Journal of Advances in Medicine and Medical Research

27(1): 1-7, 2018; Article no.JAMMR.42285
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Beta-Carotene: Positive Effect on Oxidative Stress, Lipid Peroxidation, Insulin and Leptin Resistance Induced by Dietary Fat Consumption

N. Okechukwu, Getrude[1*], N. Eteudo Albert[2], G. Akunna, Gabriel[1], Elizabeth Finbarrs-Bello[3], C. Anwara Elizabeth[2] and O. Ibegbu, Austin[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Ndufu-Alike Ikwo (FUNAI), Ebonyi State, Nigeria.
[2]Department of Anatomy, Faculty of Basic Medical Sciences, Ebonyi State University, Abakaliki (EBSU), Ebonyi State, Nigeria.
[3]Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Sciences and Technology (ESUT), Parklane, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author OIA coordinated the research. Author NOG conducted the laboratory work. Author GAG conducted the statistical analysis. Author NEA wrote the protocol. Author CAE conducted Literature search. Author EFB did the write up of the manuscripts. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/42285
Editor(s):
(1) Dr. Nurhan Cucer, Department of Medical Biology, Erciyes University, Turkey.
Reviewers:
(1) Lucia Maria Jaeger de Carvalho, Rio de Janeiro Federal University, Brazil.
(2) Fumitoshi Asai, Azabu University, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/25417

Original Research Article

Received 23rd May 2018
Accepted 25th June 2018
Published 6th July 2018

ABSTRACT

Beta-carotene (βC), an abundant natural antioxidant in fruits and vegetables may possess the Ability to modulate oxidative stress and leptin-insulin signaling. This study was designed to evaluate the biochemical effect (hormonal and oxidative enzyme markers) of βC on Wistar rats fed high dietary fat. Thirty (30) male Wistar rats were randomly divided into six (6) groups of five rats each. Group A was taken as the control and received distilled water, Group B received high-fat diet of (60% fat and 40% rat chow), Group C received 300 mg/kg body weight (bw) of βC, Group D received high-fat diet for 12 weeks and was treated with 300 mg/kg bw of βC for 2 weeks, Groups

**Corresponding author: E-mail: okeygetrude@yahoo.com, okeygetrude@gmail.com;*

E received 300 mg/kg /bw of βC for 2 weeks and then received high-fat diet for 12 weeks while Group F received high-fat diet for 12 weeks and was treated with 150 mg/kg bw of βC for 2 weeks. At the end of the experimental period of 16 weeks. The animals were humanely sacrificed, and blood samples were collected by cardiac puncture, Leptin and insulin hormonal assay and oxidative enzyme markers were evaluated using the respective standard methods. The data were analyzed with SPSS version 20.0 at a value of $P<0.05$. The result showed the mean values of leptin, insulin and Malondialdehyde (MDA)were higher for group B when compared to the rest of the groups ($P<0.05$) but this increase was reversed following βC administration. Superoxide dismutase (SOD), Catalase (CAT), has decreased values in group B, these values were increased upon administration of βC ($P<0.05$). When compared to group A, group C showed increased value for the variables SOD, CAT and is equal to A in the variable insulin. Group F is greater than group D and E for the variable leptin, MDA and equal to group D ($P<0.05$). The findings of this present study suggest that β carotene extract at high dose can be very effective in treating oxidative stress, lipid peroxidation, insulin and leptin resistance.

Keywords: Beta-carotene; dietary fat; oxidative stress; lipid peroxidation; insulin; leptin resistance.

1. INTRODUCTION

Two important anorexigenic hormones that signal adiposity and nutritional status to the hypothalamus and inhibit food intake are leptin, produced by the white adipose tissue (WAT), and insulin, produced by the pancreatic B-cells. The circulating levels of leptin and insulin are often elevated in obesity. This initiates a rapid insensitivity to both hormones which is seen as a hallmark feature of obesity. In addition to the classical target tissues for insulin action, the liver, muscle and WAT, insulin also acts on the hypothalamus where it plays a pivotal role in maintaining energy balance, regulation of peripheral lipid and glucose metabolism [1]. In contrast, leptin is a potent regulator of food intake and energy expenditure [2]. However, both hormones act synergistically in the maintenance of peripheral glucose homoeostasis [3].

