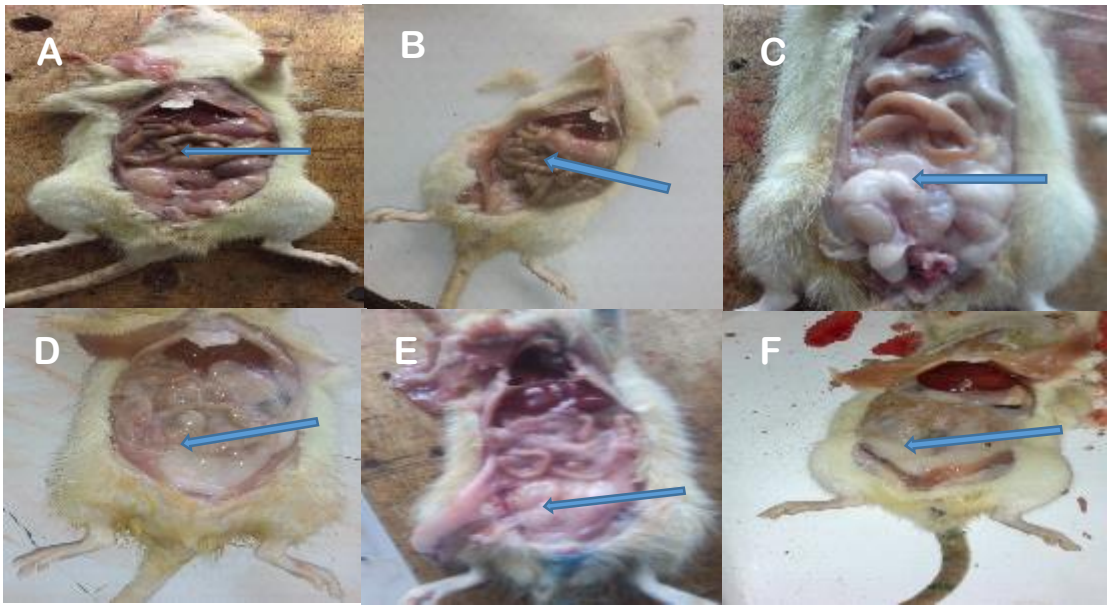


Plate 4.1: Pictorial view of the abdominal region after 17 weeks feeding

Values expressed in mean ± S.E.M of 5 rats. (A) SC (male), (B) SC (female), (C) HAFD male, (D) HAFD female, (E) HPFD male, (F) HPFD female. Where SC = Standard Chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. Arrows show areas where fat deposition could be seen.

4.6 Effect of Sexual Dimorphism on Serum Lipid profile of Rats Fed with High Fat Diets

A significant increase in $p<0.05$ total cholesterol concentration of HPFD and HAFD related with SC in both genders. Also, a significant reduction in $p<0.05$ SC but a significant rise in $p<0.05$ HPFD and HAFD of female rats related with their male counterparts respectively. (table 4.5)

A significant increase in $p<0.05$ triglyceride concentration in HPFD and HAFD related with SC in both genders respectively. A significant rise in $p<0.05$ SC, HPFD and HAFD of female rats when compared with SC, HPFD and HAFD of male. (table 4.5)

A significant increase in $p<0.05$ high density lipoprotein concentration in HPFD and HAFD when compared with SC of both genders respectively. Also, a reduction in $p<0.05$ HAFD when related with HPFD of female rats. A significant rise in $p<0.05$ HPFD of female when related with HPFD of males. (table 4.5)

A significant increase in $p<0.05$ low density lipoprotein concentration in HPFD and HAFD groups when compared with SC of both genders respectively. Also, a significant rise in $p<0.05$ HAFD groups when compared with HPFD groups of both genders. (table 4.5)

Table 4.5: Effect of Sexual Dimorphism on Serum Lipid profile of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
	TOTAL CHOLESTEROL (mg/dl)		
Male	96.0 ± 10.00	193.0 ±9.70*	191.0 ± 3.90*
Female	63.0 ±6.20*	260.0 ±19.00$^{\#, a}$	251.0 ±17.00$^{\#, c}$
	TRIGLYCERIDE (mg/dl)		
Male	72.0 ± 9.00	193.0 ±19.00*	181.0 ±16.00*
Female	102.0 ±9.90*	248.0 ±14.00$^{\#, a}$	230.0 ±12.00$^{\#, c}$
	HIGH DENSITY LIPOPROTEIN (mg/dl)		
Male	45.0 ± 1.90	16.0 ± 0.90*	17.0 ± 0.50*
Female	41.0 ± 1.40	23.0 ±1.40$^{\#, a}$	19.0 ±0.54$^{\#, b}$
	LOW DENSITY LIPOPROTEIN (mg/dl)		
Male	20.0 ±0.60	27.0 ±0.80*	35.0 ±2.00*$^{, a}$
Female	17.0 ±1.50	28.0 ±1.80$^{\#}$	40.0 ±1.50$^{\#, b}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.7 Effect of Sexual Dimorphism on Serum Cardiometabolic risk variables of Rats Fed with High Fat Diets

Aa significant rise in $p<0.05$ Apolipoprotein A concentration of HAFD related with SC in both genders. Also, there was a significant reduction in $p<0.05$ HPFD when related with SC of females. Finally, a decrease in $p<0.05$ HPFD of female rats when related with HPFD of males. (table 4.6)

A significant rise in $p<0.05$ Apolipoprotein B concentration of HPFD and HAFD when compared with SC in both genders respectively. (table 4.6)

A significant rise in $p<0.05$ Apolipoprotein B/Apolipoprotein A ratio of HPFD and HAFD when compared with SC in both genders respectively. Also, but a significant reduction in $p<0.05$ female rats when HPFD was related with HAFD was observed. Significant rise in $p<0.05$ HAFD of female rats when compared with HAFD of males. (table 4.6)

Significant rise in $p<0.05$ Lipoprotein Lipase concentration of male HAFD but a decrease in female HAFD when related with their HPFD counterpart respectively. Also, a significant rise in $p<0.05$ HPFD when compared with SC in female rats. Finally, a significant reduction in $p<0.05$ SC and HAFD of female rats when compared to SC and HAFD of male rats respectively. (table 4.6)

Table 4.6: Effect of Sexual Dimorphism on Serum Cardiometabolic risk variables of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
	Apolipoprotein A (mg/dl)		
Male	5.1 ± 0.20	4.6 ± 0.12	4.3 ± 0.19*
Female	4.6 ± 0.13	4.1 ± 0.09$^{\#}$	4.0 ± 0.14$^{\#, c}$
	Apolipoprotein B (mg/dl)		
Male	10.0 ± 0.23	12.0 ± 0.19*	13.0 ± 0.29*
Female	10.0 ± 0.34	13.0 ± 1.60$^{\#}$	12.0 ± 0.61$^{\#}$
	ApolipoproteinB/ApolipoproteinA (ratio)		
Male	2.2 ± 0.06	3.0 ± 0.13*	2.8 ± 0.07*
Female	2.1 ± 0.12	2.9 ± 0.13$^{\#}$	3.5 ± 0.46$^{\#, b, c}$
	Lipoprotein Lipase (mg/dl)		
Male	27.0 ±13.00	25.0 ± 1.30	31.0 ±1.30^{a}
Female	19.0 ±2.20*	26.0 ±1.40$^{\#}$	21.0 ± 1.40$^{b, c}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.8 Effect of Sexual Dimorphism on Serum Free Fatty Acid Concentration of Rats Fed with High Fat Diets

A significant increase in $p<0.05$ Free Fatty Acid concentration of HPFD and HAFD when compared with SC in both genders respectively. Also, significant reduction in $p<0.05$ male rats was perceived when HPFD was compared with HAFD. A significant reduction in $p<0.05$ HPFD and HAFD of females when compared with HPFD and HAFD of male rats. (figure 4.1)

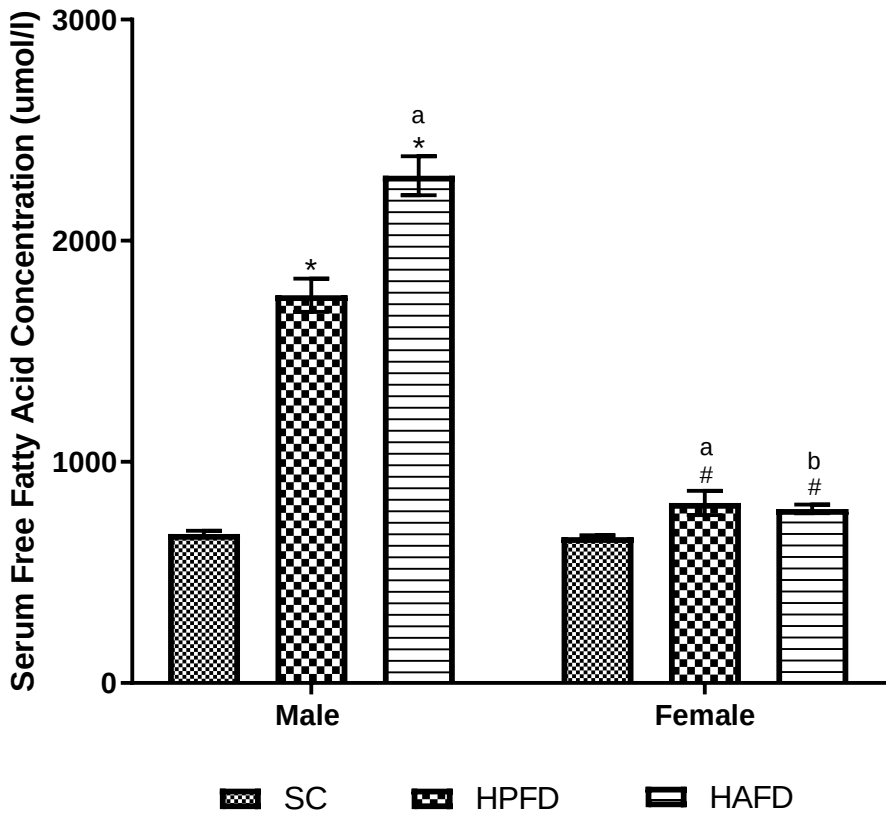


Figure 4.1: Serum Free Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.9 Effect of Sexual Dimorphism on Reproductive Hormones Concentrations of Rats Fed with High Fat Diets

A significant elevated in $p<0.05$ luteinizing hormone in HAFD when related to the HPFD group in males. A reduction in $p<0.05$ HAFD of females when compared with HAFD of male rats. (table 4.7)

In Follicular stimulating hormone, a reduction in $p<0.05$ HAFD of females when related with male HAFD of male rats. (table 4.7)

A significant increase in $p<0.05$ estrogen concentration in HAFD and HPFD related with SC in female rats. (table 4.7)

A significant decrease in $p<0.05$ Testosterone concentration in HAFD and HPFD when related with SC in male rats. (table 4.7)

Table 4.7: Effect of Sexual Dimorphism on Reproductive Hormones Concentrations of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
	Luteinizing hormone (µlU/ml)		
Male	3.5 ± 0.14	4.0 ± 0.20	3.5 ± 0.09[a]
Female	3.6 ± 0.08	3.5 ± 0.09	3.4 ± 0.08[c]
	Follicular stimulating hormone (µlU/ml)		
Male	1.5 ± 0.12	1.6 ± 0.13	1.6 ± 0.10
Female	1.3 ± 0.07	1.3 ± 0.08	1.3 ± 0.09[c]
	Estrogen (pg/ml)		
Male	0.00	0.00	0.00
Female	15.0 ± 0.91	22.0 ± 1.10[#]	22.0 ± 0.90[#]
	Testosterone (ng/ml)		
Male	15.0 ± 0.80	7.2 ± 0.50*	7.6 ± 0.53*
Female	1.5 ± 0.20*	0.6 ± 0.05[#, a]	0.5 ± 0.06[#, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); [a] $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); [c] $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.10 Effect of Sexual Dimorphism on Serum Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

A significant reduction in $p<0.05$ catalases in HPFD and HAFD when compared to SC of female rats. Also, a significant decrease in $p<0.05$ HPFD and HAFD of female rats when compared with HPFD and HAFD was observed in males. (table 4.8)

A significant reduction in $p<0.05$ reduced Glutathione level of SC, HPFD and HAFD of females when compared with their male counterpart respectively. (table 4.8)

A significant rise in $p<0.05$ Malondialdehyde concentration of HPFD and HAFD related with SC in both genders. Also, a significant rise in $p<0.05$ HAFD related with HPFD in male rats. A significant increase in $p<0.05$ SC female rats when related to SC males but a significant reduction in $p<0.05$ female HAFD related with HAFD of males was observed. (table 4.8)

A significant decrease in $p<0.05$ Superoxide Dismutase value of HPFD and HAFD related with SC and also in HAFD related with HPFD in male rats. A significant rise in $p<0.05$ HPFD when related to SC and HAFD in females. A significant reduction in $p<0.05$ female SC related with male SC but a significant rise in $p<0.05$ HPFD and HAFD of female rats related with HPFD and HAFD of male rats. (table 4.8)

Table 4.8: Effect of Sexual Dimorphism on Serum Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

Group / Variables	SC	HPFD	HAFD
	Catalase (u/ml/mins)		
Male	24±1.0	23±0.9	23±1.4
Female	21±1.0	16±0.7$^{\#, a}$	17±1.0$^{\#, c}$
	reduced Glutathione (mM)		
Male	0.6±0.06	0.6±0.05	0.6±0.03
Female	0.04±0.001*	0.04±0.002^{a}	0.05±0.003^{c}
	Malondialdehyde (mM)		
Male	11±0.2	26±0.6*	46±1.0*$^{, a}$
Female	15±1.0*	26±1.0$^{\#}$	24±1.0$^{\#, c}$
	Superoxide Dismutase (u/ml)		
Male	42±0.7	34±1.4*	29±0.9*$^{, a}$
Female	36±0.8*	39±1.0$^{\#, a}$	36±0.7$^{b, c}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.11 Effect of Sexual Dimorphism on Anthropometric variables of the Liver of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ weight of HPFD and HAFD when compared with SC in both genders. A significant rise in $p<0.05$ HPDF related with HAFD in males. Finally, a significant in $p<0.05$ SC and HAFD of female rats related with SC and HAFD of male rats. (table 4.9)

Table 4.9: Effect of Sexual Dimorphism on Anthropometric Variables of the Liver of Rats Fed with High Fat Diets

Group / Tissue	SC	HPFD	HAFD
Male	5.4±0.16	11±0.52*	7.7±0.13*, a
Female	7.8±0.29*	11±0.43#	11±0.37#, c

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); # $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); a $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); c $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.12 Effect of Sexual Dimorphism on Liver Lipid Profile of Rats Fed with High Fat Diets

In total cholesterol concentration, a significant rise in $p<0.05$ was observed in HPFD and HAFD when compared with SC in both genders respectively. There was a significant increase in $p<0.05$ in HAFD when compared with HPFD of female rats. (table 4.10)

In triglyceride concentration, a significant in $p<0.05$ was noticed in HPFD and HAFD when related with SC respectively. A significant reduction in $p<0.05$ in HAFD when compared with HPFD of male rats. Finally, a significant rise in $p<0.05$ in HPFD and HAFD of female rats when related with HPFD and HAFD of male rats. (table 4.10)

In high density lipoprotein concentration, a significant reduction in $p<0.05$ was observed in HPFD and HAFD when compared with SC in both genders respectively. A significant rise in $p<0.05$ in SC and HPFD of female rats when related with SC and HPFD of male rats. (table 4.10)

In low density lipoprotein concentration, a significant rise in $p<0.05$ was observed in HPFD and HAFD when compared with SC in both genders. A significant decrease in $p<0.05$ was noticed in HAFD when related with HPFD in males respectively. A significant reduction in $p<0.05$ was observed in SC, HPFD and HAFD of female rats when compared with SC, HPFD and HAFD of male rats. (table 4.10)

Table 4.10: Effect of Sexual Dimorphism on Liver Lipid Profile of Rats Fed with High Fat Diets

Groups / Variables	SC	HPFD	HAFD
	Total Cholesterol (mg/dl)		
Male	66.0 ±6.30	152.0 ±16.00*	187.0 ±16.00*
Female	51.0 ±4.70	114.0 ±3.60$^{\#}$	178.0 ±13.00$^{\#, b}$
	Triglyceride (mg/dl)		
Male	129.0 ± 8.50	377.0 ±21.00*	275.0 ± 13.00*$^{, a}$
Female	136.0 ± 9.40	322.0 ±11.00$^{\#, a}$	342.0 ± 8.50$^{\#, c}$
	High Density Lipoprotein (mg/dl)		
Male	22.0 ±1.50	13.0 ± 0.80*	16.0 ± 0.70*
Female	35.0 ± 1.00*	20.0 ± 1.70$^{\#, a}$	19.0 ± 1.20$^{\#}$
	Low Density Lipoprotein (mg/dl)		
Male	23.0 ± 1.90	89.0 ± 2.30*	78.0 ± 2.20*$^{, a}$
Female	16.0 ±2.10*	48.0 ±2.10$^{\#, a}$	44.0 ±1.80$^{\#, c}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.13 Effect of Sexual Dimorphism on Liver Homogenate Free Fatty Acid Concentration of Rats Fed with High Fat Diets

A significant increase in $p<0.05$ HPFD and HAFD when compared with SC in both genders respectively. There was a significant reduction in $p<0.05$ HAFD when compared to HPFD in female rats. A significant reduction in $p<0.05$ SC of females when related with SC of males but an increase in HPFD and HAFD of females when related with HPFD and HAFD of male rats respectively. (figure 4.2)

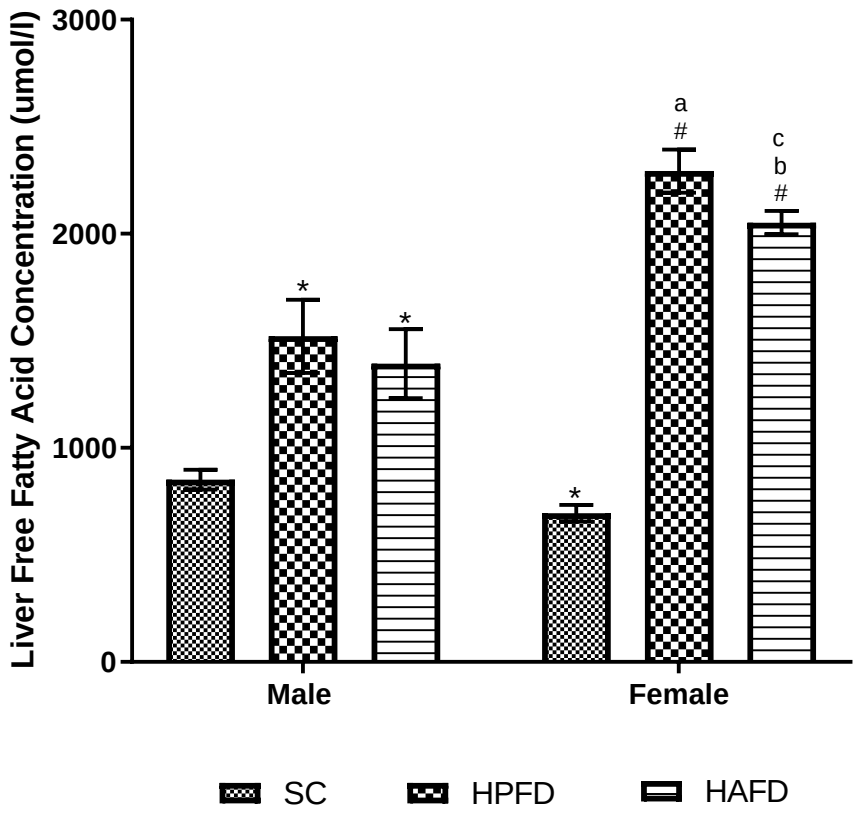


Figure 4.2: Liver Homogenate Free Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.14 Effect of Sexual Dimorphism on Liver Homogenate Saturated Fatty Acid Concentration of Rats Fed with High Fat Diets

There was a significant increase in $p<0.05$ HAFD when compared with SC in males. A significant reduction in $p<0.05$ HAFD of female rats when compared with the HAFD of male rats. (figure 4.3)

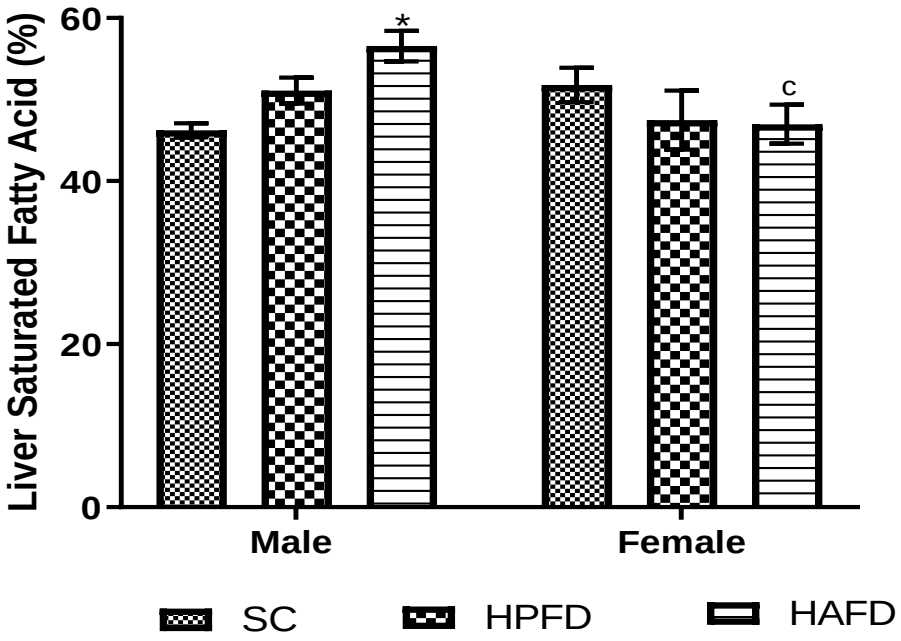


Figure 4.3: Liver Homogenate Saturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.15 Effect of Sexual Dimorphism on Liver Homogenate Monounsaturated Fatty Acid Concentration of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ HPFD and HAFD when compared with SC of female rats respectively. A significant rise in $p<0.05$ HPFD and HAFD of female rats when compared with HPFD and HAFD of male rats. (figure 4.4)

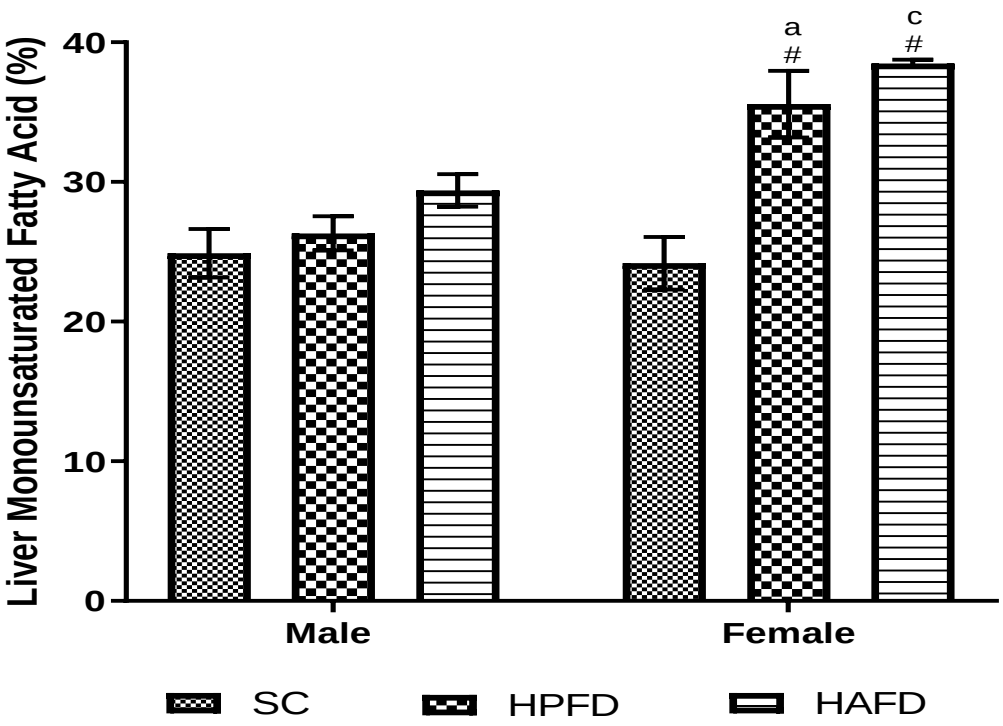


Figure 4.4: Liver Homogenate Monounsaturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.16 Effect of Sexual Dimorphism on Liver Homogenate Polyunsaturated Fatty Acid Concentration of Rats Fed with High Fat Diets

There was a significant decrease in $p<0.05$ HAFD related with SC and HPFD in male rats respectively. There was also a significant decrease in $p<0.05$ HPFD and HAFD when related with SC of female rats respectively. (figure 4.5)

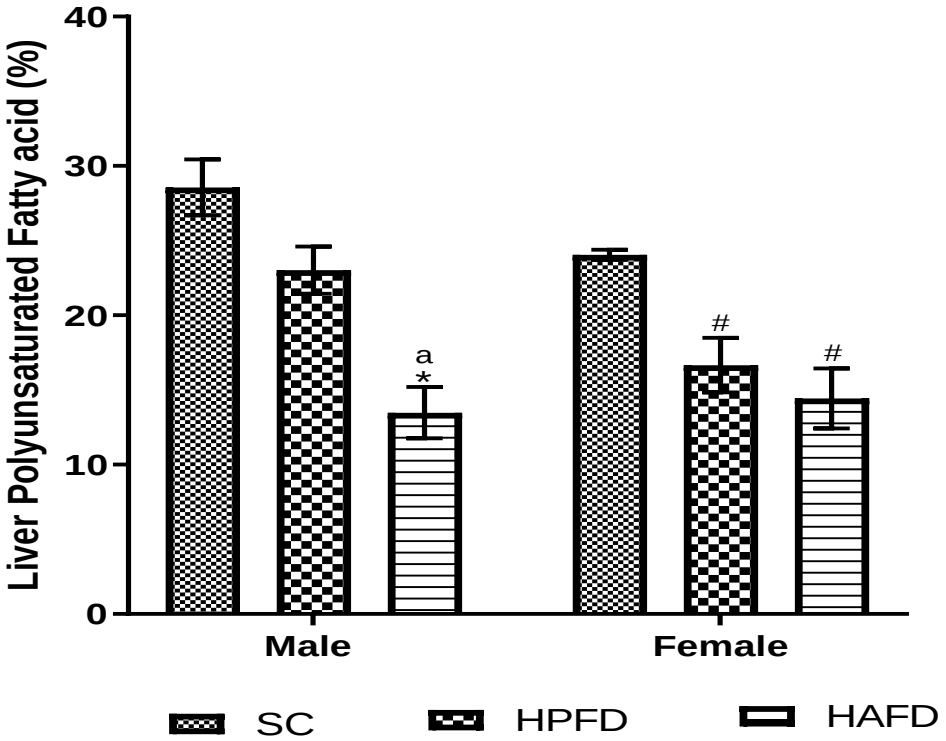


Figure 4.5: Liver Homogenate Polyunsaturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD);

4.17 Effect of Sexual Dimorphism on Liver Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

No significant variation amongst the sets in Liver Catalase was observed. (table 4.11)

In Malondialdehyde concentration, a significant increase in $p<0.05$ HPFD and HAFD related with SC in both genders and also a rise in $p<0.05$ HAFD related with HPFD in male rats were observed. Also, there was significant rise in $p<0.05$ HAFD when related with SC and also a rise in $p<0.05$ HAFD when related to HPFD in female rats. Finally, a significant increase in $p<0.05$ was observed in SC of female rats when compared to SC of males but a significant reduction in $p<0.05$ was noticed in female HPFD and HAFD when compared to HPFD and HPFD of male rats respectively. (table 4.11)

In reduced Glutathione concentration, significantly decreased in $p<0.05$ was noticed in HPFD and HAFD when related with SC in both genders respectively. Also, significantly rise in $p<0.05$ in HPFD when compared to HAFD in females. Finally, significantly rise in $p<0.05$ was observed in SC, HPFD and HAFD of female rats when compared with their male rats respectively. (table 4.11)

In Superoxide Dismutase concentration, significant reduction in $p<0.05$ was noticed in HPFD and HAFD when compared with SC respectively in female rats. (table 4.11)

Table 4.11: Effect of Sexual Dimorphism on Liver Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
	Catalase (µ/ml/mins)		
Male	24.0 ± 0.07	25.0 ± 0.12	24.0 ± 0.35
Female	25.0 ± 0.05	24.0 ± 0.08	25.0 ± 0.06
	reduced Glutathione (mM)		
Male	0.1±0.01	0.1±0.01*	0.11±0.01*
Female	0.5±0.04*	0.4±0.02$^{\#, a}$	0.27±0.02$^{\#, b, c}$
	Malondialdehyde (mM)		
Male	19.0 ± 0.70	43.0 ± 1.40*	48.0 ± 0.60*$^{, a}$
Female	26.0 ± 1.50*	30.0 ± 1.10^{a}	34.0 ± 1.30$^{\#, b. c}$
	Superoxide Dismutase (µ/ml)		
Male	44.0 ± 1.20	42.0 ±1.00	42.0 ±1.10
Female	48.0 ±1.00	40.0 ±0.80$^{\#}$	38.0 ±1.40$^{\#}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.18 Effect of Sexual Dimorphism on Histology of the Liver of Rats Fed with High Fat Diets

The black arrows point to the different levels of fat deposition on the Photomicrograph of a liver stained by H & E staining. The black arrow showed a huge deposition of fats in the HPFD and HAFD groups of both male and female rats. (plate 4.2)

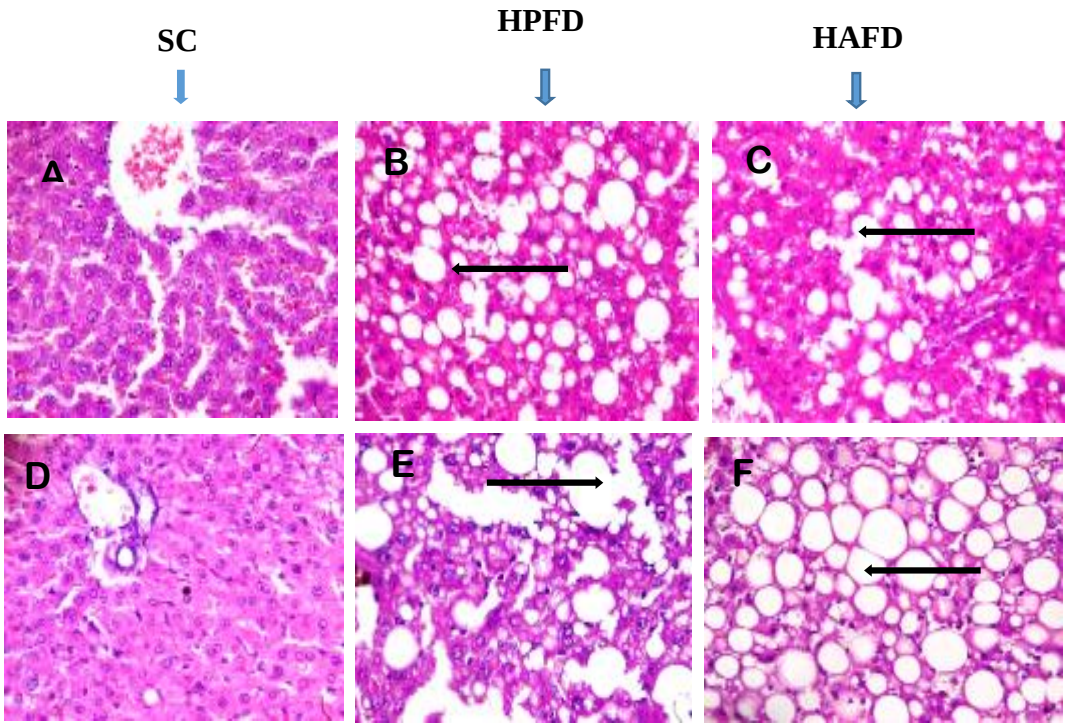


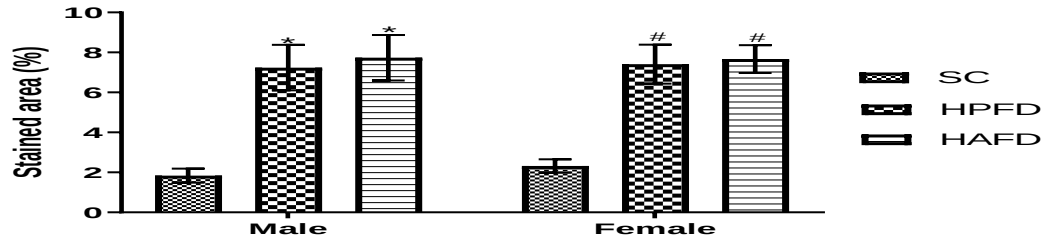
Plate 4.2: Histology of the Liver section stained by Haematoxylin and Eosin at X400

(A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver. Where SC = Control Chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. Arrows show areas where either fat deposition or congestion could be seen.

