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# Improved Cardiovascular Indices with High Fat Saturated and Unsaturated Ketogenic Diets in Rat Model

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**ABSTRACT:** The aim of this study is to ascertain the effects of different fat type ketogenic diet on some cardiovascular biomarkers in rat models. Forty Wistar albino rats were grouped into four and fed three types of ketogenic diets made of coconut oil, olive oil and butter. Ketogenic diets resulted in significant ($P<0.05$) weight loss. Total cholesterol in rats treated with coconut oil showed a significant increase ($P<0.05$) while those treated with butter showed a significant decrease ($P<0.05$) when compared with control. HDL cholesterol level was significantly increased ($P<0.05$) in rats treated with coconut oil when compared with control and also when compared with butter which showed a significant decrease ($P<0.05$) when compared with control. For LDL cholesterol and triglyceride there was no significant difference in all the treated groups when compared with control but increased significantly ($P<0.05$) in coconut oil treated rats when compared with olive oil treated rats. Plasma fibrinogen and relative plasma viscosity showed a significant decrease in rats treated with butter and coconut oil when compared with control. We therefore conclude that high fat ketogenic diet is safe for cardiovascular health.

**Keywords:** Cardiovascular, Ketogenic diet, High fat, Lipid profile, Fibrinogen, Viscosity

## Introduction

The prevalence of cardiovascular diseases (CVD) has been on the increase worldwide. Studies have shown that hypertension, obesity, cholesterol, poor nutrition, smoking, physical inactivity and psychological stress are predisposing factors to heart diseases. Rising prevalence of obesity has resulted in the increase incidence of CVD (Ng *et al*., 2013). Weight loss is one of the treatment strategies applied in the control and treatment of CVD. Recent studies have shown that diets high in carbohydrate and refined sugar have been linked with metabolic syndromes, (Mckeown *et al*., 2004; Asrihard and Jornay, 2014). Ketogenic diet can either be a high fat or high protein diet, with moderate or low carbohydrate depending of the type of ketogenic diet in question, (Veech, 2003). However, the use of high fat ketogenic diet has been on the increase, due to its therapeutic effects. High fat ketogenic diets are made with both saturated and unsaturated fats, saturated fat are fats that contain predominantly single bond fatty acid chains (Reece *et al*., 2002). Classification of the fatty acids is based on the number of carbon atoms they have, for the medium chain fatty acids, 7-12 carbon atoms, short chain fatty acids 1-6 and long chain have 13 and above (Schonfeld and Wojtczak, 2016). Long chain fatty acids can either be saturated (contains one bond) or unsaturated (contains one or more double bonds). These differences in their transport, absorption and destination can be attributed to differences in their structural makeup (Papamandjaris *et al*., 1998). Unsaturated fatty acids are further classified as mono unsaturated (containing a single bond) and poly unsaturated (containing two or more double bonds). Studies have shown that monounsaturated fats are protective against cardiovascular disease due to their impact on lipid fluidity, however monounsaturated fatty acids are more prone to lipid peroxidation, (Vessby *et al*., 2002). While it has been

observed that some monounsaturated fatty acids as well as saturated fats may promote insulin resistance, polyunsaturated fatty acids could be protective against insulin resistance. The therapeutic effects of high fat ketogenic diet in some diseases have been studied with positive findings; it has therefore become necessary to research more into these areas as the use of nutrition in the management of some diseases can reduce to a large extent our dependant on the use of drugs. This will no doubt be of benefit to the general populace both economically and socially (WHO, 2009).

Ketosis is a physiological mechanism where in the absence or low levels of dietary carbohydrate there is depletion of glucose, hence not available for normal fat oxidation; the central nervous system begins to utilize an alternative source of energy, derived from acetyl coenzyme A. Prolong fasting and intake of high fat or low carb diet result in increased concentration of ketone bodies like acetoacetate, beta- hydroxybutyric acid and acetone. This process is referred to as ketogenesis and it occurs principally within the mitochondria matrix of the liver (Fukao *et al*., 2004). According to Veech (2004), ketone bodies produce more energy when compared with glucose as a result of its high chemical potential of S-beta- hydroxybutyrate, in ATP hydrolysis.

While some studies have shown the benefits of saturated fat in the heart, other contradictions have also been reported concerning its detrimental effects to the heart in comparison with other fat types (De Souze *et al*., 2015). However, a common fear is that because ketogenic diets are high in fat both saturated and unsaturated, they might result in increase of total cholesterol in the body thereby clogging the arteries. It is thus the aim of this research to determine the effect of three different fat types (butter, olive oil and coconut oil) ketogenic diets on weight loss and some cardiovascular biomarkers.