In animal models of genetic and diet-induced obesity, the activation of an inflammatory response in the hypothalamus results into a molecular and functional resistance to the adipostatic hormones, leptin and insulin, resulting in a defective control of food intake and energy expenditure [4,5,6]. The reversal of these effects can be achieved by distinct genetic and pharmacological approaches, aimed at inhibiting inflammatory signaling [5,6].

Besides the aforementioned factors, a previous study shown that dietary fatty acids can influence the susceptibility of cells to oxidative stress, due secondary to changes in the fatty acid composition of the cellular membranes [7]. Diets rich in fatty acids, mainly saturated fatty acids (SFA) and TFA, as well as carbohydrate- rich diets, favor an acute increase in insulin resistance independent of adiposity [8]. Thus, high SFA intake also promotes steatohepatitis directly by modulating hepatic triacylglycerol accumulation and oxidative activity, and indirectly by affecting insulin sensitivity and postprandial triacylglycerol metabolism [9].

The HF diet types and elevation of fasting glucose level are usually accompanied by a moderate to distinct increase in fasting plasma insulin levels. As with obesity, fish oil-fed animals generally do not develop such signs of systemic insulin resistance [10].

However, fat deposits can release triglycerides and free fatty acids into the blood causing hyperlipidaemia, which is a major factor for atherosclerosis. Meanwhile, studies have shown that continuous deposits of triglycerides (fats) can result to non-alcoholic fatty liver disease (NAFLD). NAFLD represents a wide spectrum of disorders, the hallmark of which is hepatic steatosis. NAFLD was considered a benign condition, but is now increasingly recognized as a major cause of liver-related morbidity and mortality [11]. Although the exact physiopathology of NAFLD is not fully understood [12], it was described as a “two hit model”. The first hit is supposed to be the increase of free fatty acids in hepatocytes, which results in decrease β-oxidation, which aggravates accumulation of fatty acids and insulin resistance. The second step include all mechanisms contributing to the generation of proinflammatory cytokines, oxidative species and there by enhances lipid peroxidation of the

hepatocyte membrane [13], and development of inflammation and fibrosis [14].

On the other hand, β- Carotene a tetra- terpene and lipid soluble plant pigment with molecular weight of 536.873 g, which is widely distributed in nature, is reported to have a profound beneficial biological effect including antioxidant property [15][16]. It is also known as provitamin A because, it is enzymatically transformed into retinal, and then finally into retinol (vitamin A) [15]. This is found in foods rich in vitamin A such as onions, peas, spinach and carrots. Carrot particularly is known to have rich beta-carotene content. Therefore, based upon the antioxidant and pro- vitamin A functions of carrot derived beta-carotene; it is biologically plausible to extend the human lifespan [16,17].

The beta-carotene exerts its antioxidant property by scavenging radicals (peroxyl), to prevent lipid peroxidation. Thus, increased uptake of carotenoids from fruits and vegetables lower the incidence of various diseases such as cataract formation, cardiovascular diseases, macular degeneration and cancer [15]. An appropriate consumption of Carotenoids and other such nutrients (phytochemicals) can lower the mortality rate globally [18,19,20]. The present study aimed to evaluate the positive effects of beta-carotene on oxidative stress, lipid peroxidation, and insulin and leptin resistance when wistar rats are fed with high dietary fat.

2. MATERIALS AND METHODS

2.1 Preparation of the Extract

Five hundred grams (500 g) of fresh carrots was purchased from Meat Market Abakaliki, Ebonyi State, Nigeria. The carrots were dried under shade for three weeks and were grounded into powder. The pulverized carrots were wrapped with Whatman filter paper and placed into the chamber of the Soxhlet extractor. Then, 250 ml of N-Hexane was added into the Soxhlet flask and placed on a heating mantle. The solvent was heated at 500 C, the Soxhlet extractor condenses the sample in the filter paper and the content of the carrots were extracted until clear solvent started coming out of the extraction chamber. The extract was concentrated using Water bath at 500 C and was then stored in the refrigerator.

2.2 Animal Procurement and Ethical Statement

Thirty (30) Male Wistar rats of average weight of 71.05 g were procured from and were maintained in the animal house of the Department of Biological Science, Federal University Ndufu-Alike Ikwo, Ebonyi State Nigeria. The animals were housed in metal cages, fed and water was allowed *ad libitum* with acclimatization period of two week. The animals were maintained at room temperature in a ventilated cage and were allowed free access to vital growers mesh and water. All protocols used were in accordance with the ethics and research guidelines of the institution.

