Effect of Variable High Fat Diets on Heart Rate Variability and Selected Modifiable Cardiovascular Risk Factors

by

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DECLARATION

This thesis is my original work and has not been presented for a degree at any other university.

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DEDICATION

To the two new additions to our family, Kigen and Kibet. *Mum* would have been thrilled to hold you in her loving arms.

ABSTRACT

Background: Cardiovascular diseases are on the rise particularly in developing countries and multiple factors are responsible this alarming trend. Non-ideal dietary fats adversely affect the traditional cardiovascular risk factors. However, the effect on recently described cardiovascular (CV) risk factors like heart rate variability (HRV) is less studied. Unlike in the developed world, the effect of the variable locally consumed cooking fat types on CV risk profile are unexplored thus excluding a major avenue for intervention.

Objectives: The aim of the present study was two-fold. Firstly, to determine the effect of variable high fat diets on HRV and selected modifiable CV risk factors. Secondly, to determine the chemical compositions of three locally available cooking fat types.

Design: Experimental design.

Setting: Department of Medical Physiology, University of Nairobi, Kenya.

Study Animals: Male and Female Wistar rats aged 4-6 weeks.

Methods: After acclimatization, forty animals will be divided into four groups to constitute the control(C) group and experimental groups 1(E1), 2 (E2) and 3 (E3). The control group will be fed on regular rat diet for 6 weeks. Fat enrichment (20%) of rat diet was prepared using locally sourced sunflower oil, palm oil and ghee and fed to groups E1, E2 and E3 respectively. Measurement of ECG, systolic blood pressure (SBP), fasting blood glucose and plasma lipid profile was done at baseline, and at three and six weeks. Fatty acid composition of the three fat types used will be determined using gas chromatography mass spectrometry (GC-MS). The experimental data were expressed as Mean +/- SD and analysed using repeated measures ANOVA. Significance level set at $p < 0.05$.

Results: All groups were similar in characteristics at baseline. Six weeks later, E1 had lower BMI levels ($p = 0.026$) having recorded the least percentage weight gain ($p = 0.017$). Groups E1 and E3 demonstrated higher HRV as measured by RMSSD ($p = 0.006$). Similar differences were noted with NN20 ($p = 0.04$), NN50 ($p = 0.04$) and NN100 ($p < 0.001$). The HRV was inversely correlated with the heart rate ($r = -0.654$, $p< 0.001$) and the QTc interval ($r = -0.681$, $p < 0.001$).

On evaluation of ECG parameters, E1 and E3 recorded shorter P wave durations ($p < 0.05$), PR intervals ($p < 0.05$) and QT_c ($p < 0.01$). Moreover, E1 and E3 had smaller P wave amplitudes ($p < 0.01$) but larger Q wave amplitudes ($p < 0.01$). The groups were similar with regard to the QRS intervals and R, S and T wave amplitudes. For SBP, E2 and E3 ahd higher recordings ($p < 0.01$). No statistical differences were noted in the fasting blood glucose measurements. Experimental group 2 had statistically higher fasting plasma LDL-cholesterol ($p < 0.01$) and total cholesterol (TC) ($p < 0.01$). No statistical differences were noted in HDL, TGs and TChol/HDL ratio.

Fat analysis by GC_MS showed sunflower oil had more unsaturated fatty acids than palm oil (72 µg/mg vs. 50 µg/mg). They both contained significant amounts of cholesterol (21.3 µg/mg vs. 5.8 µg/mg respectively) and TFA (70.8 µg/mg vs. 47.3 µg/mg respectively). Ghee contained highest levels of cholesterol (126.4 µg/mg), SFA (2836 µg/mg) and TFA (107.2 µg/mg).

Conclusion: Heart rate variability, a marker of autonomic influence on the heart, is reduced by palm oil containing cooking fat. Sunflower oil not only maintains good HRV but also results in lower weights and BMI measurements, a shorter QT_c on ECG, lower SBP measurements and lower LDL and total serum cholesterol levels. Despite having an otherwise good cardiovascular profile, ghee resulted in higher weight gain, BMI and SBP. Promotion of healthier cooking oil would result in good HRV and a better overall cardiovascular risk profile.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANS	Autonomic nervous system
BMI	Body mass index
CVD	Cardiovascular disease
DBP	Diastolic Blood Pressure
DHA	Docosahexaenoic acid
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
FAMEs	Fatty acid methyl esters
GC_MS	Gas chromatography–mass spectrometry
HDL	High density lipoproteins
HRV	Heart rate variability
ICIPE	International Centre for Insect Physiology and Ecology
LDL	Low density lipoprotein
NAL	Nose-anus length
PHVO	Partially hydrogenated vegetable oils
pNN20	Proportion of the total beats whose RR interval differ from the previous RR interval by 20 milliseconds.

pNN50	Proportion of the total beats whose RR interval differ from the previous RR interval by 50 milliseconds.
QTc	QT interval corrected for Heart Rate
RMSSD	The square-root of the mean squared differences between adjacent normal R–R intervals
SBP	Systolic blood pressure
SDNN	The standard deviation of all normal R–R intervals
TChol	Total cholesterol
TFA	Trans-fatty acids
TGs	Triglycerides

1 INTRODUCTION

Cardiovascular diseases (CVDs) continue to cause significant morbidity and mortality globally. Indeed, CVDs are responsible for a higher disease burden than any other cause accounting for approximately one third of all deaths worldwide (GBD 2013 Mortality and Causes of Death Collaborators, 2015).

The trends are equally disheartening as shown by the steady rise in mortality secondary to non-communicable diseases and injuries between the years 1990 and 2013 (GBD 2013 Mortality and Causes of Death Collaborators, 2015). This is despite a considerable decline in the deaths secondary to communicable diseases, maternal, neonatal, and nutritional causes decreased (GBD 2013 Mortality and Causes of Death Collaborators, 2015). It is projected that by 2020 CVD will account for 73% of total global mortality and 56% of total morbidity (Murray and Lopez, 1996).

The development of CVD has been attributed to multiple risk factors. These are classified as modifiable and non-modifiable risk factors. Non-modifiable risk factors include advancing age, male gender and positive family history of cardiovascular disease. Traditional modifiable risk factors include high blood pressure, smoking, dyslipidaemia, diabetes/ insulin resistance, lack of physical exercise and overweight/obesity. Newer cardiovascular risk factors have been described including heart rate variability (HRV) and high inflammatory states. Reduced HRV, an indicator of autonomic neuropathy, has been shown to predispose one to cardiovascular disease (Novak et al., 1997).

Dietary intake of partially hydrogenated vegetable oils (PHVO) containing trans-fatty acids (TFA) adversely affects multiple cardiovascular risk factors thereby predisposing one to CVDs (Mozaffarian et al., 2009). Several countries, mainly in the developed world, have policies

fostering the replacement of PHVO with fats containing less saturated and trans-fatty acids. In Kenya and many developing countries, however, the consumption of PHVO is still highly prevalent and unchecked. The effect of PHVO on heart rate variability is largely unexplored (Soares-Miranda et al., 2012)

This thesis analyses the chemical composition of three locally consumed cooking fat types and explores their effects on selected cardiovascular risk factors notably HRV, blood pressure, plasma lipids profile and plasma fasting blood glucose.

LITERATURE REVIEW

1.1 Heart Rate Variability

Heart rate variability (HRV), refers to beat-to-beat variation in duration of the R–R interval (Novak et al., 1997). The HRV measurements can either be done over short periods for example 5 minutes or longer intervals like 24 hours. Measurements over short periods show moment to moment changes in the heart rate. Longer period measurements, in addition, show circadian patterns.

Heart rate variability has become an important risk assessment tool. It has been established as a reliable measure of cardiac electrophysiology and autonomic function. This is because the cardiac rhythm is under both minute-minute as well as circadian influence of the autonomic nervous system (ANS) (Zhang et al., 2010). A good balance between the sympathetic and parasympathetic arms of the ANS results in desirable HRV patterns. Predominance of the sympathetic outflow results not only in higher heart rates but also reduced HRV. In such instances, HRV indices record ultra-low and low frequency power measurements. Parasympathetic predominance, on the other hand, results in lower heart rates and higher frequency power measurements.

Established link has been documented between reduced HRV as measured by the standard deviation of all normal R–R intervals (SDNN) and the square root of the mean squared differences between adjacent normal R–R intervals (RMSSD) with myocardial infarction, cardiomyopathy, congestive heart failure and mortality (Kleiger et al., 2005). There's also suggested relationship between lower HRV and coronary heart disease, atrial fibrillation and heart failure (Malpas et al., 2002). These associations are in addition to the direct association between high heart rates and all-cause mortality, death from CVDs and sudden cardiac death

(Lahiri et al., 2008). Indeed heart rate measurement has been established as a marker of autonomic activity (Lahiri et al., 2008).

A number of techniques have now been developed to quantify HRV (Novak et al., 1997; Berntson et al., 1997; Grossman and Taylor, 2007; Thayler et al., 2010). Multiple techniques have been developed to evaluate different aspects of HRV. These are time domain methods, frequency domain methods, geometric methods, non-linear methods and long term correlations. (Novak et al., 1997).

The time domain measures look at changes in heart rate or the intervals between successive normal beats are determined an ECG record. Of note, only the normal QRS complexes are used for the calculation and 'N' is used to denote that only normal beats have been considered. Time domain measures can be applied on both short and long recordings. Descriptive time domain variables are used. These include the mean NN interval, mean heart rate, and the range (longest NN minus the shortest NN) (Kleiger et al., 1987). Statistical calculations like standard deviation of the NN interval (SDNN) are also calculated (Kleiger et al., 1987). In addition, measurement of adjacent pairs of normal beats that differ by more than 50 ms, NN50 has been used (Ewing et al., 1985).

1.1.1 Dietary Fat and Heart Rate Variability

Ingestion of diet rich in trans-fats has been shown to lower HRV (Soares-Miranda et al, 2012; Dyerberg et al., 2004).Beneficial effects have been seen with omega-3 fats (Hansen et al, 2014; Harris et al., 2012; Harbaugh et al., 2013) and docosahexaenoic acid (DHA) (Valera et al., 2014; Ninio et al., 2008). In particular 18:2 trans-fats and not 18:1 have been shown to have this HRV lowering effects (Soares-Miranda et al., 2012).

