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# Lipid Profiling in Sprague Dawley Rats Induced with High Fat Diet (HFD)

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**Abstract:- Hypercholesterolemia is a medical condition associated with the unbalance in lipids level due to unhealthy diets and potentially leading to various heart-related diseases if remains untreated. This study focused on lipid profiling in Sprague Dawley (SD) rats to provide more insight in future research in prognosis and treatment of cardiovascular diseases. There were 15 male Sprague Dawley (SD) rats maintained in an animal house and fed with HFD (n=10) and Normal Diet (n=5). Lipid profiling within four different timelines was conducted using 3-in-1 combo test device for a complete lipid panel (Mission cholesterol meter) and Multi-Linear Regression (MLR) analysis performed using SPSS software. The obvious effects of High Fat Diet (HFD) shown in the unbalanced fluctuation of lipids level in SD rats from day 0 to day 90. According to MLR, the average mean of Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) were in an increasing and decreasing patterns respectively. Meanwhile, triglyceride (TG) and total cholesterol (TC) were in an increasing and decreasing patterns respectively and each lipid in HFD were statistically correlated and significant. Unlike the control group, lipids level appeared to be in a constant manner. Our finding concluded that, dietary played a crucial role in the abnormality of lipid level which subsequently contributed to the occurrence of heart diseases.**

***Keywords:-*** *Cardiovascular, High-Fat Diet (HFD), Lipid, Sprague Dawley (SD).*

## I. INTRODUCTION

Diets contain unbalance level of lipids significantly contributed to the occurrence of hypercholesterolemia, affecting all level of societies around the globe (Matos et al. 2005). This medical condition associated with the irregular fluctuation of lipids profile including the Low-Density Lipoprotein (LDL), High-Density Lipoprotein (HDL), triglycerides (TG), total cholesterol (TC). These biochemical abnormalities potentially lead to the initiation and development of event such as atherosclerosis, which subsequently causing various heart-related diseases such as myocardial infraction (MI) and stroke if left untreated (Sa'adah et al. 2017). In addition, cardiovascular remodeling is a type of an inflammatory disease associated with the excessive uptakes of LDL mainly due to High Fat Diet (HFD), triggering the recruitment of immune cells leading towards the formation of atherosclerotic plaques and affecting the pathophysiology of heart muscle which potentially causing fatality (Veseli et al. 2017). This study aimed on analyzing the lipid changes in animal models induced with two different diets, HFD and Normal Diet (ND). The finding of this research will provide more insight in future studies in prognosis and treatment of heart diseases with regard the association between lipid level and cardiovascular dysfunction such as coronary artery disease (CAD).

## II. MATERIALS AND METHODS

### *A. Animal and Study Design*

15 male SD rats were purchased and kept in the animal house at the Research & Innovation Institute, MSU; SD rats were grouped into HFD (n=10) and ND (n=5) for 90 days. All measurements were collected within four timelines, day 0, day 30, day 60, and day 90 (see table 1). HFD is made from 60% of the fat mixture, 40% from standard rat's pellet, and ND is made up of 100% normal pellet. HFD was prepared using the diet dilutions method (Delorme et al. 1981) and the composition was adapted based on the food formula provided by Levin and Dunn-Meynell with the addition of cholesterol from pure duck yolk sources (Echeveria et al. 2018, Getz et al. 2006, Hintze et al. 2018, Karacor et al. 2014). Whilst, the control group was fed using standard gold pellet (Fattepur et al. 2018). All rats were maintained under a controlled environment which was between 22–24°C with 12 hours light and dark cycles inside polypropylene cages in the animal house (Fattepur et al. 2018).

| *Parameter* | *HFD (Treated/T)* | | | | *ND (Control/C)* | | | |
|---|---|---|---|---|---|---|---|---|
| | *T0* | *T30* | *T60* | *T90* | *C0* | *C30* | *C60* | *C90* |
| Body Weight (kg) | 0.077 ± 0.003 | 0.186 ± 0.008 | 0.381 ± 0.006 | 0.453 ± 0.025 | 0.072 ± 0.001 | 0.173 ± 0.003 | 0.256 ± 0.013 | 0.316 ± 0.004 |
| Body Length (m) | 0.158 ± 0.005 | 0.185 ± 0.010 | 0.260 ± 0.014 | 0.253 ± 0.019 | 0.151 ± 0.004 | 0.190 ± 0.007 | 0.228 ± 0.011 | 0.250 ± 0.015 |
| Body Mass Index ($kg/m^2$) | 0.308 ± 0.234 | 0.551 ± 0.076 | 0.570 ± 0.065 | 0.714 ± 0.077 | 0.315 ± 0.016 | 0.484 ± 0.047 | 0.495 ± 0.023 | 0.509 ± 0.056 |