4.19 Effect of Sexual Dimorphism on Fatty Acid Transporter Protein 4 (FATP4) Expression in Liver Tissue of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in the expression of FATP4 in Liver tissue of HPFD and HAFD groups when compared with SC in both genders respectively. (figure 4.6 A and B)

(A)



(B)

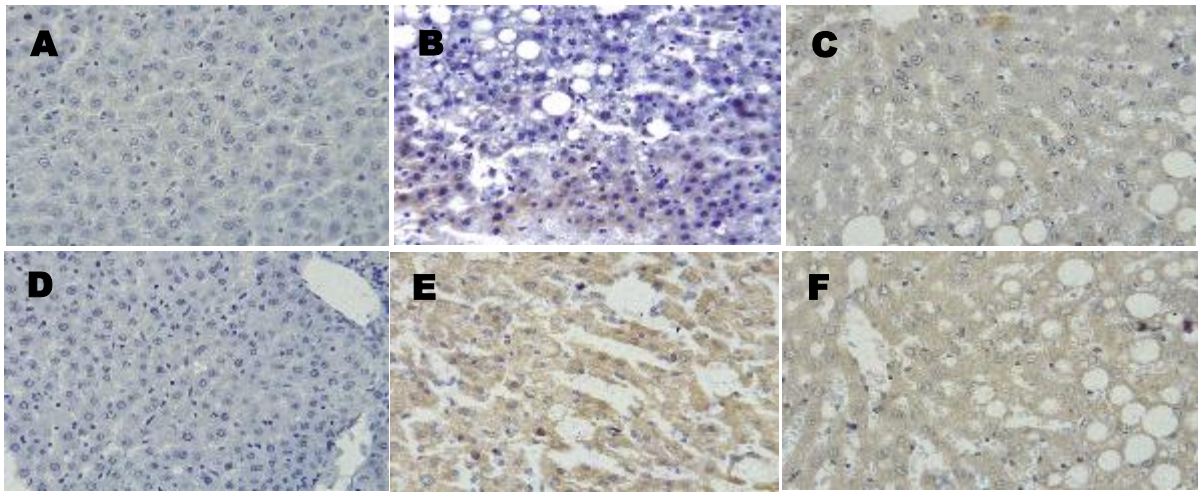


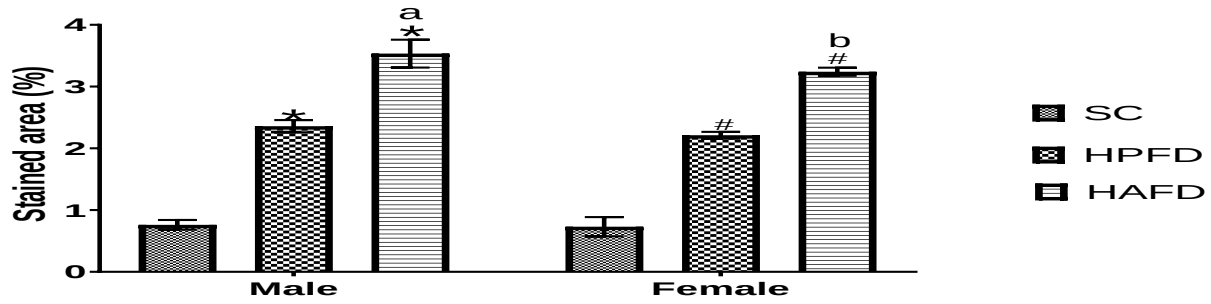
Figure 4.6: Photomicrograph and histogram of the expression of FATP4 protein in the Liver at X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.6A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); Figure 4.6B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.20 Effect of Sexual Dimorphism on Cluster of Differentiation 36 (CD36) Protein Expression in Liver Tissue of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in the expression of CD36 protein in Liver of HPFD and HAFD groups when compared with SC in both genders respectively. Also, a significant increase in $p<0.05$ was observed in the expression of CD36 protein in Liver of HAFD groups when compared with HPFD in both genders respectively. (figure 4.7 A and B)

(A)



(B)

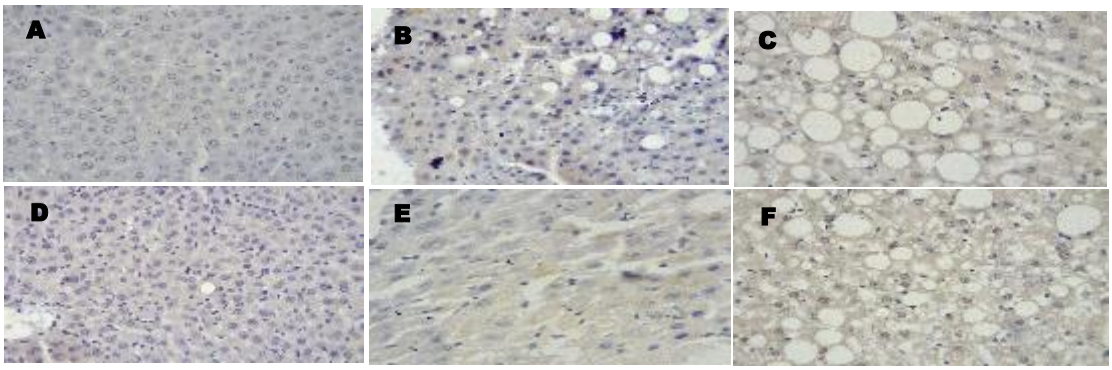


Figure 4.7: Photomicrograph and histogram of the expression of CD36 protein in the Liver at X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.7A shows the qualitative analysis of the photomicrograph detailing the level of expressions of CD36; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. *$p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}p<0.05$ Female (HPFD and HAFD) compared with Female (SC); $^{a}p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); $^{b}p<0.05$ Female (HAFD) compared with Female (HPFD); Figure 4.7B shows the physical colour changes in the photomicrograph of CD36 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.21 Effect of Sexual Dimorphism on the Weight of the Heart of Rats Fed with High Fat Diets

Significantly rise in $p<0.05$ was observed in HPFD and HAFD related with SC in both genders respectively. A significant risee in $p<0.05$ was observed in HPFD groups related with HAFD in both genders respectively. Finally, a significant reduction in $p<0.05$ in the HAFD in female rats related with HAFD in male rats separately. (table 4.12)

Table 4.12: Effect of Sexual Dimorphism on the Weight in grams of the Heart of Rats Fed with High Fat Diets

Tissue/ Gender	**SC**	**HPFD**	**HAFD**
Heart			
Male	0.6±0.01	1.1±0.03*	0.9 ±0.12*, [a]
Female	0.7±0.01	1.3±0.12[#]	0.8±0.015[#, b, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); [a] $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); [b] $p<0.05$ Female (HAFD) compared with Female (HPFD); [c] $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.22 Effect of Sexual Dimorphism on Heart Homogenate Lipid Profile of Rats Fed with High Fat Diets

Significantly rise in $p<0.05$ in total cholesterol concentration was observed in HPFD and HAFD groups when compared with SC in both genders respectively. (table 4.13)

Significantly rise in $p<0.05$ in triglyceride concentration, was noticed in HPFD and HAFD groups when compared with SC in both genders respectively. (table 4.13)

Significantly decrease rise in $p<0.05$ in high density lipoprotein concentration was observed in HPFD and HAFD when compared with SC in both genders respectively. There was also a significant increase in $p<0.05$ in HPFD of female rats when compared with HPFD of male rats. (table 4.13)

In low density lipoprotein concentration, significantly increase in $p<0.05$ was observed in HPFD and HAFD related with SC in both genders respectively. Also, a significant rise in $p<0.05$ was observed in HAFD related with HPFD in both genders respectively. A significant reduction in $p<0.05$ was observed in SC, HPFD and HAFD of female rats related with their male rats. (table 4.13)

Table 4.13: Effect of Sexual Dimorphism on Heart Homogenate Lipid Profile of Rats Fed with High Fat Diets

Groups / Variables	SC	HPFD	HAFD
	Total Cholesterol (mg/dl)		
Male	74.0 ± 8.90	113.0 ± 9.40*	123.0 ± 7.70*
Female	41.0 ± 1.90*	109.0 ± 7.40$^{\#}$	115.0 ± 11.00$^{\#}$
	Triglyceride (mg/dl)		
Male	108.0 ± 5.00	226.0 ±25.00*	211.0 ± 22.00*
Female	105.0 ± 5.80	173.0 ± 9.40$^{\#}$	186.0 ± 4.80$^{\#}$
	High Density Lipoprotein (mg/dl)		
Male	26.0 ±1.50	14.0 ±1.40*	16.0 ±1.70*
Female	29.0 ± 1.40	20.0± 1.50$^{\#, a}$	19.0 ± 1.30$^{\#}$
	Low Density Lipoprotein (mg/dl)		
Male	19.0 ± 1.60	38.0 ±2.90*	73.0 ± 1.80*$^{, a}$
Female	11.0 ±0.7*	27±1.8$^{\#, a}$	35±1.6$^{\#, b, c}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.23 Effect of Sexual Dimorphism on Heart Homogenate Free Fatty Acid concentration of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in HPFD and HAFD related with SC in both gender respectively. Also, significantly reduction in $p<0.05$ in HPFD and HAFD of females was noticed when compared with HPFD and HAFD of male rats. (figure 4.8)

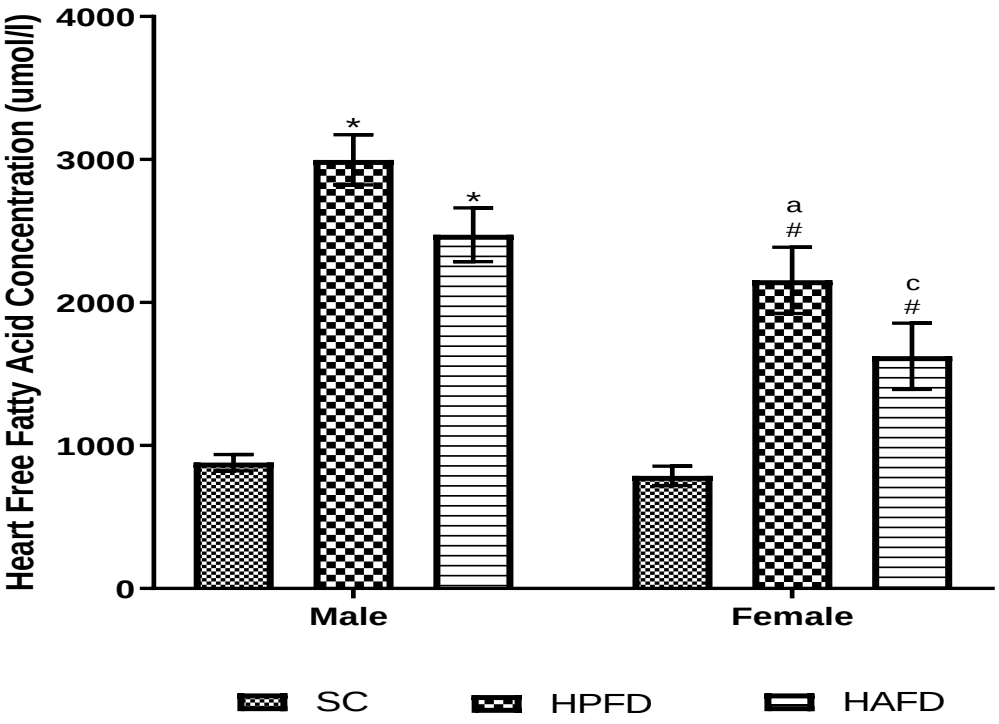


Figure 4.8: Heart Homogenate Free Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.24 Effect of Sexual Dimorphism on Heart Homogenate Saturated Fatty Acid concentration of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in HAFD when compared with SC and HPFD in female rats respectively. A significant decrease in $p<0.05$ was observed in SC and HAFD of female rats when compared with their male counterpart SC and HAFD respectively. (figure 4.9)

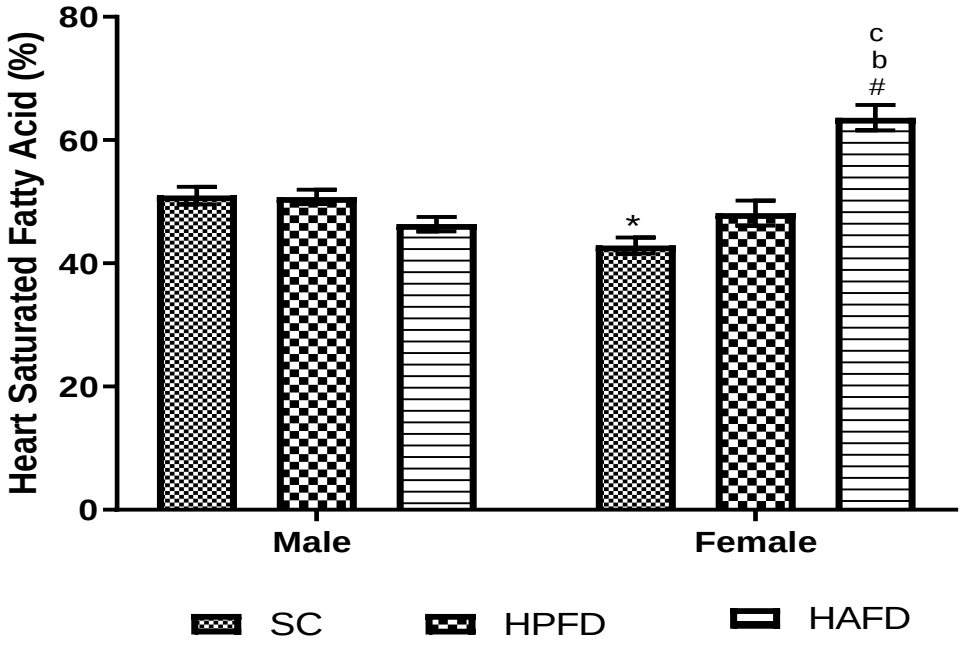


Figure 4.9: Heart Homogenate Saturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.25 Effect of Sexual Dimorphism on Heart Homogenate Monounsaturated Fatty Acid concentration of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in HPFD and HAFD related with SC in males respectively. A significant reduction in $p<0.05$ HAFD related with SC in females. A significant rise in $p<0.05$ was observed in the HPFD related with HAFD in both genders respectively. There was a significant rise in $p<0.05$ in SC but significant decreases in HPFD and HAFD of female rats related with their male counterparts respectively. (figure 4.10)

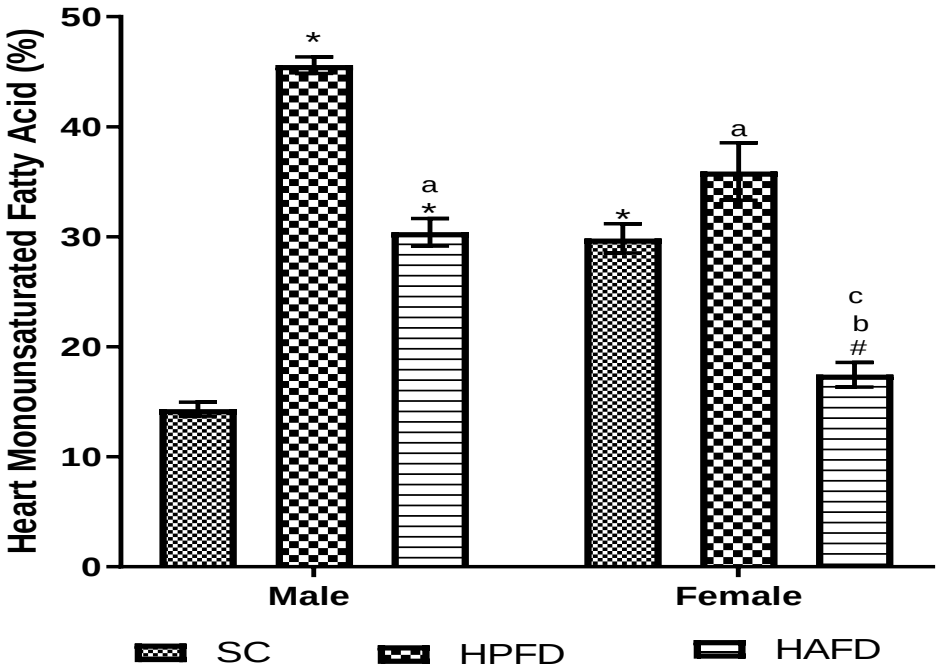


Figure 4.10: Heart Homogenate Monounsaturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.26 Effect of Sexual Dimorphism on Heart Homogenate Polyunsaturated Fatty Acid concentration of Rats Fed with High Fat Diets

A significant increase in $p<0.05$ was observed in HPFD and HAFD when compared with SC in both gender respectively. There was a significant decrease in $p<0.05$ in HPFD when compared to HAFD in male rats. Finally, a significant reduction in was observed in SC but a significant rise in $p<0.05$ in HPFD of female rats when related with their male counterparts respectively. (figure 4.11)

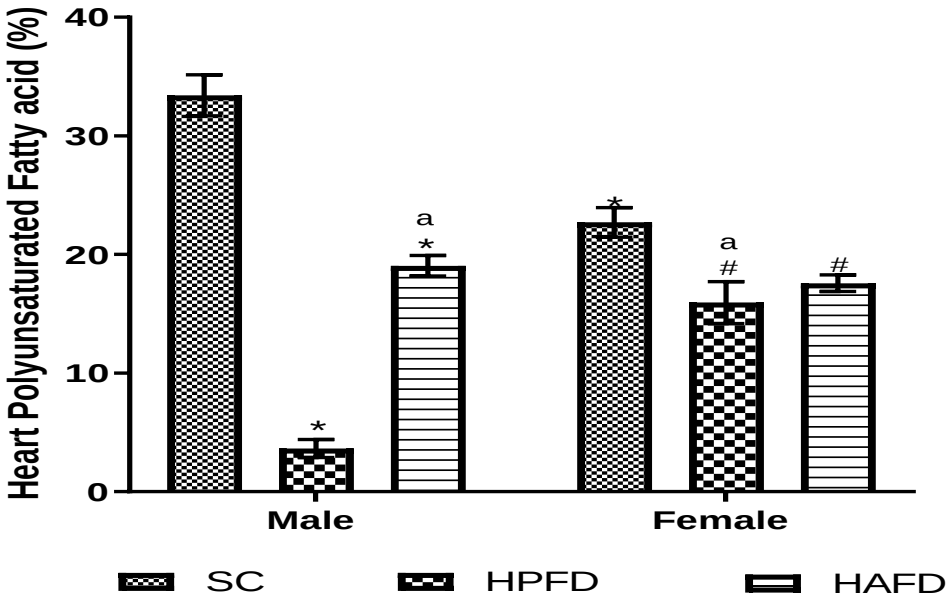


Figure 4.11: Heart Homogenate Polyunsaturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD)

4.27 Effect of Sexual Dimorphism on Heart Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

In malondialdehyde concentration, a significant rise in $p<0.05$ was observed in HAFD related with SC in both genders respectively. Also, a significant rise in $p<0.05$ in HAFD related with HPFD in male rats. Significantly increase in $p<0.05$ in SC and HPFD females was noticed when related with SC and HPFD of male rats but a significant decrease in $p<0.05$ was observed in female HAFD when compared to HAFD of male rats. (table 4.14)

In reduced Glutathione concentration, significantly reduction in $p<0.05$ in HPFD and HAFD was noticed when related with SC in females; also, there was significant decrease in $p<0.05$ in SC, HPFD and HAFD of female rats related with their male rats. (table 4.14)

In Superoxide Dismutase concentration, a significant rise in $p<0.05$ was observed in HPFD when compared with SC in female rats. (table 4.14)

No significant variation amongst groups in catalase concentration. (table 4.14)

Table 4.14: Effect of Sexual Dimorphism on Heart Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

Groups / Variables	SC	HPFD	HAFD
	Catalase (u/ml/mins)		
Male	25±0.03	25±0.07	24±0.21
Female	25±0.02	25±0.05	25±0.03
	reduced Glutathione (mM)		
Male	0.73±0.07	0.66±0.07	0.68±0.06
Female	0.48±0.03*	0.25±0.02$^{\#, a}$	0.27±0.02$^{\#, c}$
	Malondialdehyde (mM)		
Male	15±0.5	15±0.8	44±1.4*$^{, a}$
Female	22±0.7*	25±1.1^{a}	29±1.8$^{\#, c}$
	Superoxide Dismutase (u/ml)		
Male	40±1.0	42±1.4	42±1.2
Female	40±1.2	43±0.9$^{\#}$	42±0.5

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.28 Effect of Sexual Dimorphism on Histology of the Heart of Rats Fed with High Fat Diets

The black arrows point to the different levels of fat deposition on the Heart tissues on H and E staining. There was a significant level of fats seen in the myocardial and pericardial regions of the heart in all in both HPFD and HPFD groups.

The blue arrows point to the different levels of vascular congestions on the Heart tissues on H and E staining. There was a significant level of vascular congestions seen in the pericarduim and valves in all in HPFD and HPFD. (plate 4.3)

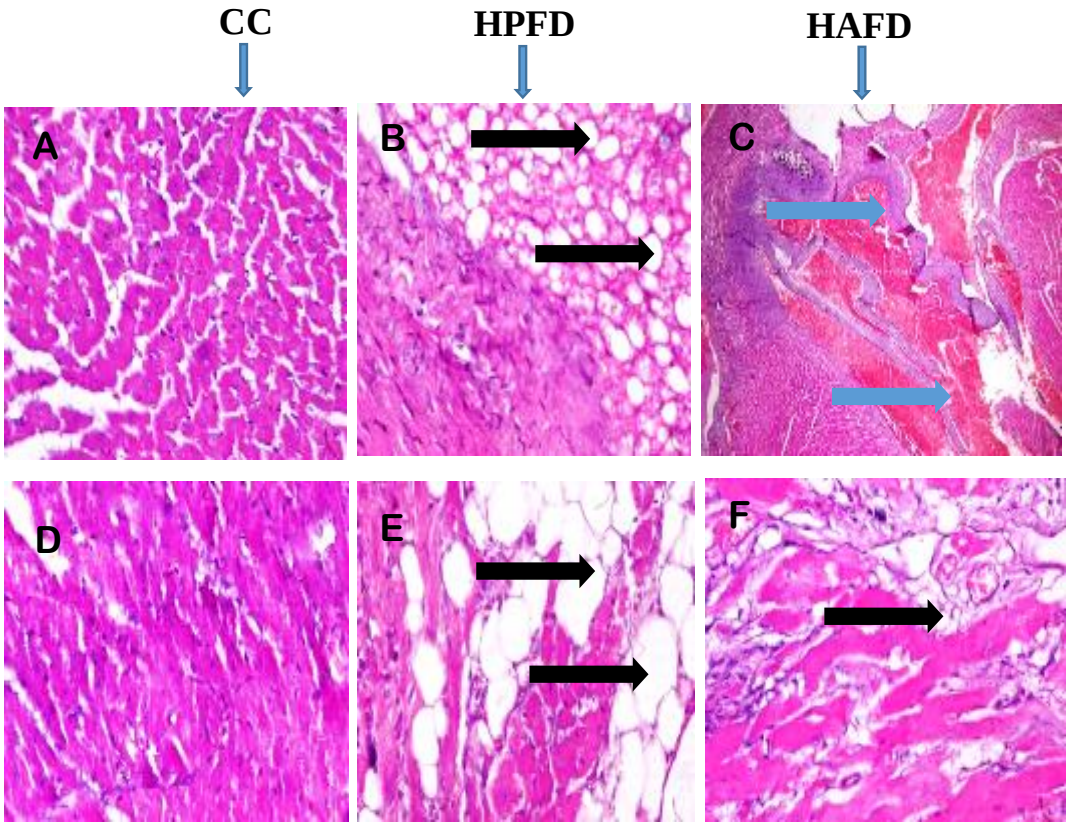


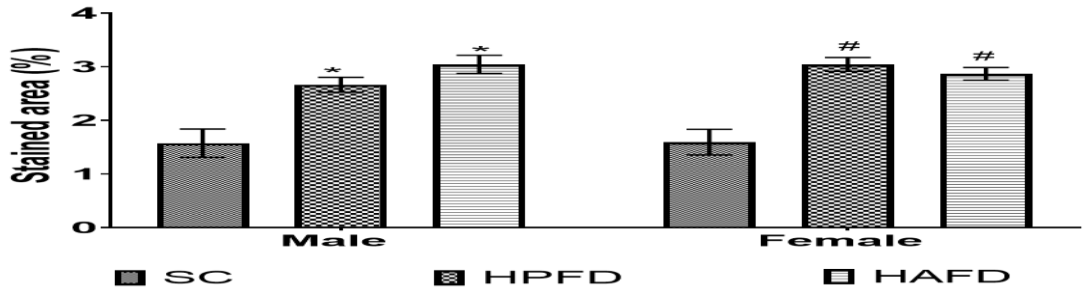
Plate 4.3: Photomicrograph of Heart Tissues Stained with X400 Magnification

(A) CC Male heart, (B) HPFD Male heart, C) HAFD Male heart, (D) CC Female heart, (E) HPFD Female heart, (F) HAFD Female heart. Where CC = Control Chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. Arrows show areas where either fat deposition or congestion could be seen.