Two hypotheses have been put forward to explain the effects of dietary fats on HRV. One, that high dietary fat increases the basal metabolic rate of an individual leading to increased sympathetic stimulation. This is associated with both higher heart rate recordings and lower heart rate variability (Millis et al, 2009). Secondly, specific fatty acids have been shown to directly alter ion conductance of on the pacemaker tissue (Billman, 2013). This alteration seems to occur during channel development with no effect on the already formed channels. Indeed, omega-3 fatty acids have been used in post-myocardial infarction patients (Villa, 2002)

1.2 Hypertension

Hypertension refers to an abnormal sustained elevation of the blood pressure. Blood pressure, defined as the force exerted on the blood vessel wall, has been a concept examined for over 100 years. There are three components to it: systolic blood pressure; diastolic blood pressure and mean arterial pressure. Systolic blood pressure is defined as the highest pressure attained within the major arteries during the cardiac cycle. This corresponds to the latter half of ventricular systole. Diastolic blood pressure, on the other hand, refers to the lowest pressure within the major arteries during the cardiac cycle. This occurs late in ventricular diastole. Mean arterial pressure refers to the average pressure within major arteries during the cardiac cycle. This is often estimated by adding one third of the pulse pressure to the diastolic blood pressure. Of the three major components, increase in the systolic blood pressure is what has been linked strongly to the development of cardiovascular diseases (Sesso et al., 2000).

Optimal human blood pressure is define as pressures <120/<80mmHg for adults of age 18 and above. For children blood pressure recording that is lower than the 90^{th} percentile for the age group is considered normal.

Hypertension is the leading risk factor for CVDs (Go et al., 2014). This is owing to its high prevalence and its high impact on disease causation. Indeed, it is the leading single contributor of adult deaths worldwide (Brundtland et al., 2002). The prevalence is on the rise and it is projected that by 2025, 40% of the adult population will be hypertensive (Kearney et al., 2004). This would be a significant rise from 25% documented in the year 2000 (Kearney et al., 2004). In 2010 a third of the US population had hypertension and 15% of all deaths were attributable to hypertension either directly or indirectly (Go et al., 2014)

In Kenya, the prevalence of hypertension is documented at between 12% and 22% (Joshi et al., 2014; De et al., 2013). Awareness among individuals with hypertension is much lower at 19.5% (De et al., 2013). Of these, only about half were actually on antihypertensive medication (De et al., 2013). Further to this, only 20% of those on medication actually achieved sufficient pressure reduction at <140/90. This implies that only 3% of hypertensive individuals are adequately treated (De et al., 2013).

Several factors have been associated with an increase in blood pressure (Go et al., 2014). These include increased age, positive family history, overweight, high blood cholesterol, diabetes, physical inactivity, smoking and diet high in salt, simple sugars and fat.

Hypertension if left untreated may result in a host of complications. These include but are not limited to atherosclerosis, acute coronary syndrome, aortic dissection, stroke, renal failure, retinal damage, erectile dysfunction, and peripheral artery disease (Sesso et al., 2000).

1.2.1 Dietary Fat and Hypertension

The ingestion of partially hydrogenated vegetable oils has been associated with elevation of the blood pressure (Bonny et al., 2008; Esmaillzadeh et al., 2011). This association is thought to be

secondary to elevated LDL cholesterol in plasma among those ingesting partially hydrogenated vegetable oils (Bonny et al., 2008).

1.3 Diabetes and Insulin Resistance

Diabetes is a chronic debilitating condition characterized by deficient or absent insulin activity. This can either be due to lack of insulin production (Type I, Insulin dependent diabetes mellitus) or deficient activity in the presence of normal or even elevated levels of insulin (Type II, Non-insulin dependent diabetes mellitus)

The "diabetes epidemic" is a great challenge to the world's health care system with a world-wide prevalence of 230 million at the end of 2006. This represented 6% of the world's population then. This is up from a prevalence of 30 million in 1985. The prevalence is expected to increase to 350 million in less than 20 years.

The situation is worse in the developing world which has 80% of the diabetes prevalence (Roglic et al., 2005). The prevalence in some countries for example India is as high as 12-20% of the adult population.

1.3.1 Dietary Fat and Diabetes

The development of insulin resistance as well as type II diabetes has been associated with the consumption of diets rich in trans-fats as well as saturated fatty acids (Simopoulos, 1994; Riccardi et al., 2004). Specifically, saturated fat worsens insulin sensitivity, while monounsaturated and omega-6 polyunsaturated fats improve it (Rivellese and Lilli, 2003). In the short term, however, consumption of variable partially hydrogenated vegetable oils has been shown not to influence insulin sensitivity or the development of diabetes (Lovejoy et al., 2002;

Esmaillzadeh et al., 2011). Indeed, this is the case with and without correcting for body mass index (BMI).

The molecular mechanism underlying varying insulin sensitivity has been shown to be secondary to fatty acid composition on muscle cell membrane (Borkman et al., 1993). Indeed, using clamp technique, insulin action has been correlated with the proportion of long-chain poly-unsaturated fatty acids (PUFA) (Borkman et al., 1993). On the contrary, insulin sensitivity was inversely correlated with the proportion of saturated fatty acids (Borkman et al., 1993)

1.4 PROBLEM STATEMENT AND JUSTIFICATION

Cardiovascular diseases are on the rise especially in developing countries including Kenya. The effect of locally consumed dietary fat on different cardiovascular risk factors is largely unexplored. This is despite the well documented role diet plays in an individual's risk profile. Even scantier, is data on the effect of our dietary fat on newer cardiovascular markers like heart rate variability. This leaves a potentially significant avenue for prevention and management unexplored. Thus, the present study tested the effects of variable high fat diets on selected cardiovascular risk factors in an animal model.

1.5 OBJECTIVES

General objective:

To determine the effect of variable high fat diets on selected modifiable cardiovascular risk factors.

Specific objectives:

To determine the effect of variable high fat diets on heart rate variability.

To determine the effect of variable high fat diets on systolic blood pressure.

To determine the effect of variable high fat diets on fasting blood glucose

To determine the effect of variable high fat diets on fasting plasma lipid profile level.

To determine the lipid composition of three locally available cooking oil/fat

2 MATERIALS AND METHODS

2.1 STUDY DESIGN

An experimental study design was employed in this study. Four groups comprising 10 animals each were used. One group served as the negative control while the other three constituted the experimental groups. (Figure 2.1.1)

2.2 STUDY ANIMALS

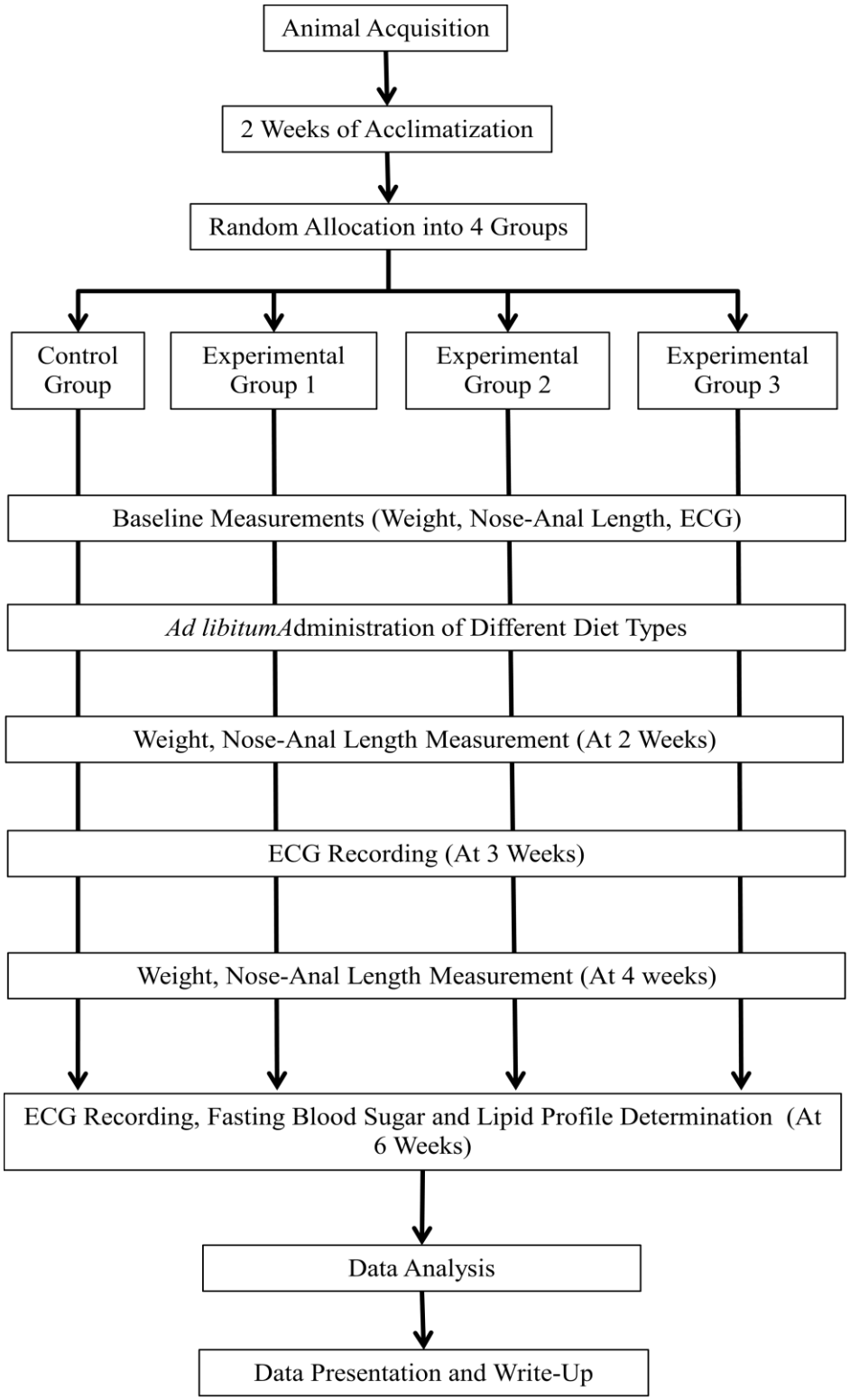
Forty (40) male and female Wistar rats aged 4-6 weeks were used. The animals were obtained from the Kabete Veterinary Laboratory of the Government of Kenya. They were housed at the Department of Medical Physiology animal house. The male and female rats were placed in separate cages.

The rats were provided with standard rat chow (Unga Feeds, Nairobi, Kenya) and drinking water *ad libitum*. Rats were habituated to handling and testing procedures for two (2) weeks prior to the start of the experiment. The animals were subjected to a 12:12-h light–dark cycle

2.3 GROUP ALLOCATION

After two weeks of acclimatization, the animals were randomly divided into four groups each containing 10 animals. These constituted the control group and three experimental groups: E1, E2 and E3.