Table 1: Baseline characteristic of SD rats

### *B. Lipid Profiling*

Before the blood collection, SD rats were fasting for 12 hours in the four different intervals, day 0, day 30, day 60, and day 90. Blood samples from all SD rats were collected from the rat's marginal tail using a 26G needle and syringe (Hillerer et al. 2018). Lipids test performed using the 3-in-1 Combo Test Device for a complete lipid panel (Mission cholesterol meter). Total cholesterol (TC), Low-Lipoprotein Cholesterol (LDL), High-Lipoprotein (HDL) cholesterol, and triglyceride (TG) were successfully obtained according to

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manufacturer protocol (user manual). LDL was calculated manually based on Fredwald's equation (Francis et al. 2019).

*C. Data Analysis*

Data processing and statistical interpretation of the Multi-Linear Regression (MLR) performed using SPSS software.

## III. RESULTS AND DISCUSSION

The obvious effects of the HFD were proven following the outcomes of the variation in the lipids level from day 0 to day 90 in both treated and control groups. Overall, the lipids level within HFD were higher compared to ND. The average LDL level found to be higher in HFD compared in ND whilst HDL were higher in the control group. In general, TG and TC within the treated group scored bigger values in corresponding to the diet duration. Additionally, the pattern of HDL, LDL, TG, and TC in both groups of HFD and ND showed a significant increase and correlated to each over time, although LDL and HDL on day 0 in the treated group were not considered as the major factor to influence the final level on day90 and the TC level on day 0 and day 30 of the treated group were not affecting the final level upon completion of HFD on the 90$^{th}$ days (see fig. 1 and fig. 2).

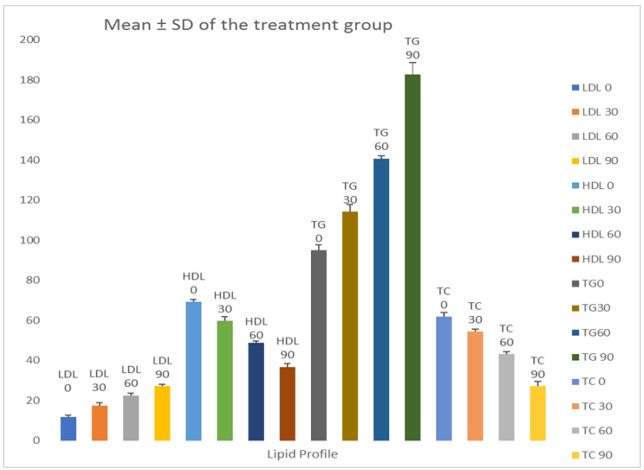


Fig. 1: Results expressed as Mean ± SD (n = 10) of different level of lipid level on day 0, day 30, day 60 and day 90

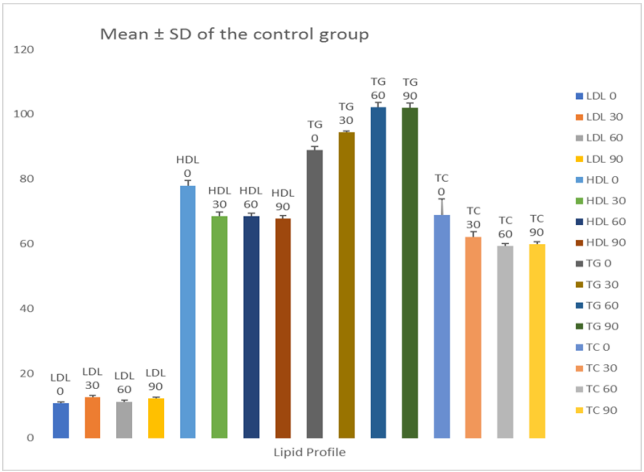


Fig. 2: Results expressed as Mean ± SD (n = 5) different level of lipid level on day 0, day 30, day 60 and day 90.

