International Journal of Research in Medical Sciences
Edith R et al. Int J Res Med Sci. 2022 Oct;10(10):2126-2131
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20222517

Original Research Article

Ketogenic diet reduces oxidative stress in obese females in Port Harcourt, Nigeria

Reuben Edith[1]*, Amah-Tariah Fortune Somiari[2]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria
[2]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Port Harcourt, Port Harcourt, Nigeria

Received: 31 March 2021
Revised: 04 August 2022
Accepted: 09 August 2022

***Correspondence:**
Dr. Reuben Edith,
E-mail: edithrng@gmail.com

ABSTRACT

Background: Sub-Saharan Africa is not insusceptible to the obesity epidemic, regardless of the continued problem of undernutrition. Increases in the rates of overweight and obesity are being identified in Sub-Saharan Africa, especially among women and people dwelling in urban populations. This study, therefore, is aimed at evaluating the effects of ketogenic diet on markers of oxidative stress (reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA)) in obese female subjects on eight weeks ketogenic diet.
Methods: A total of forty (40) participants, 10 overweight (25.0–29.9 kg/m^2) and 30 obese (≥30 kg/m^2) female subjects, were recruited and investigated via informed consent and approval obtained. The sera of the participants were collected by standard, sterile with a minimal invasive procedure for reduced glutathione, catalase, superoxide dismutase, malondialdehyde at weeks 0, 4, and 8 of ingestion of low carbohydrate ketogenic diet (LCKD).
Results: There was a statistically significant increase in mean superoxide dismutase levels of participants at the 4th and 8th week after the introduction of low carbohydrate ketogenic diet (LCKD). There were also statistically insignificant changes in catalase and malondialdehyde levels in the participants between the baseline (week 0) and 4th and 8th weeks. Mean reduced glutathione was statistically significant at week 4 when compared with the baseline.
Conclusions: Ketogenic diet reduces oxidative stress as evidenced by increased reduced glutathione and superoxide dismutase.

Keywords: Oxidative stress, Ketogenic diet, Obesity

INTRODUCTION

Obesity is a public health disorder that is reaching epidemic prevalence universally.[1] In Africa, some 27% of adults aged ≥20 years are overweight, and 8% are obese. In a systematic review of studies published from 01 January 2001, to 30 September 2012, the prevalence of overweight adults in Nigeria ranged from 20.3-35.1%, while the prevalence of obesity in adults ranged from 8.1-22.2%. Sub-Saharan Africa (SSA), is also affected by the obesity epidemic. Despite the malnutrition and undernutrition that plagued the region, obesity continues to increase.[2,3] Increases in the rates of overweight and obesity, especially among women and people dwelling in urban populations in the Sub-Saharan region, have been identified.[3,4]

The multi-factorial aetiology of obesity has been shown in several studies to include metabolic factors, diet, hormonal, genetic predisposition, environmental, socioeconomic factors.[5-7] Dietary management which is the first line in the line of management in obesity and

overweight, ranges from general advice about healthy diet choices to specific advice of caloric restriction such as low carbohydrate, low fat, high protein diets.[8]

Ketogenic diets have shown to be effective in short to medium-term use in the management of obesity, hyperlipidemia, and some cardiovascular risk factors.[10,11] The use and function of ketogenic diets in the long-term management of obesity are not well established.[10]

The term oxidative stress refers to the imbalance between free radicals (pro-oxidants) and their stabilizing agent's antioxidant enzymes in the body in favor of the oxidants, potentially leading to damage.[12,13]

Antioxidant enzymes produce reactive oxygen species (ROS); which are produced by normal cellular metabolism and subsequently react with biomolecules such as protein, lipid, and deoxyribonucleic acid (DNA) cellular damage and responsible for degenerative changes. At low concentrations, free radicals play an important role in physiological regulation, cellular signaling processes. They are responsible for some degenerative changes but the high-level ROS are known to cause deleterious transformations in the cell.[13] Differing oxidation-linked processes form the basis of most physiological and pathophysiological occurrences; they also cause direct or indirect damage in different organs and are part of most processes e.g. aging, inflammation, carcinogenesis, diabetes, cardiovascular disease, atherogenic processes, drug reaction, toxicity, and infection.[12,13]

Studies on the status of oxidative stress markers on ketogenic diet have been shown mostly in athletes and animal experiment.[14-16] However, data on oxidative stress amongst obese women appears to be limited. The literature on changes in oxidative stress markers among obese women on ketogenic diet in Nigeria has been scarcely documented. Therefore, this present study is concerned with the evaluation of the effects of ketogenic diet on markers of oxidative stress (reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA)) in obese female subjects post eight weeks ketogenic diet treatment.