4.29 Effect of Sexual Dimorphism on Fatty Acid Transporter Protein 4 (FATP4) Expression in Heart Tissue of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in FATP4 expression in the Heart tissues in HPFD and HAFD when compared with SC in both genders respectively. (figure 4.12 A and B)

(A)



(B)

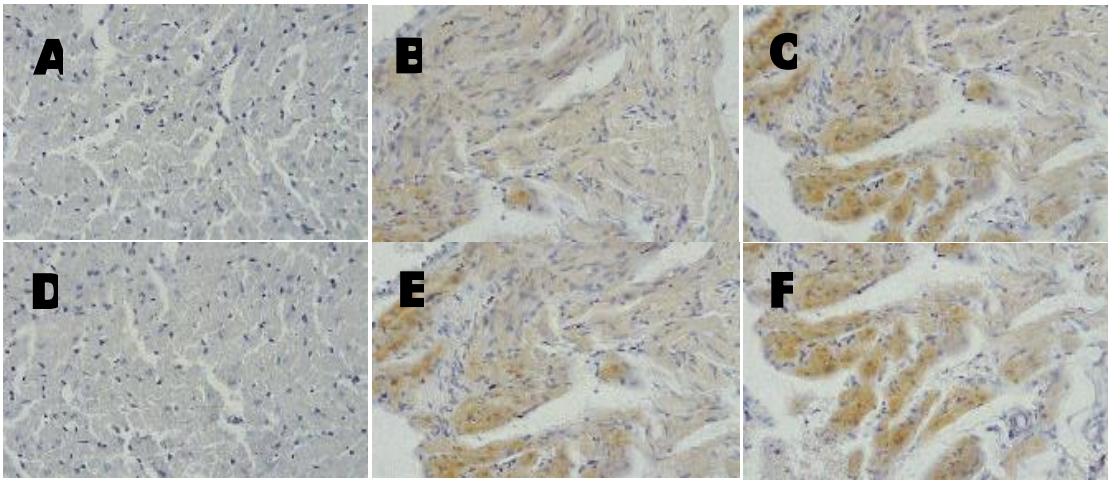


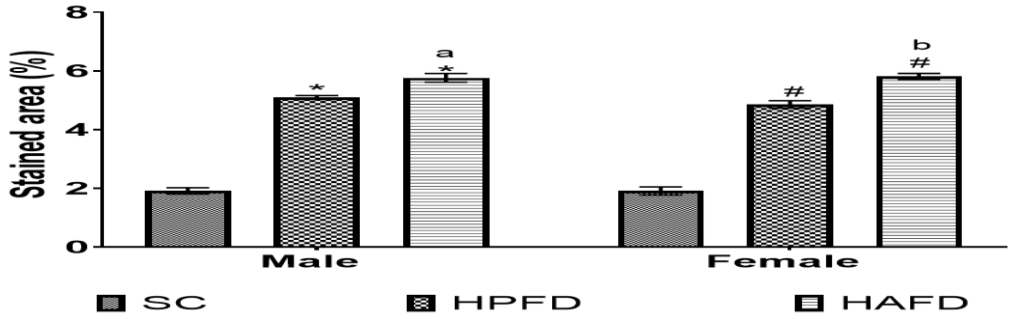
Figure 4.12: Photomicrograph with Histograph of FATP4 protein expression in the heart at X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.12A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); Figure 4.12B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.30 Effect of Sexual Dimorphism on Cluster of Differentiation 36 (CD36) Protein Expression in Heart Tissue of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in CD36 expression in the Heart tissues in HPFD and HAFD when compared with SC in both genders respectively. There was also a significant increase in $p<0.05$ in HPFD when compared with HAFD in both genders respectively. (figure 4.13 A and B)

(A)



(B)

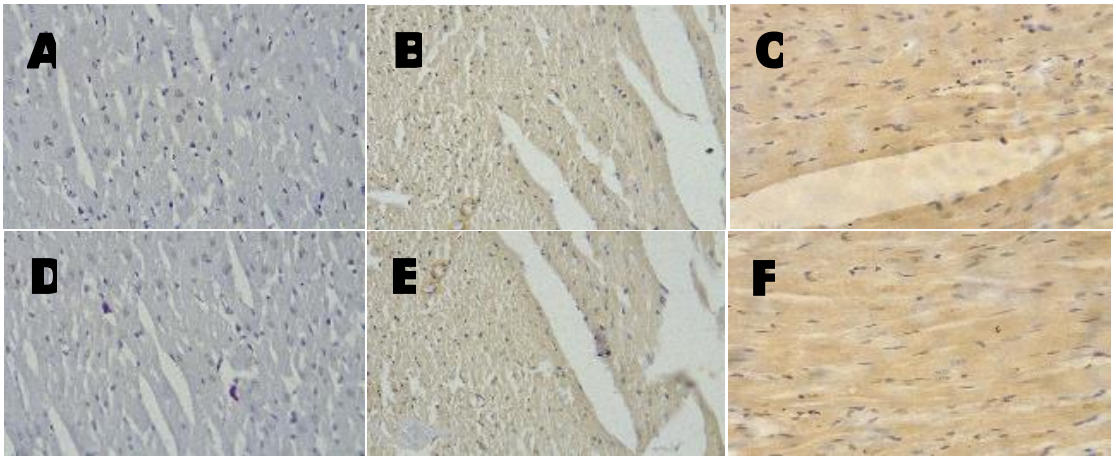


Figure 4.13: Photomicrograph and histogram of the expression of CD36 protein in the heart at X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.13A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); Figure 4.13B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.31 Effect of Sexual Dimorphism on Small Intestinal Homogenate Lipid Profile of Rats Fed with High Fat Diets

Significantly rise in $p<0.05$ in total cholesterol concentration was noted in HPFD and HAFD when compared with SC in both genders respectively. A significant reduction in $p<0.05$ in HPFD of female rats when compared with HPFD of male rats. (table 4.15)

In triglyceride concentration, a significant rise in $p<0.05$ was noted in HPFD and HAFD when compared with SC in both genders respectively. A significant reduction in $p<0.05$ in HAFD when related with HPFD of female rats. Finally, there was a significant decrease in $p<0.05$ in HAFD of female rats when compared with HAFD of male rats. (table 4.15)

Significantly reduction in $p<0.05$ in high density lipoprotein concentration in HPFD and HAFD when compared with SC in both genders respectively. (table 4.15)

In low density lipoprotein concentration, significantly increase in $p<0.05$ was noted in HPFD and HAFD when compared with SC in both genders respectively. Also, significantly reduction in $p<0.05$ was observed in HPFD and HAFD of female rats when compared with HPFD and HAFD in male rats. (table 4.15)

Table 4.15: Effect of Sexual Dimorphism on Small Intestinal Homogenate Lipid Profile of Rats Fed with High Fat Diets

Group / LIPID PROFILE	SC	HPFD	HAFD
	Total Cholesterol (mg/dl)		
Male	67.0±7.90	109.0±5.00*	128.0±13.00*
Female	52.0±8.50	85.0±8.60$^{\#, a}$	119.0±14.00$^{\#}$
	Triglyceride (mg/dl)		
Male	152.0±22.00	357.0±14.00*	374.0±17.00*
Female	112.0±4.60	361.0±17.00$^{\#}$	237.0±15.00$^{\#, b, c}$
	High Density Lipoprotein (mg/dl)		
Male	26.0±1.80	17.0±0.90*	17.0±1.10*
Female	30.0±1.70	`19.0±0.70$^{\#}$	17.0±1.40$^{\#}$
	Low Density Lipoprotein (mg/dl)		
Male	22.0±1.30	66.0±1.30*	63.0±1.30*
Female	17.0±1.60	43.0±0.99$^{\#, a}$	44.0±1.80$^{\#, c}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.32 Effect of Sexual Dimorphism on Small Intestinal Homogenate Free Fatty Acid concentration of Rats Fed with High Fat Diets

Significantly increase in $p<0.05$ was observed in HPFD and HAFD when compared with SC in both gender respectively. Significantly reduction in $p<0.05$ in SC, HPFD and HAFD of female rats when compared with their male rats respectively. (figure 4.14)

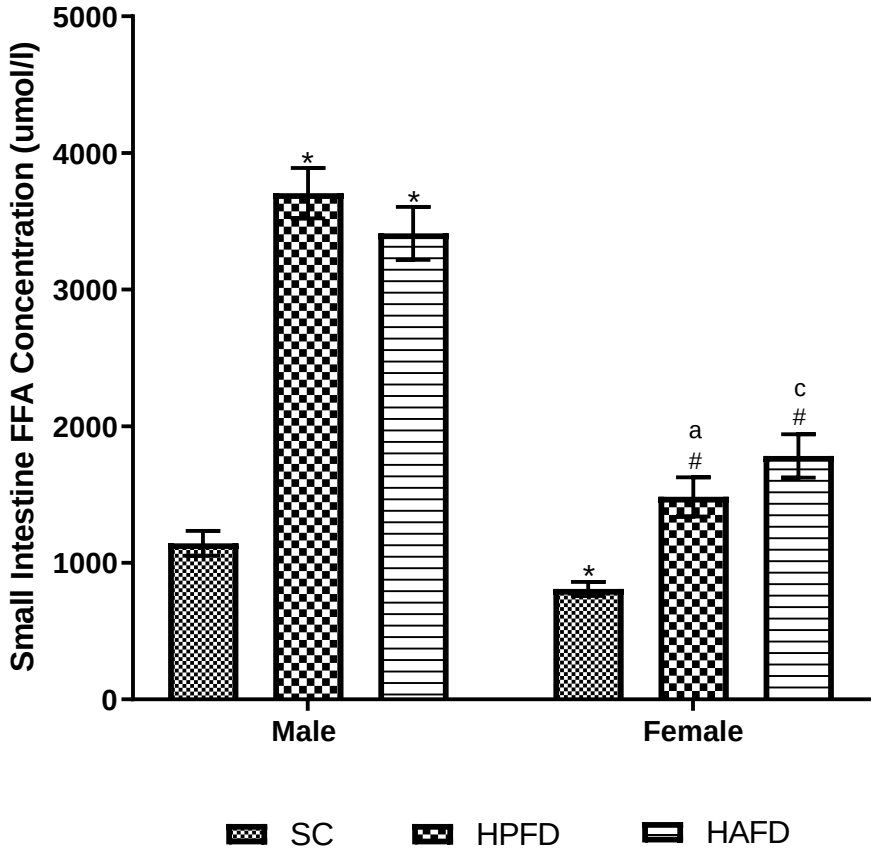


Figure 4.14: Small intestinal Homogenate Free Fatty Acid (FFA) concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.33 Effect of Sexual Dimorphism on Small Intestinal Homogenate Saturated Fatty Acid concentration of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was noted in HPFD when related with SC and HAFD respectively. A significant decrease in $p<0.05$ was noted in HPFD of female rats when related with HPFD of male rats respectively. (figure 4.15)

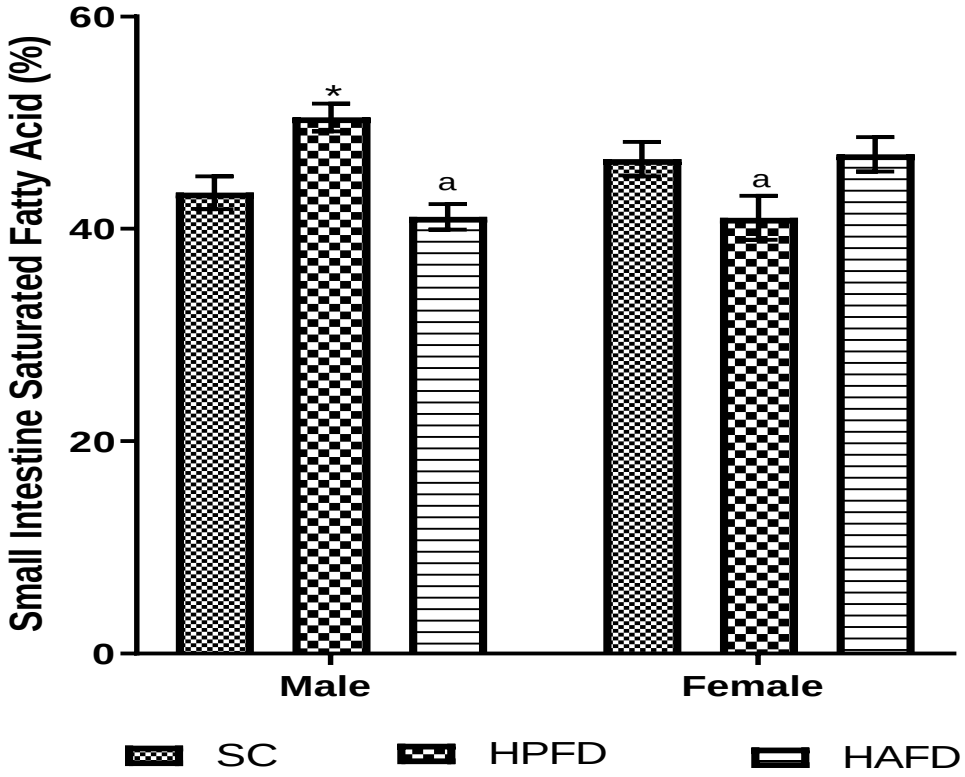


Figure 4.15: Small intestinal Homogenate Saturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); [a] $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD)

4.34 Effect of Sexual Dimorphism on Small Intestinal Homogenate Monounsaturated Fatty Acid concentration of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in HAFD when related with SC in both genders respectively. A significant rise in $p<0.05$ was noticed in HPFD when related to SC in female rats. There was a significant reduction in $p<0.05$ in HPFD when related with HAFD in males. Finally, there was a significant reduction in $p<0.05$ in HAFD of female rats when related to HAFD of male rats. (figure 4.16)

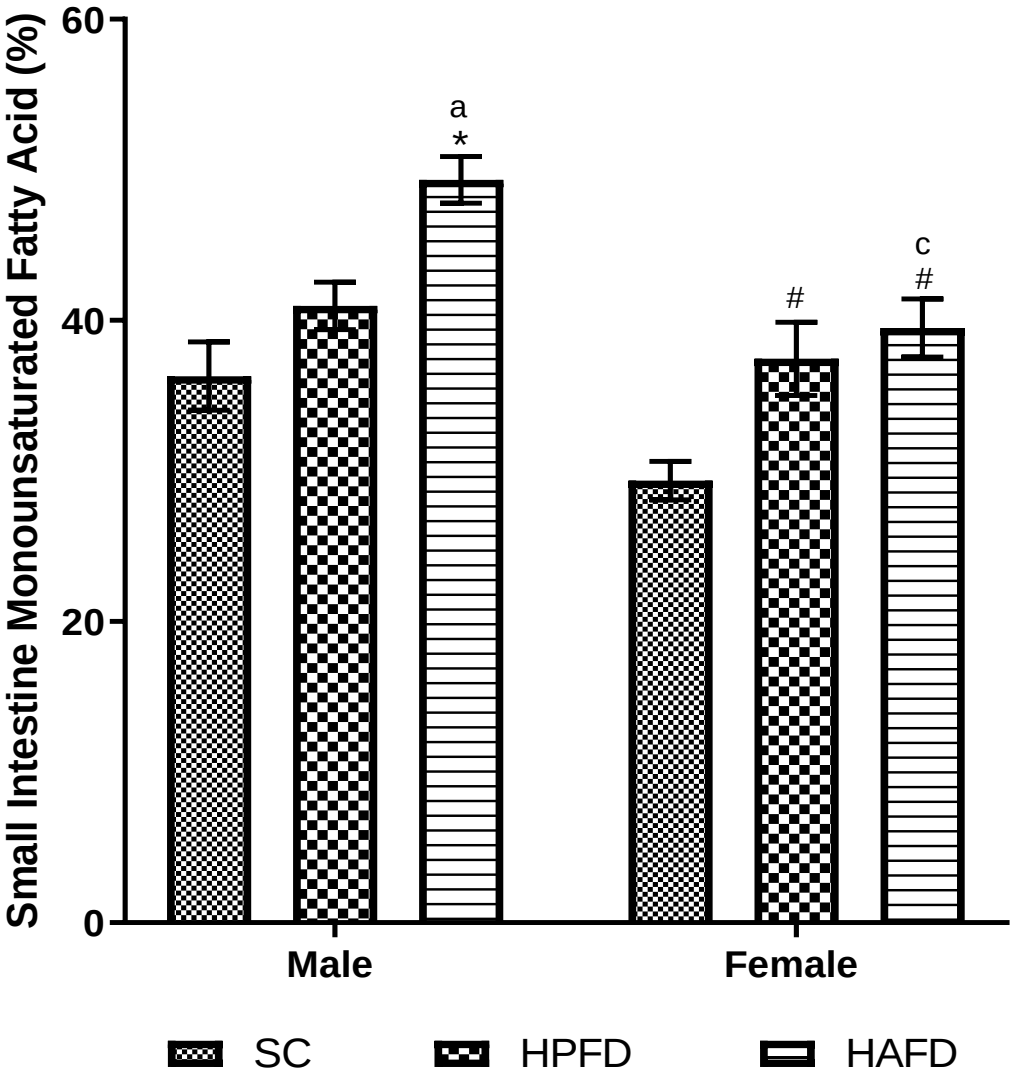


Figure 4.16: Small intestinal Homogenate Monounsaturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.35 Effect of Sexual Dimorphism on Small Intestinal Homogenate Polyunsaturated Fatty Acid concentration of Rats Fed with High Fat Diets

Significantly reduction in $p<0.05$ was noted in HPFD and HAFD when related with SC in male rats respectively. Significantly decrease in $p<0.05$ in HAFD when related to SC and HPFD respectively in female rats. Finally, significantly rise in $p<0.05$ in HPFD and HAFD of female rats when related to HPFD and HAFD of male rats respectively. (figure 4.17)

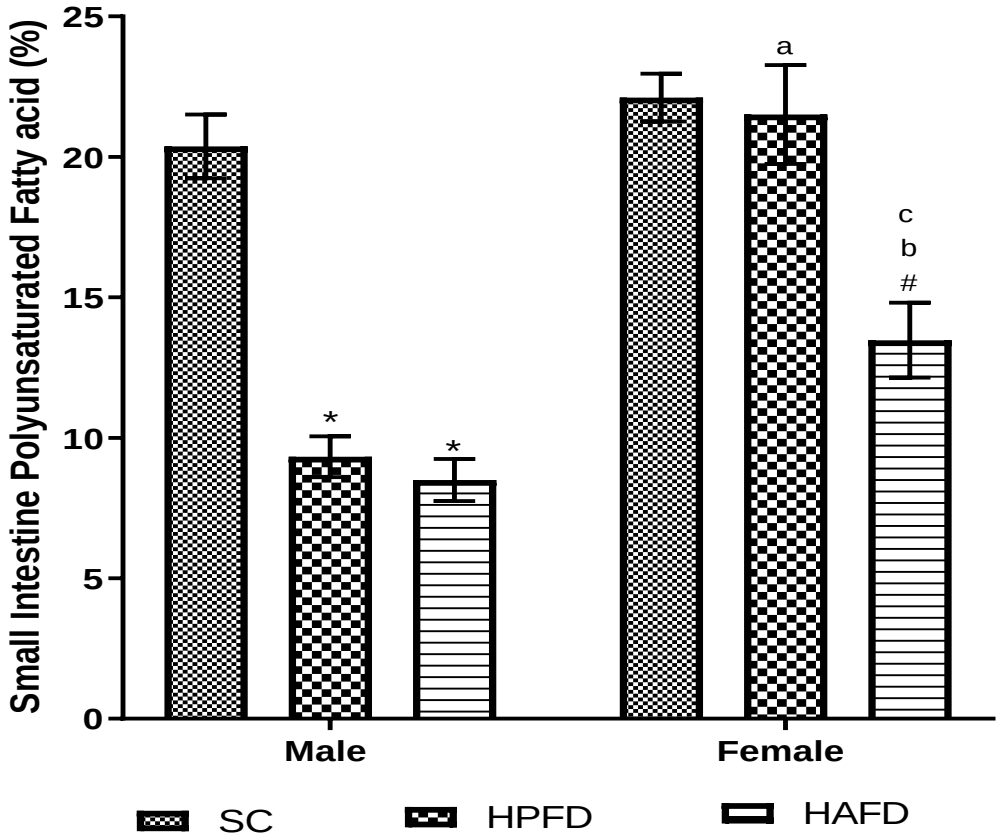


Figure 4.17: Small intestinal Homogenate Polyunsaturated Fatty Acid concentration

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{b} $p<0.05$ Female (HAFD) compared with Female (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.36 Effect of Sexual Dimorphism on Small Intestinal Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

In malondialdehyde concentration, significantly increases in $p<0.05$ was noted in HPFD and HAFD when related with SC in both genders respectively. Also, a significant increase in $p<0.05$ was noticed in HAFD when related with HPFD in male rats. Significant increases in $p<0.05$ in SC, HPFD and HAFD of female rats when related to their male rats. (table 4.16)

In reduced Glutathione concentration, significantly decreases in $p<0.05$ was noted in the intestine GSH concentration HPFD and HAFD when compared with SC of male rats. Also, significantly increases in $p<0.05$ in SC, HPFD and HAFD of female rats when related with their male rats. (table 4.16)

In Superoxide Dismutase concentration, there was a significant decrease in $p<0.05$ in HPFD and HAFD when related with SC in both genders respectively. Significantly rise in $p<0.05$ in SC of female rats when related to SC of male rats. (table 4.16)

No significant difference was noted in Catalase level. (table 4.16)

Table 4.16: Effect of Sexual Dimorphism on Small Intestinal Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed with High Fat Diets

Group / Variable	SC	HPFD	HAFD
	Malondialdehyde (mM)		
Male	12.0±0.52	22±0.57*	27±0.59*, [a]
Female	25.0±0.74*	29±1.10[#, a]	31±0.64[#, c]
	reduced Glutathione (mM)		
Male	0.1±0.01	0.07±0.01*	0.1±0.01*
Female	0.3±0.02*	0.30±0.02[a]	0.3±0.02[c]
	Superoxide Dismutase (u/ml)		
Male	51.0±1.1	46.0±1.20*	46.0±1.90*
Female	59.0±1.1*	46.0±1.00[#]	45±1.20[#]
	Catalase (u/ml/mins)		
Male	25.0±0.44	24.0±0.15	24.0±0.18
Female	25.0±0.02	24.0±0.89	24.0±0.68

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); [a] $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); [c] $p<0.05$ Female (HAFD) compared with Male (HAFD)

4.37 Effect of Sexual Dimorphism on Histology of the Small Intestinal of Female Rats Fed with High Fat Diets

Black arrows point to the different levels of fat deposition on the female small intestinal tissues on H and E staining. There was a significant level of fats seen in the lamina propria and mucosal epithelial region of female intestines in both HPFD and HPFD groups. (plate 4.4)

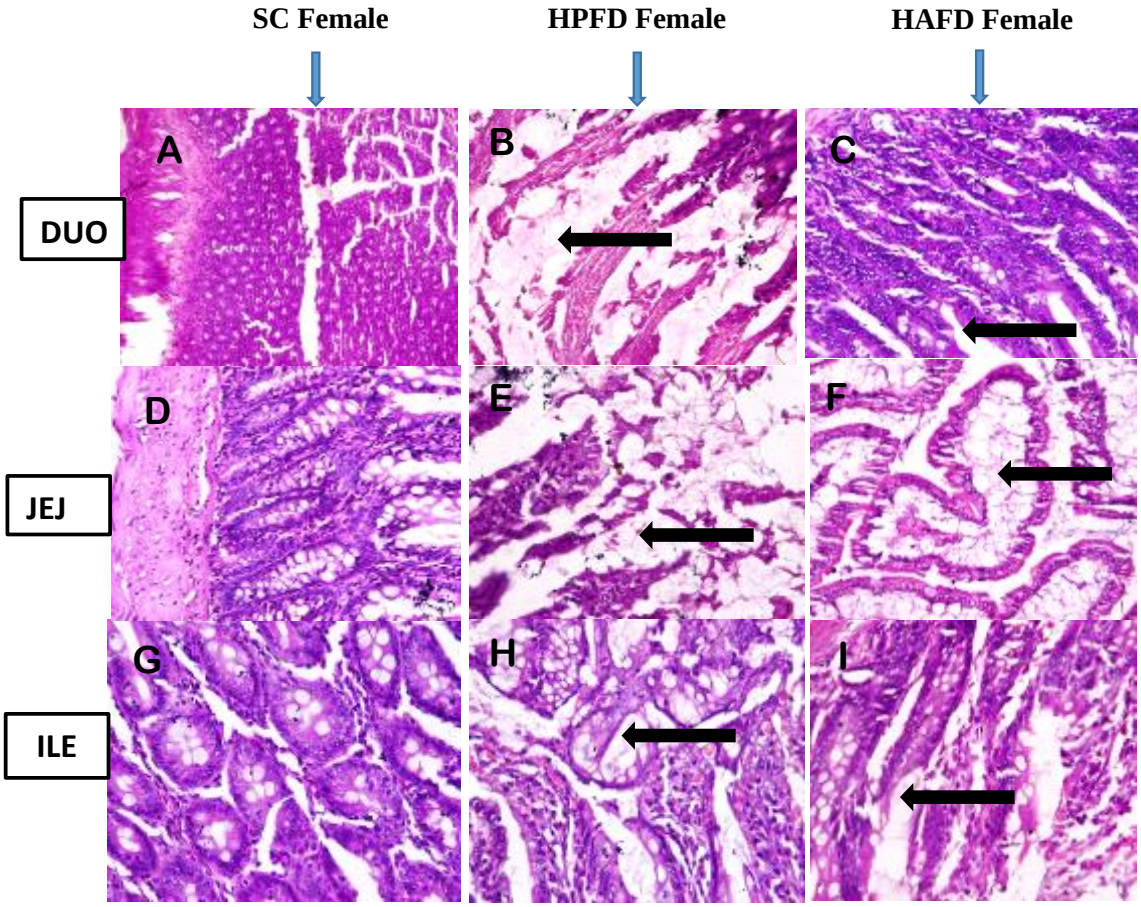


Plate 4.4: Histology of the Female Small Intestine tissues with X400 Magnification

(A) SC duodenum, (B) HPFD duodenum, C) HAFD duodenum, (D) SC jejunum, (E) HPFD jejunum, (F) HAFD jejunum, (G) SC ileum, (H) HPFD ileum, (I) HAFD ileum. Where Duo = Duodenum; Jej = Jejunum; Ile = Ileum; SC = Standard Chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. Arrows show areas where fat deposition could be seen.

4.38 Effect of Sexual Dimorphism on Histology of the Small Intestinal of Male Rats Fed with High Fat Diets

Black arrows point to the different levels of fat deposition on the male small intestinal tissues on H and E staining. There was a significant level of fats seen in the lamina propria and mucosal epithelial region of female small intestines in both HPFD and HAFD groups. (plate 4.5)

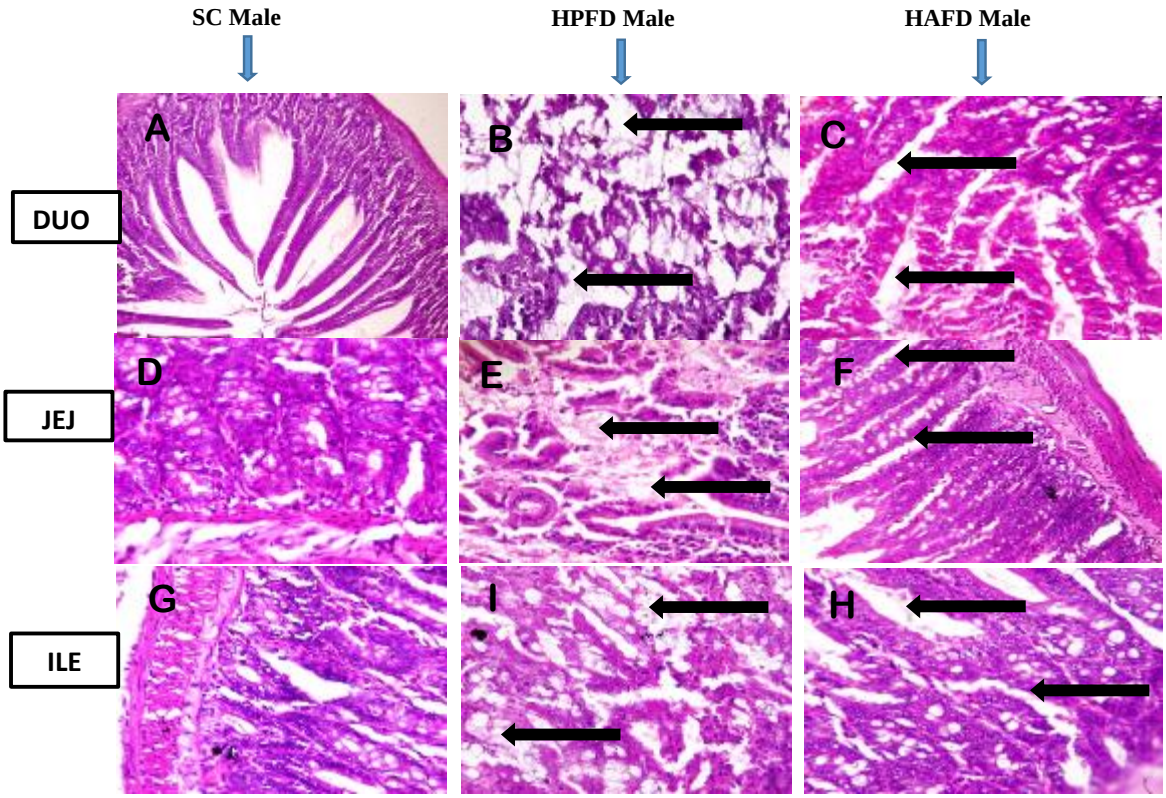


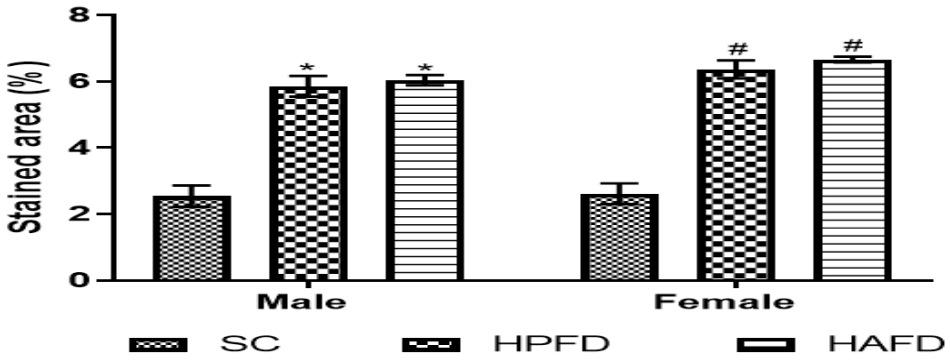
Plate 4.5: Histology of the Male Small Intestine with X400 Magnification

(A) SC duodenum, (B) HPFD duodenum, C) HAFD duodenum, (D) SC jejunum, (E) HPFD jejunum, (F) HAFD jejunum, (G) SC ileum, (H) HPFD ileum, (I) HAFD ileum. Where Duo = Duodenum; Jej = Jejunum; Ile = Ileum; SC = Standard Chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. Arrows show areas where fat deposition could be seen.

