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Research Article

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Epigallocatechin-3-Gallate (EGCG) as a potential therapeutic against cardiovascular disease risk in mice

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Abstract

Objective: *Research is focused on cardiovascular disease (CVD). CVD is the leading cause of death in the world and remains one of the major diseases strongly affected by diet and sedentary lifestyle. CVD is a class of diseases relates to the heart and or blood vessels that include, stroke, heart failure, hypertension, coronary artery disease, peripheral artery disease, and atherosclerosis caused partly due to dyslipidemia. The aim of this study was to determine the potential effect of epigallacatechin-3-gallate (EGCG) on blood lipid and glucose profiles in mice exposed to conditions that lead to CVD. Groups of male mice were subjected to different diet (standard fat diet, high fat diet and low-fat diet), exercise (voluntary, chronic and sedentary lifestyle) and EGCG supplementation. EGCG powder was dissolved in 4 ml of drinking water to deliver a daily dose of 30 mg/kg. Mice were anesthetized with isoflurane (Abbott, Cham, Switzerland) 2% (v/v) in a 20% O_2 and 80% air mixture by inhalation in a closed container, then euthanized by manual cervical dislocation and were put on the animal's bed for a blood sample from the cardiac puncture. Blood was collected for lipid profiles and glucose analysis. The data was analyzed using a multi variate approach including one-way analysis of variance test (one-way ANOVA), Tukey –Kramer multiple comparison test and pairwise correlation analysis was used to determine the significance between control and treated groups.*

Results: *The mice subjected to high-fat diet (Anova p-value = 0.00) and sedentary lifestyle (Anova p-value = 0.00) showed elevated levels for total cholesterol, low density lipoprotein's cholesterol, triglycerides and glucose. EGCG supplementation in mice undergoing high fat diet and sedentary lifestyle resulted in a significant reduction in the lipid profiles and blood glucose (Anova p-value = 0.00). Our study showed that a high fat diet and a sedentary lifestyle predispose to hyperlipidemia and high blood glucose levels in mice and also proved that EGCG fed to hyperlipidemia mice reduces lipid levels in blood.*

Keywords: Diet, Epigallocatechin-3-Gallate (Egcg), Exercise, Sedentary Lifestyle, Cardiovascular Disease (Cvd).

Introduction

To date, CVD represents a global problem for human health worldwide, especially in the western countries, where cardiovascular disease (CVD) retains a leadership as a top cause of peoples' deaths and invalidation. According to the American Heart Association, CVD accounts for around 1/3 of annual mortality in USA [1]. In 2013, an estimated 1 million deaths were attributable to CVD in sub-Saharan Africa alone, which constituted 5.5% of all global CVD-related deaths and 11.3% of all deaths in Africa [2]. By 2030, a total of 23.6 million people is expected to die from CVD worldwide [3]. CVD is a class of diseases relates to the heart and or blood vessels that include, stroke, heart failure, hypertension, coronary artery disease, peripheral artery disease, and atherosclerosis caused partly due to dyslipidemia [4].

Multiple risk factors such as high fat diets (HFD), sedentary lifestyle (SL), age, gender, behavior such as smoking, alcohol consumption, diabetes, obesity contribute to CVDs [5]. A HFD can lead to obesity due to deposition of adipose tissues in the body and increased levels of lipids such LDL and total cholesterol (TC) in circulation which forms plagues in the blood vessels causing blockade or stiffness resulting into high blood pressure and hence CVDs [5]. Lack of physical activity has been associated with obesity, increases endogenous inflammatory molecules,

impairment of the physiological balance between inflammatory and oxidative stress reactions thus contributing to lipidemia and hence CVDs [6-9].

Management of CVD may involve avoiding risk factors and the use of antioxidants [9]. Studies have suggested that consumption of green tea (GT) can prevent the incidence of CVD and this has been attributed to epigallocatechin-3-gallate (EGCG) [8]. EGCG is a biologically active polyphenolic flavonoids commonly found in GT and has been known to exert a variety of cardiovascular beneficial effects [9, 6]. The selective use of nutrients, antioxidants and regular exercise in combination which primarily protect against multiple risk factors can be designed to access their protective potential against development of vascular endothelial dysfunction (VED) due to reactive oxygen species, lipidemia that results into atherosclerosis and obesity due to lack of exercise [11-13]. The purpose of this study was to determine the potential effect of EGCG, diet and exercise on the development of hyperlipidemia which may eventually culminate into CVD in mice. Our study showed that a high fat diet and a sedentary lifestyle predispose to hyperlipidemia and high blood glucose levels; this is a risk factor for the development of cardiovascular disease in mice. Our study also proved that EGCG fed to hyperlipidemia mice reduces lipid levels in blood, which confirms that EGCG has therapeutic potential to prevent CVD.

Main text

Materials And Methods

Ethics Statement

The procedures and protocols of our study were approved by the Animal Ethics Committee of Kampala International University and Uganda national council of science and technology, Approval number is NS 645. Animals were cared for in accordance with the Guiding Principles in the Care and use of experimental animals of the European Council of the animal and the US National Institutes of Health Guide to the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) [14].

Animals And Experimental Groups

Sixty, three-month-old Albino Swiss male mice used for experimentation were acquired from animal facility of College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) of Makerere University. The mice were housed in standard cages, maintained at a normal temperature (27 ± 2°C) and humidity (55 ± 5%), and exposed to a 12-h light/dark cycle. Mice were divided into twelve groups of 5 mice each. The first six groups were exercised with normal diet and the other six received diet treatment as follows: (1) voluntary exercise (VE), (2) chronic exercise (CE), (3) sedentary lifestyle (SL), (4) VE+EGCG, (5) CE+EGCG, (2) SL+EGCG, (7) normal diet (ND) (3% fat), (8) high fat diet (HFD) (16% fat) [15]; (9), low fat diet (LFD) (0.2% fat), (10) ND+ EGCG, (11) HFD+EGCG and (12) LFD+EGCG (Figure 1 and Table S1). EGCG powder was dissolved in 4 ml of drinking water to deliver a daily dose of 30 mg/kg. For sedentary lifestyle, one mouse was reared in its own cage. For chronic and voluntary exercise, mice from each group were housed together in their respective cages.