*A. Low-Density Lipoprotein (LDL) in treated group (T)*

Multiple Linear Regression (MLR) was used to analyze if the final LDL level of treated group, LDL-T90 was in link with the LDL level on day 0, day 30 and day 60. The average mean of LDL-T90 (M= 27. 05, SD= 0.785) in corresponding to the highest mean of LDL-T60 (M= 22.580, SD= 1.12) followed by LDL-30 (M= 17. 339, SD= 1.527) and LDL-T0 (M= 11.730, SD= 0.797). There was a strong negative correlation between LDL-T60 and LDL-T90 (r= -0.784, p= 0.004) and a moderate negative correlation between LDL-T30 and LDL-T90 (r= -0.441, p= 0.101) and moderate significant correlation between LDL-T0 and LDL-T90 (r= 0.038, p= 0.000). Statistically, 69.1% of the LDLT-90 variability explained by LDLT-30 and LDL-T60 ($R^2$= 0.691, F (3, 6) = 4.465, p= 0.057).

*B. High-Density Lipoprotein (LDL) in treated group (T)*

Multiple Linear Regression Analysis (MLR) was used to test if the final HDL level at day 90 is influenced by HDL level at day 0, day 30 and day 60 within the treated group. The average mean score for HDL-T90 (M= 36.800, SD= 1.549) in corresponding to the highest mean score of HDL-T0 (M= 69.200, SD= 1.135) followed by HDLT-30 (M= 59.840, SD= 2.121) and HDL-T60 (M= 48.800, SD= 1.033). There no significant correlation between HDL-T0 and HDL-T90 (r= -0.164, p= 0.325) and there was a moderate positive correlation between HDL-T30 and HDL-T90 (r= 0.473, p= 0.084) and a moderate negative correlation between HDL-T60 and HDL-T90 (r= -0.306, p= 0.195). Statistically 54.4% of the HDL-T90 variability explained by HDL-T30 and HDL-T60 ($R^2$= 0.544, F (3, 6) = 2.390, p= 0.167).

*C. Triglyceride (TG) in treated group (T)*

Multiple Linear Regression (MLR) analysis was used to assess either the TG level at day 90 influenced by TG level on day 0, day 30 and day 60. On average the final TG level scored mean of (M=182, SD= 5.827) in corresponding to TG-T30 (M=114. 200, SD= 3.795) followed by TG-T60 (M=140.800, SD= 1.398) and TG-T0 (M= 95.000, SD= 2.625). There was moderate positive correlation between TGT60 and TGT90 (r= 0.472, p= 0.084) followed by a moderate positive correlation between TGT30 and TGT90 (r= 0.409, p= 0.120) and there was a low positive correlation between TGT0 and TGT90 (r= 0.247, p= 0.246). Statistically 53.4% of variability of the final level of TG was influenced by all TGT0, TGT30 and TGT60 ($R^2$= 0.534, F (3, 6) = 2.287, p= 0.179).

*D. Total Cholesterol (TCL) in treated group (T)*

Multiple Linear Regression (MLR) was used to analyze if the final level of Total Cholesterol (TC) at day 90 is influenced by TC level on day 0, day 30 and day 60. The average mean of TCL-T90 is (M=27.290, SD= 2.240) in corresponding to TCL-T0 which scored the highest mean (M= 61.930, SD= 0.982) followed by TCL-T30 (M= 54.439, SD= 2.000) and TCL-T60 (M= 43.220, SD= 1.228). The analysis revealed that there was a moderate negative correlation between TCL-T60 and TCL-T90 (r= -0.263, p= 0.232) and both TCL-T0 and TCL-T30 were not significantly correlated with TCL-T90 (r= -0.199, p= 0.291) and (r= 0.192, p= 0.297) respectively. Statistically 19.2% of TCLT90

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variability was influenced by TCL-T60 ($R^2$= 0.192, F (3, 6) = 0.475, p= 0.711).