METHODS

This research was carried out at the department of human physiology, University of Port Harcourt, and Biosystems Medical diagnostic center, GRA phase 1, Port Harcourt, Nigeria for a duration of three months, from October 2019 to January 2020. Informed consent was sought and obtained from the participants.

The study is longitudinal study in which the study participants acted as their own controls. A purposive sampling technique was employed. Subjects (overweight and obese females) were selected from a ketogenic club database; they were contacted via phone calls and briefed on the research study; those who granted consent were recruited into the survey at the Biosystems Medical diagnostic center.

This study involved 40 females within the age bracket of 18 to 55 years and were either overweight or obese. Participants were apparently healthy individuals who were overweight (body mass index (BMI) between 25-30 kg/m^2) or obese (BMI over 30 kg/m^2), desired to lose weight, had not participated in any other diet reduction program in the past 6 months, can follow the dietary guidelines and signed the consent form.

Participants did not include women who have previously been on a ketogenic diet or using weight loss pills or other weight-loss diets in the past six months, women with cardiovascular instability, hypo or hyperthyroidism, unstable metabolic disorders, or chronic diseases such as renal failure, liver cirrhosis, or any known etiology for liver disease, and with evidence of any other systemic or malignant diseases were excluded.

Subjects were instructed to fast overnight (8–12 hours) and to maintain proper hydration by drinking water liberally. On presentation at the centre, participants were taught how to record dietary intake. A record folder was opened for each respondent, which contained a structured questionnaire which they filled on their first visit providing information on their sociodemographic details and medical history.

During the first visit, participants were placed on a sample menu of ketogenic diet; and required to record their dietary intake (aided with handouts that demonstrate how to record daily dietary intake). The dietary invention lasted for eight weeks, and during this period, the participants made two visits to the outpatient clinic at four weeks and eight weeks, respectively. A WhatsApp support group was created, and the participant's weekly menu was shared as well as daily meals.

Dietary intervention

The intervention diet aimed to reduce carbohydrate intake and the consumption of fat with no restrictions on the type of fat. The ketogenic diet was modified using some Nigeria-based foods. The actual diet that was consumed comprised eggs, beef, chicken, turkey, catfish, mackerel fish, organ meat, crayfish, avocado mayonnaise, coconut oil, Ghee butter or butter, olive oil, coconut milk or complaint coconut milk powder, palm oil, egusi, okra, keto cereal (flax seeds/chia seeds/ sunflower seeds), almond flour or coconut flour, baking powder, psyllium husk, stevia sweetener or xylitol, monk fruit sweetener, adobo seasoning or tropical sun seasoning, Himalayan salt or lo salt, assorted Nigerian green vegetables, cabbage, cucumber, lettuce, cauliflower, eggplant, coffee, green tea, water. The subjects were instructed to eat until hunger was relieved especially using the snacks (eggs, garden eggs, cucumber, and fried meat). The caloric intake was not limited, and exercise was not included as part of the study

protocol. Serum oxidative stress biomarkers were measured before starting and at the end of the intervention.

Adherence to the diet was determined via self-reports, daily WhatsApp uploads of meals, and food records received during clinic visits. Magnesium citrate supplementation was provided, and intake was encouraged throughout the study. The supplement is not a weight-loss-inducing pill; however, the supplement helps to reduce the symptoms associated with the keto diet, such as muscle cramping, difficulty in sleeping, and irritability. Respondents returned with dietary intake records at weeks 4 and 8; also, the anthropometric measurements and biochemical screenings were conducted.

Sample collection

Five (5) ml of venous blood was collected from the subjects according to the anthropometric data below in plain tubes (Becton Dickenson, USA). The sera obtained after centrifugation for 20 min at the speed of 2000–3000 rpm using an automated chemical /ELISA machine known as Chemwell (model 2910) Automated EIA and chemistry analyser made by Awareness Technology INC. United States of America was kept at – 20 C freezer until use.