4.39 Effect of Sexual Dimorphism on Fatty Acid Transporter Protein 4 (FATP4) Expression in the Duodenum of Rats Fed with High Fat Diets

A significant increase in $p<0.05$ was observed in the expression of FATP4 protein in the Duodenum in HPFD and HAFD when compared to SC in both genders respectively in Wistar rats. (figure 4.18 A and B)

(A)



(B)

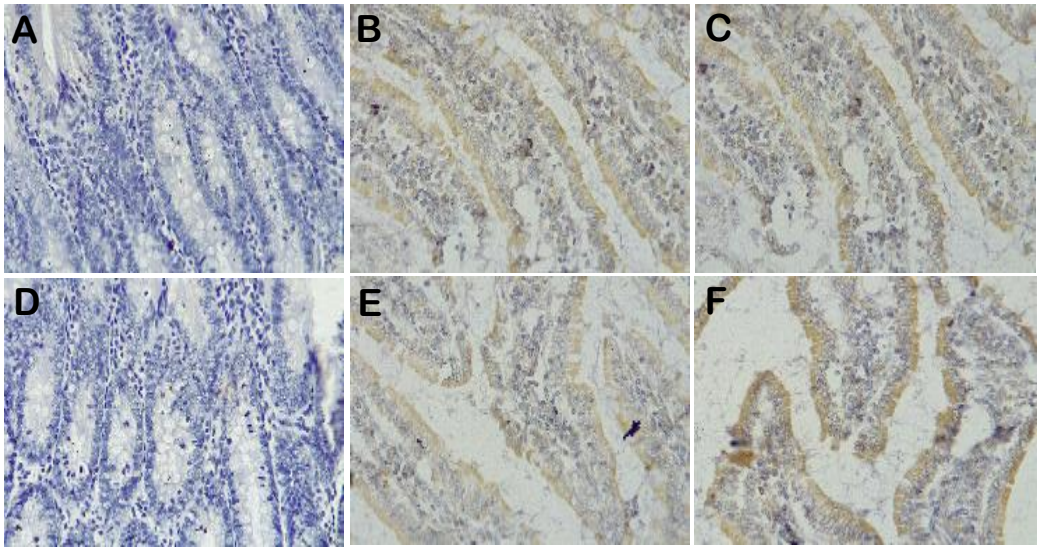


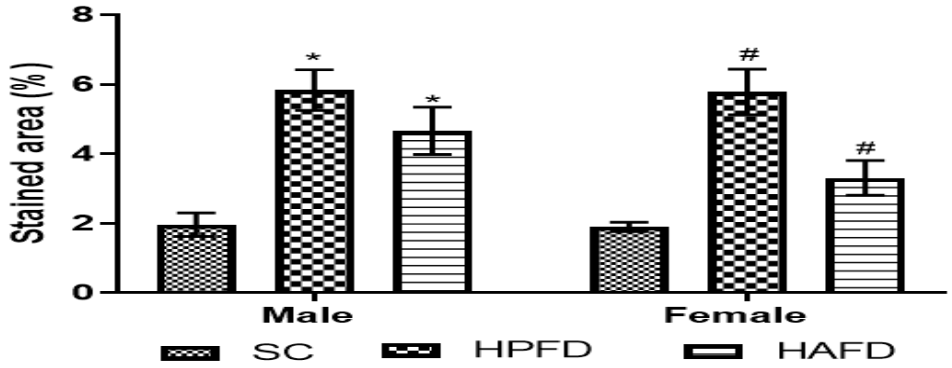
Figure 4.18: FATP4 protein expression in the Duodenum with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.18A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); Figure 4.18B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.40 Effect of Sexual Dimorphism on Fatty Acid Transporter Protein 4 (FATP4) Expression in the Jejunum of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in the expression of FATP4 protein in the Jejunum in HPFD and HAFD when compared to SC in both genders respectively. (figure 4.19 A and B)

(A)



(B)

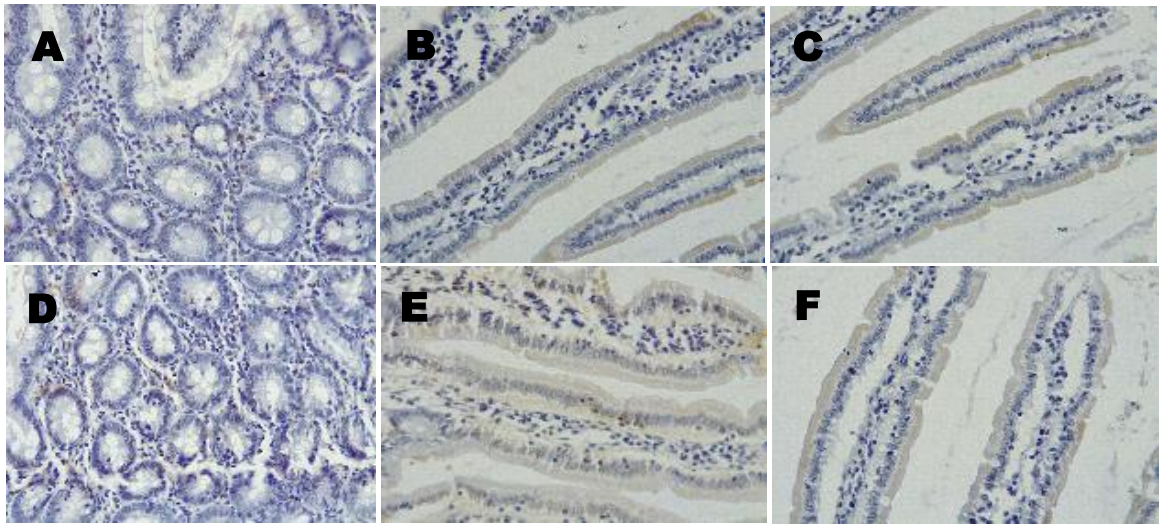


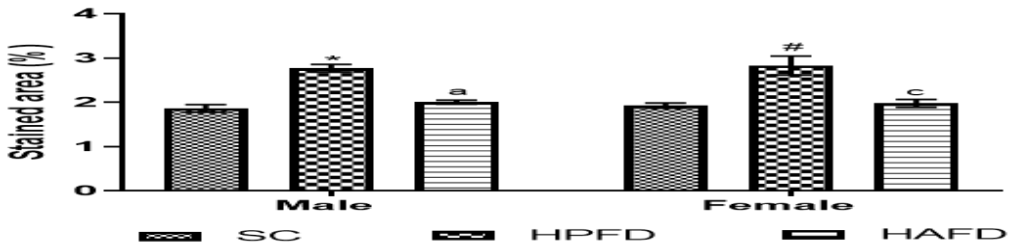
Figure 4.19: FATP4 protein expression in the Jejunum with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.19A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); Figure 4.19B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.41 Effect of Sexual Dimorphism on Fatty Acid Transporter Protein 4 (FATP4) Expression in the Ileum of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in FATP4 protein expression in the Ileum in HPFD when compared to HAFD and SC in both genders respectively. (figure 4.20 A and B)

(A)



(B)

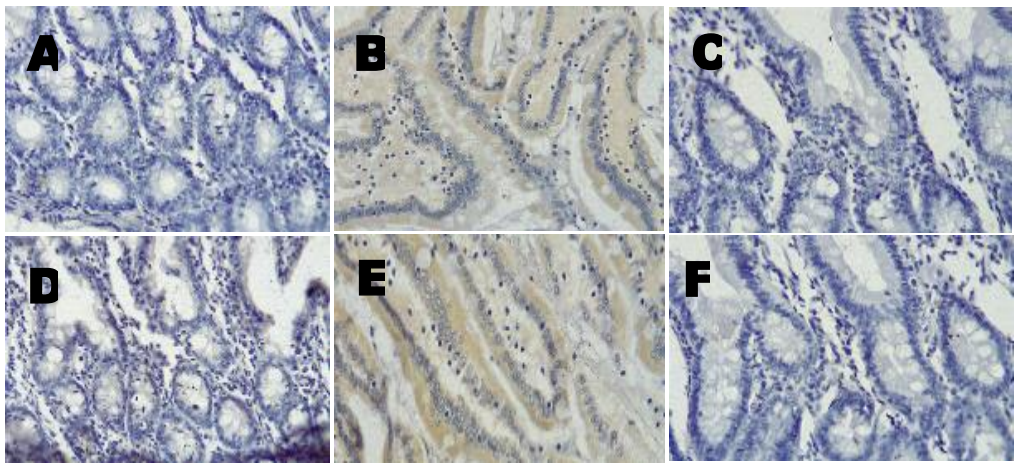


Figure 4.20: FATP4 protein expression in the Ileum with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.20A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $\alpha_{0.05}$ Female (HPFD and HAFD) compared with Female (SC); ^{a} $p<0.05$ Male (HAFD) and Female (HPFD) compared with Male (HPFD); ^{c} $p<0.05$ Female (HAFD) compared with Female (HPFD); Figure 4.20B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.42 Effect of Sexual Dimorphism on Cluster of Differentiation 36 (CD36) Protein Expression in the Duodenum of Rats Fed with High Fat Diets

A significant rise in $p<0.05$ was observed in the expression of CD36 protein in HPFD and HAFD when compared to SC in both genders respectively. A significant rise in $p<0.05$ was observed in the HAFD of female rats when related to HAFD of male rats. (figure 4.21 A and B)

(A)

(B)

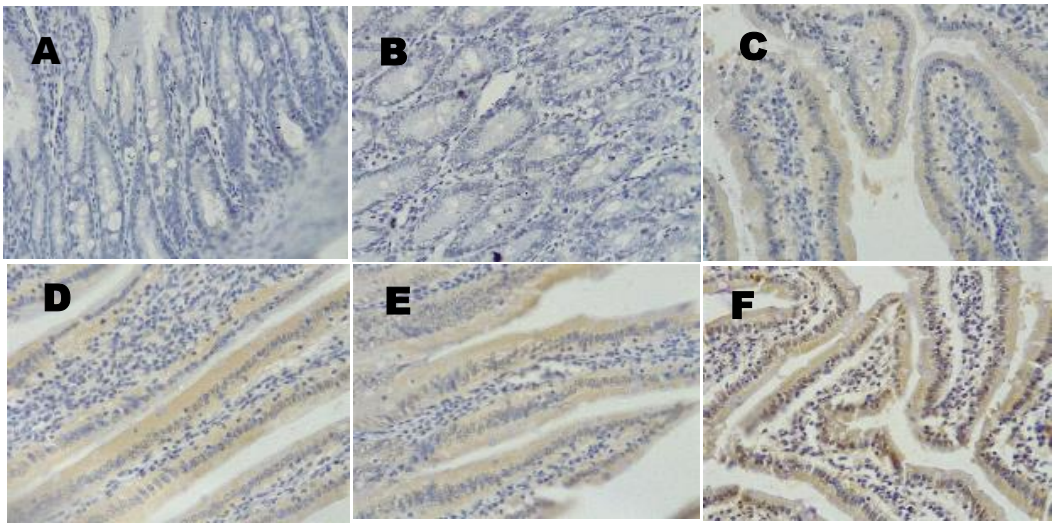


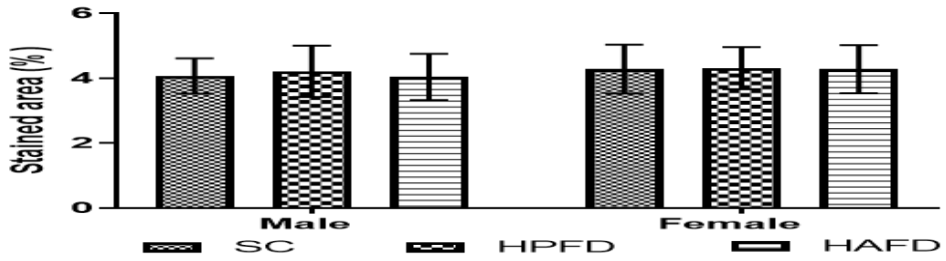
Figure 4.21: CD36 protein expression in the Duodenum with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.21A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); ^{c} $p<0.05$ Female (HAFD) compared with Male (HAFD); Figure 4.21B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.43 Effect of Sexual Dimorphism on Cluster of Differentiation 36 (CD36) Protein Expression in the Jejunum of Rats Fed with High Fat Diets

No significant changes were observed on CD36 protein expression in the Jejunum. (figure 4.22 A and B)

(A)



(B)

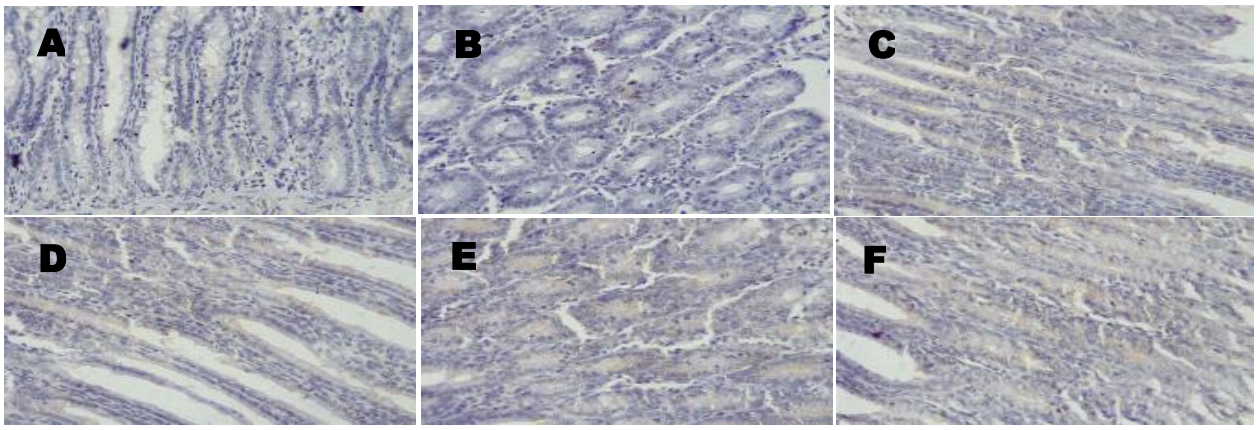


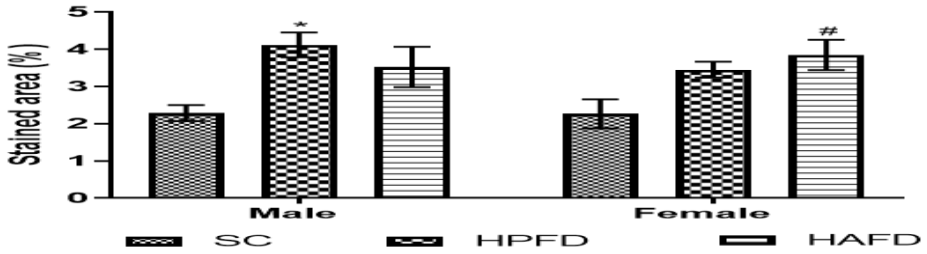
Figure 4.22: CD36 protein expression in the Jejunum with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.22A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. Figure 4.22B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

4.44 Effect of Sexual Dimorphism on Cluster of Differentiation 36 (CD36) Protein Expression in the Ileum

A significant increase in $p<0.05$ was observed in CD36 protein expression in the Ileum of HPFD when compared with SC in males. Significantly rise in $p<0.05$ in HAFD when compared to SC in female rats. (figure 4.23 A and B)

(A)



(B)

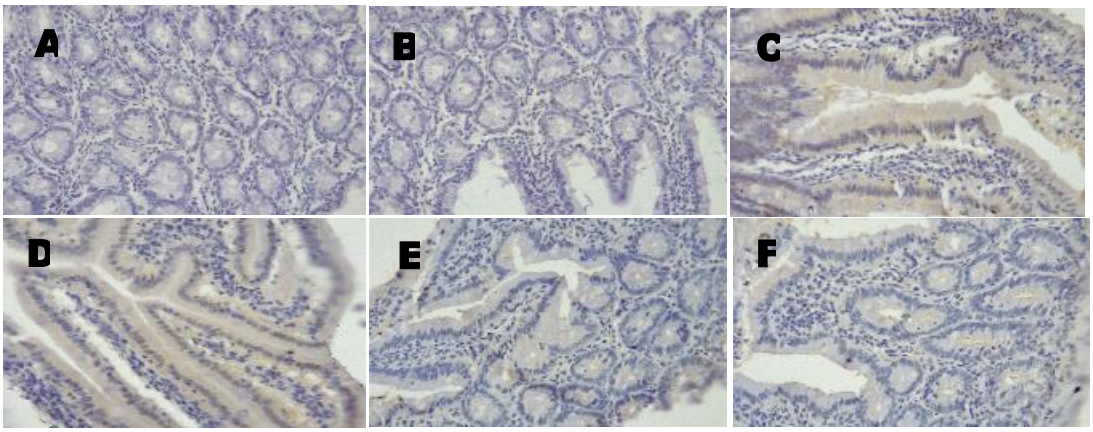


Figure 4.23: CD36 protein expression in the Ileum with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.23A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow; HPFD = High Plant Fat Diet; HAFD = High Animal Fat Diet. * $p<0.05$ Male (HPFD and HAFD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HPFD and HAFD) compared with Female (SC); Figure 4.23B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male liver, (B) HPFD Male Liver, (C) HAFD Male Liver, (D) SC Female Liver, (E) HPFD Female Liver, (F) HAFD Female Liver.

STUDY TWO

4.45 Proximate Analysis of the 3 Feeds, Gross Energy and Metabolisable Energy of the 3 Feeds, Fecal Energy and Digestible Energy of Wistar Rats in the 3 Diet Groups

There were no significant differences crude protein was relatively the same amongst the 3 feed. Significantly rise in $p<0.05$ crude fiber in the LCD feed when related to the SC and HFD feeds. Significantly rise in $p<0.05$ of the crude fat in HFD compared to SC and LCD diet. Also the gross, metabolizable, digestible and fecal energies were significantly higher in $\alpha_{0.05}$ in HFD than in SC and LCD diets. (table 4.17)

Table 4.17: Proximate analysis of the 3 feeds, Gross Energy and Metabolisable Energy of the 3 feeds, Fecal Energy and Digestible Energy of the 3 diet groups

	Groups		
PARAMETER	SC	HFD	LCD
Moisture (%)	9.28	9.39	9.81
Crude Protein (%)	22.69	22.27	23.15
Crude Fat (%)	3.63	16.27*	2.27*
Crude Fiber (%)	8.64	3.64*	20.64*
ASH (%)	6.21	5.16	6.37
NFE (%)	49.55	42.76	37.76
Gross Energy (Kcal/g)	3.30	4.46	2.66
Metabolizable Energy (Kcal/g)	2.84	3.66	2.33
Fecal Energy Male (Female) Kcal/g	1.20(1.19)	1.20(1.20)	1.11(1.11)
Digestible Energy Male (Female) Kcal/g	2.1(2.10)	3.26(3.26)	1.55(1.55)

Values expressed in mean ± S.E.M of 5 rats, where NFE = Nitrogen–free Extract or Carbohydrate portion; SC = Standard chow; HFD = High fat diet; LCD = Low caloric diet. * = $p<0.05$ when SC and HFD differ significantly compare with LCD.

4.46 Effect of Sexual Dimorphism on Anthropometric Variables of Rats Fed for 5 weeks on Low-Calorie Diet

A significantly reduction in $p<0.05$ of LCD and SC when compared with HFD in both genders. A significantly rise in $p<0.05$ was noticed in LCD when compared with SC in female groups. Finally, significantly reduction in $p<0.05$ in the female when related to the male rats respectively. (table 4.18).

Significantly reduction in $p<0.05$ in the AC of LCD and SC when compared with HFD in both genders respectively. Also, significantly reduction in $p<0.05$ was noticed in the female when related with their male rats respectively. (table 4.18)

Significantly increase in $p<0.05$ was observed in the initial BW in grams of HFD and LCD when related with SC in both genders respectively. There was significantly decreases in $p<0.05$ in female when related with males. A significant reduction in $p<0.05$ was observed in the final BW of SC and LCD when compared with HFD in both genders respectively. Also, there was significantly reduction in LCD when related to SC. Finally, there was a significant decrease in female when compared with their male rats. (table 4.18)

Table 4.18: Effect of Sexual Dimorphism on Anthropometric Variables of Rats Fed for 5 weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Body Length (cm)		
Male	21.0 ± 0.91	23.0 ± 0.77	22.0 ± 0.32
Female	21.0 ± 0.73	22.0 ± 0.42	22.0 ± 0.97
	Thoracic Circumference (cm)		
Male	6.5 ± 0.09	7.7 ± 0.27*	6.7 ± 0.21[a]
Female	4.7 ± 0.22*	6.4 ± 0.24[#, a]	5.4 ± 0.13[#, b, c]
	Abdominal Circumference (cm)		
Male	7.7 ± 0.15	9.4 ± 0.31*	7.8 ± 0.13[a]
Female	6.8 ± 0.26*	8.0 ± 0.15[#, a]	7.1 ± 0.15[b, c]
	Initial Body Weight (grams)		
Male	204.0 ± 3.90	256 ± 5.20*	250.0 ± 6.40*
Female	201.0 ± 2.30	236 ± 1.80[#, a]	232.0 ± 2.80[#, c]
	Final Body Weight (grams)		
Male	241.0 ± 2.30	300.0 ± 4.70*	224.0 ± 4.60*, [a]
Female	233.0±2.00*	274.0 ± 1.00[#, a]	210.0 ± 1.80[#, b, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.47 Effect of Sexual Dimorphism on Obesity Anthropometric indices of Rats Fed for 5 weeks on Low-Calorie Diet

A significant reduction in $p<0.05$ was observed in the Body Mass Index of SC and LCD when related to HFD in both genders respectively. Also, significantly reduction in $p<0.05$ in LCD when related with SC in female rats. Finally, a decrease in $p<0.05$ in the BMI of female LCD when related with that of males. (table 4.19)

Significantly decreases in $p<0.05$ was noticed in the Adiposity index of SC and LCD when compared with HFD in both genders respectively. Also, significantly reduction in $p<0.05$ in LCD when related with SC in both gender respectively. Finally, there was significantly increases in $p<0.05$ in the AI of female HFD when related with that of male. (table 4.19)

Significantly decreases in $p<0.05$ was noticed in the Adipose tissue weight of SC and LCD when compared with HFD in both genders respectively. Also, there was significantly reduction in $p<0.05$ in LCD when related with SC in both genders respectively. Finally, there was significantly reduction in $p<0.05$ in the adipose tissue weight of female SC when related to that of male group. (table 4.19)

Table 4.19: Effect of Sexual Dimorphism on Obesity Anthropometric indices of Rats Fed for 5 weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Body Mass Index (g/cm^2)		
Male	11.0 ± 0.07	13.0 ± 0.39*	11.0 ± 0.12^{a}
Female	11.0 ± 0.09	13.0 ± 0.05$^{\#}$	9.9 ± 0.09$^{\#, b, c}$
	Adiposity Index (%)		
Male	2.2 ± 0.10	4.6 ± 0.36*	1.2 ± 0.16*$^{, a}$
Female	1.9 ± 0.02	5.8 ± 0.19$^{\#, a}$	1.5 ± 0.05$^{\#, b}$
	Adipose tissue weight (g)		
Male	5.2 ± 0.28	14.0 ± 1.40*	2.6 ± 0.42*$^{, a}$
Female	4.4 ± 0.06*	16.0 ± 0.59$^{\#}$	3.2 ± 0.13$^{\#, b}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD); ^{c} $p<0.05$ Female (LCD) compared with Male (LCD)

4.48 Effect of Sexual Dimorphism on Feed indices of Rats Fed for 5 weeks on Low-Calorie Diet

Significantly increases in $p<0.05$ in Initial feed intake of SC and LCD when compared to HFD respectively in male rats. There was significantly reduction in $p<0.05$ in the SC, HFD and LCD of females when related to with their male rats respectively. (table 4.20)

Significantly increases in $p<0.05$ in Final feed intake of SC and LCD when compared to HFD respectively in male rats. There was significantly reduction in $p<0.05$ in the SC, HFD and LCD of females when compared with their male rats respectively. (table 4.20)

Significantly reduction in $p<0.05$ in Energy intake of SC and LCD when compared to HFD in both genders respectively. Also, there was a significant decrease in $p<0.05$ in LCD when compared to SC in both genders respectively. Finally, significantly reduction in the female rat in all groups when compared to their male rats respectively. (table 4.20)

A significant rise in $p<0.05$ was noticed in the Feed efficiency of LCD when compared with SC and HFD in both genders respectively. In female rats, significantly decreases in $p<0.05$ in HFD when related to SC. Finally, there was a significant increase in $p<0.05$ in female rats when related to their male rats respectively. (table 4.20)

Table 4.20: Effect of Sexual Dimorphism on Feed indices of Rats Fed for 5 weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Initial Feed Intake (g/day)		
Male	103.0 ± 0.49	95.0 ± 0.81*	103.0 ± 0.58[a]
Female	72.0 ± 1.70*	75.0 ± 0.40[a]	72.0 ± 0.93[c]
	Final Feed Intake (g/day)		
Male	102.0 ± 0.71	95.0 ± 0.71*	103.0 ± 0.37 [a]
Female	72.0 ± 1.20*	75.0 ± 0.86[a]	72.0 ± 0.89 [c]
	Energy Intake (KJ/day)		
Male	291.0 ± 0.71	345.0 ± 1.40*	239.0 ± 0.57*, [a]
Female	204.0 ±0.15*	272.0 ± 0.93[#, a]	167.0 ± 0.74[#, b, c]
	Feed Efficiency (%)		
Male	77.0 ± 1.90	80.0 ± 1.90	99.0 ± 1.70*, [a]
Female	107.0 ± 2.40*	94.0 ± 2.20[#, a]	133.0 ± 2.00[#, b, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.49 Effect of Sexual Dimorphism on Abdominal Region of Rats Fed for 5 weeks on Low-Calorie Diet

Decrease deposition of abdominal fat in the LCD of both genders are seen in comparison with the SC of both male and female respectively. (plate 4.6)

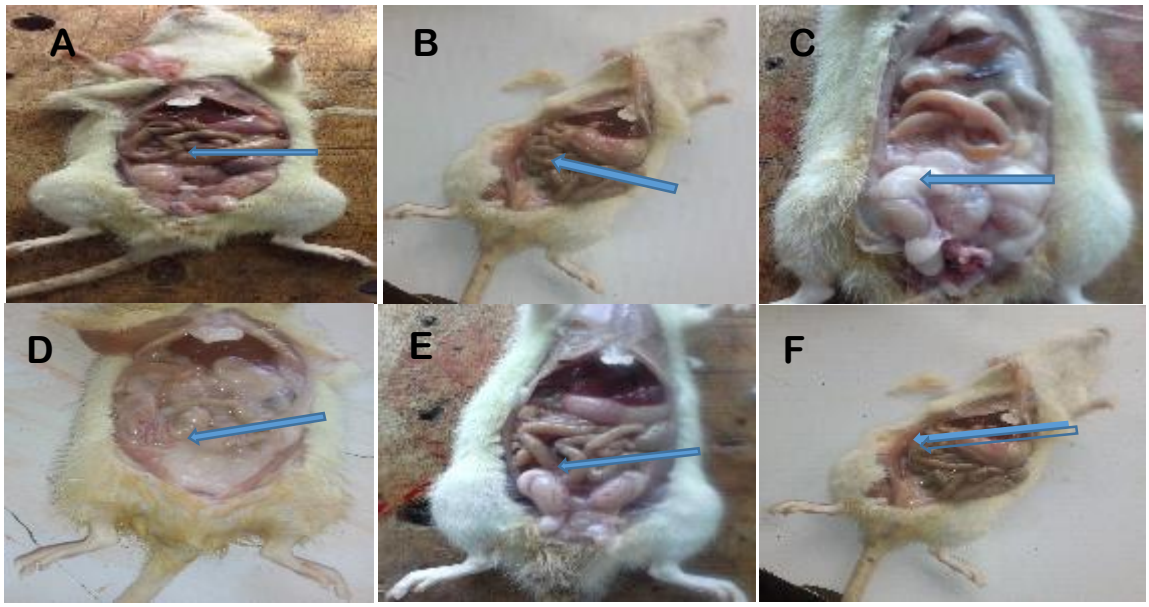


Plate 4.6: Pictorial view of the abdominal region after 5 weeks feeding of a low-calorie diet

(A) SC male, (B) SC female, (C) HFD male, (D) HFD female, (E) LCD male, (F) LCD female; after 5 weeks feeding of a low-calorie diet. Where SC = Standard chow; HFD = High fat diet; LCD = Low calorie diet. Arrows show areas where fat deposition could be seen.