Figure 2.1.1 Study Flow Chart



2.4 EXPERIMENTAL PROCEDURES

2.4.1 Food Preparation and Administration

The control group was fed on the normal rat diet and water *ad libitum.* The experimental groups were fed on three different forms of high fat (20%) diet and water *ad libitum.*

For experimental Group 1, the normal rat diet was fat enriched with sunflower oil. Experimental Group 2 had enrichment with vegetable fat from palm oil while experimental Group 3 had ghee-enriched diet. The constitution of these three lipids is summarised below in Table 2.4.1. A complete analysis of the lipids used is detailed in Table 3.7.1 in the results section.

Table 2.4.1 Table of Dietary Fat Constituents

	UNITS	GROUPS			
Constituents		**Control**	**E1**	**E2**	**E3**
Total Fat	g/100g	4	20	20	20
Saturated FA	g/100g	2.83	10.4	14.8	19.3
Monounsaturated FA	g/100g	1.1	8.4	6.1	1.91
Polyunsaturated FA	g/100g	0.17	1	0.98	0.82
Unsaturated (Monounsatured + Polyunsaturated)	g/100g	1.17	9.4	7.08	2.73
Trans - fatty acids	g/100g	1.17	9.15	6.74	0.73
Cholesterol	g/100g	0	2.8	0.83	0.86

E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

2.4.2 Determination of BMI

The weight and nose-anus length (NAL) of the rats in the control and experimental groups were measured at the start of the experiment and at weeks two, four and six. The BMI was determined

by dividing the weight in grams by the square of the nose-anus length in centimetres. Thus, obesity was defined by a BMI of greater than 0.68 g/cm^2 as previously described by Novelli et al.

2.4.3 ECG Recording

The ECG record (bipolar lead II) of each animal was taken at three weeks and six weeks. The rats were anaesthetized by use of Ketamine (0.12 mg/g bodyweight intraperitionally, IP) before recording the ECG. The ECGs were recorded by use of Power Lab Data acquisition apparatus (Model ML865, AD Instruments, Dunedin, New Zealand).

Heart rate variability (HRV) was evaluated using time domain measures: RMSSD, pNN20 and pNN50 (Novak et al., 1997). RMSSD is the square root of the mean of the sum of the squares of differences between adjacent NN/RR. This is the calculation of standard deviation of the differences between adjacent normal heart beats. pNN20 refers to the proportion of the total beats whose RR interval differs from the previous RR interval by 20 milliseconds. pNN50 refers to the proportion of the total beats whose RR intervals differ from the previous RR interval by 50 milliseconds (Novak et al., 1997).

In addition to HRV, the following standard ECG variables were analysed: heart rate, P duration, P amplitude, PR interval, QRS duration, QT interval, QTc interval, Q amplitude, R amplitude, S amplitude, T amplitude and RR interval. The QTc interval was derived from the QT interval using the Bazzet's formula: QTc= QT Interval / √ (RR interval).

2.4.4 Determination of blood glucose levels

Fasting blood glucose levels were determined at week 6.The animals are fasted overnight (12 hours) prior to the measurement. Using a disinfected pair of surgical scissors, 1-2mm of the tip

of the tail was cut. The drop of blood produced was fed into the blood glucose monitoring system (OneTouch SureStep, Milpitas, CA, USA) and the reading was recorded.

2.4.5 Blood Pressure Determination

Systolic blood pressure measurements were conducted at after six weeks. Rats were immobilized by placement into 6 cm wide bottles with ventilation holes. The animals were then warmed to between 36^{o} C and 40^{o} C. The rat tails were placed within miniature blood pressure cuffs and blood flow impeded by increasing the pressure in the cuff. Blood flow or its absence was detected using a digital pulse detector which is part of the Power Lab Data acquisition apparatus (Model ML865, 2005, ADinstruments, Dunedin, New Zealand). Pressure was recorded from a dial sphygmomanometer.

2.4.6 Lipid Profile Determination

Fasting Lipid profile was determined at the end of week 6. The profile included total cholesterol (TChol), high density lipoproteins (HDL), low density lipoproteins (LDL) and triglycerides (TGs). After 12-hours of fasting, 4 millilitres of blood was drawn via retro-orbital puncture and placed in EDTA vacutainers. Lipid profile was determined using Philips Model 23187

2.4.7 Lipid Analysis

The chemical composition of the three dietary fats used in the fat-enrichment of rat food was done using gas chromatography mass spectrometry (GC-MS). This was carried out at the International Centre for Insect Physiology and Ecology Laboratories, Nairobi Kenya.

Sample preparation

- 10 mg of was weighed from each of the three cooking fats and derivatized to form Fatty acid methyl esters which was analysed by GC-MS

- Serial dilutions of authentic standard (Octadecanoic acid; 99%, Gillingham, dorset, England) (0.2 ng/µl, 1ng/µl, 5 ng/µl, 25 ng/µl 125 ng/µl and 625 ng/µl) were prepared and analysed by GC-MS.
- The sample and the standard were analysed on an Agilent Gas Chromatograph7890A / 5975 C Mass Spectrometer in full scan mode. With the following conditions:

Machine Settings

GC Column:	HP-5 MS low bleed capillary column (30 m × 0.25mm i.d., 0.25 µm) (J&W, Folsom, CA, USA)
Flow rate,(He):	1.25 ml/min, constant flow mode
Injection Mode:	split mode
Oven temperature:	35°C (5 min.) to 280°C @10°C/min (10.5min); run time 40min
Injection volume:	1µl

Compound identities were determined using NIST'08, 05, Adams and chemical mass spectral databases (Adams, 2007)

Procedure for Derivatization

Method adopted from (Sweeney et al., 2004) summarised as follows:

1. Ten milligrams of the sample were weighed and esterification carried out. A solution of sodium methoxide in dry methanol was prepared to give a concentration of 15 mg/mL. Subsequently, 1000 µL volume of this solution the sample was added, vortexed for 1 min and sonicated for 5 min. The reaction mixture was incubated at 70 $^{\circ}$C for 1 hr.

Quenching was done by adding 200 µL deionized water and vortexed. The methyl esters were extracted using GC-grade hexane (Sigma–Aldrich, St. Louis, USA) and centrifuged at 14, 000 rpm for 5 min.

2. The supernatant was dried using anhydrous Na_2SO_4 and analyse using GC-MS.

2.5 STATISTICAL ANALYSES

The results are expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to test for difference between the different groups. Alpha (α) value was set at 0.05. ANOVA was followed by the Tukey's post hoc test in instances where significant differences were observed.

2.6 ETHICAL CONSIDERATIONS

The study protocol was approved by the Postgraduate Research Committee, Department of Medical Physiology, School of Medicine, University of Nairobi. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and care. Animals were handled with care and in accordance with the FELASA guidelines.

3 RESULTS

3.1 Weight and Body Mass Index

At baseline, there were no significant differences in the weights (127.3 ± 28.1 (control) vs 129.6 ± 24.1 (E1) vs 129.7 ± 19.7 (E2) vs 121.6 ± 17.5 (E3); p= 0. 972) and BMI (0.60 ± 0.04 (control) vs 0.61 ± 0.02 (E1) vs 0.61 ± 0.04 (E2) vs 0.60 ± 0.03 (E3);p=0.885) of the animals in the different groups. The data are illustrated in Table 3.1.1.

Table 3.1.1 Baseline Anthropometric Measures

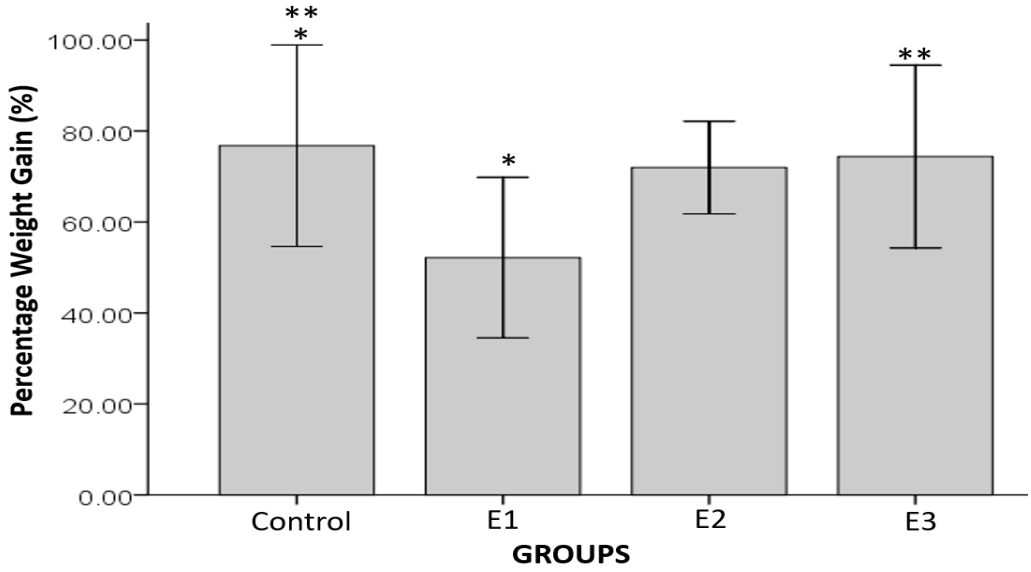
GROUPS	Weight (g)	BMI[a] (g/cm^2)
Control	127.3 ± 28.1*	0.60 ± 0.04†
E1	129.6 ± 24.1*	0.61 ± 0.02†
E2	129.7 ± 19.7*	0.61 ± 0.04†
E3	121.6 ± 17.5*	0.60 ± 0.03†

Values are Mean ± SD
*p = 0.972; †p = 0.885
[a]BMI= Body mass index
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

Experimental group 1 recorded the least percentage weight gain over the six weeks of the study (p = 0.022) while the experimental group 3 recorded the highest percentage weight gain (p = 0.044). The percentage weight gained was (76.8 ± 22.2(control) vs. 52.2 ± 17.6 (E1) vs. 72.0 ± 10.2 (E2) vs. 74.4 ± 20.1 (E3) %; p = 0.017). This is illustrated in Figure 3.1.1.