2.3 High Fat Diet Preparation

Cow fat was purchased from Meat Market Abakaliki, Ebonyi State, Nigeria. The fat was dissolved by heating, collected in metal containers and stored in the refrigerator. High Fat Diet was prepared by mixing 60% of cow fat and 40% of normal rat chow as described by [8] and then was stored in the refrigerator.

2.4 Animal Experimentation

The rats used were randomly divided into 6 groups. Group A received normal rat chow for 14 weeks. Group B received high fat diet daily for 14 weeks. Group C received 300 mg/kg body weight (bw) of β- Carotene daily for 14 weeks. Group D received High fat diet (HFD) daily for 12 weeks and then 300 mg/kg bw of β- Carotene daily for 2 weeks. Group E received 300 mg /kg bw of β- Carotene daily for 2 weeks, and then HFD daily for 12 weeks. Group F received HFD daily for 12 weeks and then 150 mg/kg bw of β- Carotene daily for 2 weeks.

2.5 Biochemical Study

After 14 weeks, the animals were starved for 24 hours and then sacrificed by cervical dislocation. The rats were dissected and blood sample collected by cardiac puncture and centrifuged. Lipid peroxidation in the tissue was estimated calorimetrically by thiobarbituric acid reactive substances (TBARS) method [21] Catalase activity was measured according to the Aebi's method [22]. Superoxide dismutase activity was measured according to the method of Winterbourn [23] as described by Rukmini [24]. The concentrations of insulin and leptin in the

serum were measured respectively with rat insulin ELISA kit (Alpco Diagnostics, Salem, USA), rat leptin ELISA kit (BioVendor, Brno, Czech Republic).

2.6 Data Analysis

Data obtained were expressed as mean ± SD. The level of homogeneity among the group was tested using students t-test. A value of $p < 0.05$ was considered significant using Statistical Package for Social Sciences (version 20.0).

3. RESULTS

The biochemical study shows a significant ($P<0.05$) increase in the variables: insulin and leptin in animals in group B. These effects were reversed in beta carotene treated group, but not to a great extent in group F (beta carotene low dose) ($P<0.05$) for insulin and group D (high dose beta carotene) ($P<0.05$) for leptin. It also shows significant increase in Malondialdehyde (MDA) and a significant decreased Catalase (CAT) and Superoxide dismutase (SOD) in group B ($P<0.05$) although these effects were reversed significantly, in beta carotene treated group, for the whole parameter.

Table 1. T-test analysis showing the hormone leptin

Variable (groups)	Mean ± SD	*P* value	Remarks
A-B	159.37±0.00 390.12±11.46	0.0130	Significant
A-C	159.37±0.00 125.81±6.467	0.0004	Significant
B-D	390.12±11.46 250.21±15.70	0.0022	Significant
B-E	390.12±11.46 225.74±21.90	0.0038	Significant
B-F	390.12±11.465 305.27±5.84	0.0004	Significant

Values are expressed as Mean ± SD;N=5, P<0.05

4. DISCUSSION

Significant increase in the level of circulating insulin ($p< 0.05$) and in the level of circulating leptin respectively was observed in the animal given HF diet for 14 weeks, this is in agreement with the studies carried out by [25,26] respectively. These changes were reversed after the administration of beta carotene for 2 weeks but this reverse was not significant ($p<0.05$) when group A was compared to B for the variable insulin, although this reverse was significant ($p<0.05$) when group B was compared to D, B to E and B to F respectively. Studies have proven that oxidative stress is implicated in the pathophysiology [27,28,29] of neurodegenerative diseases. In addition to the above findings, our study showed that there was elevated oxidative stress and enhanced lipid peroxidation ($p<0.05$) in the animals fed with dietary fats.

Table 2. T-test analysis showing hormone insulin

Variable (Groups)	Mean ± SD	*P value*	Remarks
A-B	0.12±0.00 1.81±0.14	0.0720	Significant
A-C	0.12±0.00 0. 12±0. 01	0.0001	Significant
B-D	1.81±0.14 0.66±0.07	0.0172	Significant
B-E	1.81±0.14 0.43±0.12	0.0343	Significant
B-F	1.81±0.14 1.31±0.13	0.0011	Significant

Values are expressed as Mean ± SD; N=5, P<0.05

Table 3. T-test analysis showing SOD

Variable (groups)	Mean ± SD	*P-value*	Remarks
A-B	28.18± 2.91 13.79±2.30	0.0082	Significant
A-C	28.18± 2.91 32.78±3.73	0.0003	Significant
B-D	13.79±2.30 20.94±2.23	0.0023	Significant
B-E	13.79±2.30 22.46±1.48	0.0031	Significant
B-F	13.79±2.30 11.58±2.04	0.0007	Significant

Values are expressed as Mean ± SD; N=5, P<0.05.