4.50 Effect of Sexual Dimorphism on Serum Lipid Profile of Rats Fed for 5 weeks on Low-Calorie Diet

Significantly rise in $p<0.05$ was observed in the serum triglyceride concentration of LCD when compared with SC but a decrease in $p<0.05$ was observed when compared with HFD in both genders. There was significantly rise in $p<0.05$ in SC, HFD and LCD of females when related to their males respectively. (table 4.21)

Significantly increases in $p<0.05$ in the serum total cholesterol concentration of LCD was noticed when compared with SC but a decrease was observed when compared with HFD in both genders. A significant reduction in $p<0.05$ was observed in the SC of female rats when compared to the SC of male rats but significantly rise in the HFD of female rats when related with the HFD. (table 4.21)

A significant reduction in $p<0.05$ was observed in the High density lipoprotein concentration of LCD when compared with SC but a significant increase in $p<0.05$ was noticed when compared to HFD. There was a significant increase in $p<0.05$ in Female HFD when compared with HFD of male. (table 4.21)

A significant reduction in $p<0.05$ was observed in the serum Low density lipoprotein value of LCD when compared with HFD in Wistar rats. (table 4.21)

Table 4.21: Effect of Sexual Dimorphism on Serum Lipid Profile of Rats Fed for 5 weeks on Low-Calorie Diet

Gender \ Group	SC	HFD	LCD
		Triglyceride (mg/dl)	
Male	114.0±4.40	202.0±6.90*	146.0±4.30*, a
Female	57.0±6.00*	272.0±19.00#, a	137.0±12.00#, b, c
		Total Cholesterol (mg/dl)	
Male	80.0±6.90	217±9.8*	111.0±1.40*, a
Female	109.0±6.40*	258±11#, a	130.0±9.70#, b
		High Density Lipoprotein (mg/dl)	
Male	45.0±1.70	17±0.67*	32.0±0.97*, a
Female	41.0±1.40	24±0.60#, a	32.0±1.40#, b
		Low Density Lipoprotein (mg/dl)	
Male	20.0±0.80	31.0±2.30*	22.0±0.52a
Female	18.0±1.10	34.0±3.20#	21.0±0.63b

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); # $p<0.05$ Female (HFD and LCD) compared with Female (SC); a $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); b $p<0.05$ Female (LCD) compared with Female (HFD); c $p<0.05$ Female (LCD) compared with Male (LCD)

4.51 Effect of Sexual Dimorphism on Serum Cardiometabolic risk variables of Rats Fed for 5 weeks on Low-Calorie Diet

In Apolipoprotein A level, there was no significant difference observed. (table 4.22)

In Apolipoprotein B level, significantly reduction in $p<0.05$ was noticed in LCD when related with HFD in both genders. Also, a significant rise in $p<0.05$ was observed in HFD when compared with SC and LCD respectively. (table 4.22)

In Apolipoprotein B/Apolipoprotein A ratio, a significant decreased in $p<0.05$ was noticed in LCD when compared with HFD in both genders. Also, a significant decreased in $p<0.05$ was observed in HFD when related with SC in both genders. A significant increase in $p<0.05$ in LCD of females when compared to SC was also observed. Finally, there was significantly rise in $p<0.05$ in female LCD when related to LCD male rats. (table 4.22)

In Lipoprotein lipase level, no significant difference amongst the groups. (table 4.22)

Table 4.22: Effect of Sexual Dimorphism on Serum Cardiometabolic risk variables of Rats Fed for 5 weeks on Low-Calorie Diet

Group / Atherogenic Index	SC	HFD	LCD
		Apolipoprotein A (mg/dl)	
Male	5.0±0.3	4.8±0.03	4.5±0.35
Female	4.5±0.18	4.3±0.08	4.1±0.13
		Apolipoprotein B (mg/dl)	
Male	10.0±0.28	13.0±0.20*	11.0±0.45[a]
Female	10.0±0.16	14.0±1.40[#]	10.0±0.20[b]
		ApolipoproteinB/ApolipoproteinA (ratio)	
Male	2.2±0.08	3.1±0.09*	2.1±0.05[a]
Female	2.1±0.08	3.1±0.14[#]	2.5±0.06[#, b, c]
		Lipoprotein Lipase (mg/dl)	
Male	24.0±1.90	26.1±3.00	28.0±3.20
Female	21.0±2.60	25.0±2.20	23.0±1.70

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.52 Effect of Sexual Dimorphism on Serum Free Fatty Acid Concentration of Rats Fed for 5 weeks on Low-Calorie Diet

Significantly decreases in $p<0.05$ of free fatty acid in LCD when compared with HFD in both genders. Also, a significant rise in $p<0.05$ was observed in HFD when compared with SC in both genders. There was significantly decrease in $p<0.05$ in female HFD when related to HFD male rats. (figure 4.24)

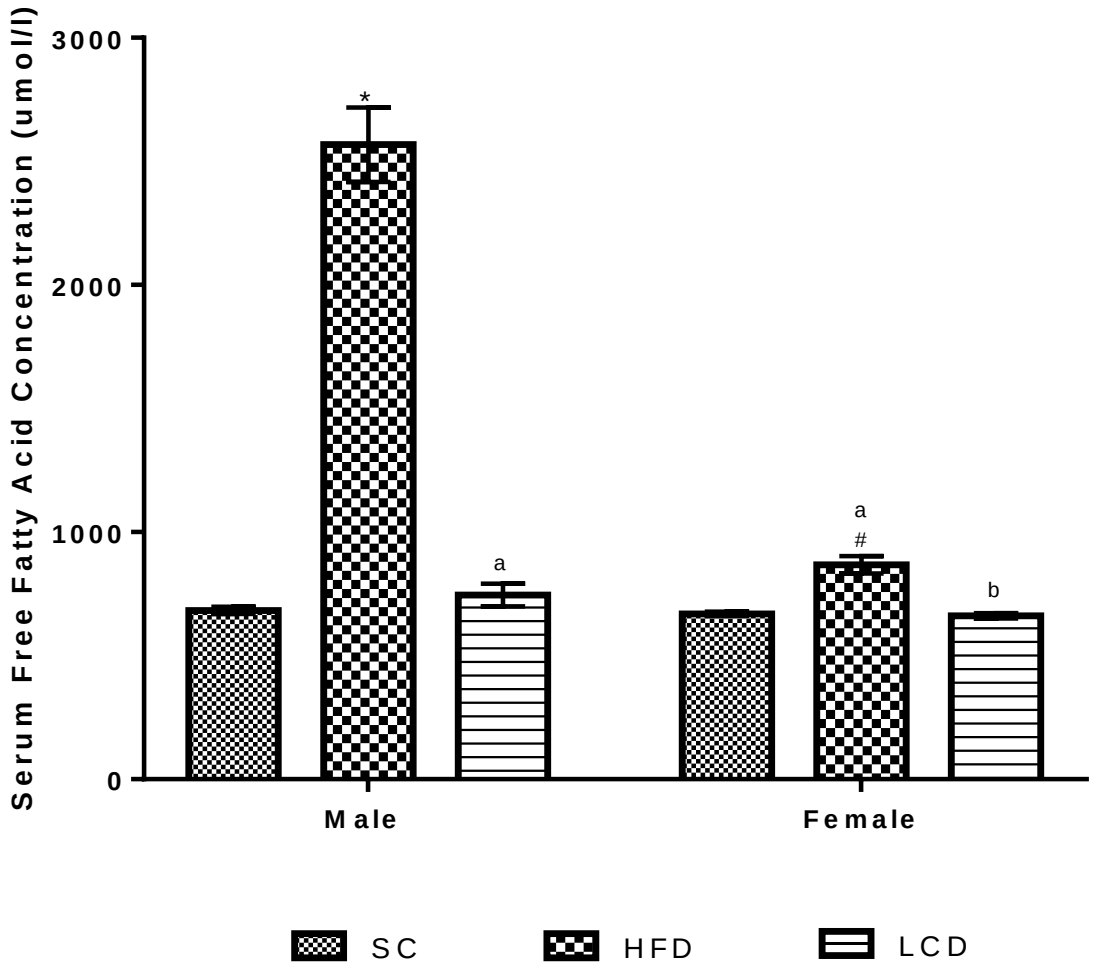


Figure 4.24 Serum Free Fatty Acid Concentration after Low calorie diet treatment for 5 weeks

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Male (LCD)

4.53 Effect of Sexual Dimorphism on Reproductive hormones of Rats Fed for 5 weeks on Low-Calorie Diet

There was no significant difference observed in Luteinizing hormone level. (table 4.23)

A significant rise in $p<0.05$ was observed in follicular stimulating hormone concentration in LCD when related to SC in female rats. (table 4.23)

In Estrogen concentration, a significant decrease in $p<0.05$ was noticed in LCD when compared with HFD in female rats. Also, there was a significant rise in $p<0.05$ in HFD when compared with SC in female rats. (table 4.23)

Finally, in testosterone concentration, a significant decrease in LCD when related to SC but a significant increase in $p<0.05$ when compared with HFD in male rats was noticed. There was a significant decrease in $p<0.05$ in HFD and LCD when related to SC in female rats was observed. (table 4.23)

Table 4.23: Effect of Sexual Dimorphism on Reproductive hormones of Rats Fed for 5 weeks on Low-Calorie Diet

Variable \ Group	SC	HFD	LCD
	Luteinizing hormone (µlU/ml)		
Male	3.5±0.17	3.9±0.24	3.6±0.12
Female	3.5±0.19	3.5±0.09	3.5±0.12
	Follicular Stimulating Hormone (µlU/ml)		
Male	1.5±0.11	1.6±0.13	1.6±0.06
Female	1.3±0.07	1.4±0.15	1.5±0.04$^{\#}$
	Estrogen (pg/ml)		
Male	0.00	0.00	0.00
Female	16.0±0.26	23.0±2.00$^{\#}$	16.0±1.50^{b}
	Testosterone (ng/ml)		
Male	16.0±0.37	7.9±0.57*	11.0±0.77*$^{, a}$
Female	2.4±0.13	1.0±0.08$^{\#}$	1.12±0.10$^{\#}$

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD)

4.54 Effect of Sexual Dimorphism on Serum Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 weeks on Low-Calorie Diet

A significant rise male rats in $p<0.05$ was noticed in the Malondialdehyde concentration of HFD and LCD when related with SC, also, a significant decrease in $p<0.05$ in LCD when compared to HFD was observed. In female rats, there was a significant increase in $p<0.05$ in HFD when compared with SC but there was a significant decrease in $p<0.05$ in LCD when compared with HFD. Finally, significantly reduction in $p<0.05$ in the HFD of females when compared with the HFD of male rats. (table 4.24)

In male rats, significantly decreases in $p<0.05$ was noticed in the reduced Glutathione level of LCD when related to SC and HFD respectively. In female rats, a significant increase in $p<0.05$ in LCD when compared to SC and HFD respectively was observed. Also, there was a significant decrease in $p<0.05$ in SC and HFD of female rats when compared with SC and HFD of male rats respectively. (table 4.24)

A significant reduction in $p<0.05$ was noticed in the SOD concentration of HFD and LCD when compared with SC in male rats. A significant rise in $p<0.05$ was observed in HFD when compared to SC but a decrease in $p<0.05$ in LCD when compared to SC in female rats. Finally, significantly reduction in $p<0.05$ in the SC and LCD of females when compared with the SC and LCD of male rats but significantly rise in $p<0.05$ in HFD of female rats when related to that of male rats. (table 4.24)

A significant reduction in $p<0.05$ was observed in the catalase concentration of HFD when compared to SC in female rats. Also, a significant reduction in $p<0.05$ was observed in HFD of females when related with HFD of male rats. (table 4.24)

Table 4.24: Effect of Sexual Dimorphism on Serum Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Malondialdehyde (mM)		
Male	11.0±0.30	31.0±1.20*	14.0±0.48*, a
Female	14.0±0.92	27.0±0.88#, a	16.0±0.71
	reduced Glutathione (mM)		
Male	0.6±0.04	0.6±0.02	0.4±0.03*, a
Female	0.4±0.01*	0.4±0.01a	0.5±0.04#, b
SOD (u/ml)			
Male	42.0±0.63	35.0±0.84*	38.0±0.66*
Female	36.0±0.96*	39.0±0.64#, a	31.0-±0.28#, c
Catalase (u/ml/mins)			
Male	24.0±1.00	23.0±1.40	24.0±1.30
Female	21.0±0.95	18.0±0.83#, a	20.0±0.34

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); # $p<0.05$ Female (HFD and LCD) compared with Female (SC); a $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); b $p<0.05$ Female (LCD) compared with Female (HFD); c $p<0.05$ Female (LCD) compared with Male (LCD)

4.55 Effect of Sexual Dimorphism on Weight of Liver of Rats Fed for 5 Weeks on Low-Calorie Diet

Significantly rise in $p<0.05$ in weight was noticed in LCD when compared with SC but a significant reduction in $p<0.05$ was observed when related with HFD in both genders. Finally, there was a significant decrease in SC and LCD in female rats when compared with SC and LCD of males respectively. (table 4.25)

Table 4.25: Effect of Sexual Dimorphism on Weight (grams) of Liver of Rats Fed for 5 Weeks on Low-Calorie Diet

Tissue/ Gender	**SC**	**HFD**	**LCD**
Male	5.4 ± 0.14	11.0 ± 0.48*	6.9 ± 0.11*, [a]
Female	8.0 ± 0.18*	12.0 ± 0.21[#]	9.5 ± 0.12[#, b, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.56 Effect of Sexual Dimorphism on Liver Lipid Profile of Rats Fed for 5 weeks on Low-Calorie Diet

In total cholesterol concentration, significantly decreases in $p<0.05$ was noticed in LCD when related with HFD respectively. A significant rise in $p<0.05$ was noticed in LCD when related with SC in female rats. Also, significantly rise in $p<0.05$ was observed in HFD when related to SC respectively. (table 4.26)

In triglyceride concentration, significantly reduction in $p<0.05$ was noticed in LCD when related with HFD respectively. Significantly rise in $p<0.05$ was observed in HFD when related to SC respectively. Also, significantly reduction in $p<0.05$ in HFD of female rats when compared with HFD of male rats respectively. (table 4.26)

In high density lipoprotein concentration, when compared with SC, a significant reduction in $p<0.05$ was observed in HFD and LCD in both genders respectively. Significantly rise in $p<0.05$ was observed in SC, HFD and LCD of female rats when related to their male rats respectively. (table 4.26)

In low density lipoprotein concentration, a significant decrease in $p<0.05$ was related in LCD when compared with HFD respectively. A significant rise in $p<0.05$ was observed in HFD when compared with SC in both genders respectively. Finally, a significant reduction in $p<0.05$ was observed in the HFD and LCD of female rats related to the HFD and LCD of male rats respectively. (table 4.26)

Table 4.26: Effect of Sexual Dimorphism on Liver Lipid Profile of Rats Fed for 5 weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Total Cholesterol (mg/dl)		
Male	73.0±6.8	196.0±13.00*	70.0±6.10[a]
Female	57.0±12	177.0±13.00[#]	72.0±5.70[#, b]
	Triglyceride (mg/dl)		
Male	142.0±9.20	395.0±17.00*	153.0±9.40[a]
Female	140.0±10.00	350.0±6.30[#, a]	165.0±9.70[b]
	High Density Lipoprotein (mg/dl)		
Male	20.0±1.50	15.0±0.80*	14.0±0.76*
Female	35.0±0.83*	21.0±1.10[#, a]	23.0±1.30[#, c]
	Low Density Lipoprotein (mg/dl)		
Male	26.0±0.95	82.0±3.60*	31.0±1.60[a]
Female	17.0±1.50	48.0±2.30[#, a]	14.0±0.85[b, c]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.57 Effect of Sexual Dimorphism on Liver Homogenate Free Fatty Acid Concentration of Rats Fed for 5 weeks on Low-Calorie Diet

A significant reduction in $p<0.05$ was noticed in LCD when compared with HFD in both genders respectively. Significantly rise in $p<0.05$ was observed in HFD when related to SC in both genders respectively. Also, significantly rise in $p<0.05$ in LCD when related to SC in females. Finally, significantly reduction in $p<0.05$ in SC of females when related to SC of males but an increase in $p<0.05$ in female HFD when compared with HFD male rats respectively. (figure 4.25)

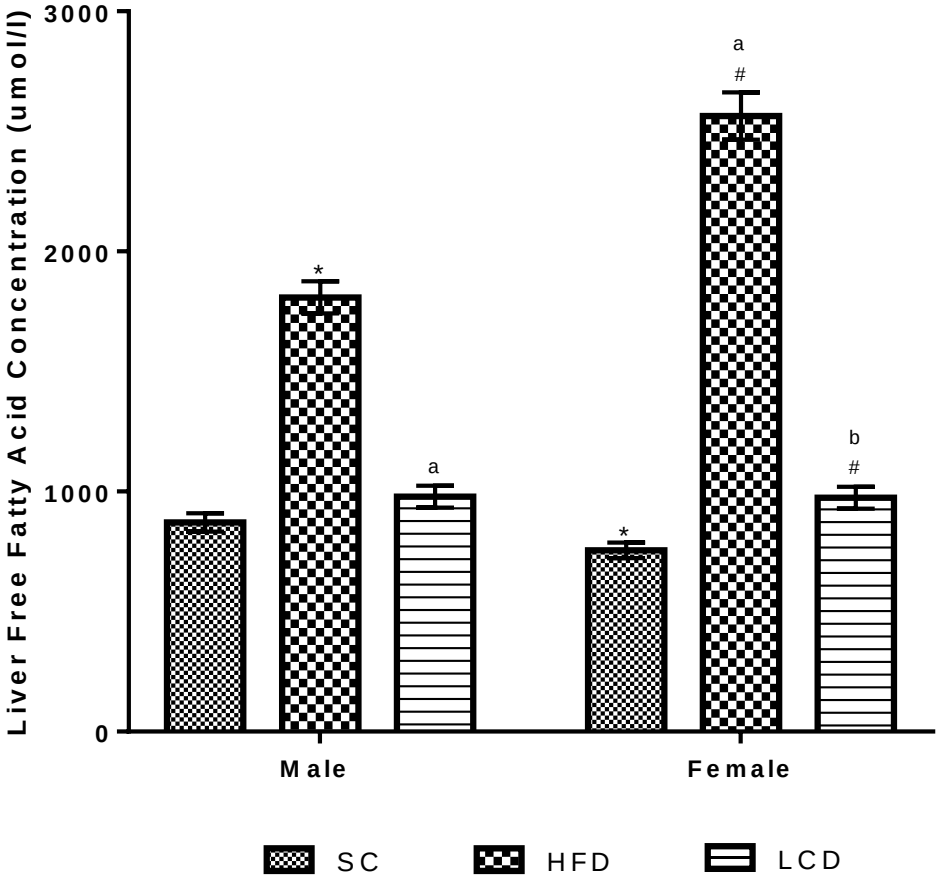


Figure 4.25: Liver Homogenate Free Fatty Acid Concentration after Low calorie diet treatment for 5 weeks

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD)

4.58 Effect of Sexual Dimorphism on Liver Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 weeks on Low-Calorie Diet

In Malondialdehyde concentration, a significant rise in $p<0.05$ was observed in HFD when related to SC but significantly reduction in LCD when compared to HFD was observed. Also, significantly reduction in $p<0.05$ was noticed in LCD when compared with SC of female rats. Finally, a significant rise in $p<0.05$ was observed in the SC in female rats in comparison with the SC of males but a reduction in $p<0.05$ in HFD of females when related to HFD of male rats. (table 4.27)

In reduced Glutathione concentration, a significant decrease in $p<0.05$ was noticed in HFD and LCD when related with SC respectively in female rats and a significant increase in $p<0.05$ in LCD when compared to HFD in males. Also, there was significantly rise in $p<0.05$ in SC, HFD and LCD of female rats when compared with their male rats respectively. (table 4.27)

In Superoxide Dismutase concentration, significantly reduction in $p<0.05$ was noticed in HFD when related with SC but an increase in $p<0.05$ in LCD when compared to HFD all in female rats. (table 4.27)

In catalase, no significant change was observed in tissue homogenates. (table 4.27)

Table 4.27: Effect of Sexual Dimorphism on Liver Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 weeks on Low-Calorie Diet

Variable \ Group	SC	HFD	LCD
	Malondialdehyde (mM)		
Male	19.0±1.10	53.0±0.92*	21.0±1.00[a]
Female	24.0±1.00*	37.0±1.20[#, a]	19.0±1.10[#, b]
	reduced Glutathione (mM)		
Male	0.1±0.01	0.1±0.01	0.2±0.01[a]
Female	0.5±0.04*	0.3±0.01[#, a]	0.3±0.01[#, c]
	Superoxide Dismutase (µ/ml)		
Male	43.0±1.10	43.0±1.20	45.0±1.50
Female	48.0±2.20	38.0±0.93[#]	49.0±1.10[b]
	Catalase (µ/ml/mins)		
Male	24.0±0.07	24.0±0.36	25.0±0.03
Female	25.0±0.53	24.0±0.06	24.0±0.04

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.59 Effect of Sexual Dimorphism on Histology of the Liver Rats Fed for 5 Weeks on Low-Calorie Diet

The black arrows point to the different levels of fat deposition while the blue arrow shows areas where congestion occurred on the liver on H and E staining. There was very mild infiltration of fat into the cytoplasm and moderate congestion of the central venules in animals fed with LCD. Also, there was severe cytoplasmic infiltration of fat – macrovesicular hepatic steatosis in animals fed with HFD. (plate 4.6)

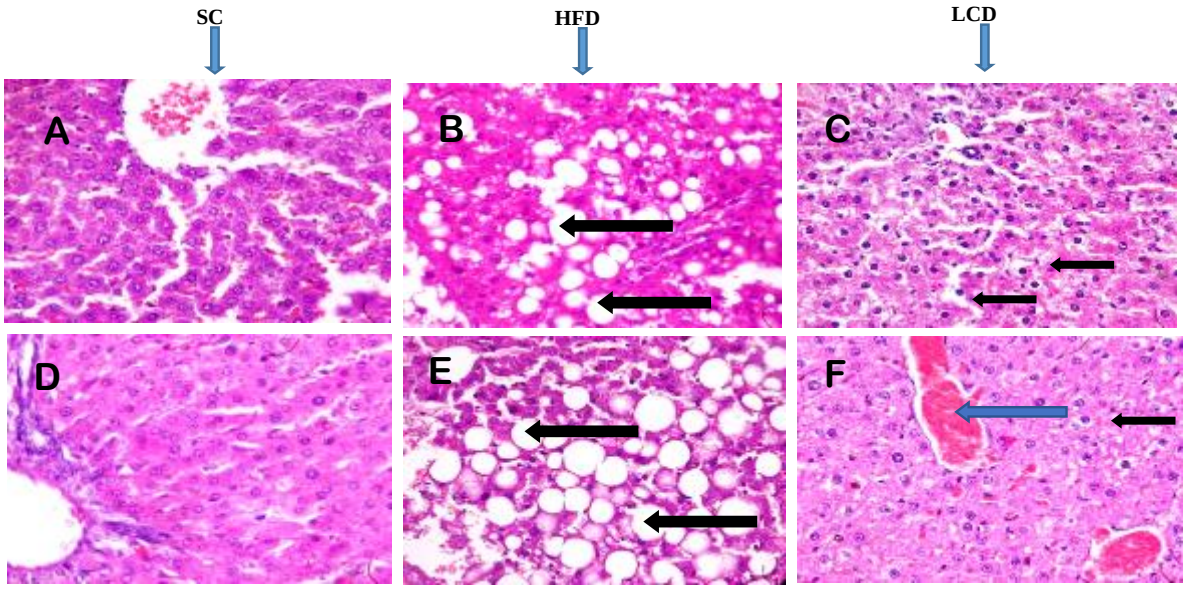


Plate 4.7: Histology of the Liver of rats fed with a low calorie diet with X400 Magnification

(A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment. Where SC = Standard chow; HFD = High fat diet; LCD = Low calorie diet. Arrows show areas where either fat deposition or congestion could be seen.

4.60 Effect of Sexual Dimorphism on the Expression of Liver Fatty Acid Transporter Protein 4 (FATP4) of Rats Fed for 5 weeks on Low-Calorie Diet

A significant increase was observed in FATP4 expression in HFD when compared with SC but a significant decrease in $p<0.05$ in LCD was observed when compared to HFD. (figure 4.26 A and B)

(A)

(B)

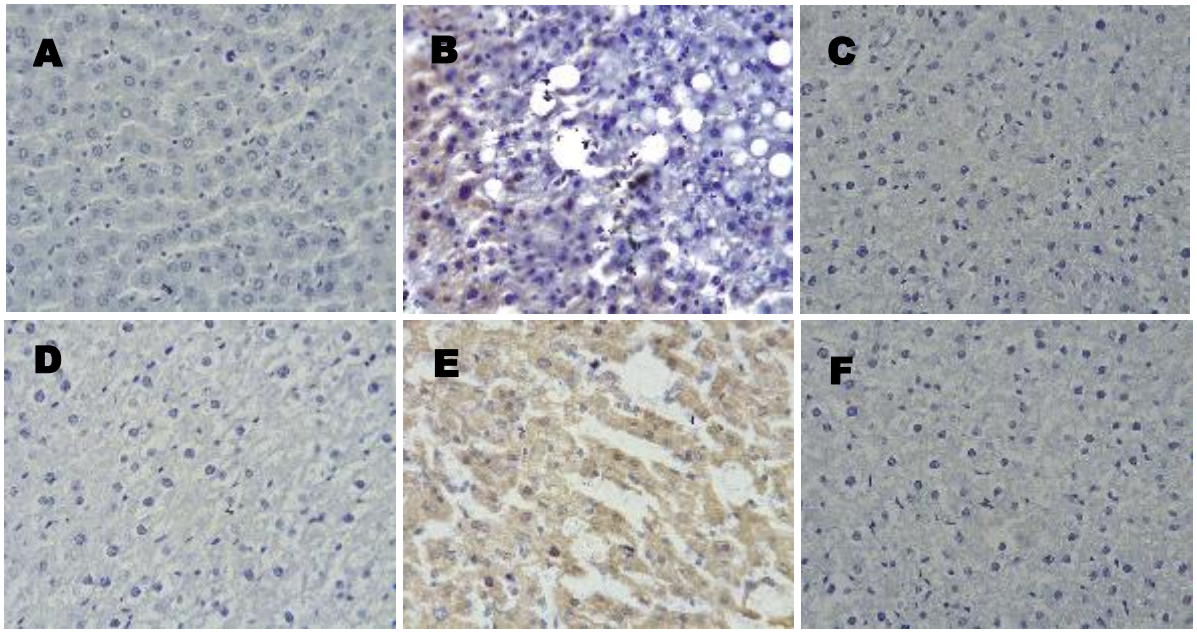


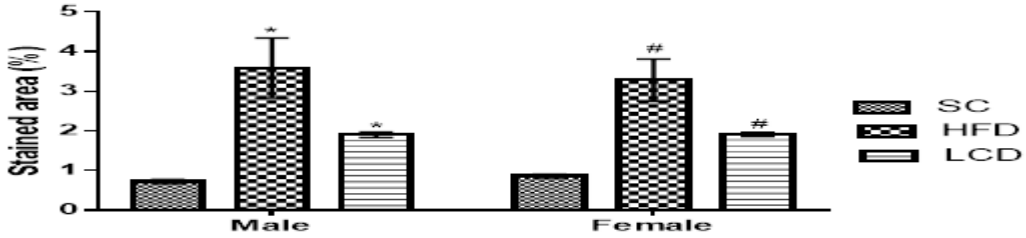
Figure 4.26: Liver FATP4 expression of rats, fed with a low calorie diet with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.26A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD); Figure 4.26B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.61 Effect of Sexual Dimorphism on the Expression of Liver Cluster of Differentiation 36 (CD36) of Rats Fed for 5 weeks on Low-Calorie Diet

Significantly increase in $p<0.05$ was noticed in CD36 expression in HFD and LCD when compared with SC in both genders respectively in Wistar rats. (figure 4.27 A and B)

(A)



(B)