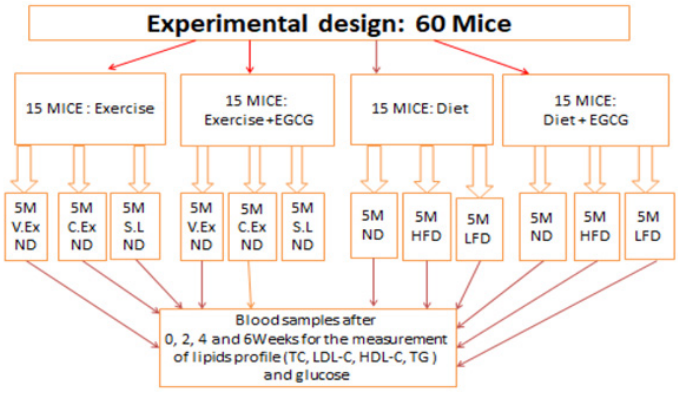

Figure 1: Experimental design for the study.

CE is chronic exercise, EGCG is epigallocatechin-3-gallate, HDL-C is high density lipoprotein cholesterol, HFD is high fat diet, LDL-C IS low density lipoprotein cholesterol, LFD is low fat diet, M is mice, ND is normal diet, SL is sedentary lifestyle, TC is Total cholesterol, TG is triglycerides and VE is voluntary exercise.

Table S1: Percentage composition of the amount of nutrients used in feed formulation fed to mice

Nutrient name	Quantity of nutrient for a diet containing 16% in fat	Quantity of nutrient for a diet containing 3% in fat	Quantity of nutrient for a diet containing 0.2% in fat
1. Maize Bran	39	80	80
2. Soya Bean-Full	45	4	0
3. Sunflower	5	4	0
4. Cotton Seed Cake	5	2	0
5. Rice Bran	3	2	0
6. Shell	1	4	6
7. Fish	2	4	6
8. Cassava	0	0	8
Total composition	100	100	100

Animals, Preparation, Anesthesia, Euthanasia, Blood Collection, Glucose and Lipid Profile Measurements

The experiments were carried out in compliance with European animal protection laws. A three-month-old male Swiss Albino mouse for each group was studied. These mice were anesthetized with isoflurane (Abbott, Cham, Switzerland) 2% (v/v) in a 20% O2 and 80% air mixture by inhalation in a closed container, then euthanized by manual cervical dislocation and were put on the animal's bed for a blood sample from the cardiac puncture [16-17]. A total of 48 mice were sacrificed (12 mice after 0, 2, 4 and 6 weeks). The blood sample was taken for each treatment in order to analyze the lipid and glucose profile. Plasma lipid profile and glucose levels were examined using a plasma glucose (GO) Assay Kit (Catalog Number GAGO20), cholesterol quantitation Kit (Catalog Number MAK043), HDL and LDL quantitation Kit (Catalog Number MAK045), and triglycéride quantitation kit (Catalog Number MAK266A) (Sigma-Aldrich) and were measured with an analyzer automatic biochemical (AR-S300) from China.

Statistical Analysis

All data were shown as mean ± standard deviation (SD). Results were analyzed using one-way analysis of variance test (one-way ANOVA), Graph Pad Prisms Software version 6 followed by Tukey –Kramer multiple comparison test and pairwise correlation. P-value was used to determine the significance level of various diets and exercise in lipid profile and glucose in mice. Most of the statistical tests, the level of significance was fixed at $p<0.05$.

Results

Evaluation Of the Effects of Egcg on Glucose and Lipid Profile After Diet Treatment

In normal and HFD treated mice, we observed elevated levels of total and LDL cholesterol; with twice the concentration in HFD by the 4th and 6th week (Figure 2, Figure S1A&B). However, treatment of these mice with the EGCG resulted in a significant reduction in the levels of total cholesterol and LDL by weeks 4 and 6 in mice undergoing HFD treatment (Supplementary data: Tables S2 and S3).

Results on the means of TC and LDL cholesterol over the entire treatment period (Figure S2: A & B) showed that there is a statistically significant relationship between diets supplemented with or without EGCG on total cholesterol (Anova p-value = 0.01) and LDL cholesterol (Anova p-value = 0.01).

The levels of triglyceride and glucose levels were increased as the duration of experiment increased (Figure 2, Figure S1 D&E). EGCG decreased serum triglyceride and glucose levels (Figure 2, Figure 1S and Figure S2: D&E). There was statistically significant association between EGCG treatment and the levels of triglycerides in the serum (Anova p = 0.00). Similarly, we observed high likelihood of development of CVD in untreated mice in comparison to those treated with EGCG (Anova p-value = 0.00).

Table S2. Anova p-values for lipid and glucose profile in diets and exercise treatments. A. Anova p-values for lipid and glucose on diets supplemented with or without EGCG. B. Anova p-values for lipid and glucose under exercise supplemented with or without EGCG

A. Relationship between diet on lipid and glucose profile						
	High Fat Diet (HFD)		Normal Diet (ND)		Low Fat Diet (LFD)	
	HFD	HFD+EGCG	ND	ND+EGCG	LFD	LFD+EGCG
P-value	0.0039	< 0.0001	0.0018	< 0.0001	0.0001	< 0.0001
Global P-value	< 0.0001		< 0.0001		< 0.0001	
B. Relationship between physical exercise on lipid and glucose profile						
	Sedentary lifestyle (SL)		Voluntary Exercise (VE)		Chronic Exercise (CE)	
	SL	SL+EGCG	VE	VE+EGCG	CE	CE+EGCG
P-value	0.0016	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Global P-value	< 0.0001		< 0.0001		< 0.0001	