In this present study, SOD and CAT activities were significantly decreased ($p<0.05$) in the group that received dietary accordance with [30] who reported that β- Carotene plays an important role in protecting cell membrane against oxidative damage because of its property of scavenging lipid and peroxyl radicals. Moreover, Shih [31] observed that β- the diet-induced oxidative stress in rats fed with high fat, a high-cholesterol diet, due in part to up-regulated antioxidant defenses. At the same time, the significant decrease in MDA level in the animals fed with dietary fats after β-Carotene administration herein is in agreement with [32] who reported that the addition of β-Carotene returned MDA levels back to normal diabetic rats.

Table 4. T-test analysis showing MDA

Variable (groups)	Mean ± SD	*P-value*	Remarks
A-B	0.57±0.06 3.12±0.01	0.0436	Significant
A-C	0.57±0.06 0.30±0.02	0.0065	Significant
B-D	3.12±0.01 1.86±0.21	0.0033	Significant
B-E	3.12±0.01 1.66±0.74	0.0074	Significant
B-F	3.12±0.01 2.82±0.27	0.0001	Significant

Values are expressed as Mean ± SD; N=5, P<0.05

Table 5. T-test analysis showing CAT

Variable (Groups)	Mean ± SD	*P-value*	Remarks
A-B	19.61±0.85 8.82±0.44	0.0100	Significant
A-C	19.61±0.85 20.42±0.01	0.0001	Significant
B-D	8.82±0.44 17.21±1.27	0.0065	Significant
B-E	8.82±0.44 15.81±0.57	0.0045	Significant
B-F	8.82±0.44 11.32±1.27	0.0006	Significant

Values are expressed as Mean ± SD; N=5, P<0.05

5. CONCLUSION

It could to conclude that beta Carotene has been proven to be an attractive target for therapeutics of the HF diet-induced fluctuation of insulin and leptin level. It also attenuated the diet-induced oxidative stress and lipid peroxidation.

CONSENT

It is not applicable.

ETHICAL APPROPVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lam TK, Schwartz GJ, Rossetti L. Hypothalamic sensing of fatty acids. Nature Neuroscience. 2005;8:579–584.
2. Farooqi IS, O'Rahilly S. Leptin: A pivotal regulator of human energy homeostasis. American Journal of Clinical Nutrition. 2009;89:980S–984S.
3. Koch C, Augustine RA, Steger J, Ganjam GK, Benzler J, Pracht C, Schwartz MW, Shepherd PR, Anderson GM, Grattan DR, Tups A. Leptin rapidly improves glucose homeostasis in obese mice by increasing hypothalamic insulin sensitivity, Journal of Neuroscience. 2010;30:16180–16187.
4. Carvalheira JB, Ribeiro EB, Arau´ jo EP, Gui `mara˜es RB, Telles MM, Torsoni M, Gontijo JA, Velloso LA, Saad MJ. Selective impairment of insulin signalling in the hypothalamus of obese Zucker rats, Diabetologia. 2003;46:1629 –1640.
5. Howard JK, Cave BJ, Oksanen LJ, Tzameli I, Bjørbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet- induced obesity in mice with haploinsufficiency of Socs3. Nature Medcine. 2004;10:734–738.
6. De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ, Velloso LA. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology. 2005; 146:4192–4199.
7. Nakbi A, Tayeb WA, Grissa A, et al. Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2,4- Dichlorophenoxyacetic acid-treated rats. Nutrition and Metabolism. 2010; 7(80).
8. Lima MLR, Leite LHR, Goida CR, Leme FOP, Couto CA, Coimbra CC, Leite VHR, Ferrari TCA. 2016 A novel Wistar Rat model of obesity-related nonalcoholic fatty liver disease induced by sucrose-rich diet. Journal of Diabetes Research. 2010;1-10.
9. Papandreou D, Rousso I, Malindretos P, Moudiou T, Pidonia I, Pantoleon A, Economou I, Mavromichalis I. Are saturated fatty acids and insulin resistance associated with fatty liver in obese children? Clinical Nutrition. 2008;27(2): 233–240.