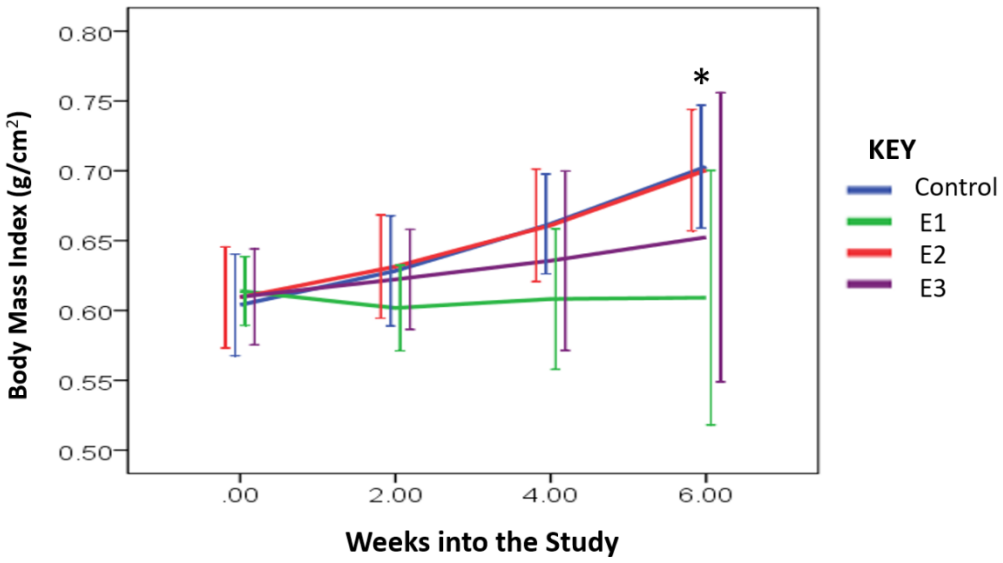
Accordingly, E1 had the lowest body mass index (BMI) measurements at six weeks (p = 0.041). The BMI measurements at six weeks were (0.70 ± 0.04(control) vs 0.61 ± 0.09 (E1) vs 0.70 ± 0.04 (E2) vs 0.65 ± 0.10 (E3); p = 0.026). This is illustrated in Figure 3.1.2.

Figure 3.1.1 Bar Graph of Percentage Weight Gain



Bars represent means ± SD.
*p = 0.022
**p = 0.044
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

Figure 3.1.2 Line Graph of Body Mass Index over the duration of study



Error bars represent ±SD.
*E1 vs Control *p* = 0.041
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

3.2 Heart Rate Variability

RMSSD (Standard Deviation of Adjacent Normal RR Intervals)

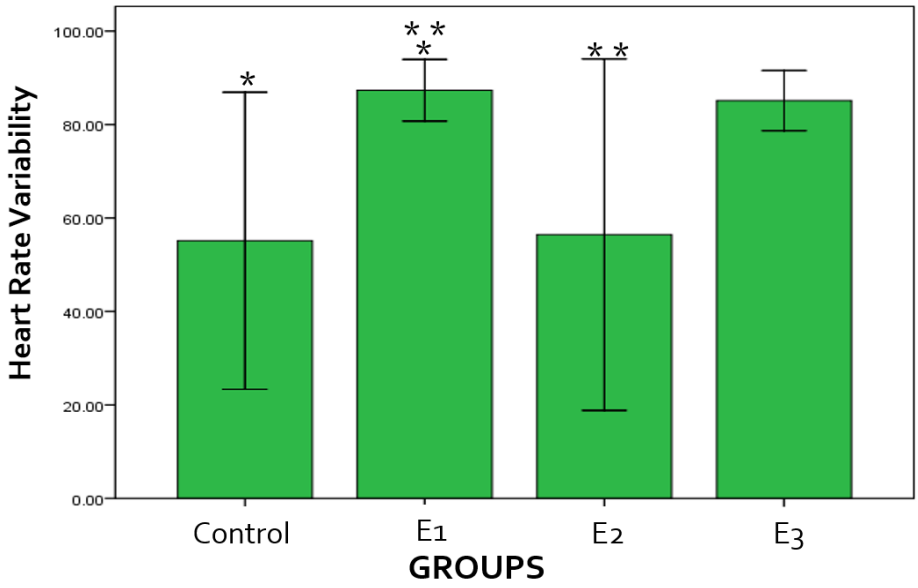
There were no statistically significant differences in RMSSD measure of HRV between the different group at baseline (5.64 ± 5.5 (control) vs 5.59 ± 2.6 (E1) vs 5.04 ± 4.1 (E2) vs 4.41 ± 2.1 (E3); p = 0.878). Similarly, no statistically significant differences were noted in measurements recorded at three weeks (5.46± 6.3 (control) vs 10.07 ± 13.2 (E1) vs 8.29 ± 7.3 (E2) vs 4.24 ± 4.5 (E3); p = 0.41). At week six, the RMSSD measurements demonstrated statistical difference between the groups with higher variability recorded in groups E1 (p = 0.005) and E3 (p = 0.003). The RMSSD recordings were (55.13 ± 31.8 (control) vs 87.340 ± 6.6 (E1) vs 56.43 ± 37.6 (E2) vs 85.12 ± 6.4 (E3); p = 0.006). This is illustrated in Table 3.2.1 and Figure 3.2.1.

Table 3.2.1 Table of Heart Rate Variability (RMSSD) Measured over Time

GROUPS	Heart Rate Variability (ms)		
	Baseline	Week 3	Week 6
Control	5.64 ± 5.5	5.46± 6.3	55.13 ± 31.8*
E1	5.59 ± 2.6	10.07 ± 13.2	87.340 ± 6.6*
E2	5.04 ± 4.1	8.29 ± 7.3	56.43 ± 37.6*
E3	4.41 ± 2.1	4.24 ± 4.5	85.12 ± 6.4*

Values are Mean ± SD
*p< 0.01
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

Figure 3.2.1 Line Graph representing Heart Rate Variability (RMSSD) at Six Weeks



Bars represent means ± SD.
*p < 0.05
* * p < 0.05
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

NN20, NN50, NN100

At baseline, there were no statistical differences between the groups in the HRV measurements of NN20 1.6 ± 3.5 (control) vs 1.4 ± 2.9 (E1) vs 1.7 ± 2.8 (E2) vs 0.8 ± 1.6 (E3); p = 0.67), NN50 1.1 ± 1.8 (control) vs 1.3 ± 2.1 (E1) vs 1.3 ± 2.2 (E2) vs 0.7 ± 1.3 (E3); p= 0.87) and NN100 0.0 ± 0.0 (control) vs 0.0 ± 0.1 (E1) vs 0.1 ± 0.1 (E2) vs 0.0 ± 0.0 (E3); p = 0.87). This is summarized in Table 3.2.2.

Similarly, no statistical difference was noted at three weeks:NN20 1.8 ± 4.6 (control) vs 1.7 ± 3.0 (E1) vs 2.1 ±3.2 (E2) vs 0.6 ±1.1 (E3); p = 0.746), NN50 0.7 ± 1.9 (control) vs 1.4 ± 2.5 (E1) vs 1.6 ± 2.6 (E2) vs 0.5 ± 1.1 (E3); p = 0.594) and NN100 0.0 ± 0.0 (control) vs 0.2 ± 0.6 (E1) vs 0.0 ± 0.0 (E2) vs 0.0 ± 0.0 (E3); p = 0.404) as shown in Table 3.2.2.

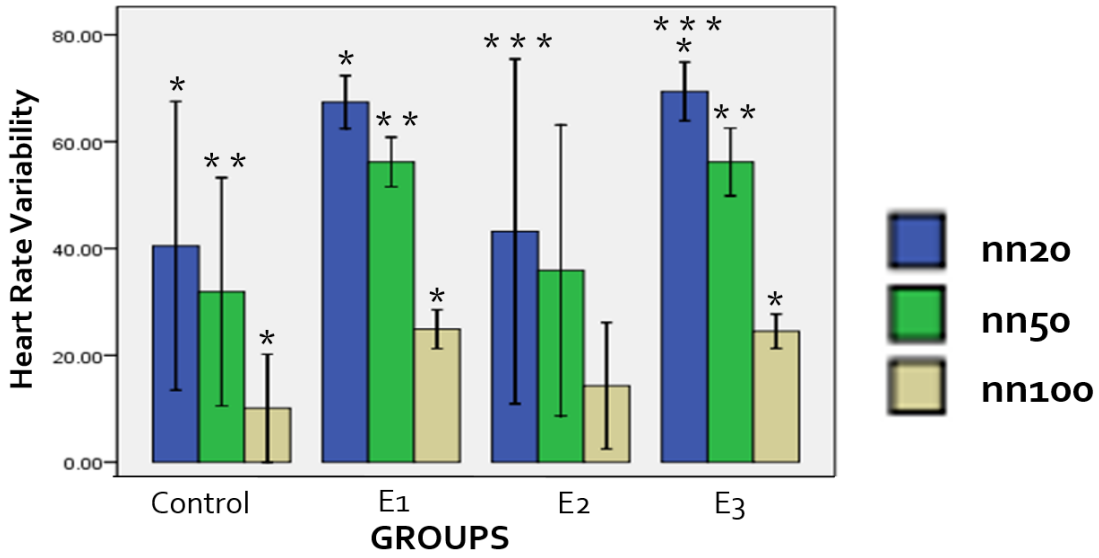
Measurements of NN20, NN50 and NN100 done at six weeks all recorded higher values for groups E1 and E3. For NN20 40.5 ± 27.0 (control) vs 67.4 ± 4.9 (E1) vs 43.2 ± 32.2 (E2) vs 69.4 ± 5.5(E3); $p = 0.04$), NN50 31.9± 21.4 (control) vs 56.2 ± 4.6 (E1) vs 35.9 ± 27.2 (E2) vs 56.2 ± 6.3 (E3); $p = 0.04$) and NN100 10.1 ± 10.1 (control) vs 24.9 ± 3.6 (E1) vs 14.3 ± 11.8 (E2) vs 24.5 ± 3.2 (E3); $p < 0.001$) respectively. These results are illustrated in Table 3.2.2 and Figure 3.2.2.