Table S3. Anova p-values of EGCG effect on lipid and glucose in mice on diet and exercise. A.Anova p-values of lipid and glucose in mice on diet supplemented with or without EGCG. B. Anova p-values of lipid and glucose under exercise supplemented with or without EGCG. in mice. C. Anova p-values for lipid and glucose in association between diet and exercise with or without EGCG

A. Lipids and glucose profile after Diet treatment										
	TC	TC+EGCG	LDL-C	LDLC+EGCG	HDLC	HDLC+EGCG	TGs	TGs+EGCG	Glu	Glu+EGCG
P-value	0.0418	0.0365	0.0435	0.0394	0.4193	0.0015	0.0091	0.0014	0.0218	0.0094
Global P-value	0.027	0.0193	0.0001	0.0001	0.0005					

B. Lipids and glucose profile after exercise treatment										
	TC	TC+EGCG	LDL-C	LDLC+ EGCG	HDLC	HDLC+ EGCG	TGs	TGs+ EGCG	Glu	Glu+ EGCG
P-value	0.0421	0.0180	0.0372	0.0185	0.3055	0.0377	0.0068	0.0022	0.0193	0.0342
Global P-value	0.0062	0.0059	0.1314	0.0001	0.0008					
C. Correlation with Diet and Exercise										
	TC	TC+EGCG	LDL-C	LDLC+ EGCG	HDLC	HDLC+ EGCG	TGs	TGs+ EGCG	Glu	Glu+ EGCG
P-value	0.025	0.0008	0.013	0.0013	0.057	0.0021	0.0001	< 0.0001	0.0001	< 0.0001

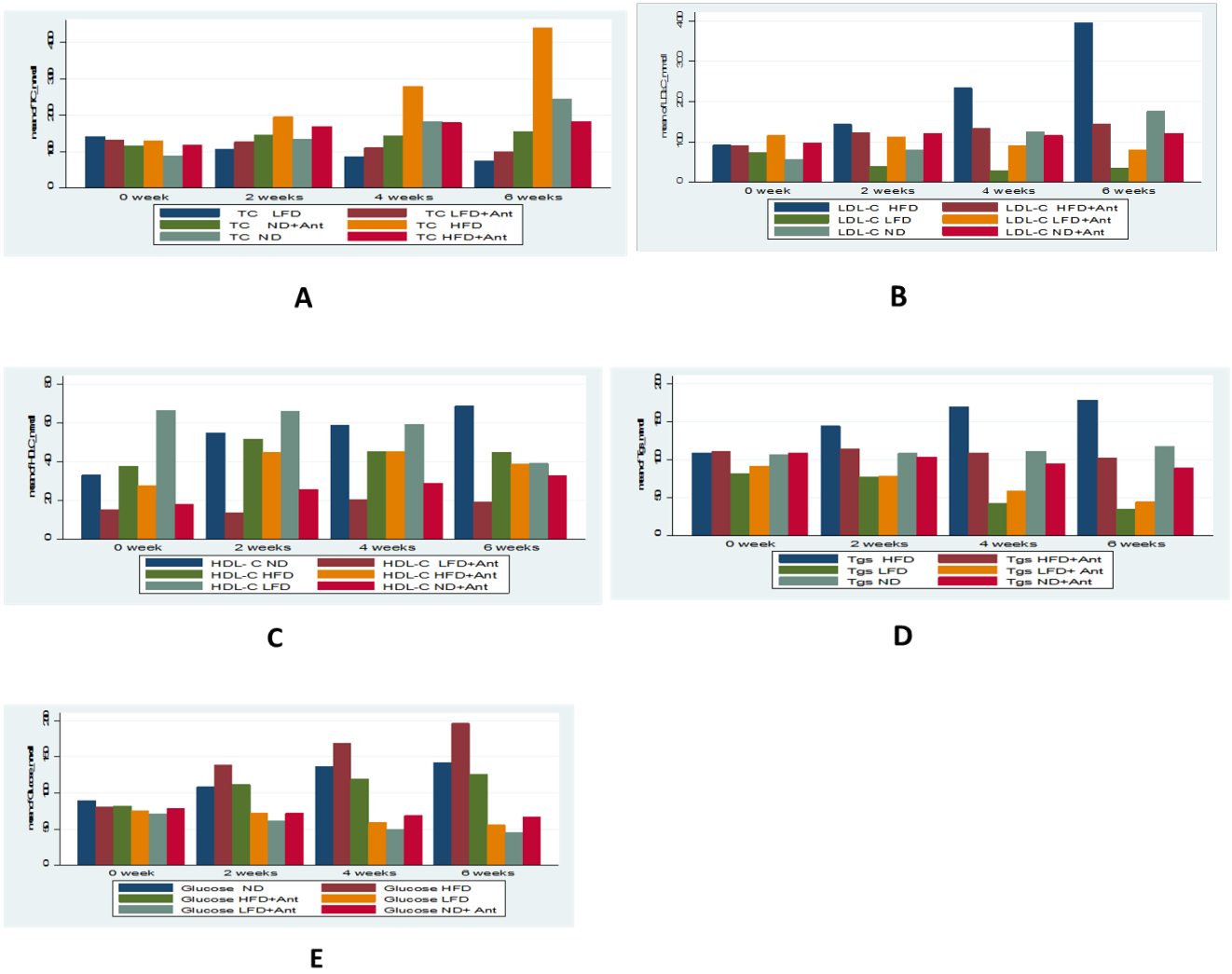

Figure S1: Effect of EGCG on lipids and glucose in mice on diets supplemented or not with EGCG
Relationship between total cholesterol in ND, HFD and LFD with or without EGCG(A), low density lipoprotein cholesterol in ND,HFD and LFD with or without EGCG(B), high density lipoprotein cholesterol in ND, HFD and LFD with or without EGCG(C), triglycerides in ND,HFD and LFD with or without EGCG(D) and glucose in ND, HFD and LFD with or without EGCG(E).