Assay methods of GSH, CAT, SOD, and MDA

The levels of human serum GSH, CAT, SOD, and MDA were analysed. The MDA concentrations in the sera were determined by the colorimetric assay by the Ohkawa and Ohishi method using the principle that under acidic conditions, 2-thiobarbiturate to yield a pink-coloured complex which is measured at 532 nm. The SOD concentrations in the sera were determined using the Misra and Fridovich Method, in which SOD inhibits the autooxidation of adrenaline to adrenochrome in a basic medium. CAT activity was measured by Clairborne method with the principle of catalase in the sample split hydrogen peroxide, which was measured at 240 nm. One unit of Catalase activity equals the amount of protein used to convert 1 µmol H_2O_2/min.

Determination of GSH based on the principle of development of a relatively stable yellow colour when dithiobis 2-nitrobenzioc acid- DNTB (Elliman's reagent) when added to suphahydryl-compounds measured at 412nm. All reagents, dilutions, and calculations were applied according to the manufacturer's instructions at Biosystems laboratory, GRA phase 1, Port Harcourt.

Statistical analysis

Data were analysed using statistical package for social science (SPSS) version 25.0. The descriptive statistics were presented in percentages (for categorical variables) and means (for continuous variables), including graphs. Inferential statistics involved comparisons between the pre-intervention values and post-intervention values using Wilcoxon signed-rank test. The correlation test was using Spearman's correlation.

RESULTS

The proportion of overweight and obesity in this study was 10 (25.0%) to 30 (75.0%). The mean age of participants was 36.0±4.49 years, and the mean BMI of overweight and obese participants was 27.98±1.38 kg/m^2 and 37.67±4.46 kg/m^2, respectively.

The BMI of overweight and obese participants decreased significantly from 27.98±1.38 kg/m^2 and 37.67±4.46 kg/m^2 at week 0 to 26.83±1.76 kg/m^2 and 36.55±4.47 kg/m^2 at week 4 and 24.67±1.68 kg/m^2 and 34.53±5.08 kg/m^2 at week 8.

Changes in mean SOD, CAT, GSH., and MDA after ketogenic diet

Table 2 shows the mean values for SOD, CAT, GSH, and MDA at weeks 0, 4, and 8, respectively. There was a statistically significant increase in SOD levels of participants at the 4th and 8th weeks after the introduction of LCKD. CAT and MDA levels increased, though statistically insignificant, between the baseline and 4th and 8th weeks. GSH level increased and was statistically significant between the baseline and 4th week.

There was a positive correlation between BMI and SOD at week 4.

Table 1: Socio-demographic characteristics of the study population.

Characteristics	Frequency (n)	Percent (%)
Age bracket (years)		
<35	14	35.0
>35	26	65.0
Mean age (mean±SD)=36.0±4.49 years		
Marital status		
Single	5	12.5
Married	30	75.0
Separated	3	7.5
Divorced	2	5.0
Educational level		
Primary	0	0
Secondary	2	5.0
Tertiary	38	95.0
Profession/occupation		
Business/entrepreneur	16	40.0
Civil servant	6	15.0
Health worker	8	20.0
Public servant	6	15.0
Others	4	10.0

Table 2: Body mass index (BMI) of pre diet (week 0) and post diet (week 4 and week 8).

Variable	BMI (kg/m^2)
Overweight	
Week 0	27.97±1.38
Week 4	26.83±1.76*
Week 8	24.67±1.68*#
Obese	
Week 0	37.67±4.46
Week 4	36.55±4.47
Week 8	34.53±5.08
Total	
Week 0	35.24±5.77
Week 4	34.12±5.81
Week 8	32.06±6.21

Values represent mean±SD; %MD=percentage mean difference, *statistically significant change in value between baseline and week 4 and, #statistically significant change in value between week 4 and week 8 using Wilcoxon signed rank test

Table 3: Mean values of oxidative stress biomarkers in the study population.