Table 3.2.2 Table of Heart Rate Variability (NN20, NN50 and NN100)

	GROUPS	Heart Rate Variability		
		NN20	**NN50**	**NN100**
Baseline	Control	1.6 ± 3.5	1.1 ± 1.8	0.0 ± 0.0
	E1	1.4 ± 2.9	1.3 ± 2.1	0. ± 0.1
	E2	1.7 ± 2.8	1.3 ± 2.2	0.1 ± 0.1
	E3	0.8 ± 1.6	0.7 ± 1.3	0.0 ± 0.0
Week 3	Control	1.8 ± 4.6	0.7 ± 1.9	0.0 ± 0.0
	E1	1.7 ± 3.0	1.4 ± 2.5	0.2 ± 0.6
	E2	2.1 ±3.2	1.6 ± 2.6	0.0 ± 0.0
	E3	0.6 ±1.1	0.5 ± 1.1	0.0 ± 0.0
Week 6	Control	40.5 ± 27.0*	31.9± 21.4*	10.1 ± 10.1†
	E1	67.4 ± 4.9*	56.2 ± 4.6*	24.9 ± 3.6†
	E2	43.2 ± 32.2*	35.9 ± 27.2*	14.3 ± 11.8†
	E3	69.4 ± 5.5*	56.2 ± 6.3*	24.5 ± 3.2†

Values are mean ± SD
*$p < 0.05$, †$p < 0.001$
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

Figure 3.2.2 Bar Graph representing Heart Rate Variability (NN20, NN50 and NN100) at Six Weeks



Bars represent means ± SD.
*p < 0.05
* p < 0.05
* * * p < 0.05
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

3.3 ECG Changes

Electrocardiographic measurements at baseline revealed no statistically significant differences in the heart rate, RR interval, P duration, PR interval, QRS interval, QT interval, QTc, P amplitude, Q amplitude, R amplitude, S amplitude and T amplitude (p> 0.05).

At six weeks, E1 had the shortest PR intervals (p = 0.03) while E2 had the longest (p = 0.04). The PR intervals were (44.4 ± 3 (control) vs 41.4 ± 1.6 (E1) vs 45.7 ± 4.9 (E2) vs 42.2 ± 1.7 (E3); p = 0.016). This is illustrated in Table 3.3.1 and Figure 3.3.1.

Similarly, P wave duration was shortest is E1 (p = 0.02) and longest in E2 (p = 0.03). The measurements were (20.5 ± 2.0 (control) vs 18.4 ± 1.3 (E1) vs 21.1 ± 2.9 (E2) vs 18.8 ± 1.5 (E3); p = 0.013).

QT_C interval, QT interval corrected for different heart rates using the Bazzet's formula, was also shortest in E1 (p = 0.022) and longest in E2 (p = 0.014). The measurements were (20.5 ± 2.0(control) vs 18.4 ± 1.3 (E1) vs 21.1 ± 2.9 (E2) vs 18.8 ± 1.5 (E3); p = 0.004).

The heart rate measurements between the groups demonstrated no statistical differences (369.3 ± 39.9 (control) vs 344.3 ± 14.6 (E1) vs 366.3 ± 67.3 (E2) vs 355.6 ± 40.6 (E3);p = 0.314). Considering the descriptive statistics however, experimental groups 1 and 3 had lower mean heart rates than both the control group and experimental group 2 as depicted in Table 3.3.1.

The QRS intervals measured from the different groups had no statistical differences (20.5 ± 2.0(control) vs 18.4 ± 1.3 (E1) vs 21.1 ± 2.9 (E2) vs 18.8 ± 1.5 (E3); p = 0.348).

On evaluation of the wave amplitudes, E1 recorded the least P wave amplitude (p = 0.028) while E2 the highest (p = 0.023). The P wave amplitudes were (20.5 ± 2.0(control) vs 18.4 ± 1.3 (E1) vs 21.1 ± 2.9 (E2) vs 18.8 ± 1.5 (E3); p< 0.01).

On the contrary, Q wave amplitude was highest in E1 (p = 0.0003) and least in E2 (0.008). The Q wave amplitudes were (-12.4 ± 19.9(control) vs -63.6 ± 34.0 (E1) vs -18.4 ± 28.6 (E2) vs -38.8 ± 22.3 (E3); p< 0.01).

Lastly, no statistical differences were noted between R, S, and T amplitudes of the different groups: R (208.8 ± 107.6(control) vs 157.5 ± 62.1 (E1) vs 192.8 ± 90.6 (E2) vs 139.6 ± 29.1 (E3); p> 0.05); S (-62.9 ± 54.6(control) vs -64.8 ± 33.2 (E1) vs -126.1 ± 125.8 (E2) vs -38.3 ±

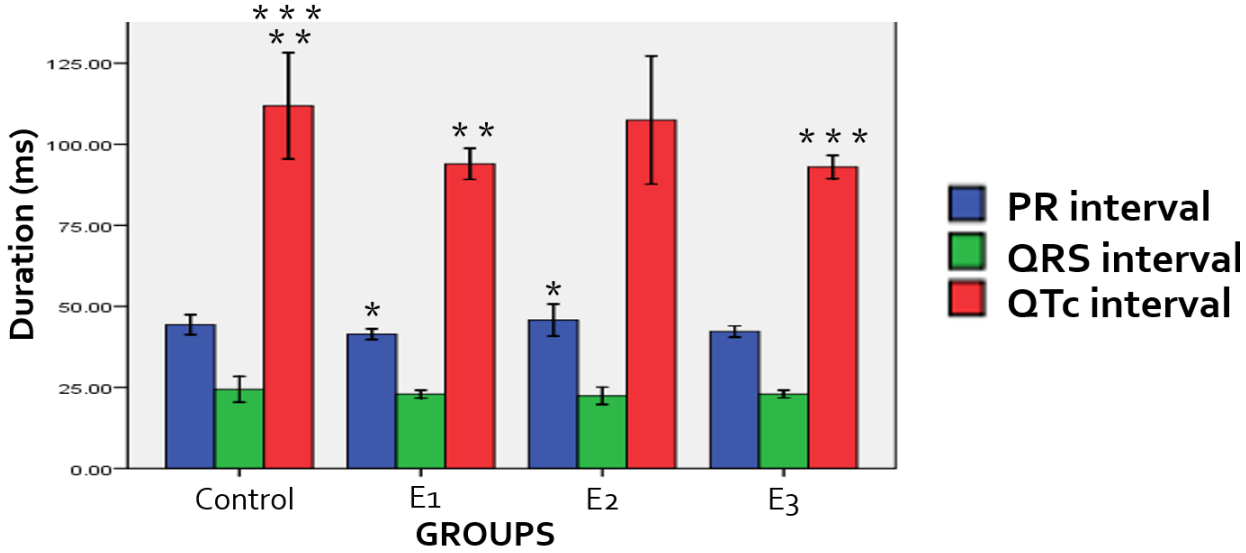
19.0 (E3); $p > 0.05$); T (61.9 ± 50.5(control) vs 17.4 ± 6.2 (E1) vs 55.3 ± 82.7 (E2) vs 17.3 ± 10.0 (E3); $p > 0.05$). These are demonstrated in Table 3.3.1.

Table 3.3.1 Table of ECG Parameters

ECG Parameter	GROUPS			
	Control	**Exp Group 1**	**Exp Group 2**	**Exp Group 3**
Heart Rate (beats/min)	369.3 ± 39.9	344.3 ± 14.6	366.3 ± 67.3	355.6 ± 40.6
RR Interval (msec)	167.8 ± 20.7	180.4 ± 9.5	176.0 ± 44.3	182.7 ± 11.1
P Duration (msec)	20.5 ± 2.0*	18.4 ± 1.3*	21.1 ± 2.9*	18.8 ± 1.5*
PR Interval (msec)	44.4 ± 3*	41.4 ± 1.6*	45.7 ± 4.9*	42.2 ± 1.7*
QRS Interval (msec)	24.4 ± 4.0	22.9 ± 1.2	22.4 ± 2.7	23.0 ± 1.2
QT Interval (msec)	45.1 ± 4.9†	39.4 ± 1.7†	44.0 ± 6.9†	39.1 ± 1.5†
QTc (msec)	111.9 ± 16.4†	93.9 ± 4.8†	107.5 ± 19.8†	93.0 ± 3.6†
P Amplitude (µv)	49.5 ± 45.1†	3.3 ± 9.3†	49.7 ± 46.6†	6.7 ± 6.4†
Q Amplitude (µv)	-12.4 ± 19.9†	-63.6 ± 34.0†	-18.4 ± 28.6†	-38.8 ± 22.3†
R Amplitude (µv)	208.8 ± 107.6	157.5 ± 62.1	192.8 ± 90.6	139.6 ± 29.1
S Amplitude (µv)	-62.9 ± 54.6	-64.8 ± 33.2	-126.1 ± 125.8	-38.3 ± 19.0
T Amplitude (µv)	61.9 ± 50.5	17.4 ± 6.2	55.3 ± 82.7	17.3 ± 10.0

*****$p < 0.05$
†$p < 0.01$
ECG, Electrocardiogram; RR, P, Q, R, S, T electrocardiographic waves/ time intervals

Figure 3.3.1 Bar Graph representing P Duration, PR, QRS and QTc Intervals at Week 6

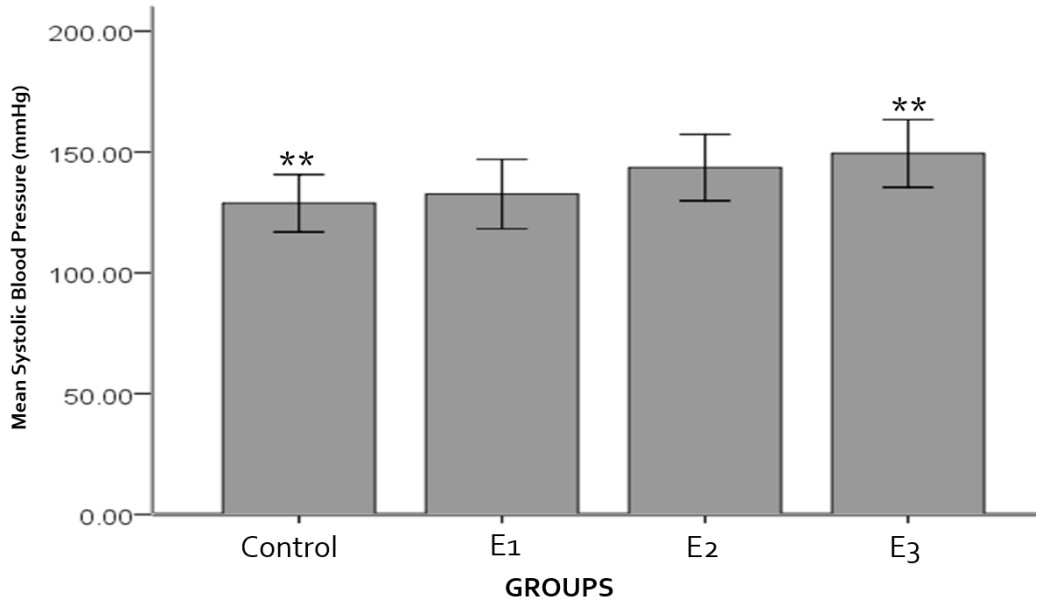


Bars represent means ± SD.
*p < 0.02
** p < 0.022
* * * p < 0.014
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

3.4 Systolic Blood Pressure

One-Way ANOVA analysis of the systolic blood pressure (SBP) measurements done at six weeks demonstrated E3 had significantly higher measurements (p = 0.009). The SBP measurements were (128.8±11.9 mmHg (control) vs 132.6±14.4 mmHg (E1) vs 143.6±13.7 mmHg (E2) vs 149.4±14.0mmHg; p<0.01). This data is illustrated in Figure3.4.1.