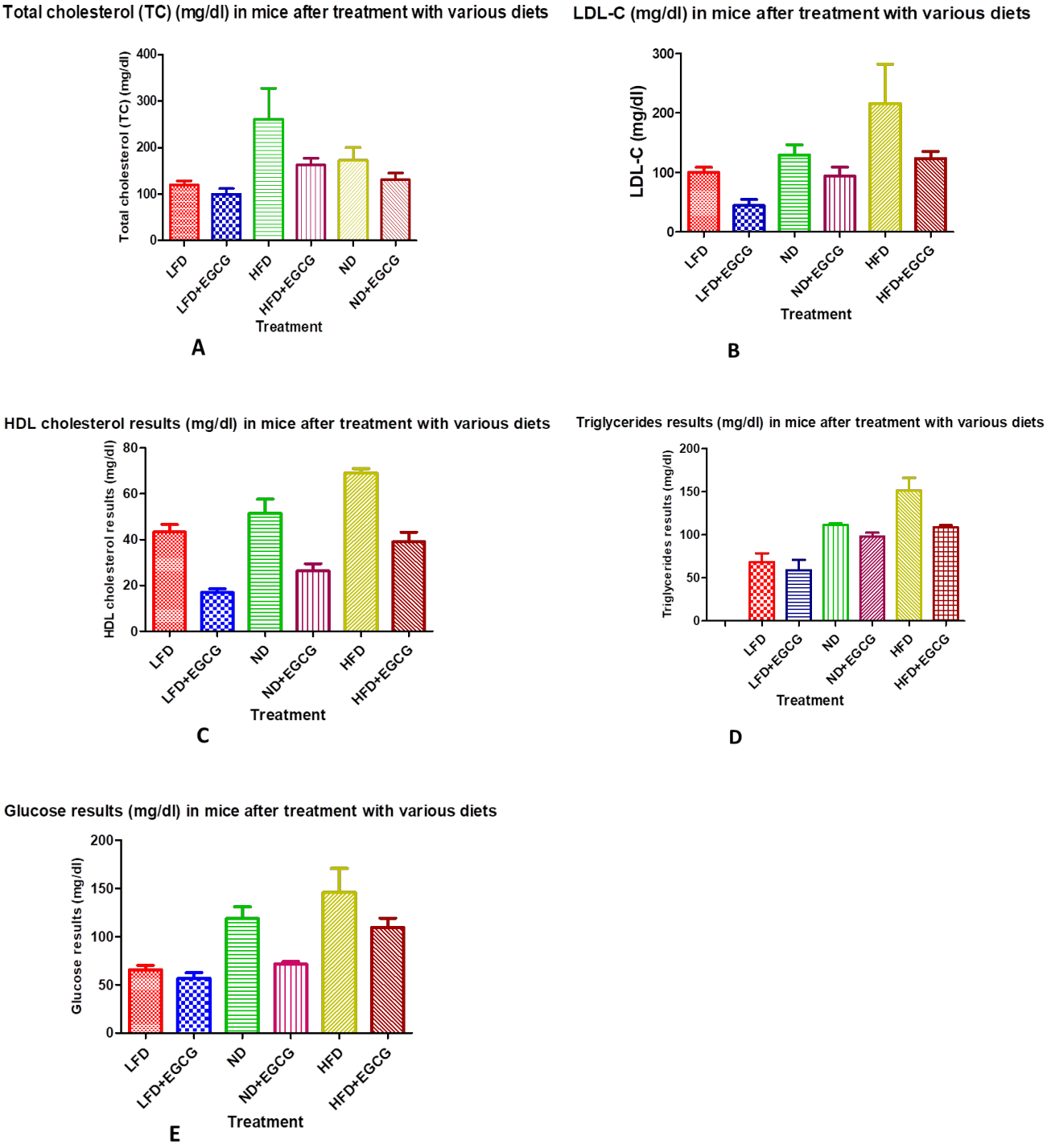

Figure S2: Effect of EGCG on lipid and glucose means in mice on diets supplemented with EGCG
Relationship between total cholesterol in ND, HFD and LFD with or without EGCG(A), low density lipoprotein cholesterol in ND,HFD and LFD with or without EGCG(B), high density lipoprotein cholesterol in ND, HFD and LFD with or without EGCG(C), triglycerides in ND,HFD and LFD with or without EGCG(D) and glucose in ND, HFD and LFD with or without EGCG(E).

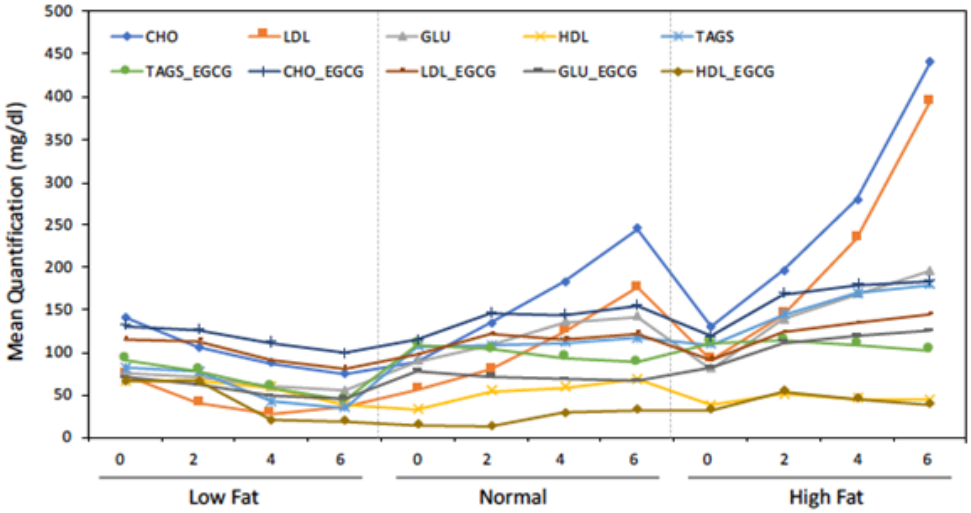

Figure 2: Effect of EGCG on lipids and glucose of serum in mice after diet treatment.