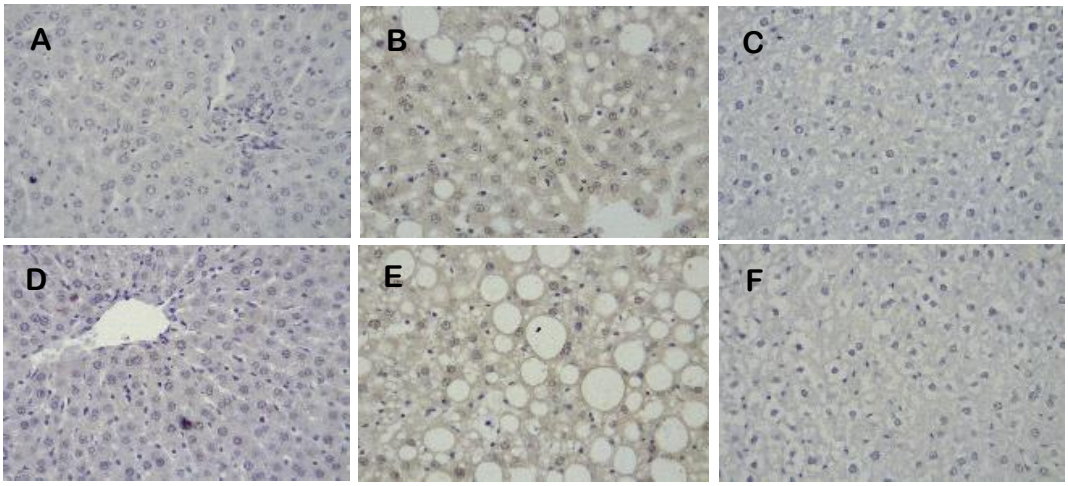


Figure 4.27: Liver CD36 protein expression of rats, fed with a low calorie diet at X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.27A shows the qualitative analysis of the photomicrograph detailing the level of expressions of CD36; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}p<0.05$ Female (HFD and LCD) compared with Female (SC); Figure 4.27B shows the physical colour changes in the photomicrograph of CD36 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.62 Effect of Sexual Dimorphism on Weight of Heart of Rats Fed for 5 Weeks on Low-Calorie Diet

Significantly rise in $p<0.05$ in grams in LCD was noted when compared with SC but significantly reduction was observed when related to HFD in both genders. Also, significantly rise in $p<0.05$ was observed in HFD of Heart when related to SC in both genders. (table 4.28)

Table 4.28: Effect of Sexual Dimorphism on Weight of Heart of Rats Fed for 5 Weeks on Low-Calorie Diet

Tissue/ Gender	**SC**	**HFD**	**LCD**
Heart			
Male	0.6±0.02	1.1±0.04*	0.8±0.02*, [a]
Female	0.7±0.02	1.2±0.10[#]	0.8±0.01[#, b]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD)

4.63 Effect of Sexual Dimorphism on Heart Lipid Profile of Rats Fed for 5 Weeks on Low-Calorie Diet

Significantly reduction in total cholesterol concentration in $p<0.05$ was noticed in LCD when related with HFD in both genders. Also, a significant rise in $p<0.05$ was observed in HFD when compared with SC and LCD in both genders. There was a significant rise in $p<0.05$ in LCD when related to SC in females. A significant reduction in $p<0.05$ in the SC of females when related with the SC of male rats. (table 4.29)

In triglyceride concentration, a significant reduction in $p<0.05$ was observed in LCD when compared with HFD in both genders. Also, a significant rise in $p<0.05$ was observed in HFD when compared with SC in both genders. Also, significantly reduction in $p<0.05$ in HFD of females when compared with HFD of male rats respectively. (table 4.29)

In high density lipoprotein concentration, significantly reduction in $p<0.05$ was observed in HFD and LCD when compared with SC in both genders. Also, a significant increase in $p<0.05$ was noticed in LCD when compared to HFD in female rats. (table 4.29)

In low density lipoprotein concentration, a significant reduction in $p<0.05$ was observed in LDL when compared with HFD in both genders. Also, significantly rise in $p<0.05$ was observed in HFD when compared with SC in both genders. A significant reduction in $p<0.05$ was observed in the HFD of female rats when compared to the HFD of male rats. (table 4.29)

Table 4.29: Effect of Sexual Dimorphism on Heart Lipid Profile of Rats Fed for 5 Weeks on Low-Calorie Diet

Variable \ Group	SC	HFD	LCD
	Total Cholesterol (mg/dl)		
Male	78.0±6.70	123.0±11.00*	69.0±6.20[a]
Female	43.0±2.90*	121.0±11.00[#]	73.0±5.10[#, b]
	Triglyceride (mg/dl)		
Male	110.0±3.20	257.0±18.0*	121.0±5.40[a]
Female	109.0±5.50	187.0±8.40[#, a]	127.0±6.20[b]
	High Density Lipoprotein (mg/dl)		
Male	27.0±1.60	16.0±1.20*	20.0±1.20*
Female	28.0±1.70	18.0±0.86[#]	23.0±0.95[#, b]
	Low Density Lipoprotein (mg/dl)		
Male	22.0±2.50	57.0±5.90*	24.0±1.80[a]
Female	13.0±1.80	33.0±2.00[#, a]	15.0±1.60[b]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD)

4.64 Effect of Sexual Dimorphism on Heart Homogenate Free Fatty Acid Concentration of Rats Fed for 5 Weeks on Low-Calorie Diet

Significantly rise in $p<0.05$ was observed in HFD when related to SC in both genders. Also, a significant decrease in $p<0.05$ was observed in LCD when compared with HFD. There was a significant decrease in $p<0.05$ in female HFD when related to HFD male rats. (figure 4.28)

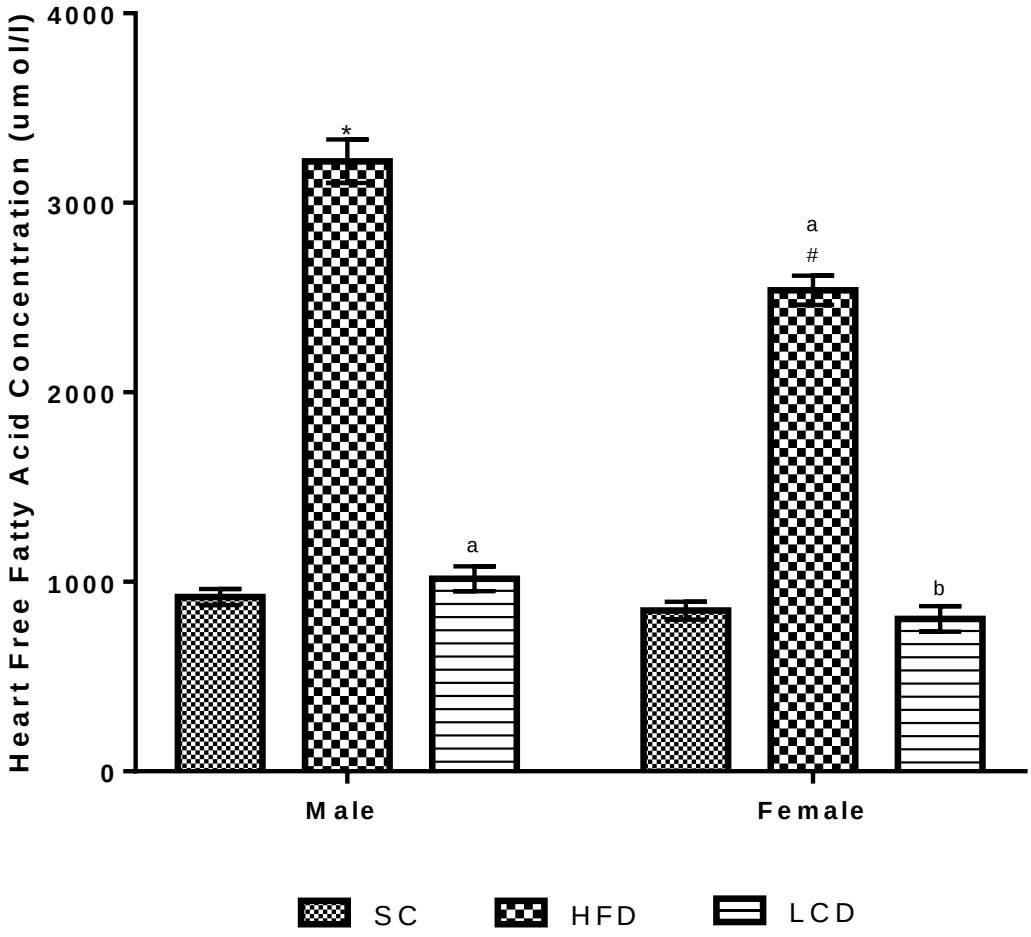


Figure 4.28: Heart Homogenate Free Fatty Acid Concentration after Low calorie diet treatment for 5 weeks

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD)

4.65 Effect of Sexual Dimorphism on Heart Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 weeks on Low-Calorie Diet

In Malondialdehyde concentration, a significant rise in $p<0.05$ was noticed in HFD when compared with SC but a significant decrease in $p<0.05$ in LCD was noticed when compared to HFD in both genders respectively. Also, significant reduction in $p<0.05$ was noticed in LCD when related with SC of female rats. Finally, there was a significant decrease in the SC and HFD of female rats when compared with the SC and HFD of male rats. (table 4.30)

In reduced Glutathione concentration, a significant reduction in $p<0.05$ was noticed in HFD and LCD when compared with SC respectively in female rats. Also, there was significantly reduction in $p<0.05$ in SC, HFD and LCD of females when compared with their male rats respectively. (table 4.30)

A significant rise in $p<0.05$ was noticed in the heart homogenate Superoxide Dismutase level of HFD when related with SC in male and female rats respectively. (table 4.30)

No significant change was observed in Catalase level. (table 4.30)

Table 4.30: Effect of Sexual Dimorphism on Heart Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 weeks on Low-Calorie Diet

Variable \ Group	SC	HFD	LCD
	Malondialdehyde (mM)		
Male	15.0±0.79	24.0±1.40*	17.0±0.70[a]
Female	22.0±0.74*	30.0±0.90[#, a]	13.0±1.00[#, b]
	reduced Glutathione (mM)		
Male	0.7±0.07	0.8±0.05	0.7±0.07
Female	0.5±0.02*	0.3±0.02[#, a]	0.3±0.03[#, c]
	Superoxide Dismutase (u/ml)		
Male	40.0±0.95	45.0±0.94*	42.0±0.97
Female	40.0±1.10	46.0±0.76[#]	43.0±0.98
	Catalase (u/ml/mins)		
Male	25.0±0.03	25.0±0.02	24.0±0.46
Female	25.0±0.02	25.0±0.07	25.0±0.02

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.66 Effect of Sexual Dimorphism on Histology of the Heart of Rats Fed for 5 Weeks on Low-Calorie Diet

The black arrows point to the different levels of fat deposition while the blue arrow shows areas where congestion occurred on the heart on H and E staining. There was mild fat deposit seen within the inter cardiac muscles and arterial wall in SC male group. There were moderate to severe fat deposits within the inter cardiac muscle in HFD female group and in an area of the myocardium in the HFD male group. There were moderate to severe congestion at the epicardial layer and myocardial layer in LCD of both male and female groups. (plate 4.7)

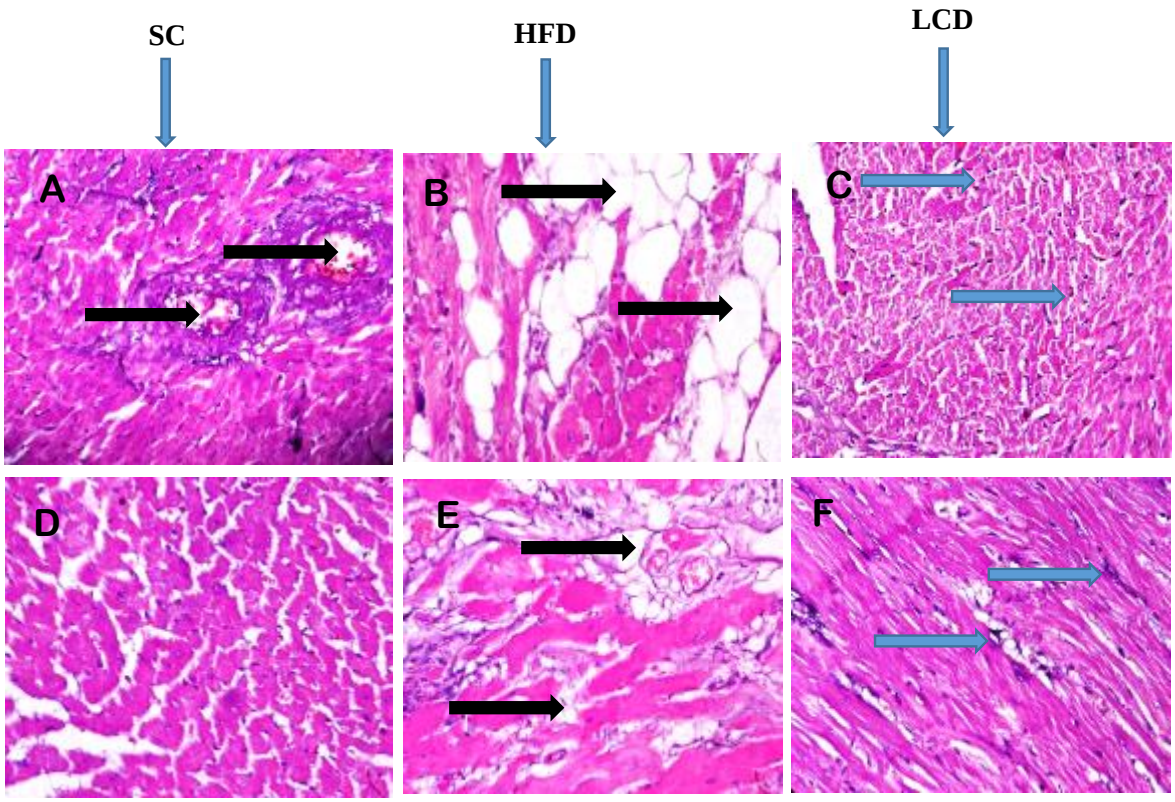


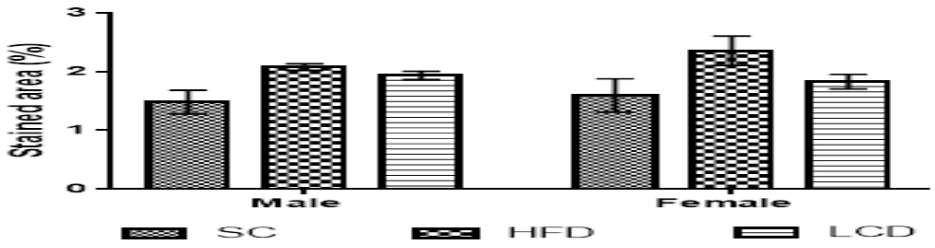
Plate 4.8: Histology of the Heart of rats fed with a low calorie diet with X400 Magnification

(A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment. Where SC = Standard chow; HFD = High fat diet LCD = Low calorie diet. Arrows show areas where either fat deposition or congestion could be seen.

4.67 Effect of Sexual Dimorphism on the Expression of Heart Fatty Acid Transporter Protein 4 (FATP4) of Rats Fed for 5 weeks on Low-Calorie Diet

No significant change was observed on FATP4 expression. (figure 4.29 A and B)

(A)



(B)

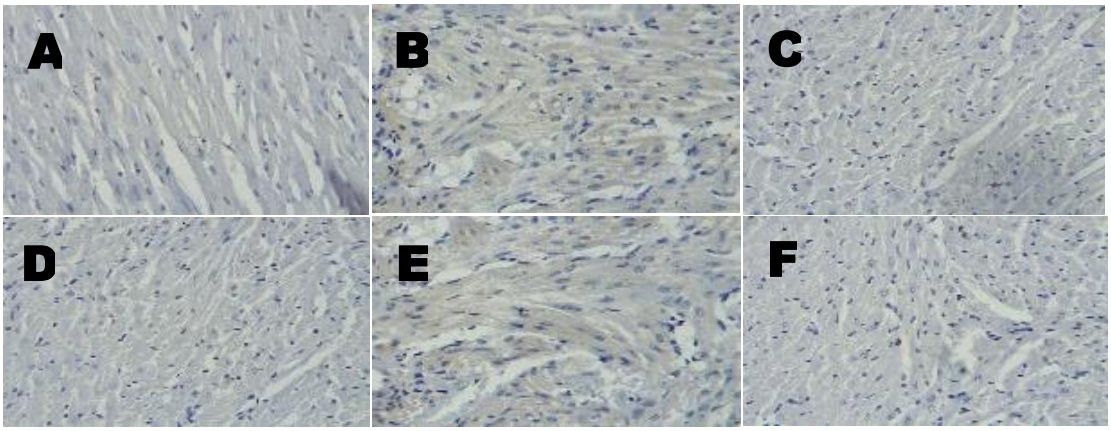


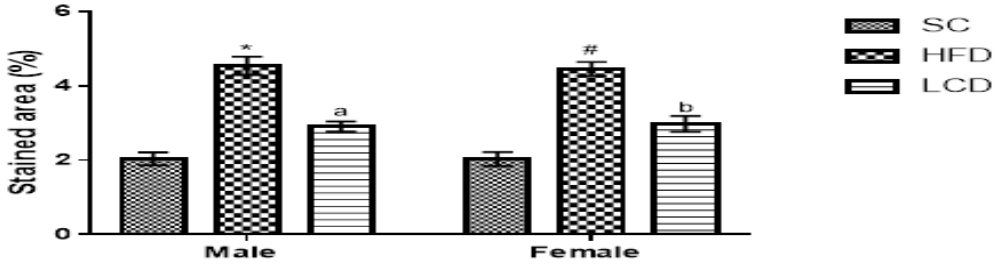
Figure 4.29: FATP4 expression in the Heart of rats fed LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.29A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. $p<0.05$ Figure 4.29B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.68 Effect of Sexual Dimorphism on the Expression of Heart Cluster of Differentiation 36 (CD36) of Rats Fed for 5 weeks on Low-Calorie Diet

A significant increase in $p<0.05$ was observed in the expression of CD36 in the heart of HFD when compared to SC and LCD in both genders respectively. (figure 4.30 A and B)

(A)



(B)

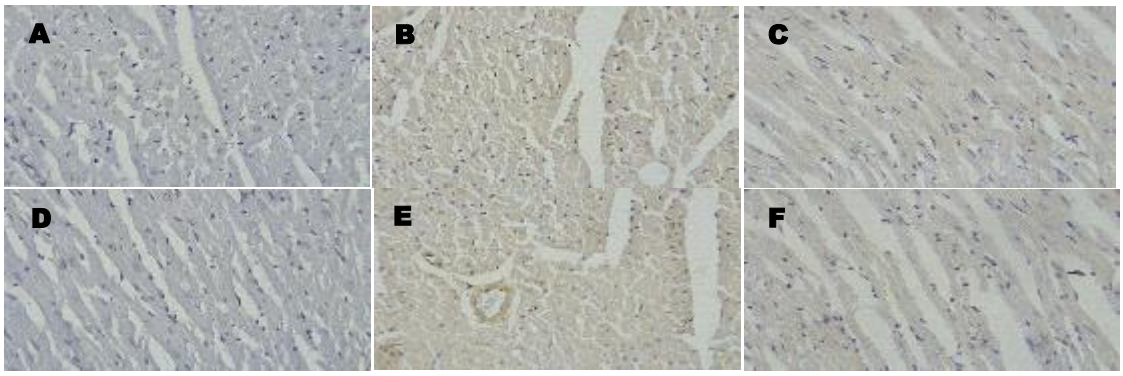


Figure 4.30: CD36 protein expression in the Heart of rats fed LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.30A shows the qualitative analysis of the photomicrograph detailing the level of expressions of CD36; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD); Figure 4.30B shows the physical colour changes in the photomicrograph of CD36 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.69 Effect of Sexual Dimorphism on Small Intestine Homogenate Lipid Profile of Rats Fed for 5 Weeks on Low-Calorie Diet

In total cholesterol concentration, a significant rise in $p<0.05$ was noticed in HFD when compared with SC in both genders. Also, a significant decrease in $p<0.05$ was noticed in LCD when related with HFD in both genders. (table 4.31)

In triglyceride concentration, a significant rise in $p<0.05$ was observed in HFD when compared with SC in both genders. Also, significantly reduction in $p<0.05$ was noticed in LCD when related with HFD in both genders. Significantly reduction in $p<0.05$ in LCD when related to SC in females. Finally, significantly reduction in $p<0.05$ in SC and LCD of female rats when compared with their male rats respectively. (table 4.31)

Significantly reduction in high density lipoprotein concentration in $p<0.05$ was observed in HFD and LCD when related with SC in both genders respectively. (table 4.31)

In low density lipoprotein concentration, significantly rise in $p<0.05$ was observed in HFD when compared with SC in both genders. Also, a significant reduction in $p<0.05$ was observed in LCD when compared with HFD in both genders. A significant reduction in $p<0.05$ in the HFD of female rats when compared to the HFD of male rats. (table 4.31)

Table 4.31: Effect of Sexual Dimorphism on Small Intestine Homogenate Lipid Profile of Rats Fed for 5 Weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Total Cholesterol (mg/dl)		
Male	69.0±7.00	127.0±5.70*	60.0±6.10[a]
Female	66.0±8.00	118.0±15.00[#]	66.0±8.60[b]
	Triglyceride (mg/dl)		
Male	170.0±17.00	379.0±15.00*	182.0±5.80[a]
Female	120.0±4.40*	365.0±14.00[#]	109.0±2.10[#, b, c]
	High Density Lipoprotein (mg/dl)		
Male	26.0±1.20	18.0±0.48*	19.0±1.20*
Female	30.0±1.70	20.0±0.65[#]	22.0±1.10[#]
	Low Density Lipoprotein (mg/dl)		
Male	20.0±1.40	64.0±1.70*	25.0±1.20[a]
Female	18.0±1.90	44.0±1.80[#, a]	19.0±1.50[b]

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD)

4.70 Effect of Sexual Dimorphism on Small Intestine Homogenate Free Fatty Acid Concentration of Rats Fed for 5 Weeks on Low-Calorie Diet

Significantly rise in $p<0.05$ was noticed in HFD when compared with SC in both genders. Furthermore, significantly reduction in $p<0.05$ was noticed in LCD when related with HFD in both genders. Also, significantly rise in $p<0.05$ in LCD when relation to SC in male rats. Finally, there was a significant reduction in $p<0.05$ in SC, HFD and LCD of female rats when compared with their male rats respectively. (figure 4.31)

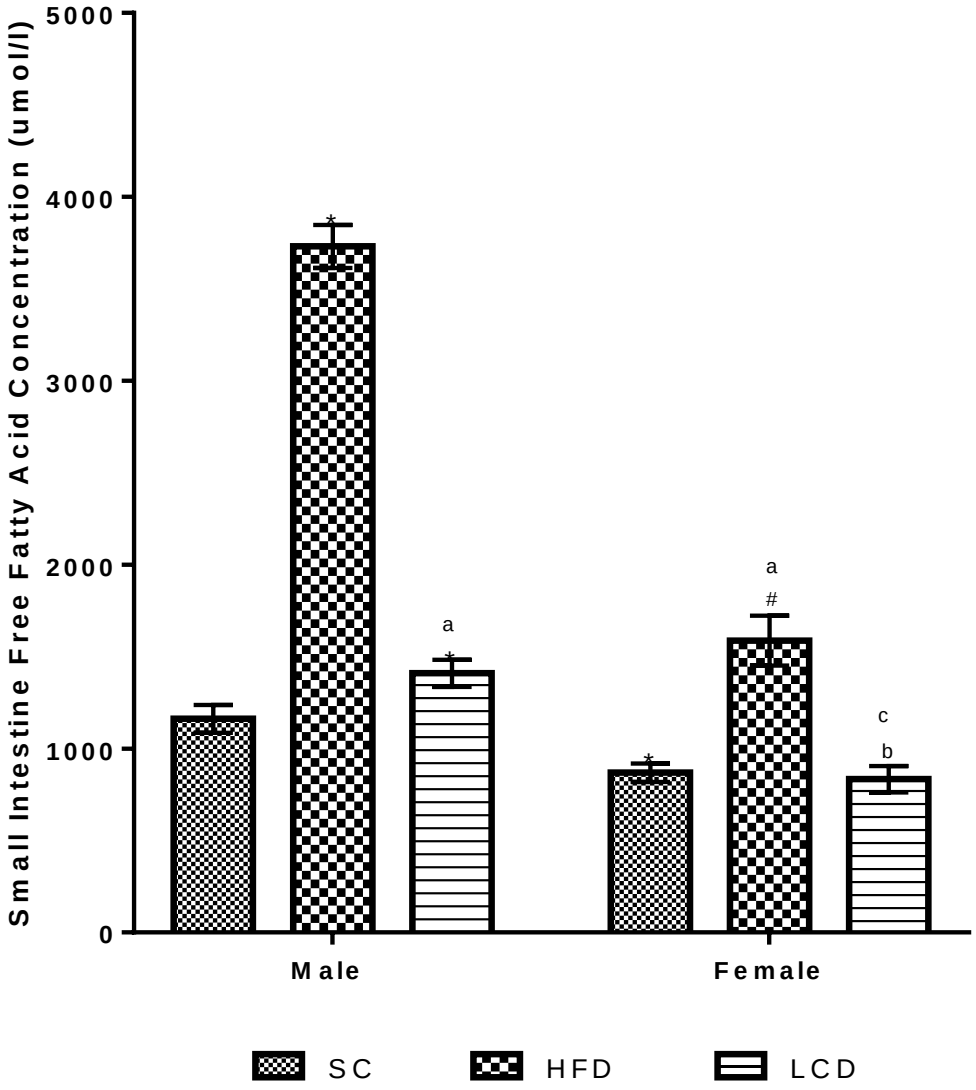


Figure 4.31: Small Intestine Homogenate Free Fatty Acid Concentration after Low calorie diet treatment for 5 weeks

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD); ^{c} $p<0.05$ Female (LCD) compared with Male (LCD).

4.71 Effect of Sexual Dimorphism on Small Intestine Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 Weeks on Low-Calorie Diet

In Malondialdehyde concentration, a significant increase in $p<0.05$ was observed in HFD when compared with SC but a significant decrease in $p<0.05$ was noticed in LCD when compared to HFD in both genders respectively. Finally, a significant increase in $p<0.05$ was observed in the SC, HFD, and LCD of female rats when compared with their male rats respectively. (table 4.32)

In reduced Glutathione concentration, a significant reduction in $p<0.05$ was observed in HFD and LCD when compared with SC respectively in female rats and a significant increase in $p<0.05$ in LCD when compared to HFD in males. Also, significantly rise in $p<0.05$ in SC, HFD, and LCD of female rats when compared with their male rats respectively. (table 4.32)

In Superoxide Dismutase concentration, a significant reduction in $p<0.05$ was observed in HFD when compared with SC but a significant increase in $p<0.05$ was noticed in LCD when compared to HFD in female rats. Significantly rise in $p<0.05$ in SC and LCD of female rats when related to SC and LCD of male rats. (table 4.32)

No significant change was observed in catalase measurement. (table 4.32)

Table 4.32: Effect of Sexual Dimorphism on Small Intestine Homogenate Oxidative and Anti-Oxidative Stress Markers of Rats Fed for 5 Weeks on Low-Calorie Diet

Group / Variable	SC	HFD	LCD
	Malondialdehyde (mM)		
Male	12.0±0.30	29.0±1.40*	11.0±0.25[a]
Female	25.0±0.95*	36.0±0.89[#, a]	27.0±0.75[b, c]
	reduced Glutathione (mM)		
Male	0.1±0.01	0.1±0.01	0.2±0.01[a]
Female	0.5±0.04*	0.3±0.01[#, a]	0.3±0.01[#, c]
	Superoxide Dismutase (u/ml)		
Male	49.0±1.60	45.0±1.30	45.0±1.30
Female	58.0±1.40*	47.0±0.39[#]	60.0±1.80[b, c]
	Catalase (u/ml/mins)		
Male	25.0±0.44	25.0±0.07	25.0±0.05
Female	25.0±0.02	25.0±0.02	24.0±0.22

Values expressed in mean ± S.E.M of 5 rats where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); [#] $p<0.05$ Female (HFD and LCD) compared with Female (SC); [a] $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); [b] $p<0.05$ Female (LCD) compared with Female (HFD); [c] $p<0.05$ Female (LCD) compared with Male (LCD).