Figure 3.4.1 Bar Graph of Systolic Blood Pressure Measurements



Bars represent means ± SD.
**p = 0.009
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

3.5 Fasting Blood Glucose

Fasting blood Glucose levels done at the end of week six showed no statistically significant difference between the different groups (5.6±0.8 mmol/dL(control) vs 5.6±1.2 mmol/dL (E1) vs 5.7±0.6 mmol/dL (E2) vs 5.9±1.2 mmol/dL (E3); p =0.866)as shown in Table 3.5.1.

Table 3.5.1Table of Fasting Blood Glucose Measurements

GROUPS	Fasting Blood Sugar (mmol/dL)
Control	5.6±0.8*
E1	5.6±1.2*
E2	5.7±0.6*
E3	5.9±1.2*

Values represented as mean ± SD
*p = 0.866
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

3.6 Lipid Profile

The lipid profile results comprised of four parameters; Total Cholesterol, High Density Lipoprotein, Low Density Lipoprotein and Triglycerides.

Total Cholesterol measurements demonstrated statistical differences between the groups with E2 having the highest levels (p = 0.001). The TChol measurements were (1.73 ± 0.18 mmol/L (control) vs 1.97 ± 0.46mmol/L (E1) vs 2.26 + 0.26mmol/L (E2) vs 1.95 ± 0.12mmol/L (E3); p<0.01*)* as illustrated in Table 3.6.1 and Figure 3.6.1.

Similarly, E2 recorded highest LDL cholesterol levels (p = 0.001). The LDL measurements were (0.49 ± 0.22 mmol/L(control) vs 0.69 ± 0.30 mmol/L (E1) vs 0.92 ± 0.22 mmol/L (E2) vs 0.70 ± 0.15 mmol/L (E3); p<0.01*)*.

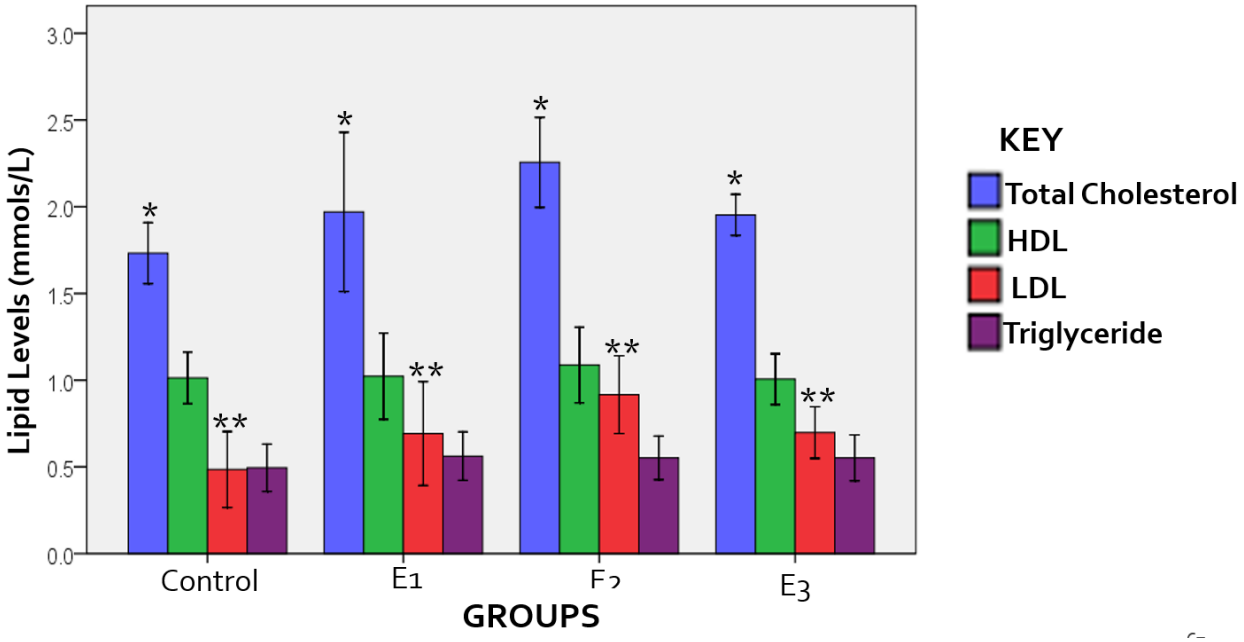
No statistical differences were observed between measurements of HDL(1.01 ± 0.15 mmol/L(control) vs 1.02 ± 0.25 mmol/L (E1) vs 1.09 ± 0.22 mmol/L (E2) vs 1.00 ± 0.15 mmol/L (E3); p=0.781*)* and TGs(0.50 ± 0.14 mmol/L(control) vs 0.56 ± 0.14 mmol/L (E1) vs 0.55 ± 0.13 mmol/L (E2) vs 0.55 ± 0.13 mmol/L (E3); p=0.669)as illustrated in Table 3.6.1 and Figure3.6.1.

Table 3.6.1 Table of Lipid Profile Measurements

GROUPS	Total Cholestrol	HDL	LDL	Triglycerides
Control	1.73 ± 0.18*	1.01 ± 0.15	0.49 ± 0.22**	0.50 ± 0.14
E1	1.97 ± 0.46*	1.02 ± 0.25	0.69 ± 0.30**	0.56 ± 0.14
E2	2.26 + 0.26*	1.09 ± 0.22	0.92 ± 0.22**	0.55 ± 0.13
E3	1.95 ± 0.12*	1.00 ± 0.15	0.70 ± 0.15**	0.55 ± 0.13

Values represented as mean ± SD
*p = 0.003; **p = 0.002
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

Figure 3.6.1 Bar Graph of Lipid Profile Measurements



Bars represent Mean ± SD
*p = 0.003; **p = 0.002
E1= Experimental Group 1, E2= Experimental Group 2, E3= Experimental Group 3

3.7 GC-MS Analysis of Fat

The three cooking oil/fat types used to fat-enrich the rat pellets fed to experimental groups 1, 2 and 3 were analysed using Gas Chromatography-Mass Spectrometry (GC-MS). The compounds and their concentrations are listed alphabetically in Table 3.7.1. Eighty five percent of all the compounds were classified according to chemical structure and summarized in Table 3.7.2.

Table 3.7.1 Chemical Composition of Three Cooking Oil Types

	Chemical Library ID	Sample 1 (µg/mg)	Sample 2 (µg/mg)	Sample 3 (µg/mg)
1	.alpha.-Amyrin	3.5901	2.3441	14.2467
2	.alpha.-Methylstyrene			8.9560
3	.beta.-Pinene	0.8725		
4	.beta.-Sitosterol		1.7059	
5	.gamma.-Ergostenol	6.3061		
6	.gamma.-Sitosterol			18.6200
7	1-Heptacosanol			23.7352
8	1-Octanol, 2-butyl-	0.8838		12.6048
9	1R-.alpha.-Pinene	0.8602	0.8590	9.0409
10	2,2-Dimethoxybutane	0.9627		10.3355
11	2,4-Dimethyl-1-heptene	0.9504	0.8974	9.8021
12	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	1.0890		
13	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	1.6810	1.8910	21.8602
14	2,6-Diisopropylnaphthalene	0.9001		
15	2-Bromo dodecane	1.5023		
16	2-Heptanone			8.9465
17	2-Hepten-4-one, 6-hydroxy-2-methyl-6-(4-methyl-3-cyclohexen-1-yl)-	0.8753		
18	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-			12.5941
19	2-Pentadecanone			13.7343
20	2-Pentadecanone, 6,10,14-trimethyl-	0.9807		
21	3.17 Octane<n->	1.8191		
22	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one		3.2528	14.6060
23	4.alpha.,14-Dimethyl-5.alpha.-ergosta-8,24(28)-dien-3.beta.-ol	3.3580		
24	5.alpha.-Cardanolide, 2.alpha.,3.beta.,14-trihydroxy-		1.4895	
25	7,10,13-Eicosatrienoic acid, methyl ester			16.7949

26	7,9-Dimethyl-1,4-dioxa-7,9-diazacycloundecane-8-thione		0.9638	
27	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	3.3674		
28	9,19-Cyclolanost-6-ene-3,7-diol, diacetate	1.9109		
29	9-Hexadecenoic acid, methyl ester, (Z)-		0.8935	121.8241
30	9-Octadecenoic acid (Z)-, methyl ester			25.7210
31	9-Octadecenoic acid, methyl ester, (E)-		38.4445	
32	Bacchotricuneatin c	0.8755		
33	Butanoic acid, 2-methyl-, methyl ester	0.8626		
34	Campesterol	2.5069	1.0852	
35	Cholest-7-en-3-ol-15-one, 14-methyl-	1.6066		12.2473
36	Cholesterol		1.5233	95.5765
37	cis-10-Nonadecenoic acid, methyl ester			32.9053
38	cis-11-Eicosenoic acid, methyl ester	1.7914		44.2784
39	cis-13-Eicosenoic acid, methyl ester		1.5230	
40	Cyclohexane, 1,2,4,5-tetraethyl-			9.1109
41	Cyclohexane, methyl-	1.3483	2.2170	
42	Cyclopentane, 1,2,4-trimethyl-		1.7210	26.1000
43	Cyclopentane, ethyl-	0.8662	0.8747	
44	Cyclopentane, undecyl-	0.8867		
45	Cyclotetradecane	0.9566		
46	Decane, 2,3,6-trimethyl-	4.0000		
47	Decane, 2-methyl-		0.8873	46.7900
48	Decane, 3,6-dimethyl-		1.9210	
49	D-Limonene	0.8661		
50	Docosane	3.5300	2.6100	50.8400
51	Dodecane	2.7300	3.8020	30.3300
52	Eicosane	2.7800	3.4480	44.0600
53	Ethyl 5,8,11,14-eicosatetraenoate			16.0564
54	Germacyclopent-3-ene, 1,1,3,4-tetramethyl-		0.9011	
55	Heneicosane	3.1600	1.2768	19.1723
56	Heneicosane, 3-methyl-		0.9095	
57	Hentriacontane		1.0663	
58	Heptacosane	1.4891	0.8811	12.8732
59	Heptadecane	3.3300	2.9850	
60	Heptadecanoic acid, methyl ester			58.6374
61	Heptane	32.6700	30.0100	343.8000
62	Hexacosane		2.5890	34.3000
63	Hexadecane	3.6100	2.6070	
64	Hexadecane, 2-methyl-			15.1280
65	Hexane, 2,3,5-trimethyl-	1.7300		64.5000
66	Hexane, 3,3-dimethyl-		1.7350	