CHO is total cholesterol, CHO_EGCG is total cholesterol supplemented by EGCG, LDL is low density lipoprotein cholesterol, LDL_EGCG is low density lipoprotein cholesterol supplemented by EGCG, GLU is glucose, GLU_EGCG is glucose supplemented by EGCG, HDL is high density lipoprotein cholesterol, HDL_EGCG is high density lipoprotein cholesterol supplemented by EGCG, TAGS is triglycerides and TAGS_EGCG is triglycerides supplemented by EGCG.

Evaluation Of the Effects of Egcg on Glucose and Lipid Profile After Exercise Treatment

We observed that total and LDL cholesterol levels (Figure 3 Figure S3 A&B) increased gradually in mice subjected to the SL. There was a significant difference in the levels of TC (Anova p-value = 0.00) and LDL cholesterol (Anova p-value=0.00) between EGCG-treated and untreated mice (Figure S3 A&B). It was also observed that HDL cholesterol, triglycerides and glucose were also increased for the SL (Figures 3, S4).

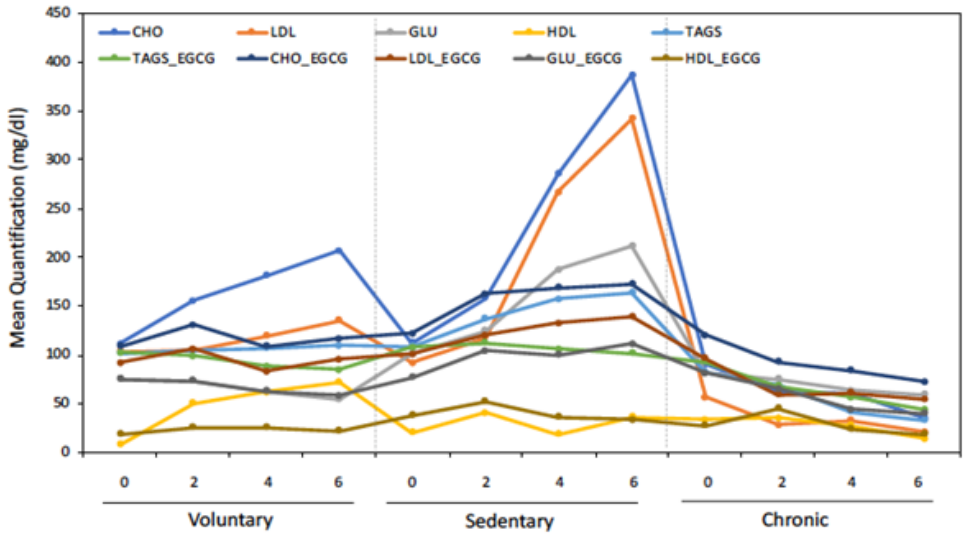

Figure 3: Effect of EGCG on lipids and glucose of serum in mice after exercise treatment.

CHO is total cholesterol, CHO_EGCG is total cholesterol supplemented by EGCG, LDL is low density lipoprotein cholesterol, LDL_EGCG is low density lipoprotein cholesterol supplemented by EGCG, GLU is glucose, GLU_EGCG is glucose supplemented by EGCG, HDL is high density lipoprotein cholesterol, HDL_EGCG is high density lipoprotein cholesterol supplemented by EGCG, TAGS is triglycerides and TAGS_EGCG is triglycerides supplemented by EGCG.

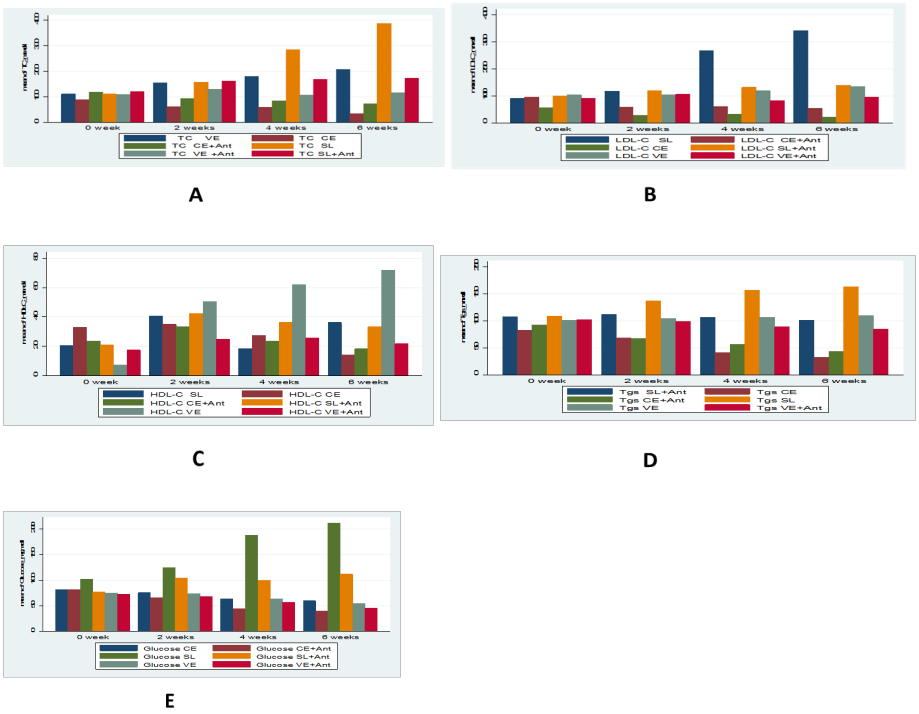

Figure S3: Glucose and lipide profiles results in mice subjected to exercise supplemented by EGCG.
Relationship between total cholesterol in VE, SL and CE with or without EGCG(A), low density lipoprotein cholesterol in VE, SL and CE with or without EGCG(B), high density lipoprotein cholesterol in VE, SL and CE with or without EGCG(C), triglycerides in VE, SL and CE with or without EGCG(D) and relationship between glucose in VE, SL and CE with or without EGCG(E).