Variable	SOD (µ/ml)	CAT (µ/g)	GSH (µg/ml)	MDA (µmol/l)
Overweight				
Week 0	0.44±0.16	3.76±1.54	2.80±0.37	0.82±0.75
Week 4	0.43±0.20	3.92±1.37	2.74±0.48	0.84±0.76
Week 8	0.42±0.18	4.03±1.03	2.83±0.49	0.86±0.65
Obese				
Week 0	0.33±0.13	3.50±0.93	2.72±0.56	0.68±0.16
Week 4	0.45±0.14*	3.49±0.97	3.04±0.61*	0.68±0.16
Week 8	0.47±0.16*	3.48±1.06	2.92±0.59	0.71±0.16
Total				
Week 0	0.36±0.14	3.57±1.10	2.74±0.52	0.71±0.39
Week 4	0.44±0.15*	3.60±1.08	2.97±0.59*	0.72±0.40
Week 8	0.46±0.17*	3.62±1.07	2.90±0.56	0.75±0.34

Values represent mean±SD; *statistically significant change in value between baseline and week 4/week 8 using Wilcoxon signed rank test, reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MD)

DISCUSSION

There is a need for a proper understanding of oxidative stress markers including SOD, CAT, MDA, and GSH in ketogenic diet as regards to its clinical significance, especially in Nigeria where this is a rising lifestyle for women who want to lose weight. Caloric restriction, as seen in ketogenic diet, has been proposed to reduce oxidative stress and apoptosis, inhibit pro-inflammatory mediators, such as the cytokines tumour necrosis factor-a and interleukins.[17] In an animal model of Alzheimer's disease, transgenic mice who ingested ketogenic diet showed better mitochondrial function and less oxidative stress.[18]

There is evidence that obesity decreases the ability of the body to produce protective antioxidants, thereby increasing systemic oxidative stress. Susceptibility to oxidative damage receptiveness is seen to increase in obese individuals due to decreased antioxidant sources, such as SOD, and CAT, vitamin A, vitamin E, vitamin C, and β-carotene.[19,20] Reactive oxygen species are known to increase in obesity, and also, the production of antioxidants is seen to be impaired in obesity.[21] However, after the introduction of the ketogenic diet, the production of ketone bodies modifies the tricarboxylic acid cycle, thereby increasing GABA synthesis in the brain and limiting reactive oxygen species (ROS) production, which leads to a reduction in ROS and boosts energy production by increasing energy reserves.[22]

Based on our findings, the oxidative stress enzymes increase after the introduction of the ketogenic diet. There was a significant increase in SOD and GSH between week 0, week 4, and week 8 after the introduction of a low carbohydrate ketogenic diet (LCKD). However, MDA and CAT had no significant change. In contrast with the findings of the present study, Nazarewicz et al also reported that after the introduction of the ketogenic diet, MDA, CAT, and SOD content did not change. However, the antioxidative capacity of healthy subjects increased after the introduction of a low carbohydrate ketogenic diet.

Roh et al reported no significant change in MDA and SOD, while in this study, there was a statistically significant change in SOD but no statistically significant change in MDA in our study. In animal model studies, showed that the ketogenic diet increased GSH biosynthesis and revealed that the ketogenic diet firstly produces mild oxidative and electrophilic stress, which leads to improvement of the mitochondrial redox state.[24] McAllister et al report is in contrast with our report as there was a significant change in MDA and no significant change in SOD.

The research on the effect of ketogenic diet on oxidative stress has been shown to report conflicting results; however, this research involved subjects with different disease entities.[20] This study was not without limitations. First, the number of subjects was small, and this study did not include a control group. Despite thorough instructions to the participants not to change any variable other than the diet, we can thus not completely exclude the influence of possible changes in physical activity due to weight loss.

CONCLUSION

The finding of this present study revealed that there was an increase in oxidative stress enzymes, especially those with antioxidant capacity SOD and CAT as well as GSH and MDA. LCKD is, therefore a safe and effective dietary intervention in patients with obesity and can aid in reduction in oxidative stress associated with obesity.