67	Hexatriacontane		1.5368	
68	i-Propyl 7,10,13,16,19-docosapentaenoate			23.4067
69	Methyl 10-trans,12-cis-octadecadienoate	2.8037	2.5810	
70	Methyl 13-methyltetradecanoate			33.6286
71	Methyl 15-methylhexadecanoate			58.0093
72	Methyl 8,11,14,17-eicosatetraenoate			15.7396
73	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	2.9980	2.3870	
74	Methyl butanoate			34.6346
75	Methyl decanoate			95.3301
76	Methyl dodecanoate			121.6301
77	Methyl eicosa-5,8,11,14,17-pentaenoate			14.5424
78	Methyl hexadec-9-enoate	1.1563	1.0973	20.0346
79	Methyl linoleate	2.9455		11.4838
80	Methyl linoleate			37.6999
81	Methyl myristoleate			60.9441
82	Methyl nonanoate			10.2135
83	Methyl octadecanoate	5.2791	1.3514	448.7678
84	Methyl octadecanoate		6.8700	
85	Methyl octanoate			49.1129
86	Methyl palmitate (Methyl hexadecanoate)	8.1108	34.0051	686.6352
87	Methyl tetradecanoate		1.9993	312.6476
88	Methyl undecanoate			11.7287
89	n-Hexadecanoic acid			12.4119
90	Nonadecane	2.1500	1.0163	43.0900
91	Nonane, 2,6-dimethyl-		1.9400	20.0400
92	Nonane, 4,5-dimethyl-	1.9390		
93	Octacosane	3.1670	2.7530	14.3214
94	Octadecane (C18)	3.4190	2.1850	42.6100
95	Octane, 4,5-diethyl-		4.5000	37.7400
96	Octane, 4-methyl-	1.8870		
97	Pentacosane	2.0466	2.6890	13.5193
98	Pentadecane	0.9289	1.9430	64.1500
99	Pentane, 3-ethyl-2,4-dimethyl-		0.8644	
100	Phellandrene<beta->		0.8757	
101	Phenol, 2,4-bis(1,1-dimethylethyl)-	1.0779	1.8590	
102	p-Xylene		0.8615	
103	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	3.3701		
104	Stigmasterol	2.3118		
105	Tetracosane	4.1750		
106	Tetracosanoic acid, methyl ester		1.7779	21.9794
107	Tetradecane (C14)	0.8773	0.8675	11.3007

108	Tetratriacontane			12.5737
109	trans-13-Octadecenoic acid, methyl ester	60.2936		26.1290
110	trans-2-Undecen-1-ol	0.8724		
111	Triacontane	1.5261	2.1389	
112	Tricosane	1.3637		55.9200
113	Tridecane		0.8882	
114	Tridecane, 5-propyl-			25.3500
115	Tridecanoic acid, 12-methyl-, methyl ester	1.1528	1.6659	
116	Tritriacontane	1.8111		11.6160
117	Tungsten, cyclopentadienyl-cinnamoyltricarbonyl	1.6967		
118	Undecane, 4,6-dimethyl-	1.8610		9.3021
119	Undecane, 4-methyl-		0.9047	

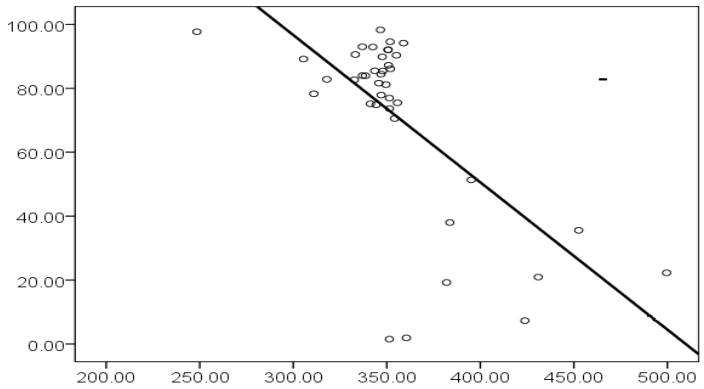
Table 3.7.2 Summary of Chemical Composition of Three Cooking Oil Types

	Chemical class	**Sample 1 (µg/mg)**	**Sample 2 (µg/mg)**	**Sample 3 (µg/mg)**
1	Amyryn	3.5901	2.3441	14.2467
2	Cholesterol	21.3704	5.8039	126.4438
3	Mono-Unsaturated	65.064	42.858	280.6945
4	Poly-Unsaturated	7.4827	6.859	120.9943
5	Saturated	80.3216	103.4271	2836.984
6	Cis fats	1.7914	2.4166	294.4739
7	Trans fats	70.7554	47.2982	107.2150

3.8 Correlations

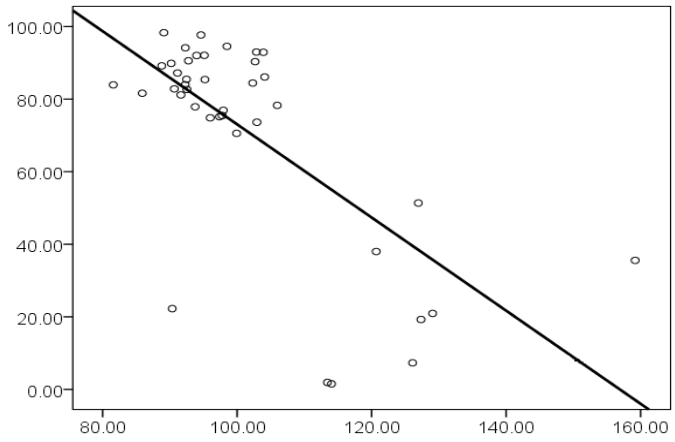
With bivariate analysis, RMSSD measure of HRV, was negatively correlated with the heart rate (r = -0.654, p<0.001) as depicted on Figure 3.8.1.

Figure 3.8.1 Scatter Plot Graph of RMSSD and Heart Rate



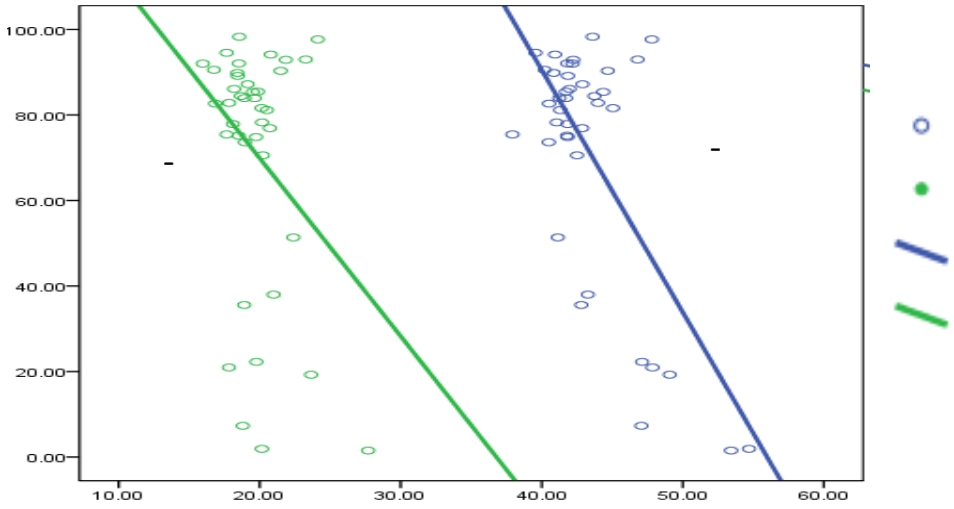
The heart rate variability at week 6 as measured by RMSSD was also negatively correlated with the QTc interval (r = -0.681, p< 0.001) as illustrated in Figure 3.8.2.

Figure 3.8.2 Scatter Plot Graph of RMSSD and QTc



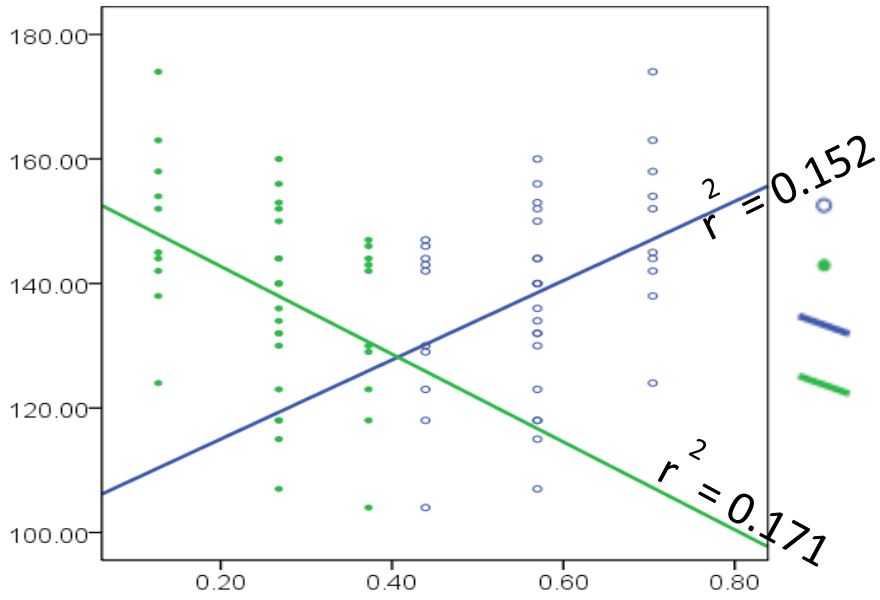
A negative correlation was also noted between the heart rate variability at week 6 as measured by RMSSD and both the P duration and PR interval (r = -0.325, $p< 0.05$ and r = -0.690, $p< 0.001$ respectively) as depicted in Figure 3.8.3.

Figure 3.8.3 An Overlay Scatter Plot of RMSSD with both P Duration and PR Interval



The systolic blood pressure, was positively correlated with the proportion of saturated fatty acids in the diet (r = 0.391, $p< 0.05$) and inversely correlated with the proportion of unsaturated fatty acids (r = -0.413, $p< 0.05$) as shown on Figure 3.8.4.