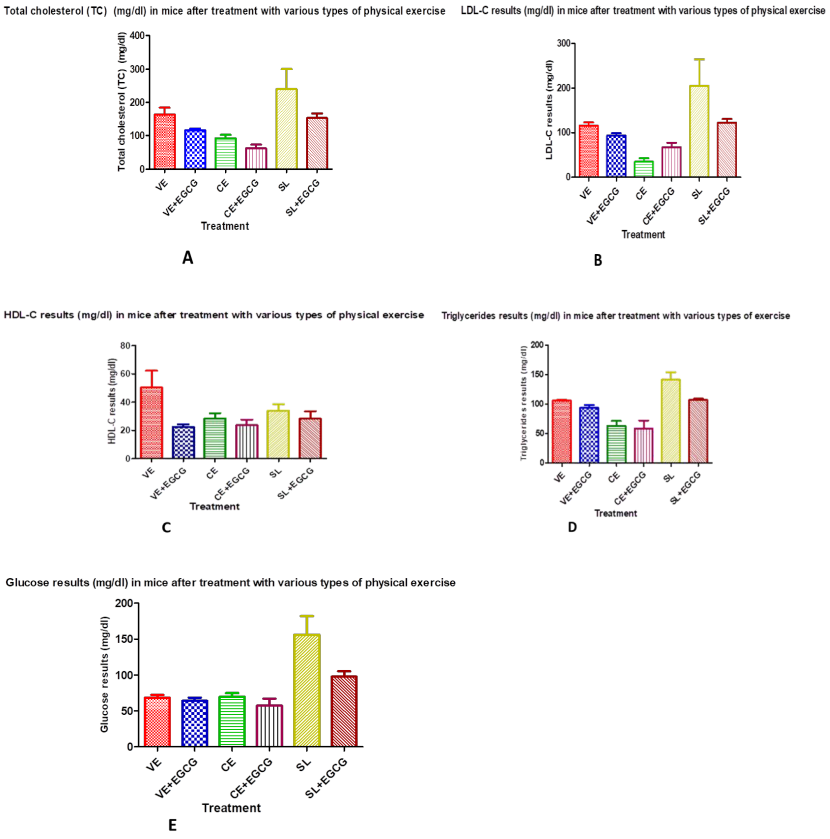

Figure S4: Effect of EGCG on lipid and glucose means in nice under exercise supplemented with EGCG
Relationship between total cholesterol in VE, CE and SL with or without EGCG(A), low density lipoprotein cholesterol in VE, CE and SL with or without EGCG(B high density lipoprotein cholesterol in VE, CE and SL with or without EGCG(C), Tgs in VE, CE and SL with or without EGCG(D) and Glucose in VE, CE and SL with or without EGCG(E).

Correlation Between Diet and Exercise on Lipid and Glucose Profile in Mice

Study of correlation between diet and exercise revealed that there exists significant association between diets and exercise in mice when treated with or without EGCG for TC, LDL cholesterol, triglycerides and glucose. The level of significance of mean value for all treatments is less than 5% level of significance (Table S3).

We observed high concentration of total and LDL cholesterol in HFD and SL. However, these reduced significantly when the mice subjected to CE were treated with EGCG (Anova p-value = 0.00 for total cholesterol and 0.001 for LDL cholesterol: Figures S5 and S6).