4.72 Effect of Sexual Dimorphism on Small Intestine Histology of Male Rats Fed for 5 Weeks on Low-Calorie Diet

The black arrows point to the different levels of fat deposition while the blue arrow shows areas where congestion occurred on the male small intestine on H and E staining. There was moderate fat infiltration in the mucosal epithelium villi in the duodenum and ileum of HFD and lamina propria in the Duodenum, Jejunum, and ileum of HFD. There was mild fat infiltration in the mucosal epithelium villi in the ileum of SC group. (plate 4.8)

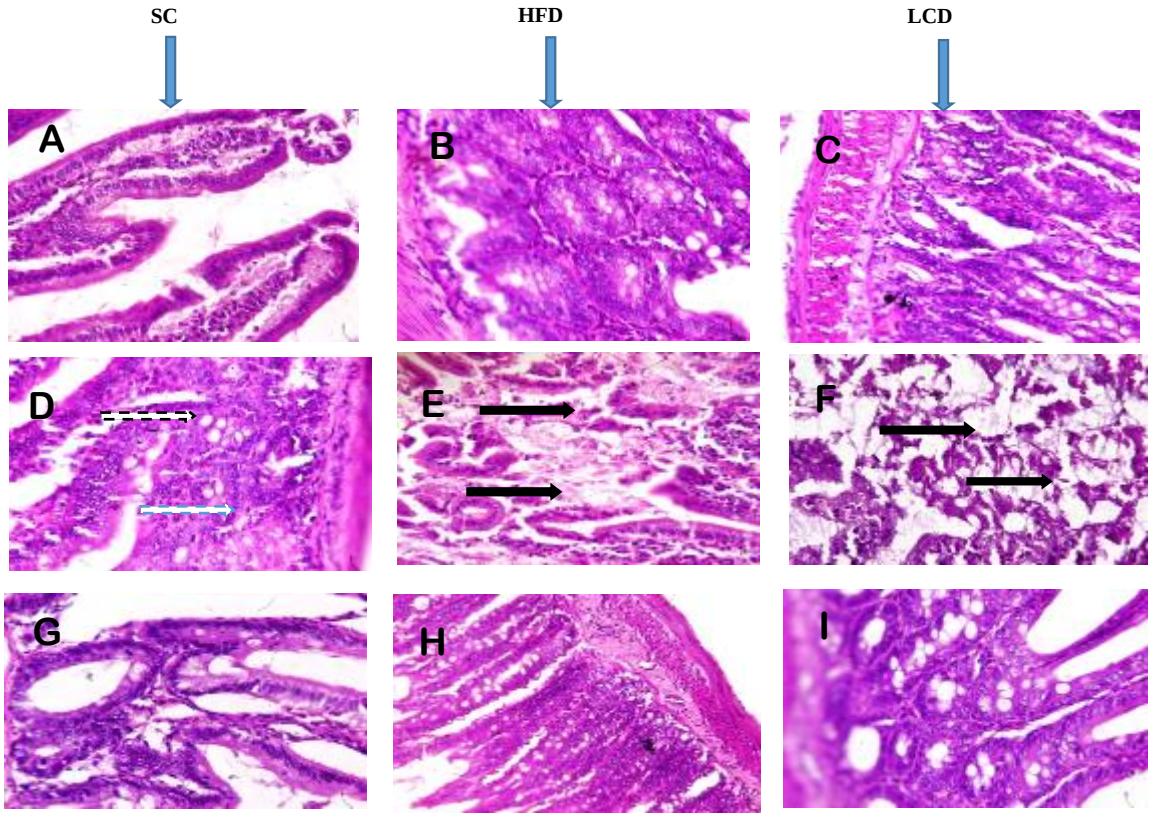


Plate 4.9: Histology of the male small intestine fed with an LCD with X400 Magnification

(A) SC Duodenum, (B) HFD Duodenum, (C) LCD Duodenum, (D) SC Jejunum, (E) HFD Jejunum, (F) LCD Jejunum, (G) SC Ileum, (H) HFD Ileum, (I) LCD Ileum; after 5 Weeks of Low Calorie Diet treatment. Where SC = Standard chow; HFD = High fat diet; LCD = Low caloric diet. Arrows show areas where either fat deposition or congestion could be seen

4.73 Effect of Sexual Dimorphism on Small Intestine Histology of Female Rats Fed for 5 Weeks on Low-Calorie Diet

The black arrows point to the different levels of fat deposition while the blue arrow shows areas where congestion occurred on the male small intestine on H and E staining. There were several fat deposits in the villi and lamina propria of HFD jejunum and Ileum. There was only a mild deposit of fat in the Ileum of LCD group. (plate 4.9)

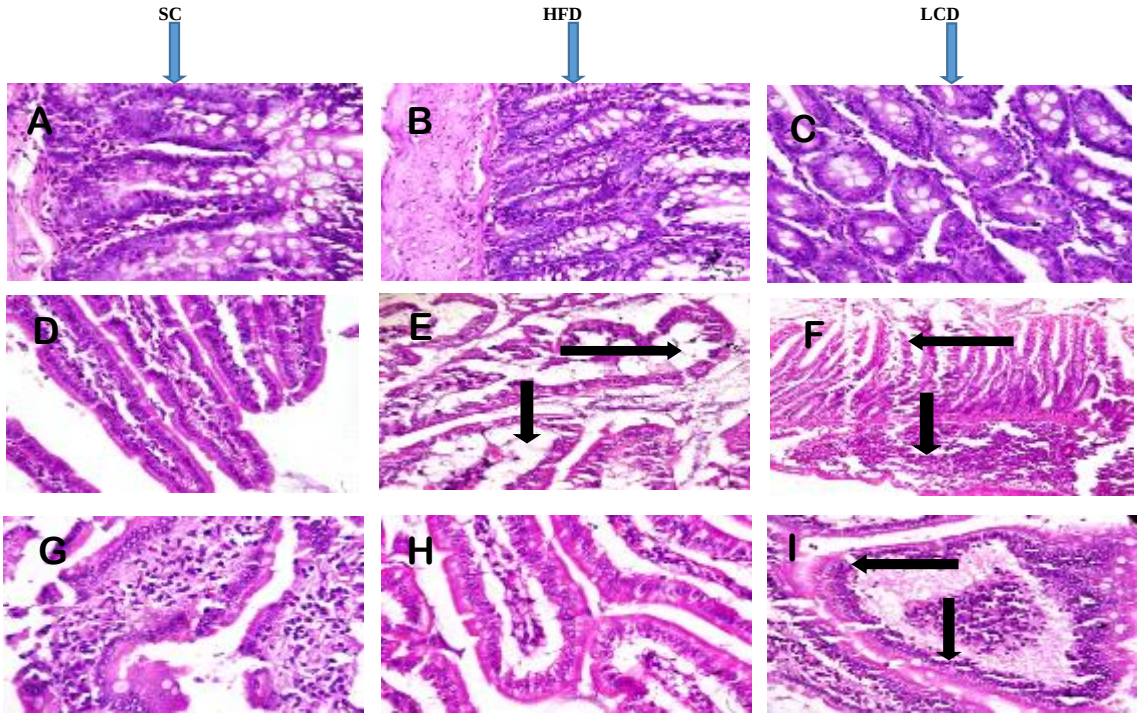


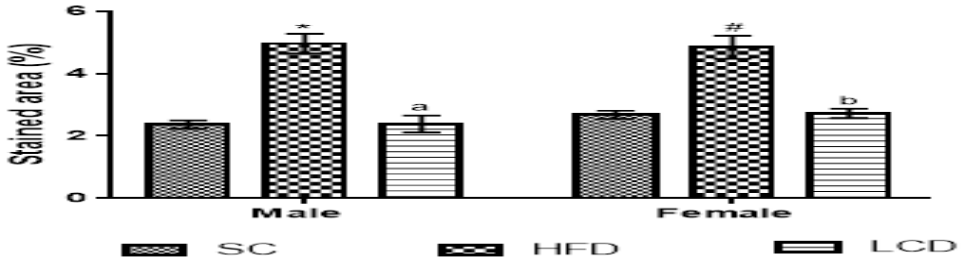
Plate 4.10: Histology of the female small intestine fed with an LCD with X400 Magnification

(A) SC Duodenum, (B) HFD Duodenum, (C) LCD Duodenum, (D) SC Jejunum, (E) HFD Jejunum, (F) LCD Jejunum, (G) SC Ileum, (H) HFD Ileum, (I) LCD Ileum; after 5 Weeks of Low Calorie Diet treatment. Where SC = Standard chow; HFD = High fat diet; LCD = Low caloric diet. Arrows show areas where either fat deposition or congestion could be seen

4.74 Effect of Sexual Dimorphism on the Expression of Duodenal Fatty Acid Transporter Protein 4 (FATP4) of Rats Fed for 5 weeks on Low-Calorie Diet

Significantly increase in $p<0.05$ was noticed in the expression of FATP4 in HFD when compared to their SC and LCD in both genders respectively. (figure 4.32 A and B)

(A)



(B)

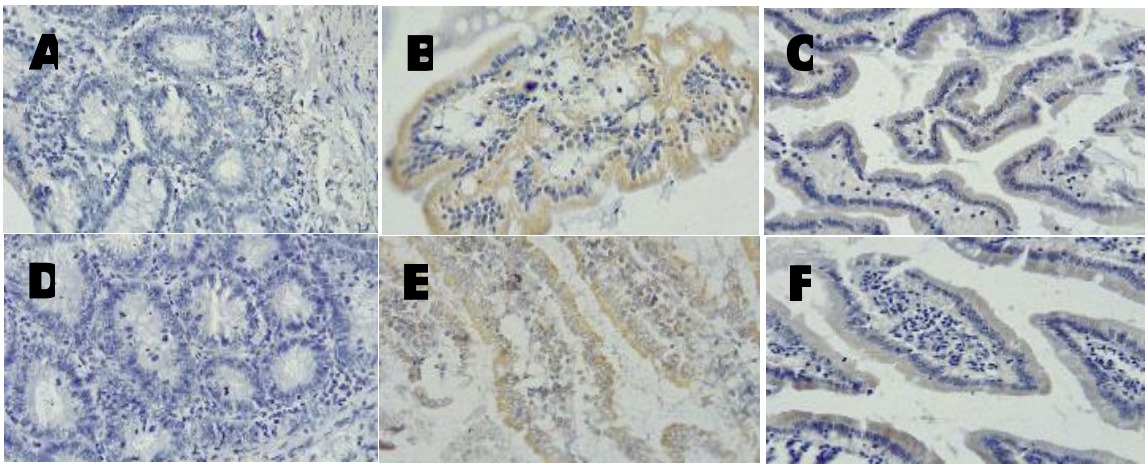


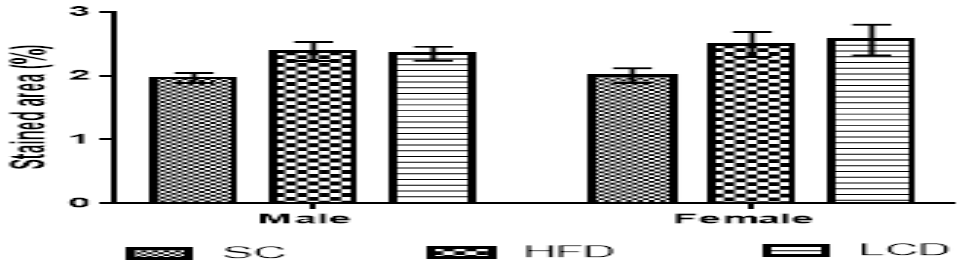
Figure 4.32: Duodenal FATP4 expression in rats fed with LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.32A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{b} $p<0.05$ Female (LCD) compared with Female (HFD); Figure 4.32B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.75 Effect of Sexual Dimorphism on the Expression of Jejunum Fatty Acid Transporter Protein 4 (FATP4) of Rats Fed for 5 weeks on Low-Calorie Diet

No significant change was observed in the expression of FATP4 protein in the jejunum amongst groups. (figure 4.33 A and B)

(A)



(B)

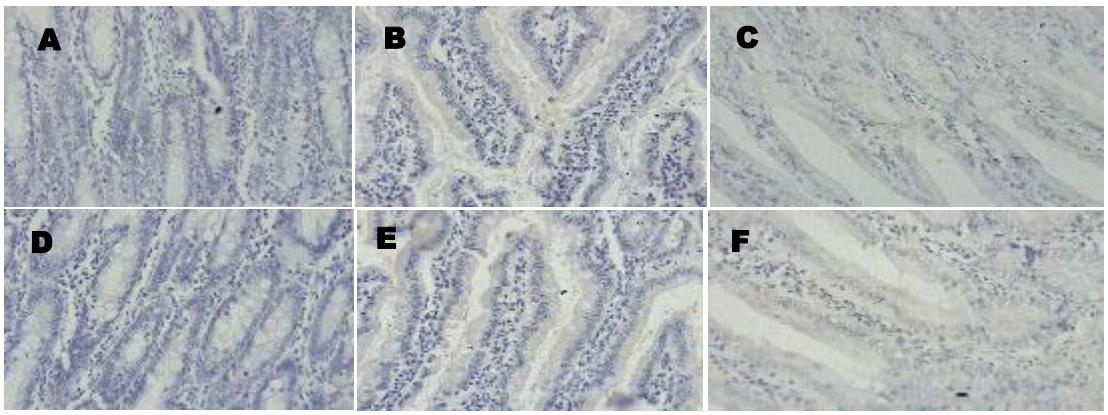


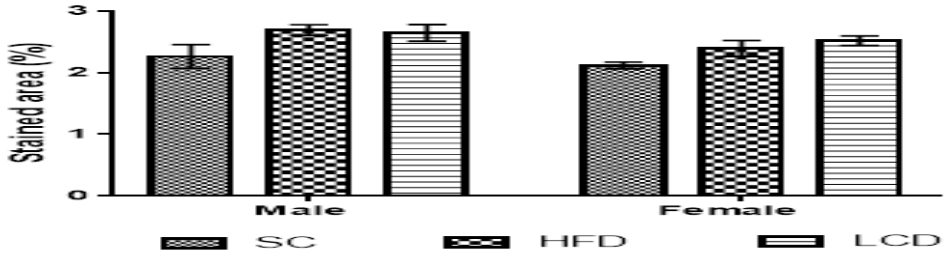
Figure 4.33: Jejunum FATP4 expression in rats fed with LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.33A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. $p<0.05$; Figure 4.33B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.76 Effect of Sexual Dimorphism on the Expression of Ileum Fatty Acid Transporter Protein 4 (FATP4) of Rats Fed for 5 weeks on Low-Calorie Diet

No significant change was observed in the expression of FATP4 in the ileum amongst groups. (figure 4.34 A and B)

(A)



(B)

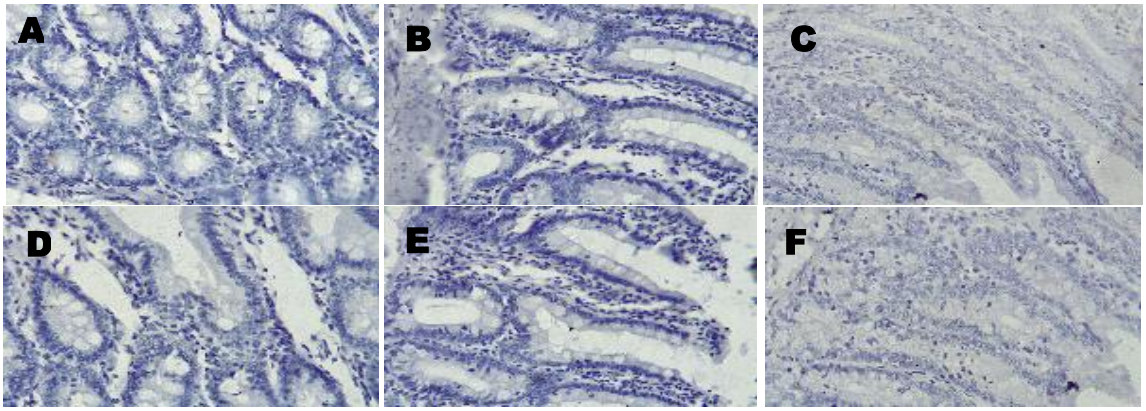


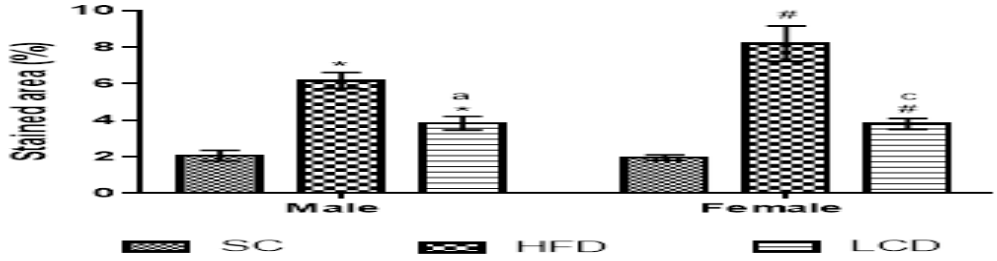
Figure 4.34: Ileum FATP4 expression in rats fed with LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.34A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. $p<0.05$; Figure 4.34B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.77 Effect of Sexual Dimorphism on the Expression of Duodenum Cluster of Differentiation 36 (CD36) of Rats Fed for 5 weeks on Low-Calorie Diet

A significant increase in $p<0.05$ was observed in the expression of CD36 protein in the duodenum of HFD and LCD when related to their SC in both genders respectively. A significant decrease in $p<0.05$ in LCD when compared to their HFD in both genders respectively. (figure 4.35 A and B)

(A)



(B)

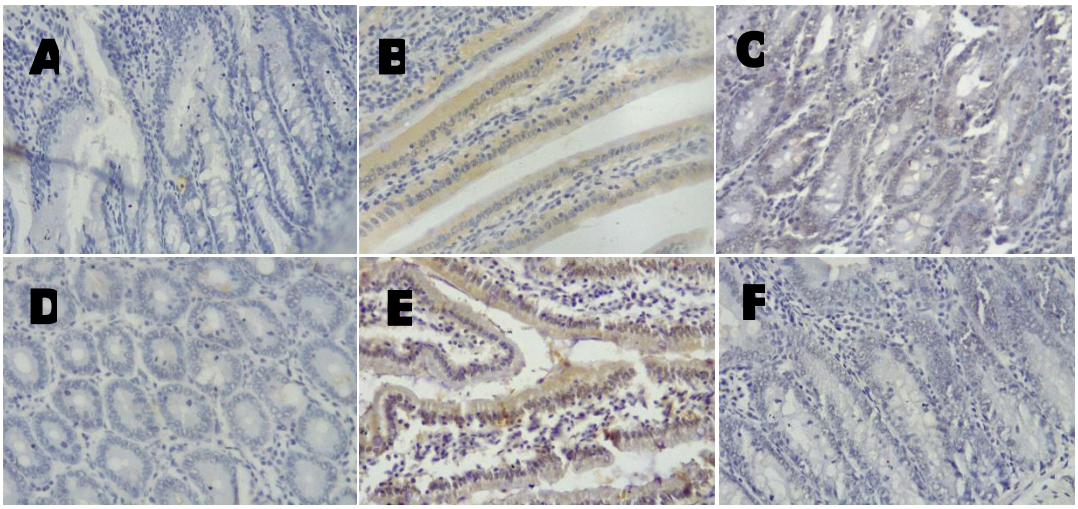


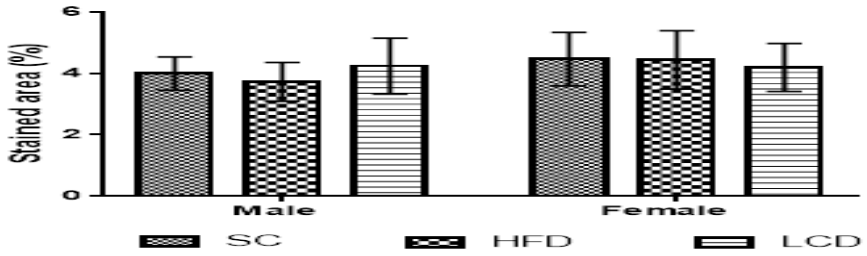
Figure 4.35: Duodenal CD36 protein expression in rats fed with LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.35A shows the qualitative analysis of the photomicrograph detailing the level of expressions of CD36; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); $^{\#}$ $p<0.05$ Female (HFD and LCD) compared with Female (SC); ^{a} $p<0.05$ Male (LCD) and Female (HFD) compared with Male (HFD); ^{c} $p<0.05$ Female (LCD) compared with Female (HFD); Figure 4.35B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.78 Effect of Sexual Dimorphism on the Expression of Jejunum Cluster of Differentiation 36 (CD36) of Rats Fed for 5 weeks on Low-Calorie Diet

No significant change was observed in the expression of CD36 protein in the jejunum amongst groups. (figure 4.36 A and B)

(A)



(B)

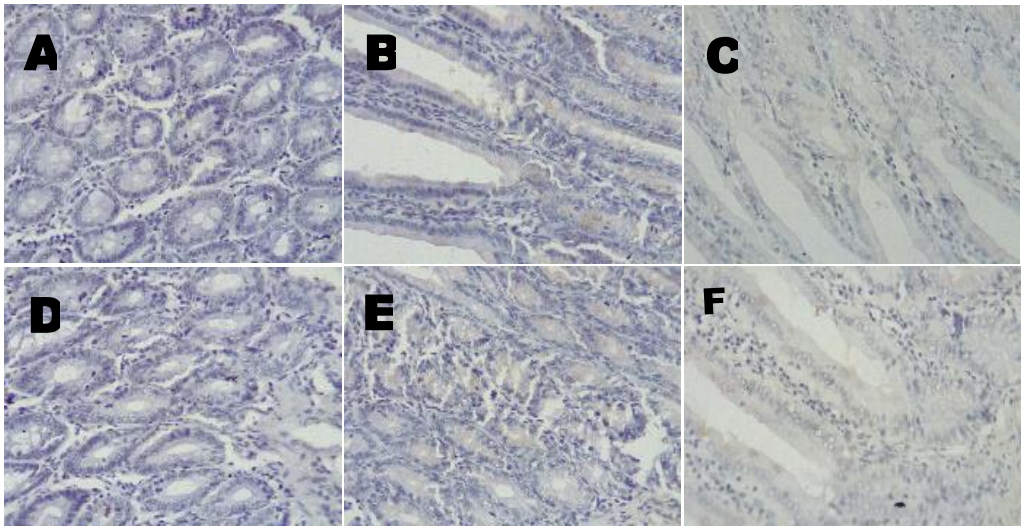


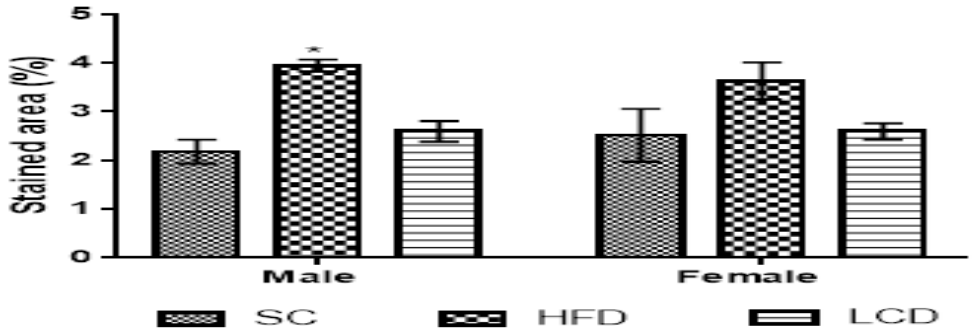
Figure 4.36: Jejunum CD36 protein expression in rats fed with LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.36A shows the qualitative analysis of the photomicrograph detailing the level of expressions of CD36; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. $p<0.05$; Figure 4.36B shows the physical colour changes in the photomicrograph of CD36 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment.

4.79 Effect of Sexual Dimorphism on the Expression of Ileum Cluster of Differentiation 36 (CD36) of Rats Fed for 5 weeks on Low-Calorie Diet

A significant increase in $p<0.05$ was observed in the expression of CD36 protein in the ileum of HFD when compared to SC in Wister rats. (figure 4.37 A and B)

(A)



(B)

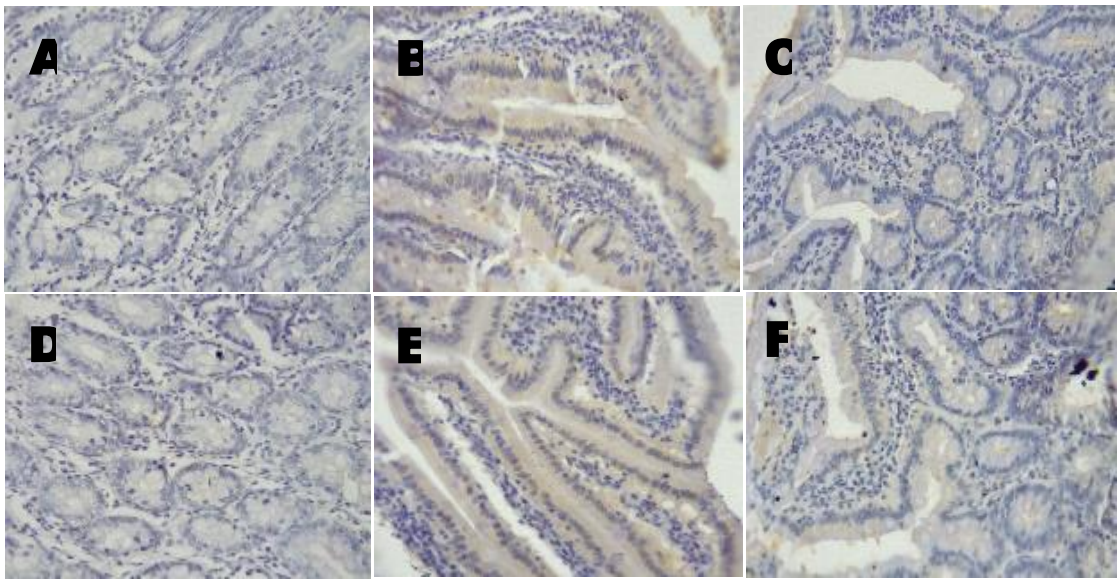


Figure 4.37: Ileum CD36 protein expression in rats fed with LCD with X400 Magnification

Values expressed in mean ± S.E.M of 3 rats. Figure 4.37A shows the qualitative analysis of the photomicrograph detailing the level of expressions of FATP4; where SC = Standard chow HFD = High fat diet; LCD = Low caloric diet. * $p<0.05$ Male (HFD and LCD) and Female (SC) compared with Male (SC); Figure 4.37B shows the physical colour changes in the photomicrograph of FATP4 where (A) SC Male, (B) HFD Male, C) LCD Male, (D) SC Female, (E) HFD Female, (F) LCD Female; after 5 Weeks of Low Calorie Diet treatment

CHAPTER FIVE

5.1 DISCUSSION

Substitution of nutritive saturated fatty acid and trans-fat with monounsaturated fatty acid and polyunsaturated fatty acid have been suggested for a long time to prevent cardiovascular disease [CVD] (Kritchevsky, 1998). Even with the change in diet (which in majority of cases is the cutting down on high fat diets especially those high saturated fats) and increased exercise, the frequency of occurrence of obesity still progressed at a rapid rate of 12% as observed in a study in 2008 (Nascimento *et al.,* 2008).

There are a lot of interventions aimed at weight reductions such as pharmaceutical measures, surgical, exercise and dietary interventions. The pharmaceutical measures due to its adverse effects is highly discouraged while the surgical intervention is very expensive and could results in complications while exercise which has been shown to be effective comes with its own challenges such as time factor especially for working class individual and health complications for those who are very obese. There have been growing interest in the used of dietary manipulations for the amelioration of obesity and its accompanying comorbidities, although, a wrong administration of LCD often promotes metabolic disorders.

The studies highlighted in the previous paragraph have given rise to the likelihood of unsaturated fats, on one hand, predisposing the body to cardiovascular risk and inappropriate LCD formulation on the other hand, leading to metabolic disorders. Also, most non gender specific preclinical studies investigating obesity and CVD are usually carried out in male subjects alone but are being implemented in men and women (Sondergaard *et al.,* 2012). This has created scarcity of information regarding the sexually dimorphic responses to obesity and CVD in the literature (Sondergaard *et al.,* 2012). This study focused on providing a sound physiological experimental basis on sexual dimorphic response of metabolic variables in high fatty diet induced obesity in Wistar rats on low calorie diet.

The entire body mass is a sum total of water, protein, bone and adipose tissue weights in the body (Kyle *et al.,* 2003). Further increment in one of these factors could result in elevated body weight. Studies have shown that increased consumption of fat over prolonged time instigates an increase in weight, which invariably increases the prevalence of obesity (Popkin *et al.,* 1995). This means that the more fats the body stores as a result of overconsumption of fatty diets the more the body drift to obesity. Although excessive intake of carbohydrates and protein can predispose the body to obesity, this study focused on fats because of the enormous experimental and epidemiological evidences implicating fats in both obesity and public health. The well-established variations in weight between the fatty and low calorie dietary groups in our study shows the great influence dietary manipulations can have on weight.

The weight gain observed in this study with rats fed with diets rich in plant fat exhibited the greatest increment in weight while rats fed with low-calorie diet had the lowest weight gain when compared with the control and obese diet groups respectively. Although, the bone mass and protein mass were not evaluated which would have also contributed to the increment in body weight, the increased weight may be due to variations in adipose tissue mass observed amongst the different groups with rats fed with diets rich in plant fat accumulating the greatest amount of fats while rats fed with LCD exhibited the lowest amount of fats.

Increment in weight as a result of long-term consumption of diets dense in fats is well documented in numerous research work (Pinnick *et al.,* 2012). The explanation for the increment in weight was as a result of diminution of protein mass and an elevation in fatty tissue weight (Wong *et al.,* 2016). However, findings from different studies, observed no difference in weight in groups that ate diets rich in fat in relations to rats fed on a standard chow over an extensive duration (Angéloco *et al.,* 2012). Few hypotheses were made in an attempt to elucidate the similarity in weight amongst animals who consumed standard chow and fatty chow. The most striking hypothesis is from the study which observed that normal chow diet is usually rich in protein (21–23 %) but low in fat (3 %) [Rodriguez *et al.,* 2005], aiding in the elevated muscle mass observed in typical control rats. Although the fat component of the various chow in our study varied, the protein percentage mass was fairly equal across the four groups.

Excessive intake of feed with surplus energy predisposes the body to developing obesity if consumption is prolonged (Swinburn and Egger, 2002). Previous studies have shown that energy intake rather than a simple increase in fat dietary intake is a major factor that contributes to weight gain (Prentice, 1998). Although in most diets increase energy intake is accompanied with increased feed intake (Woods *et al.,* 2003). Feed intake in numerous studies have been shown to have greater influence on weight gain than the energy content alone. In a particular study, rats fed with high fat diet *ad lib* and another rat group that were fed with diet containing lesser fat content also *ad lib* were both found to be relatively obese (Woods *et al.,* 2003).