**Materials and methods**

**Experimental design:** Forty (40) Albino Wistar rats (acquired from pharmacology animal house, Faculty of Pharmacy, University of Benin) of either sex were used in this study. The weights of the rats were taken on arrival after which the rats were grouped into 4 according to the type of fat used in their diets; Group A (10 rats) were fed with butter constituted diet, Group B (10 rats) were fed with olive oil constituted diet, Group C (10 rats) were fed with coconut oil constituted diet. Control group D (10 rats) were fed with the normal rat chow. The rats were first acclimatized for two weeks after which all groups were kept in their respective diet for 8 weeks and they were allowed free access to feeds and water during the duration of the experiment. Daily intake of feeds was weighed, and the rats were weighed weekly. After the first one month, the rats' urine samples were taken to ascertain ketosis, which is the presence of ketone bodies in urine.

**Collection of samples:** The rats were made to fast overnight prior to sample collection, they were then put to sleep using chloroform in a closed chamber. Fasted blood samples were collected from the abdominal aorta and cardiac puncture into plain tubes and citrate test tubes for the estimation of Lipid profile, Plasma fibrinogen, and Plasma viscosity. Lipid profile was estimated spectrophotometrically using *Randox* reagent kits. Total cholesterol was analyzed by the method of Allain *et al*. (1952), HDL by the method of Lopes-Virella *et al*. (1977**).** Low density was extrapolated from the result obtained from triglycerides, cholesterol and high density lipoprotein cholesterol (Friedewald *et al*., 1972).

The method described by Schnieder (1952) was used to determine the plasma fibrinogen using 0.25 % aqueous methylene blue. The principle is based on the premise that the plasma fibrinogen titre reflects the available coagulable protein semiquantitatively. Parallel quantitative determination of fibrinogen protein nitrogen has shown that when a titre of 128 or higher is obtained, fibrinogen content is in excess of 200 mg/100 ml. A titre of 64 indicates fibrinogen present in a concentration of 100-200 mg/100 ml. A titre of 32 or less indicates fibrinogen under 100 mg/100 ml.

The method described by Reid and Ugwu (1986) was used to determine the relative plasma viscosity. This procedure was carried out based on comparison of flow rate of plasma and distilled water under equal pressure and constant temperature. The result was then expressed as plasma viscosity of plasma relative to that of water.

**Statistical analysis:** Data were presented in mean ±standard error of mean (SEM). Analysis of Variance (ANOVA) was used for the comparison within the groups while Turkey test was considered as the post-hoc test and $P<0.05$ was considered as the statistical significance. The Graph pad prism version 5.0 statistical packages were used for the result analysis.

## Results

The mean values of body weights of rats fed with coconut oil, olive oil and butter for 8 weeks are presented in Table 1. There were a significant (P<0.05) decrease in body weight when the various groups were compared with control, but when initial weight of the treated groups were compared with the final weight there was a significant (P<0.05) reduction in weight after treatment, except in the butter treated group, while in the control group there was a significant (P<0.05) increase in final weight when compared with the initial weight.

**Table 1:** Body weights of rats fed with coconut oil, olive oil and butter

| Parameters | Control | Butter | Coconut oil | Olive oil |
|---|---|---|---|---|
| Initial body wt (g) | 179.2 ± 5.39 | 217.5 ± 44.28 | 203.0 ± 17.93 | 214.0 ± 21.06 |
| Final body wt (g) | 262.5 ± 9.90 | 166.3 ± 20.55 | 151.0 ± 12.19 | 137.0 ± 13.00 |
| P-values | P < 0.05 | P > 0.05 | P < 0.05 | P < 0.05 |

**Table 2:** Lipid profiles, relative plasma viscosity and fibrinogen concentration in rats fed with coconut oil, olive oil and butter

| Parameters | Control | Butter | Coconut oil | Olive oil |
|---|---|---|---|---|
| Total cholesterol (mg/dl) | 77.83 ± 4.15 | 58.25 ± 3.90* | 95.00 ± 7.52[a] | 66.20 ± 5.05[b] |
| HDL (mg/dl) | 83.00 ± 2.48 | 71.00 ± 0.91* | 98.00 ± 6.13*[a] | 91.60 ± 6.83[a] |
| LDL (mg/dl) | 18.17 ± 4.66 | 12.30 ± 4.49 | 38.44 ± 9.29 | 12.08 ± 3.75[b] |
| TG (mg/dl) | 98.50 ± 12.28 | 92.75 ± 9.21 | 83.80 ± 5.53 | 87.40 ± 16.04 |
| Plasma fibrinogen (g/dl) | 2.48 ± 0.22 | 2.68 ± 0.29 | 1.32 ± 0.23*[a] | 3.10 ± 0.19[b] |
| Relative plasma viscosity | 0.57 ± 0.009 | 0.51 ± 0.024* | 0.50 ± 0.025* | 0.54 ± 0.034 |

*P < 0.05 indicates significant difference at the different treatments compared with the control.
[a]P < 0.05 indicates significant difference when coconut or olive oil treated are compared with butter treated.
[b]P < 0.05 indicates significant difference when coconut treated is compared with olive oil treated.