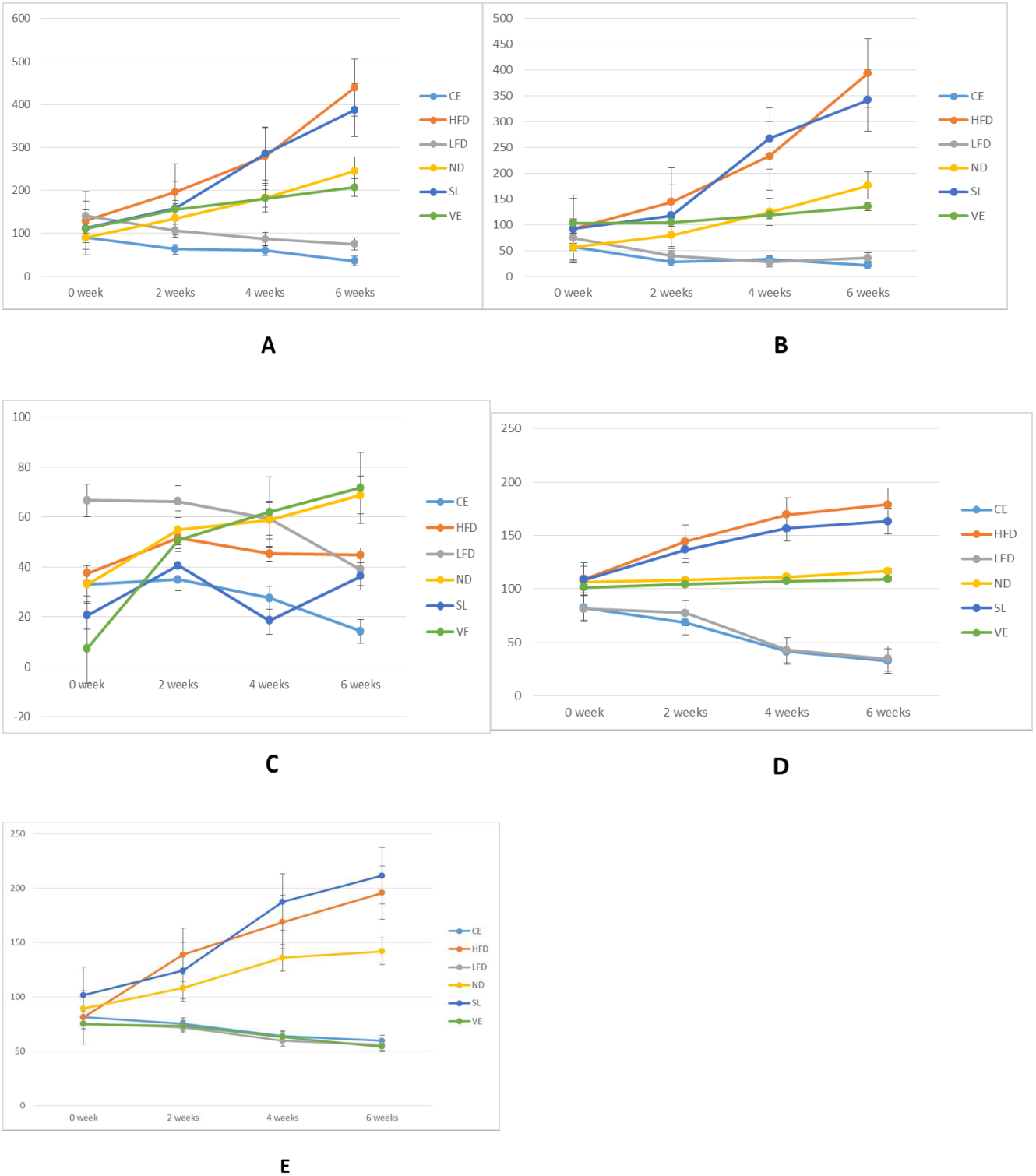

Figure S5: Correlation between diet and exercise supplemented with EGCG on lipid and glucose profile in mice
Total cholesterol (A), low density lipoprotein cholesterol (B), high density lipoprotein cholesterol (C), triglycerides (D) and glucose (E)

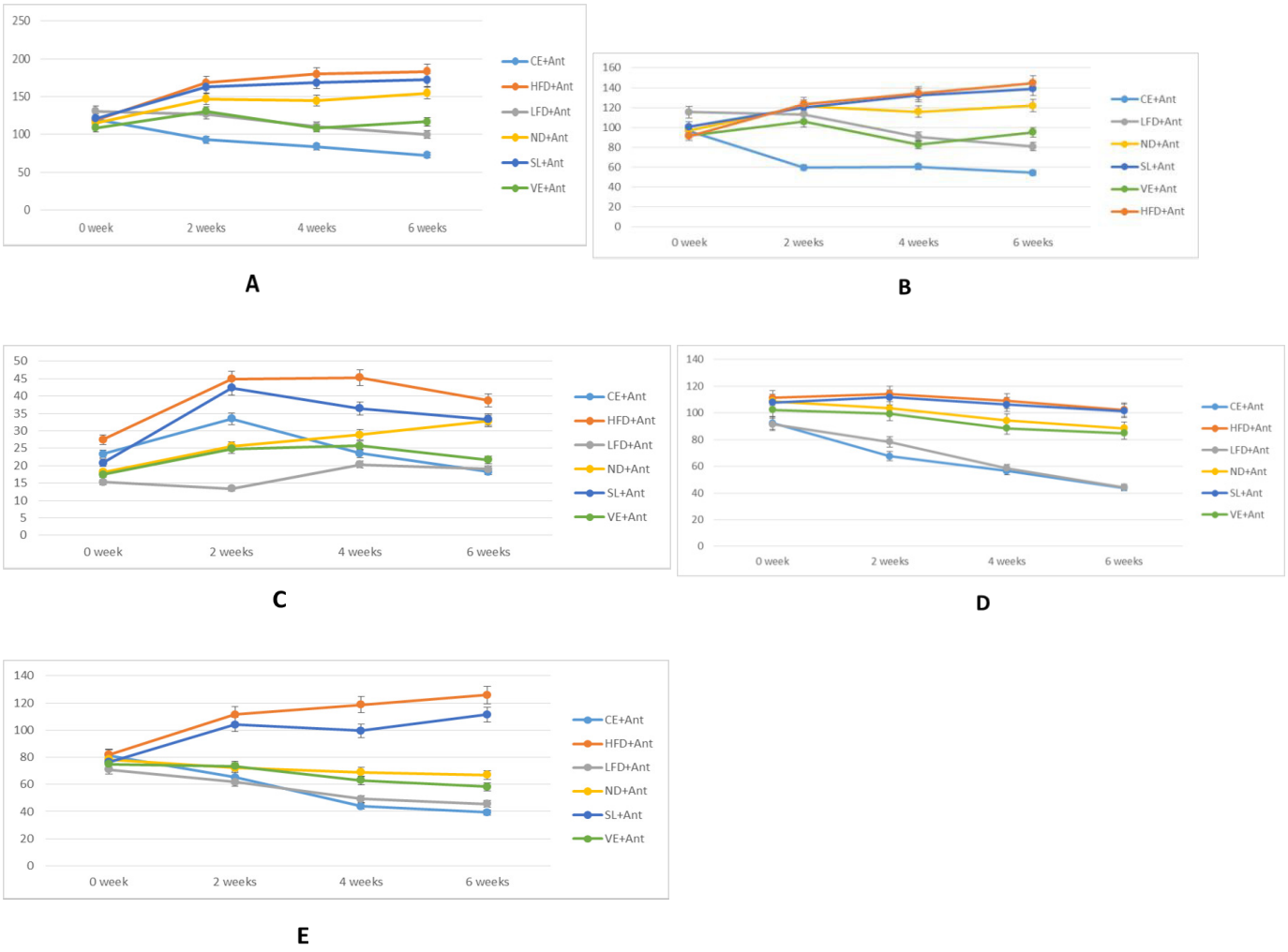

Figure S6: Correlation between diet and exercise supplemented with EGCG on lipid and glucose profile in mice.
Total cholesterol (A), Low density lipoprotein cholesterol (B), High density lipoprotein cholesterol (C), Triglycerides (D) and Glucose (E).

Feed Formulation and Pallet Preparation

The formulation of the feeds and the preparation of the pallets were carried out at the nutrition laboratory of COVAB, Makerere University. Three types of feeds were prepared: a high fat diet (16% fat), a normal diet (3% fat) and a low-fat diet (0.2% fat) as shown in table S1. To obtain the different food proportions, the following nutrients were mixed: maize bran which is rich in carbohydrate, soy bean-full which is rich in fat and protein, sunflower which is rich in fiber and protein, cotton seed cake which is rich in fat and protein, rice bran that is rich in carbohydrate and fiber, shell that is rich in minerals, fish that is rich in fat and animal protein and cassava that has a very low nutritional value. To obtain the rations/percentages of these three types of food, we weighed an amount for each nutrient, carefully mixed them and then proceeded to extract the fat contained in the mixture. After formulation of the feeds, pellets were made with pallet making machine.