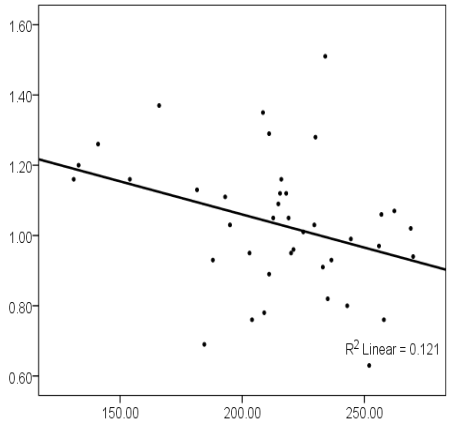
Figure 3.8.4 An Overlay Scatter Plot of Systolic Blood Pressure and Proportion of both Saturated Fatty Acid and Unsaturated Fatty Acid



SBP = Systolic Blood Pressure
FA = Fatty Acid

The levels of plasma HDL cholesterol were inversely correlated with the weights at six weeks (r = -0.347, p = 0.028) as shown in Figure 3.8.5.

Figure 3.8.5 A Scatter Plot of Plasma HDL Cholesterol and Weights at Six Weeks



r = -0.347,
p = 0.028
r^2 = 0.121

4 DISCUSSION

Cardiovascular diseases (CVD) including hypertension, stroke, and the range of acute coronary syndrome are responsible for approximately 30% of the global disease burden. The development of CVD is multifactorial and gradual taking between 10-18 years. During this crucial time, cardiovascular risk factors and markers are present and reliably predict future events.

Dietary fat imparts significantly on these cardiovascular risk factors and together with physical exercise, account for approximately 60% of the total preventable attributable risk. Diet has both beneficial and detrimental effects on the cardiovascular health. Fatty acid from dairy products have been shown to be atherogenic due to the presence of myristic and lauric acids. This was basis of the recommendation for use of low fat dairy products (Lokuruka, 2007).

The current study evaluated the effect of variable high fat diet on heart rate variability, ECG parameters, systolic blood pressure, fasting blood glucose and fasting plasma lipid profile. The fat-enrichment was carried out using three locally consumed fat types made from sunflower oil, palm oil and ghee. Chemical composition of the selected fat types was subsequently determined using gas chromatography-mass spectrometry.

4.1 Heart Rate Variability

Heart rate variability (HRV) is an established indicator for cardiovascular health. Increased variability has been associated with better cardiovascular outcomes in healthy subjects as well as in post-myocardial infarction patients (Kleiger et al., 2005; Malpas et al., 2002). The current study demonstrated increased HRV in rats fed on rat chow enriched with either sunflower oil or ghee. Consumption of solid vegetable fat made from palm oil resulted in reduced HRV. Our

findings, although in rats, compare with those of Soares-Miranda et al. (2012) and Millis et al. (2009). The HRV was negatively correlated with heart rate. This is in keeping with findings by Millis et al. (2009) as well as Vaseghi and Shivkumar (2008). Poirier et al. (2003) demonstrated that reduction in dietary fat results in better HRV indicative of higher parasympathetic outflow. Higher heart rates as well as low frequency heart rate variability would both result from increased sympathetic outflow (Pongchaidecha et al., 2009). These two factors are both associated with poorer cardiovascular health. In addition, Lasisi et al (2012) in Nigeria demonstrated that ambulant patients with chronic heart failure have reduced heart rate variability factors that worsens the prognosis of these patients. In our study, HRV was inversely correlated with the percentage weight gain although statistical significance was not achieved. Our findings compare with those of Millis et al. (2010) who demonstrated an inverse association between HRV and body fat percentage. Similarly, Mouridsen et al. (2012) in a human study showed that weight loss resulted in improved HRV parameters.

4.2 ECG Changes

Evaluation of the electric cardiac function has reliably been studied using the ECG. This study demonstrated inter-group differences in several ECG parameters.

Firstly, palm oil resulted in higher heart rates as compared to sunflower oil. Obesity induced increase in heart rate has been previously shown by our research team (Mutiso et al., 2014) and others (Verwaerde et al., 1997). This is postulated to be a consequence of increased sympathetic outflow that is seen in obesity (Verwaerde et al., 1997).

Secondly, the study demonstrated prolongation of the PR interval in the rats fed on palm oil compared to sunflower oil. This implies a slower conductance between the atria and ventricles.

Such PR prolongation has been documented in humans with metabolic syndrome (Granér et al., 2014) as well as in rats (Axelsen et al., 2015). Metabolic syndrome refers to a cluster of the most dangerous heart attack risk factors: diabetes and raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure (Alberti et al., 2005)

QT_c, QT interval corrected for the heart rate using the Bazzet's formula, has been linked to increased propensity for arrhythmias. In our study, palm oil ingestion resulted in QT_c prolongation as compared to both the control group and the group on sunflower oil. Similar prolongation of the QT_c has previously been documented by our research team (Mutiso et al., 2014) and others (Zarzoso et al., 2013). This prolongation of the QT_c is indicative of ventricular remodelling that is seen in metabolic syndrome (Axelsen et al., 2015) among other conditions. Ventricular remodelling refers to the changes in size, shape, structure, and function of the heart (Mihl et al, 2008). This can happen physiologically as a result of exercise (Fernandes et al., 2015) or pathologically after injury to the heart muscle (Burchfield et al., 2013). These changes are however not secondary to changes in either Na^+ or K^+ conductance (Axelsen et al., 2015).

4.3 Systolic Blood Pressure

Blood pressure elevation has been associated with high salt ingestion, advancing age, increased body weight and increased plasma LDL- cholesterol (World-heart-federation, 2015). Our study demonstrated elevated systolic pressure in rats fed on ghee-enriched as well as palm oil-enriched diets. Our findings are similar to those of Esmaillzadeh et al. (2011) who demonstrated in humans increased blood pressure secondary to diets rich in fats.

In our study, the systolic blood pressure was positively correlated with the weight and body mass index. A rise in blood pressure is expected with increase in weight as was demonstrated by Drøyvold et al. (2005). This rise in blood pressure seen in obesity is thought to be secondary to increased sympathetic activation as well as activation of the renin-angiotensin-aldosterone system (Rahmouni et al., 2004). In a Kenyan study, leading cardiovascular risk factors among hypertensive patients were found to be obesity, dyslipidaemia and ventricular hypertrophy (Yonga et al., 1993). Of these, obesity and dyslipidaemia are linked to dietary fat intake.

4.4 Fasting Blood Glucose

Fasting blood glucose, as a marker of insulin resistance, is a recognized cardiovascular risk factor. Indeed, poorly controlled diabetes increases the risk for cardiovascular disease. This study demonstrated no statistical difference in the fasting blood glucose levels between the groups on variable high fat diets. Similar results have been documented from other short term studies looking at the effect of variable high fat diets on blood glucose levels (Lovejoy et. al, 2002; Esmaillzadeh et. al, 2011). Studies with longer follow up, however, demonstrated increased insulin resistance following high fat ingestion (Riccardi et. al, 2004; Rivellese and Lilli, 2003). Ingestion of a high fat diet results in obesity which has been shown to cause insulin resistance (Kahn, Hull and Utzschneider, 2006). Several hypotheses have been put forward to explain the link between obesity and insulin resistance. These are classified into endocrine, inflammatory, neural, and intracellular signalling pathways (Steppan et al., 2001; Qatanani and Lazar, 2007).

4.5 Plasma Lipid Profile

The profile of plasma lipids that is often measured includes the low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides and total cholesterol. An additional subclass of

very low-density lipoproteins is at times included. Of these, HDL is anti-atherogenic and therefore protective against myocardial infarction, stroke and other cardiovascular diseases. The cholesterol: HDL ratio is often calculated and it magnifies the measured effect by having an atherogenic measure as the numerator and an anti-atherogenic measure as the denominator.

This study demonstrated increased levels of both total cholesterol and LDL following ingestion of palm oil derived cooking fat. These were higher than those of both the control group and the experimental group on sunflower oil. The differences in HDL and TGs were not statistically significant. These results were expected and compare with those from other studies by Clevidence et al. (1997) and Siri-Tarino et al. (2010) that showed unfavourable plasma lipid profile after ingestion of fats rich in saturated and trans fatty acids.

Two subpopulations of high-density lipoprotein have been characterized based on the lipoprotein in the molecule. These are apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II), comprising about 70% and 20% of the total HDL protein mass, respectively (Schultz et al., 1992). Of the two, the (apoA-I) is approximately 15-fold more anti-atherogenic (Schultz et al., 1992). Several mechanisms explaining this cardio-protective property have been demonstrated including causation of cholesterol efflux from cell membranes and mediation of anti-oxidation, and anti-inflammatory (Rye et al., 2008).

On the contrary, low-density lipoprotein cholesterol is atherogenic and has been linked to increased cardiovascular risk (Carmena, 2004; Grundy, 1997). Other atherogenic lipoproteins are the small, dense and oxidized LDL particles (Carmena, 2004). Studies looking at the role of LDL in atherogenesis have demonstrated the stimulation of macrophages leading to phagocytocis of

oxidized LDL particles on the endothelium. This is initiates further deposition of lipids resulting in a positive-feedback phenomenon (Young et al., 1994)

Lastly, a rise in LDL is often associated with elevated levels of TGs and reduced HDL levels all of which result in increased cardiovascular risk (Jeppesen et al., 1997).

5. CONCLUSION AND RECOMMENDATION

In summary, heart rate variability, a marker of autonomic influence on the heart was reduced by palm oil containing cooking fat. Sunflower oil as well as ghee consumption resulted in high degree of heart rate variability.

Secondly, the systolic blood pressure measurements were elevated following ghee ingestion. Both palm oil and sunflower oil did not result in statistically significant elevations of the systolic blood pressure.

The fasting blood glucose in the different groups were statistically similar.

Ingestion of both sunflower oil and ghee resulted in favourable fasting lipid profiles. Palm oil derived cooking fat ingestion resulted in unfavorable lipid profile characterized by high levels of LDL-cholesterol and total cholesterol.

Locally available cooking fat types contain variable amounts of saturate fatty acids and trans fatty acids.

The study recommends a shift to healthier cooking oil in our setting. This may be achieved by either public sensitization or preferential taxation making healthier fat types more affordable. Secondly, further studies to investigate the long-term effects of locally available cooking fat types on cardiovascular risk factors are recommended.

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