Table 2 shows the lipid profiles, relative plasma viscosity and fibrinogen concentration in rats fed with cocnut oil, olive oil and butter after 8 weeks of feeding.

**Total Cholesterol:** There was a significant (P < 0.05) reduction in total plasma cholesterol concentrations in rats fed olive oil and butter constituted diets when compared with control while for rats fed with the coconut oil constituted diet, showed a significant (P < 0.05) increase in total cholesterol levels.

**High density lipoprotein**- There was a significant (P < 0.05) increase in HDL cholesterol concentrations in rats fed with coconut oil and olive oil when compared with butter and control rats, while rats fed with butter constituted diets was significantly lower when compared with control.

**LDL Cholesterol**- There was a significant (P < 0.05) reduction in LDL cholesterol concentration in rats fed olive oil constituted diet when compared with coconut oil, there was however no significant difference between the control group and others.

**Triglyceride**- There was no significant difference in the triglycerides values of rats fed with the three ketgenic diets when compared with control.

**Plasma fibrinogen**- There was a significant (P < 0.05) reduction in fibrinogen concentration in rats fed coconut oil constituted diet when compared with control and olive oil values, while there was no significant difference in butter constituted diet when compared with control.

**Relative plasma viscosity**- There was a significant (P < 0.05) reduction in relative plasma viscosity in values of rats fed the butter and coconut oil constituted diets when compared with control; however rats fed with the olive oil diet did not show any significant difference with control and other groups.

### Discussion

Rats fed the various ketogenic diets showed a reduction in weight (P<0.05) when compared with control, Also, when compared within groups, rats fed olive oil constituted diets were observed to have lost more weight when compared with rats fed butter constituted diets. Until now, there has been a general worry, in the use of ketosis in medicine, however recent findings in nutritional studies have shown that ketosis is of great benefits in medicine, hence it's been embraced both by those in the medical world and the general public. In our previous study we observed a significant reduction in weights of rats fed high fat constituted diet, (Eiya and Osakue, 2018), In humans, ketone bodies are the only source of energy after glucose (Amiel, 1995; Yehuda *et al.*, 1999). There has been disagreement on the intake of high fat ketogenc diets, while some school of thoughts believe that it can result to obesity due to high calorie content when compared to low fat diets and high carbohydrates diets,

as consumption of high fat diet have been associated with high energy intake (Prentice, 1998). Conversely, recent researches (Foster *et al.,* 2003) and this current study has observed and proven that ketogenic diet can be used as a treatment strategies for weight control in obese patients, this is due to the driving of the body into ketosis; a physiological mechanism in which insulin activates key enzymes in the pathway, storing up energy derived from carbohydrate which results in low amounts of insulin, leading to a reduction in lipogenesis and fat gathering.

Rats fed with coconut oil constituted diets showed a significant increase in total cholesterol level, their HDL cholesterol level and LDL cholesterol levels when compared with control. Coconut oil fed rats also showed a significant increase in their total cholesterol, HDL and LDL cholesterol levels when compared within groups. Results in this study agrees with findings of Mensink *et al*. (2003), who explained that coconut oil contains a large proportion of lauric acid which raises total cholesterol level by increasing both HDL and LDL cholesterol. This fact has over the years, brought a number of controversies among researchers as it has been thought that for normal or optimum cardiovascular health, HDL cholesterol which is the good cholesterol has to be high while LDL cholesterol commonly known as the bad cholesterol has to be low (Barter *et al*., 2004). This fact is however not contested, but the fact that the type of LDL present goes a long way in ascertaining cardiovascular health. Recent studies have proven that there are two types of LDL cholesterol particles; the small dense (type B) and the large fluffy (type A), where the small dense are known to be more harmful than the large fluffy ones. The prevalence of the small dense LDL particles is correlated with increased risk of cardiovascular disease (Austin *et al*., 1994). Hence the type of LDL particles goes a long way in determining cardiovascular health. Also, trending controversies in the importance of cholesterol have it that lowering cholesterol is not necessary or healthy, as cholesterol are of great importance and could cause dementia or Alzheimer's disease when they are in little quantity because since cholesterol is carried to the brain via the LDL receptor and is a building block for neurons (Ray *et al*., 2010), reducing LDL levels too much starves the brain of cholesterol and increases the workload on the liver to produce and synthesize more cholesterol. Also Mensink *et al*., (2003) further stated that the persistent consumption of coconut oil does not exclude the possibility of it increasing the risk of cardiovascular disease. However, a high percentage of coconut oil consist of medium chain fatty acids and can be oxidized immediately for energy by the liver as there are no need for re-esterification (Guillot *et al.,* 1993), as a result of this specific characteristics of medium chain fatty acids, it is believed that coconut oil cannot be regarded as hypercholesterolemic.