Discussion

In the present study, we investigated the protective or enhancing effects of EGCG for HFD and for a SL in mice in relationship of development of CVD. We formulated a HFD [15] and we designed a SL model for mice that were fed for 6 weeks and then given EGCG. Mice fed a HFD and mice subjected to a SL showed increased lipid and glucose levels in blood. Study suggests that a high cholesterol diet influences the deposition of cholesterol in the aorta and other tissues as cholesterol esters [19]. Another study has shown that HDL cholesterol levels were found to be decreased in rats fed a pro-atherogenic diet [20]. The increased levels of total cholesterol and LDL cholesterol observed in mice fed a HFD and sedentary mice may be due to a decrease in LDL receptor activity which reduces LDL catabolism in animals fed cholesterol. This hypothesis is also pointed out by other studies [21]. Yu et al. reported that serum total cholesterol and triglycerides increased significantly in rabbits receiving an HFD [22]. Risk of CVD has been reported to be related to increased consumption of saturated fatty acids and percentage of calories from fat, which are positively associated with cholesterol intake [23]. Our relationship demonstrated a risk of developing CVD in mice fed a HFD and in mice subjected to a SL, since we found high levels of total cholesterol, LDL cholesterol, triglycerides and glucose. Our results are confirmed by other studies [24].

Study assessed the effects of exercise and diet first and secondly exercise and diet supplemented with EGCG. We determined whether the plasma lipid and glucose levels left by these procedures were maintained in the presence or absence of a HFD and a SL. Cardiovascular risk factors alter endothelial function but

physical exercise have the potential to slow down the endothelial damage associated with aging and CVD [24, 25]. The study reported that regular voluntary exercise and chronic physical exercise protects the installation and development of CVD in mice unlike HFD and SL [27].

A study in inactive mice showed that consumption of the HFD between 9 and 12 months led to obesity, hyperglycemia, hyperinsulinemia and hypercholesterolemia [15]. The found effects almost similar to those in the study above. We have noticed an increase in plasma lipid and glucose levels. A 3-month HFD induced obesity tripled the level of circulating cholesterol, induced oxidative plasma stress induced hyperglycemia and hyperinsulinemia in mice [26-28].

Mice that are fed a HFD and mice that are SL supplemented with EGCG compared to those that were not supplemented with EGCG had low total cholesterol, LDL cholesterol, triglycerides and glucose, which confirmed the preventive role of EGCG on CVD. Xu et al. demonstrated the effectiveness of EGCG (100 mg/kg) in an atherosclerosis disease model induced by feeding atherogenic diet to Wistar rats [29]. Results of the tissue morphometric analysis and lipid profile of the EGCG treated atherogenic diet fed rats showed that there was a reduction in total cholesterol, triglycerides, low-density and very low-density lipoprotein cholesterol fractions as compared to those untreated atherogenic diet fed rats [30]. In clinical and animal model experiments consumption of green tea resulted in weight reduction. GTP extracts significantly lowered total visceral and liver fat weight [31-34]. Furthermore, GTP considerably improves total serum cholesterol, triglycerides, and LDL cholesterol [35]. Our findings support a role for regular consumption of dietary EGCG such as green tea in day-to-day life will reduce the risk of CVD, and that represents a potential therapeutic agent for the prevention of atherosclerosis and related CVD.

Conclusion

The main topics on which my research is based on CVDs and mechanisms that characterize them: diet, physical exercise and EGCG, with particular attention for its benefits on cardiovascular health. Study showed that a HFD and a SL predispose to hyperlipidemia and high blood sugar. Study also proved that EGCG administered to hyperlipidemia mice reduced blood glucose and lipid levels, confirming that EGCG has therapeutic potential to prevent CVD.

Limitations

The limitation of this study was the experimental process. The mice were placed in large cages for chronic exercise and forced to run three sessions a day for 30 minutes each. It would have been interesting to put the mice on treadmills and compare the effects of exercise training between cages and treadmills on cardiovascular health, but the cage model used gave inconclusive results.

Abbreviations

CE: Chronic exercise
CoVAB: College of Veterinary Medicine, Animal Resources and Biosecurity (Makerere University)
CVD: Cardiovascular disease
EGCG: Epigallocathin-3-gallate
GT: Green tea
GTP: Green tea polyphenol
HDL: High density lipoprotein
HFD: High-fat diet
LFD: Low fat diet
LDL: Low-density lipoprotein
ND: Normal diet
SL: Sedentary lifestyle
TC: Total cholesterol
TG: Triglycerides
VE: Voluntary exercise
VED: Vascular endothelial dysfunction
VLDL: Very low-density lipoprotein cholesterol.

Author Contributions

Banzubaze Emmanuel is the research designer. In addition to the design of the research, this Banzubaze Emmanuel did the experimentation, the analysis of the results, the discussion and the drafting of the manuscript. The other authors were the directors of the research. To do this, they read and made corrections to errors of substance and form in the manuscript.

Declaration

In research, there is no competing interest as the research has been experimental on laboratory animal models (mice).

Ethics Approval

The research has been approved by the Animal Ethics Committee of Kampala International University and the Uganda national council of science and technology; Approval number is NS 645.

Consent To Publish

Before submitting the manuscript, there was consent of the authors for its submission

Founding Source

This work was supported by National Institute of Public Health of Bujumbura, Burundi

Availability of Data and Materials

I declare on my honor that the data and materials used in the writing of this manuscript are available in the file entitled "manuscript data submit". I also declare that this data may be made public by the scientific community for research purposes or during review of the manuscript by editors or reviewers.

Acknowledgements

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