10. Buettner R, Scholmerich J, Bollheimer LC, High fat diets: Modelling the metabolic disorders in human obesity in rodents, Obesity. 2007;15: 798-808.
11. Xiao J, Fai SK, Liong EC, Tipoe GL. Recent advances in the herbal treatment of non-alcoholic fatty liver disease. Journal of Tradititional and Complementary Medicine 2013;3:88–94.
12. Kaser S, Ebenbichler CF, Tilg H. Pharmacological and non-pharmacological treatment of non-alcoholic fatty liver disease. International Journal of Clinical Practce. 2010;64:968–983.
13. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biology and Medicine. 1991;11:81–128.
14. Day CP, James OF. Steatohepatitis: A tale of two “hits”? Gastroenterology. 1998;114: 842–845.
15. Zahra N, Alim-Un-Nisa Arshad F, Malik MS, Kalim I, Hina S, 91 Javed A, Inan SM. Comparative study of beta-carotene determination by various methods: A review. Bio Bulletin. 2016;2(1):96-106.
16. Zhoa LG, Qing-Li Z, Ji-Li Z, Hong-Lan L, Wei Z, Wei Guo T, Yong-Bing X. Dietary circulating beta carotene and risk of all-cause motality: A meta-analysis from prospective studys. Scientific Report 6, 2016;26983.
17. Prince MR, Prisoli JK. Beta carotene accumulation in the serum and skin, American Journal of Clinical Nurition. 1993;57(2):175-81.
18. El-Habit OHM, Saada HN, Azab KS, Abdel-Rahman, M.and El- Malah, D.F. The modifying effect of β-carotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats, Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2000;466: 179-186.
19. Elliott R. Mechanisms of genomic and nongenomic actions of carotenoids, Biochimica et Biophysica Acta (BBA)-Molecular Basis Disease. 2005;1740:147-154.
20. Khoo HE, Prasad KN, Kong KW, Jiang Y. Ismail A. Carotenoids and their isomers: color pigments in fruits and vegetables.Molecules. 2011;16:1710-1738.
21. Buege JA, Aust SD. Microsoma lipid peroxidation. Method in enzymology. Open Access and Academic Publisers. 1978; 52:302-310.
22. Aebi HE, Catalase. In: Bergmeyer HU, Bergmeyer J, Garbi M. (edS) Methods of enzymatic Analysis, Weinheim, Germany: Verlag Chemical, 3rd edition, volume III, 1983;273-286.
23. Winterbourne CC, Hawkins RE, Brain M, Carrel RW. The estimation of red cell superoxide dismutase activity. Journal of Laboratory Chemistry and Medicine. 1975;85:337-341.
24. Rukumimi MS, D’Souza B, D’Souza V. Supeoxide dismutase and catalase activity and their coleration with malondialdehyde in schizophrenic patients. India Journal of Clinical Biochemistry. 2004;19(2):114-118
25. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature Medcine. 1995;1:1155–1161
26. Adam TC, Toledo-Corral C, Lane CJ, Weigensberg MJ, Spruijt-Metz D, Davies JN, Goram MI. 2009.
27. Gandhi S. Abramov AY. Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev. 2012;2012:428010.
28. Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E. Mitochondria, oxidative stress and neurodegeneration. Journal of Neurol Science. 2012;322:254–262.
29. Patten DA, Germain M, Kelly MA, Slack, RS. Reactive oxygen species: Stuck in the middle of neurodegeneration. Journal of Alzheimers Disease. 2010;20(Suppl2): S357– S367.
30. El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: Protective role of vitamin E and β-carotene, Biochemistry. 2004; 42(10):1563-71.
31. Shih T, Vourvahis M, Singh M, Papay J. Pharmacogenetics: From bench science to the bedside. Therapeutic Innovation and Regulatory Science. 2008;42:503–513.

32. Berryman AM, Maritim AC, Sanders RA, Watkins JB. Influence of treatment of diabetic rats with combinations of pycnogenol, β-carotene, and alfa-lipoic acid on parameters of oxidative stress. Journal of Biochemical and Molecular Toxicology. 2004;18:345–352.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25417