Rats treated with butter showed a significant decrease ($P<0.05$) in their total cholesterol level and HDL cholesterol level when compared with control. It however also showed a decrease in the LDL level, although not significant when compared with control and when compared within groups. This is in agreement with our previous study, where rats fed with 65% butter constituted diet were observed to have significant reduction in total cholesterol and LDL cholesterol levels, while HDL cholesterol increased though not significant, (Eiya and Osakue, 2018). Stearic acid in butter fat has a higher level than in coconut oil that is less absorbed than other fatty acids, thus the bioavailability of butter is low (Weisburger, 2001), and since butter showed a significant increase ($P<0.05$) in their fibrinogen level when compared with coconut oil, stearic acid in butter may thus be responsible to predispose platelet aggregation and facilitate thrombosis.

Rats treated with olive oil showed a reduction in their total cholesterol level and LDL cholesterol level which was however not significant when compared with control. There was also no significant difference in the level of HDL cholesterol and triglycerides when compared with control. However, there was a significant reduction ($P<0.05$) in the LDL cholesterol level of olive oil when compared with coconut oil, but there was no significant difference in the total cholesterol and HDL cholesterol level when compared within groups. Olive oil was shown to decrease the plasmatic level of LDL cholesterol, thus lessen the likelihood of coming down with heart diseases. It has thus been recommended that high intake of monounsaturated fatty acid instead of saturated and polyunsaturated fatty acids will reduce the sensitivity of circulating lipoproteins to peroxidation, thus diminishing the onset of atherosclerosis (Alarcón de la Lastra *et al*., 2001). This could be due to the fact that saturated fatty acid consumption significantly increases the plasma concentration of low density lipoprotein cholesterol, potentially increasing the risk of cardiovascular heart disease (Ridker, 2014). All treated groups showed no significance in their level of triglycerides when compared with control and when compared within groups.

The relationship between high triglyceride concentrations and cardiovascular disease has long been established (Austin *et al*., 1998; Sarwar *et al.,* 2007). The degree to which triglycerides advances cardiovascular disease or being a biomarker of risk is still been debated for ages now. Studies have shown that triglycerides are crucial in lipid metabolism as it has been established that they promote the formation of fatty deposits in arteries thus making it an important biomarker in CVS. Also overweight and sedentary lifestyle has also been associated with metabolic syndrome a risk factor for CVS, thus the urgent need to encourage an improved lifestyle (Tirosh *et al.,* 2007).

Fibrinogen concentration showed a significant decrease ($P<0.05$) in the rats fed coconut constituted diets, when compared with control and also when compared with butter and olive oil treated rats. Butter and coconut oil

treated groups showed a significant decrease ($P<0.05$) in their relative plasma viscosity level when compared with control, but there was no significant difference in olive oil treated group when compared with control and when compared within groups. Lowe; *et al*. (1981), considered the fact that blood viscosity augments cardiovascular health. Also fibrinogen is a key contributor for plasma viscosity; high plasma viscosity has been associated with poor oxygen delivery to the ischaemic myocardium.

## Conclusion

Findings from this study has shown that high fat ketogenic diet made from saturated fatty acids and unsaturated fatty acids are both effective for weight loss, however this study was done for just 8 weeks hence there is need to ascertain its effect on long term use. This study has also shown that as long as carbohydrate level is low, both saturated and unsaturated fats are not hazardous to cardiovascular health but the use of olive oil was observed to result in significant reduction in LDL cholesterol which makes it more heart friendly compared to the other fats, however we observed an increase in total cholesterol, HDL and LDL in rats fed coconut oil constituted diet while fibrinogen levels was low, even though previous studies have tried to explain reasons for this, there is need for further detailed study to be done to ascertain the safety of coconut oil on cardiovascular health.

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