ACKNOWLEDGEMENTS

Authors would like to thank the participants for voluntarily participating and the dietician, without them this study would not have been possible.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Golubic R, Laur C, Kelsey M, Livesy A, Hoensch J, Park A, et al. The Cambridge Intensive Weight Management Programme Appears to Promote Weight Loss and Reduce the Need for Bariatric Surgery in Obese Adults. Front Nutr. 2018;5.
2. Bleich SN, Cutler D, Murray C, Adams A. Why is the developed world obese? Annu Rev Public Health. 2008;29:273-95.
3. Scott A, Ejikeme S, Clottey EN, Thomas JG. Obesity in sub-Saharan Africa: development of an ecological theoretical framework. Health Promot Int. 2012;28(1):4-16.
4. Afolabi WAO, Addo AA, Sonibare MA. Activity pattern, energy intake and Obesity among Nigerian urban market women. Int J Food Sci Nutr. 2004;55(2):85-90.
5. Weinsier RL, Hunter GR, Heini AF, Goran MI, Sell SM. The etiology of Obesity: Relative contribution of metabolic factors, diet, and physical activity. Am J Med. 1998;105(2):145-50.
6. Jé E, Tappy L. Regulation of Body Weight in Humans. Physiol Rev. 1999;79(2):451-80.
7. Stunkard AJ, Foch TT, Hrubec Z. Study of Human Obesity. J Am Med Assoc. 2010;1986(256):51-4.
8. Somasundaram N, Rajaratnam, Henry Wijeyarathne CKS, De, Shamya P, Wickramasinghe P, et al. Management of Obesity. Sri Lanka J Diabetes, Endocrinol Metab. 2014;1(20):55-70.
9. Raben A, Macdonald I, Astrup A. Replacement of dietary fat by sucrose or starch: Effects on 14 d ad libitum energy intake, energy expenditure and body weight in formerly obese and never-obese subjects. Int J Obes. 1997;21:846-59.
10. Bueno NB, De Melo ISV, De Oliveira SL, Da Rocha Ataide T. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: A meta-analysis of Randomised controlled trials. Br J Nutr. 2013;110(7):1178-87.
11. Al-Khalifa A, Mathew TC, Al-Zaid NS, Mathew E, Dashti HM. Therapeutic role of low-carbohydrate ketogenic diet in diabetes. Nutrition. 2009;25(11-12):1177-85.
12. Betteridge DJ. What is oxidative stress? Metabolism. 2000;49(2):3-8.
13. Leverve X. Oxidative, stress and antioxidants? Cah Nutr Diet. 2009;44(5):219-24.
14. Huang Q, Ma S, Liu C, Tominaga T, Suzuki K. An 8-Week, Low Carbohydrate, High Fat, Ketogenic Diet Enhanced Exhaustive Exercise Capacity in Mice Part 2: Effect on Fatigue Recovery, Post-Exercise Biomarkers, and Anti-Oxidation Capacity. Nutrients. 2018;10:1-16.
15. McAllister MJ, Basham SA, Smith JW, Fountain BJ, Krings BM, Waldman HS. Effect of carbohydrate restriction on oxidative stress response to high-intensity resistance exercise. Transl Sports Med. 2019;2(6):334-40.
16. Roh H-T, Rhyu H-S, Cho S-Y. The effects of ketogenic diet on oxidative stress and antioxidative capacity markers of Taekwondo athletes. Exer Rehab. 2014;10(6):362-6.
17. Maalouf MA, Rho JM, Mattson MP. The Neuroprotective Properties Of Calorie Restriction, The Ketogenic Diet, And Ketone Bodies. Brain Res Rev. 2009;59(2):293-315.
18. Paoli A, Rubini A, Volek JS, Grimaldi KA. Beyond weight loss: A review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. Eur J Clin Nutr. 2013;67(8):789-96.
19. Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. Is Obesity Associated With Increased Plasma Lipid Peroxidation And Oxidative Stress In Women? ARYA Atheroscler. 2010;2(4).
20. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, et al. Oxidative stress in Obesity: A

critical component in human diseases. Int J Mol Sci. 2015;378-400.
21. Knight SF, Imig JD. Obesity, insulin resistance, and renal function. Microcirculation. 2007;14(4-5):349-62.
22. Bough KJ, Rho JM. Anticonvulsant Mechanisms of the Ketogenic Diet. Epilepsia. 2007;48(1):43-58.
23. Nazarewicz RR, Ziolkowski W, Vaccaro PS, Ghafourifar P. Effect of short-term ketogenic diet on redox status of human blood. Rejuvenation Res. 2007;10(4):435-40.
24. Milder JB, Liang L-P, Patel M. Acute Oxidative Stress and Systemic Nrf2 Activation by the Ketogenic Diet. Neurobiol Dis. 2010;40(1):238-44.

Cite this article as: Edith R, Somiari ATF. Ketogenic diet reduces oxidative stress in obese females in Port Harcourt, Nigeria. Int J Res Med Sci 2022;10:2126-31.