*E. Low-Density Lipoprotein (LDL) in control group (C)*

Average mean scored in LDL-C90 is (M= 12.520, SD= 0.263) in corresponding to the highest mean of LDL-C30 (M=12.560, SD= 0.555) followed by LDL-C60 (M= 11.240, SD= 0.513) and LDL-C0 (M= 10.760, SD= 0.378). According to correlation score, there was no significant correlation between LDL-C0 and LDL-C90 (r= -.0.029, p= 0.481) but there was a moderate negative correlation between LDL-C30 and LDL-C90 (r= -0.466, p= 0.214) and a low negative correlation between LDL-C60 and LDL-C90 (r= -0.274, p= 0.328). Statistically 98.9% of the variability in LDLC-90 explained by LDL-C30 and LDLC60 ($R^2$= 0.989, F (3, 1) = 30.876, p= 0.131).

*F. High-Density Lipoprotein (HDL) in control group (C)*

Average mean scored of HDL-C90 is (M= 67.800, SD= 0.837) in corresponding to the mean scored in HDL-C0 (M= 78.000, SD= 1.581) followed by HDL-C30 (M= 68.600, SD= 1.140) and HDL-C60 (M=59.400, SD= 0.628). According to correlation analysis, there was a strong positive correlation between HDL-C0 and HDL-C90 (r= 0.945, p= 0.008) and there was a strong negative correlation between HDL-C30 and HDL-C90 (r= -0.629, p= 0.128) and there was a strong positive correlation between HDL-C60 and HDL-C90 (r= 0.713, p= 0.088). Statistically, 96.1% of the variability in HDL-C90 explained by HDL-C0, HDL-C30 and HDL-C60 ($R^2$= 0.961, F (3, 1) = 8.249, p= 0.249).

*G. Triglyceride (TC) in control group (C)*

Average mean scored of TG-C90 is (M= 102.200, SD= 1.4832) in corresponding to the mean score of TG-C0 (M= 88. 600, SD= 1.140) followed by TG-C30 (M= 94.400, SD= 1.14) and the highest mean score of TG-C60 (M= 102.200, SD= 0.447). The correlation test revealed that there was a strong positive correlation between TG-C0 and TG-C90 (r= 0.798, p= 0.053) followed by a strong negative correlation between TG-C30 and TG-C90 (r= -0.798, p= 0.053) and a moderate positive correlation between TG-C60 and TG-C90 (r= 0.302, p= 0.311). Statistically, 75.2% of the variability in TG-C90 is explained by TG-C0, TG-C30 and TG-C60 ($R^2$= 0.752, F (2, 2) = 3.033, p= 0.248).

*H. Total Cholesterol (TC) in control group (C)*

The average mean score of TCL-C90 is (M= 59.880, SD= 0.819) in corresponding to the average mean score of TCL-C0 (M= 68.880, SD= 4,913), followed by TCL-C30 (M=62.180, SD= 1.629) and average mean of TCL-C60 is (M= 59.400, SD= 0.628). The correlation test show that there was a strong positive correlation between TCL-C60 and TCL-C90 (r= 0.844, p= 0.036) and a very low positive correlation between TCL-C0 and TCL-C90 (r= 0.229, p= 0.356) but a moderate negative correlation between TCL-C30 and TCL-C90 (r=-0.459, p= 0.218). Statistically 98.1% of the TCL-C90 variability explained by TCL-C0, TCL-C30 and TCL-C60 ($R^2$= 0.981, F (3, 1) = 17.496, p= 0.174).

## IV. CONCLUSION

This study concluded that the variation in lipid level was associated with the type of diet intake; HFD revealed significant unbalance fluctuation in lipid level Thus, lipid profiling considered important tool in the assessment of cardiovascular remodelling. However, wider range of parameters with advanced research technology remains paramount to provide a solid justification of our finding.

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## V. CONFLICT OF INTEREST

No conflict of interest.

APPENDIX

Equation 1

Body mass index (BMI) 〖kgm〗^(-2) =(Body weight (kg))/(Body length ( 〖m〗^2))

Equation 2

Lee index ($\sqrt[3]{}$kg/m) = ($\sqrt[3]{}$ (Body) weight (kg))/ (Body Lenght (m)) Equation 3

LDL cholesterol =TC - (HDL cholesterol) - (TG/5)

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