Foods exhibiting enormous energy values beyond the needs of the body gradually results in a buildup of surplus calorie in the form of accumulated adipose tissue. The resulting effect of caloric intake on body weight stimulated some noteworthy modifications in adipose tissue, adipose tissue mass, body mass index and adiposity index amongst the groups in this study. Adiposity index enables experimental studies determine the extent to which dietary consumption buildup adipose tissue. This index is very necessary in experimental studies since it is known from studies that body weight alone greatly underestimate the actual degree of obesity that develops in obese rats (Leopoldo *et al.,* 2016). This study observed that body mass index, adiposity index, thoracic and abdominal circumferences, body fat mass and energy intake were highest in the fatty groups and lowest in the low calorie dietary groups. This makes our study to be in line with other works that focused on dietary manipulations (Shinagawa *et al.,* 2015).

Researches have also revealed the sensitivity of obese and lean rats to the scrumptiousness of diets (Castonguay *et al.,* 1982) with diets rich in fats being more appetizing in relations to other dietary varieties. Nascimento *et al.* (2008) credited this scrumptiousness or increased fed intake to fatty diets instigating reduced contentment and subsequently an elevated diet consumption (Nascimento *et al.,* 2008). Other research works suggest that palatability has no positive correlation to fatty diet intake, that they are lesser palatable compared to normal diets (Dourmashkin *et al.,* 2005). There was a reduction in consumption rate in rats fed diets rich in fat when compared to the standard models (Angéloco *et al.,* 2012). With the exception of the HAFD group which exhibited lower feed intake, there was no significant difference noticed in the consumption of food amongst the SC, HPFD, and LCD groups. The reduction in the

HAFD might have been as a result of the lard used as the fat source which may have been less palatable to the rats.

Findings from this study gives indications that palatability may have no positive correlation to fatty diets considering the fact that the low calorie diet which were rich in fiber and low in fat were highly palatable. This might partly explain why the HAFD had reduced weight despite the high energy content of the feed. Feed efficiency from previous studies were observed to increase in groups where feed intake and energy intake were high (Novelli *et al.,* 2007). Similar outcomes were observed in this study. Women for decades were observed to possess a greater risk of obesity compared to males (National Center for Health Statistics, 1990). However, recent studies do show males possessing greater body weight in relation to females (Duong *et al.,* 2014; Khalid *et al.,* 2017). This study also observed dimorphism in body weight but were diet specify. For example, males showed increased weight gain in HPFD and LCD groups while females showed increased weight gain in HAFD, and SC having no dimorphic changes.

A number of factors other than fat deposit as discussed earlier could be responsible for this dimorphic disparity in fat storage with respect to this study and also considering the fact that dimorphism on fat depot only occurred in the diet rich in plant fat and low calorie diet with female having higher rate in the HPFD group and males having higher rate in the LCD group. Numerous studies have observed males exhibiting higher dietary intake than females and this positively correlated with increased intake of calories when related to females (Khalid *et al.,* 2017). Similar outcomes were observed in this study in all groups which showed males exhibiting greater consumption rates, although the mechanism for these observations is still unknown. Men typically exhibit greater ThC and AC in comparison with their feminine equivalents (Adedoyin *et al.,* 2012).

This gender variation in ThC might have resulted from women possessing smaller lung volume values when compared with men of similar stature and age (Duong *et al.,* 2014). This research work also observed male rats exhibiting elevated ThC and AC compared to their female counterpart in every group. Studies by Kautzky-Willer *et al.* in 2012, observed an elevated adiposity rate as shown by elevated body mass index (BMI) and adiposity index (AI), to be gender-precise and was greater in men when compared with women. The AI was higher in females compared to males in both high fat diet groups.

Gender-variation in BMI was only observed in the calorie restriction group in this study with males have higher rate. The effects of gender on BMI has been debatable, with a couple of researches such as Duong *et al.,* (2014) and other studies observing males possessing reduced BMI compared to females, whereas studies such as Khalid *et al.,* (2017) observed men possessing elevated BMI compared to women.

High fatty diets are known to influence circulating hormonal levels. LH or FSH from studies were shown to have a reduced concentration in subjects that have consumed high-fat diets for a long period of time, and this resulted in impaired ovulation or follicular stimulations and other LH or FSH linked activities (Gouveia *et al.,* 2004). This could be the likely explanation for the increased level of LH, firstly in the HAFD group when compared with HPFD considering the observations from this study that the dietary consumption rate was higher in the plant fat based diet and secondly, in the sexual dimorphic variation in males when compared to females in the HAFD group since the consumption rate were higher in the male subject. This study observed FSH concentration to be independent of consumption of high fatty diet concentration and this was pronounced in LCD group which had high FSH level despite its low level of fat. Contrary to previous study, sexual dimorphism in FSH was dependent on the consumption of high fatty diet as our study showed males in HAFD group having higher level than its female counterpart.

Estrogen levels were higher in the female fatty diet groups compared to the control and low calorie diet groups in our study. High-fat diet intake does stimulate increase oestrogen production in females (Holmes *et al.,* 2000) on one hand and reduces testosterone production (Holmes *et al.,* 2000) in men on the other hand. A massive research work carried on postmenopausal ladies observed no positive correlation between fat consumption and estrogen levels (Wu *et al.,* 2002). Another study observed an inverse correlation of estrogen with fatty consumption (Holmes *et al.,* 2000). Long-term calorie restriction has been reported in numerous studies to reduce oestrogen and testosterone (Holmes *et al.,* 2000). A particular meta-analysis of intervention observed a meaningfully reduced estradiol level in a group given a low-fat diet (Wu *et al.,* 1999). Similar observations were noticed in our obese group that were treated with a low-calorie diet for five weeks in this study.

Lipoprotein lipase (LPL) is vital in the metabolism of lipids, where study has shown it to be involved in the hydrolysis of triglyceride in triglyceride-rich lipoproteins such as chylomicrons and VLDL (Magkos et al., 2009). There were lots of inconsistencies with LPL concentration that made it difficult making a definitive inference in this study. Similar observations have been reported from study which showed LPL to be high irrespective of the calorie content (Magkos et al., 2009).

Apolipoprotein A and B are very essential apolipoproteins used for cardiovascular diseases assessment since they are connected with atherogenic lipid particles mainly LDL-C (for apolipoprotein B) and anti-atherogenic particles such as HDL-C (for apolipoprotein A) [Hirschler et al., 2015]. In our study, serum ApoA concentration were reduced while ApoB increased concentration of HAFD and HPFD reduced when compared with SC indicating that the atherogenic lipids were higher than the non atherogenic lipids in the fatty dietary group. This assessment was further reinforced by the ApoB/ApoA ratio increasing in both fatty dietary groups when compared to the control. ApoB/ApoA ratio have been shown from studies to be a more potent CVD event indicator than other indicators like LDL-C/HDL-C or TC/HDL-C (McQueen *et al.*, 2008).

The low calorie diet in this study was able to an extent to ameliorate the atherogenic tendency of the obesogenic diet. Although the low calorie diet was able to reduce the apoB level, it was not able to reverse the ApoB/ApoA ratio which the obesogenic diet induced. Previous studies have been inconclusive on the ameliorative properties of low calorie diets (Hirschler *et al.,* 2015). These inconsistencies might have been due to the composition of the diet and the experimental design. Apolipoproteins A and B were found to be higher in males compared with females (McQueen *et al.,* 2008). In this study, sexual dimorphism was more of variable dependent for example, apoB were lower in females compared to males in HPFD group while ApoB/ApoA ratio increased in females compared to males in HAFD and LCD groups.

Also, this study showed that animals exposed to fatty diets for a long period of time increased the triglyceride, TC and LDL level but reduced high density lipoprotein in all tissues and when low calorie diet were introduced the reverse occurred in most tissues. High consumption of food dense in fats is characteristically connected with a raise in

plasma LDL-c, total cholesterol and triglyceride and aggravation of heart problems (Brouwer *et al.,* 2010, Leopoldo *et al.,* 2016). Furthermore, the lipid level between the fatty diets in this study had a lot of inconsistencies which makes a proper inference on which diet is more lipogenic very difficult to make. For example, the diet rich in animal fats had higher low density lipoprotein in the serum and heart of male rats, high liver total cholesterol in female rat, low liver triglyceride and low density lipoprotein in male rat and low triglyceride level in small intestine of male rat when compared to HPFD.

Previous studies observed LDL-c to be higher in some studies (Hassanzadeh-Taheri *et al.,* 2018) and lower in others (Sharma *et al.,* 2010), and in few studies no difference (Shukla *et al.,* 2012) especially in rats fed with plant fats when compared to rats fed with animal fats. LDL-c from studies usually shows an inverse relationship with HDL-c, where an increase in LDL-c results in a decrease in HDL-c (Hassanzadeh-Taheri *et al.,* 2018). We recorded similar observations, in which organs which had increased LDL-c had a decrease in HDL-c. Although triglyceride is the form in which dietary fats are absorbed from the intestine and stored, it should be noted here that it is the LDL-c that is easily susceptible to oxidative modifications.

Atherogenesis is widely accepted to be instigated by the oxidation of lipids in the LDL-c (Chen *et al.,* 2003), thereby making it a great cardio-risk factor. Although most studies that investigated changes in lipid profile after consumption of low-calorie diet mostly investigated the effect on the serum and in most observations a reduction in TC, TG, LDL were noticed (Gawecka *et al.,* 2014). We studied the effects of low-calorie diets on lipid profile on the serum, liver, heart and small intestine and observed reductions in TC, TG and LDL in all tissues when compared to the high fat diet group. Similar observations were noticed from previous works (Gawecka *et al.,* 2014). In recent times, there have been great interest aimed at understanding the sexual dimorphic pattern of lipid profile. Males have a tendency to possess reduced HDL-c and TG concentrations than females (Barrett-Connor, 1997). Also, females were observed from studies to exhibit elevated TC and HDL-c but reduced TG concentration in the plasma in comparison to males (Kolovoua *et al.,* 2009).

Even though males usually exhibit elevated concentrations of TG than females, likelihood of TG concentrations increasing during mid-age (between 40-69 years) in

humans have been observed in females with males not having such increase (Razay *et al.,* 1992). With these observations in humans, TG is being regarded as a better forecaster of CVD in females compared with males (Hokanson and Austin, 1996). Studies showed females possessing higher level of TG compared to males (Magkos and Mittendorfer, 2009). Studies have shown TG having a higher level in both lean and obese males compared to females (Magkos *et al.,* 2009). This observation in males were believed to be due to the low clearance rates from plasma of TG. When calorie restriction was introduced, males were observed to have lower (Magkos *et al.,* 2007) or similar (Gormsen *et al.,* 2006) TG level compared to females showing that males had higher plasma clearance rates of TG than females.

In human studies, TC and LDL concentrations in ladies after menopausal were greater than that in men (Roeters van Lennep *et al.,* 2002). This was believed to be mediated by reduced estrogen level which is known to exert effect on LDL receptor thereby increasing LDL concentrations. There have been diverging view on the dimorphic nature of LDL-c with some studies showing males with higher levels (Georgopoulos and Rosengard, 1989) and others showing women having higher LDL-c levels (Smiderle *et al.,* 2014). Also, females were shown to have higher levels of HDL-c (Magkos and Mittendorfer, 2009). In this study, both fatty diets showed the females having higher total cholesterol in the serum, higher triglyceride in the serum and liver but reduced low density lipoprotein in liver, heart and small intestine when compared to males. We also observed dimorphism to be diet specific.

For example, females fed with diet rich in plant fats had higher high density lipoprotein in the serum, liver and heart but decreased total cholesterol in the small intestine than the male rats while females fed with diets rich in animal fats had decreased triglyceride in the small intestine than the male rats. The LDL-c was higher in males compared to females in tissues such as the liver, heart and small intestine in our study. These differences may be as a result of the type of model or reproductive age of the model especially that of the female model. Sexual dimorphic patterns were observed in more groups in HDL-c measurement with females having higher concentration in this study and was in accordance with studies by Magkos and Mittendorfer, (2009).

The fat deposition in the heart, liver and small intestine tissue being higher on the fatty groups as shown in the histological analysis is likely as a result excessive influx of fatty acids into the respective tissues and this could be seen reduced to an extent when low calorie diet were introduced to ameliorate the obesogenic effects. This deposition of fat in the tissue likely influenced the changes in tissue weight as discussed earlier. This study went further to investigate the amount of fatty acids (FFA, SFA, MUFA, PUFA) present in each tissue which contributed greatly to the observed elevation of fat deposition in the tissues.

This study showed elevated FFA level in the fatty diet group in all tissues and also when the obese groups were treated with low-calorie diet, there was reverser of the high levels to that of the control. The levels of FFA have been conflicting in studies. For example, few works exhibited no significant alteration in the serum FFA levels amongst groups fed with fatty diets and standard chow (McLaughlin *et al.,* 2001). Another study that worked on the progression in liver FFA at different weeks building up to week 24 observed an increased in FFA as the weeks increases and the highest peak was noticed at weeks 24 (Liu *et al.,* 2016). From our studies, we have been able to show, just like some previous studies, that FFA levels are influenced by the amount of fat available. In post-absorptive state, males and females do have similar Plasma FFA (Jensen, 1995), but once dietary fat consumption occurs, the plasma FFA concentration increases in male and the decrease in females is likely due to insulin (Jensen, 1995). This suggest that females are more susceptible to accumulating more fats. With the exception of fatty diet groups in the liver, male had higher FFA levels in this study including the treatment groups and this is in opposition with other studies that show females having higher values (Soeters *et al.,* 2007).

Most studies attributed these observations to the greater lipolytic rates as a result of fat accumulation. Earlier research works concentrated only on the dimorphic nature of FFA in the plasma and liver but our study extended it to the heart and small intestine and observed that the dimorphic nature of the FFA were tissue specific. Numerous research works have shown that a fast reduction in weight can result in unnecessary breaking down of fat which will result in a notable rise in FFA level (Lu *et al.,* 1998). There was a reduction in serum, liver, heart and small intestinal FFA of obese rats treated with LCD compared to the HFD in this study. FFA levels in the obese subject treated with LCD

has generated a lot of controversies with some observing an elevation (Lu *et al.,* 1998) while others observed no difference (Fan *et al.,* 2003). In all these studies the composition of the HFD diets used in inducing obesity and the LCD diet composition were not consistent and this might be the reason for the variations in the findings in each study.

The American Heart Association/American College of Cardiology guideline made recommendations of decreasing the intake of SFA to about 5-6% of the daily caloric intake as a measure of averting increased occurrence of CVD (Eckel *et al.,* 2013). The scientific reasons for reducing SFA in the diet emanated from its deep-rooted abilities in increasing low-density lipoprotein (LDL) cholesterol together with the decrease in high-density lipoprotein (HDL) cholesterol which is a very potent cardiovascular risk factor [Mensink , 2016].

This study which showed SFA increasing in the liver, heart and small intestinal groups when compared with the control and SFA also increasing in the heart but reducing in the small intestine of HAFD when compared with HPFD were contrary to previous studies. Although LDL-c and TG were elevated in the fatty dietary groups in this study which from previous study attributed it to increased SFA, our study attributes the increased atherogenic lipids to higher consumption of dietary fat irrespective of the level of saturation. Also, SFAs are known to stimulate metabolic syndrome while unsaturated fatty acids exert opposite effects (Galgani *et al.,* 2008; Kennedy *et al.,* 2009). For example, the elevation of SFA is connected with low-grade inflammation in obese subjects while unsaturated fatty acid has been shown to prevent pro-inflammatory cytokine assembly in cells and tissues (Jia *et al.,* 2008). Other studies observed SFA and MUFA to be elevated in obese and diabetic subjects especially in the liver tissues (Park *et al.,* 2013). Another research observed a reduction in SFA and MUFA but a rise in PUFA in the fatty dietary group in comparison to the standard in both fed and fasted state in serum (Liu *et al.,* 2015).

In this study, MUFA and PUFA increased in the fatty groups in the liver, heart and small intestine tissues when compared with the control. Also, MUFA increased in small intestine tissue but reduced in the heart while PUFA increased only in the heart tissue of HAFD when compared to HPFD. Polyunsaturated FA (PUFA) are considered to

improve lipid markers by decreasing the concentration of TG and ApoB which in turn decreases LDL-c [Benes *et al.,* 2016] while monounsaturated FA (MUFA) have been shown to reduce the oxidization of LDL, and TG levels [Alsina *et al.*, 2016]. This study clearly shows that both PUFA and MUFA have contributed to increasing the atherogenic lipids as SFA did. Variations in the previous studies and this study could be as a result of composition of the diet or experimental model. There is paucity of information on the dimorphic nature of fatty acids. Few studies observed females having higher fatty acids (MUFA, PUFA, and SFA) levels than males (Terrade *et al.*, 2013) but these variations were observed in the placenta. Although dimorphism occur in this study, we could not clearly ascertain if male or females had higher fatty acids level since dimorphism varied with tissue and diet. For example, female rats had increased liver MUFA, small intestine PUFA and SFA but decreased heart PUFA and SFA and small intestine MUFA compared with male rats.

Short to medium chain fatty acids are transported through cystolic fatty acid binding protein because of their ability to penetrate the membrane easily. But for long chain fatty acids a transport protein (such as CD36, FATP4 and so on) at the plasma membrane is required to facilitate this process since they are not readily permeable to the cell membrane. The FATP4 and CD36 are membrane proteins required for the uptake or transportation of fatty acids especially the long-chain fatty acid from the extracellular environment into the cell (Hajri *et al.,* 2003). The uptake of these fatty acids by the transport protein depends on their availability which varies from cell to cell (Hames *et al.,* 2014). The first step is the conversion of this fatty acids to acyl-CoA through the enzyme acyl-CoA synthases then binding to the transport membrane protein occurs. These acyl-CoA are then converted to complex lipids such as triglycerides for transportation to needed energy sites. The level of expression of these transport proteins determine the quantity or amount of fatty acids present in that tissue. CD36 could be described as a glycoprotein found in the surface of the cell and plays a role of a transporter or a receptor for long chain fatty acids. CD36 is found to be expressed in numerous cell lines which includes the liver, heart and small intestinal cells (Zhang *et al.,* 2003).

This study focused on understanding the expression levels of FATP4 and CD36 on 3 tissues (liver, heart and small intestine) which are very important in fatty acid

metabolism. Duodenum and ileum had increased expression of FATP4 and CD36 while jejunum only had FATP4 expression in both fatty tissues when compared with the control. When diets were changed to a low calorie diet, expressions of FATP4 and CD36 were only observed in the duodenum and were reduced when compared to the high fatty diet. Also, CD36 expression in the duodenum were observed to be higher in the females compared to the males.

Series of studies have been done regarding the expression level of fatty acid transporters in different segments of the small intestine with conflicting results. A study observed no significant differences were found between any of the segments of SI in FATP4 expression (Masson *et al.,* 2010). Also, CD36 protein level were observed to be significantly higher in ileum when compared to other part of the small intestine (Masson *et al.,* 2010). Another study showed CD36 protein expression to be highest in the jejunum followed by the duodenum and least at the ileum in rats (Nassir *et al.,* 2007) while another study showed humans CD36 expression to be restricted to the duodenum and jejunum (Lobo *et al.,* 2001).

Another study found CD36 expression level to be highest in the duodenum with the expression reducing as we go more distal (Nassir *et al.,* 2007). FATP4 expression was high in the jejunum and ileum but were found in minute quantity in the duodenum (Lobo *et al.,* 2001). CD36 is principally expressed in the brush border membrane of the proximal portion of the small intestine (Nassir *et al.,* 2007) where it helps in the absorption of fats and in facilitating the transportation of fatty acids across the cell membrane (Masson *et al.,* 2010). FATP4 and CD36 are one of the 3 major fatty acids transporters in the small intestine. CD36 protein were highly expressed in the proximal portion but in the middle and distal portion there was no difference (Nassir *et al.,* 2007). CD36 is important for very long chain fatty acid (especially 18 carbon atoms and above) metabolism in the intestine (Lobo *et al.,* 2001). CD36 has were expressed in the duodenum and jejunal epithelium in humans (Nassir *et al.,* 2007). CD36 were expressed in low concentration in the ileum but with high concentrations in the jejunum followed by the duodenum of rats (Masson *et al.,* 2010).

There was increased expression of CD36 in the liver and heart of animals fed with diet rich in animal fats when compared to groups fed with diets rich in plant fat. This

observation indicates that the HAFD diet group had greater fatty acids activities than HPFD. The low-calorie treatment resulted in reduced expression of CD36 and FATP4 in the liver, heart and small intestine and this could be due to reduced lipids and fatty acids concentration observed in LCD. One of the principal aim of using low calorie diet in ameliorating the obesogenic tendencies was achieved in this study with reduction in the transport proteins which invariably reduced the transportation of long chain fatty acid.

Over 70-80% of the energy for heart muscles contractile activities are facilitated by long chain fatty acid (Stanley *et al.,* 2005). The heart muscles are not capable of storing much lipids, therefore the uptake and oxidation of long chain fatty acid are firmly coupled to transport proteins (Stanley *et al.,* 2005). The heart had increased expression of FATP4 and CD36 in both fatty tissues when compared with the control in this study. Also CD36 in the heart had reduced expression in the group fed with animal fats when compared with the group fed with plant fats and when the diet was changed to a low calorie diet, only CD36 showed reduced expression when compared to the high fatty diet. Factors that likely regulate the activities fatty acids transporters in the heart muscles are; (1) availability of fatty acids in the blood; (2) hormone milieu particularly insulin which favours LCFA uptake through CD36 (Luiken *et al.,* 2003); (3) the energy requirement of cardiac cells (Luiken *et al.,* 2003); and (4) an adequate oxygen supply.

In oxidative metabolism, oxygen is required in the energy generating process and this results in the production of free radical or reactive oxygen species and increased caloric intake is an important factor in decreasing mitochondrial fluidity, thus generating free radical. It must be emphasized here that free radical itself is not harmful to the body but in circumstances were it is produced in large quantity, weakening of the body's defense system occurs resulting in oxidative stress which is the imbalance of oxidant and antioxidant defense system (Novelli *et al.,* 2007). Oxidative stress is a state that occurs when the amount of reactive oxygen species (ROS) development surpasses the amount of the antioxidant defence system (Kunsch and Medford, 1999). Oxidative stress is regarded as a vital tool in the progression of cardiovascular diseases since it is both associated with type 2 diabetes and hyperlipidaemia (Kunsch and Medford, 1999). Oxidative stress was observed to increase in fatty groups (Novelli *et al.,* 2007). The results from this study showed that the animals in the groups fed with high fatty diets

could not establish the balance between the oxidative-antioxidative homeostatic systems due to the uncontrollable influx of fatty acids into the tissues.

This was evidence with MDA which is a biomarker for oxidative stress being elevated in the serum, liver and small intestine of both fatty groups while in the heart tissues MDA only elevated in the animal fat dietary group in comparison to the control. Even amongst the fatty groups MDA were higher in groups that consumed diets rich in animal fats in the serum, liver, heart and small intestine tissues. One of the greatest risk of an elevated oxidative stress is its tendency to exacerbate atherogenesis through the oxidation of lipids in LDL-c (Chen *et al.,* 2003). This means that increase MDA is directly proportional to increased LDL-c and this trend was also observed in this study. Under physiological state, tissues are shielded from Oxidative stress by antioxidant defense mechanisms which includes SOD, catalase and glutathione peroxidase (Kunsch and Medford, 1999).

Results from our study show that oxidative degradation from excessive emitted free radicals weaken the anti-oxidative defensive system of most tissues with only few exceptions in our study especially in the fatty groups for example, SOD was reduced in serum, liver and small intestine; GSH reduced in heart, liver and small intestine; catalase reduced in serum while SOD was elevated in the heart only in the HPFD group. Observations in MDA and GSH levels with exception to the heart homogenates in our study were similar to the observations of Wu et al. (2002) who found out an elevation in MDA levels but a reduction in GSH levels in fatty groups compared to the control (Chen *et al.,* 2003). But in the heart, we observed GSH being elevated and MDA reduced. This could imply that the oxidative or antioxidant levels could be tissue-dependent.

The SOD levels in this study were reduced in the fatty tissues compared to the control. The antioxidant defense system of subject fed high-fat diets has been highly debatable with some studies showing an increase (Maciejczyk *et al.,* 2018) and others decrease (Noeman *et al.,* 2011). One of the limitations of these studies was that it was not carried out in the same tissue type. The ameliorative ability of low-calorie diets in revising the obesogenic outcomes of consuming diets rich in fat on the antioxidative defence system has been impressive from pieces of literature with most literature showing reduction in MDA levels (Sartori-Valinotti *et al.,* 2007). Similar results were achieved in this study,

showing LCD as therapeutically viable in ameliorating oxidative stress as a result of obesity induced by a high-fat diet.

Due to the fact that oxidative stress has a role in the progression of numerous diseases, therefore a great understanding of the sexual dimorphic changes associated with oxidative-antioxidative system is very vital. In this study, the dimorphic nature of the oxidative-antioxidative system were very inconclusive and greatly depended on tissue type and dietary group. For example, in females' MDA increased in the small intestine, reduced in the liver, increased in the heart in the group fed diets rich in plant fats but reduced in the group fed diet rich in animal fats when compared with males. Generally, males do have elevated oxidative stress than females in rats (Barp *et al.,* 2002) and humans (Dantas *et al.,* 2004).

Although, in a physiological state, there is inconsistency on the levels of oxidative stress between males and females and studies done on either animal or human subjects exhibit conflicting findings [Sartori-Valinotti *et al.,* 2007]. Numerous works have showed that males possess greater plasma concentrations of oxidative stress markers than females (Brunelli *et al.,* 2014). In a study females were observed to have a mild level of oxidative stress that was meaningfully greater than their male counterpart (Brunelli *et al.,* 2014). In disparity, a different work showed that the same oxidative stress markers were meaningfully greater in women (Sartori-Valinotti *et al.,* 2007). These variations could be as a result of methodology used in the determination of oxidative stress biomarkers in the body.

Also, hormonal factor could have a play on the dimorphic disparity observed especially the protective shield against oxidative stress. Numerous documents from studies show that, the concentrations of anti-oxidants (such as SOD, GSH, and catalase) are meaningful reduced in males compared females (Malorni *et al.,* 2008). A study is of the view that estrogen could be the cause of the observed increases in female antioxidant level since estrogen has been showed to induce the over expression of antioxidant enzymes (Barp et al., 2002). This study noticed that in the anti-oxidative system, females had increased levels of GSH level in the heart but reduced level in the liver and small intestine while catalase and SOD were only increased in the serum when compared with

males. Both experimental and clinical data show that females have higher antioxidant defense system than males (Malorni *et al.,* 2008).

There is no evenly agreement amongst researchers on the effect of sexual dimorphism on SOD of subjects that consumed high fatty diets. For example, some studies show no meaningful variation in SOD activity levels amongst male and female mice in the heart (Azevedo *et al.,* 2001). Another study found females possessing elevated level of SOD than males in the heart (Barp et al., 2002). But when both female and male rats were castrated, SOD level decreased in comparison with their respective control (Barp et al., 2002). This last observation shows that sex hormones could have an influence on SOD activities. Just as in SOD, catalase enzyme concentration did not vary significantly in the heart between both genders in a study (Azevedo *et al.,* 2001) while another study showed males having lower catalase than females (Barp *et al.,* 2002). GSH activities were reduced in females in comparison to males (Azevedo *et al.,* 2001) but in the macrophages no dimorphic changes was observed (Barp *et al.,* 2002). Even when both genders were castrated, no reasonable variation were observed in GSH, suggesting that sex hormones might not have a role to play (Barp *et al.,* 2002).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Excessive intake of fatty diet was observed to predispose rats to obesity and possibly its comorbidities, regardless of the nature of the fatty diet or energy content of the fatty diet. The obesogenic nature of fatty diets depends also on the consumption rate and calorie content of the diets in addition to the level of saturation. Low calorie diet in this study ameliorated the obesogenic effects as observed in majority of metabolic variables. Although male rats were more prone to metabolic risk and increased oxidative stress than females, sexual dimorphism were more metabolic variable and diet dependent.

6.2 Research Findings

This study showed that the obesogenic effects as a result of high fat diet consumption is a combination of both *quality* (type/calorie content of fat) *and quantity* (amount of fat) of the fat consumed

This study also showed that sexual dimorphism was more pronounced in *high fat diets* than in *low fat diets*

This study showed that outcomes of sexual dimorphism varies with the *metabolic variable* or *diet.*

6.3 Conclusion

The sexual dimorphic response that was more pronounced in males on low calorie diet was enhanced by sexually distinct hormonal differences between the sexes

6.4 Contributions to Knowledge

This study showed that the obesogenic effects of high-fat diets are not dependent only on the level of saturation and calorie content but also on the quantity of feed consumed

This study is one of the few studies that has extensively exploited the sexual dimorphic pattern in which rat response to diets (especially fatty and low caloric) on metabolic variables in several body organs.

This study sheds more light on the need to include both genders in research designs for better understanding of the body system

6.5 Recommendations for Future Studies

More mechanistic studies are required to understand the molecular basis of the obesogenic effects of plant and animal diets

Low calorie diet could be a potential treatment for obesity and its related metabolic disorders in the future with more research interest developed.

Increased knowledge of sexual dimorphism might contribute significantly to disease prevention and treatment, such as optimizing dietary recommendations and pharmacological protocols in a sex-specific way. Therefore, sex differences should be considered in future research designs that are not sex dependent, since their responses have been shown to differ.

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