Journal of Advances in Medicine and Medical Research

26(12): 1-7, 2018; Article no.JAMMR.42282
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Tumor Necrosis Factor Alpha (TNF-α) and Plasma Glucose Level Changes Due to Dietary Fat and Beta Carotene in Wistar Rats

Okechukwu, N. Getrude[1], Nweke, B. Ofobuike[1], Uchewa O. Obinna[1], Ezemagu, K. Uchenna[1] and Ibegbu, O. Augustine[1*]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Federal University Ndufu-Alike Ikwo (FUNAI), Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AOI designed the study and supervised all aspects of the study. Author ONG wrote the protocol and the first draft of the manuscript. Author NBO performed all the Laboratory studies. Author UOO managed the analyses of the study and author EKU managed the literatures. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/42282
Editor(s):
(1) Dr. E. Umit Bagriacik, Department of Immunology, Gazi University, Turkey.
Reviewers:
(1) Mark Agatemor, University of Nigeria, Nigeria.
(2) Tabe Franklin Nyenty, University of Ngaoundere, Cameroon.
Complete Peer review History: http://www.sciencedomain.org/review-history/25346

Original Research Article

Received 13th April 2018
Accepted 18th June 2018
Published 30th June 2018

ABSTRACT

Background: Beta-carotene (βC), an antioxidant present in fruits and vegetables responsible for the yellow coloration of these plants has been used for ages as remedies against different ailments. The present study was aimed at investigating tumor necrosis factor (TNF-α) and plasma glucose level changes due to dietary fat and β-carotene in Wistar rats.
Methods: Thirty (30) male Wistar rats were grouped into 6. Group A was 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 (bwt) of βC, Group D received high-fat diet for 12 weeks and was treated with 300 mg/kg bwt of βC for 2 weeks, Group E received 300mg/kg bwt of βC for 2 weeks and then high-fat diet for 12 weeks while Group F received high-fat diet for 12 weeks and then treated with 150

**Corresponding author: E-mail: austine.ibegbu@funai.edu.ng;*

mg/kg bwt of βC for 2 weeks. At the end of 14 weeks, the animals were sacrificed; blood samples collected and the livers were harvested, homogenised and assayed.
Results: The results showed a significant decrease in plasma glucose level in Groups C and D when compared to Groups A, B and F (P≤0.05). The result of TNF-α assay showed that the TNF-α was significantly higher in Group B compared with the other Groups and was significantly lower in Group E compared to the treatment Groups (P≤0.05).
Conclusion: The results of the present study suggest that β carotene can be effective in reducing blood glucose and that consumption of dietary fats sequel the increase in TNF-α and as such could predispose to dietary obesity and cancer in humans and animals.

Keywords: Beta-carotene; dietary fat; tumor necrosis factor; plasma glucose; wistar rats.

1. INTRODUCTION

Dietary fats are essential source of essential fatty acids, and it has been reported that uncontrolled intake of dietary fats *ad libitium* could lead to obesity, type 2 diabetes mellitus, dyslipo-proteinaemia, hypertension and metabolic syndrome including coronary heart disease, stroke and cancer [1,2]. It has been reported that rats fed with a diet containing 70% fat have developed obesity and elevated basal and postprandial blood sugar values [1,3]. Such high fat diet effect noticed when the fat content is well above 30% energy, have been subsequently specified for different animal strains, fat types, and diet lengths [4,5].

High-fat diet effects on blood glucose levels are described discrepantly, while normoglycemia, slight hyperglycaemia, and the development of type 2 diabetes have been reported with different diet regimes [1,6]. From the data published so far, one can conclude that prolonged feeding with both animal and plant fat-enriched diets would eventually lead to moderate hyperglycaemia in most rat and mouse strains of which the most widely used are Wistar rats and Sprague-Dawley rats [3,4]. With the High-Fat diet types, the elevation of fasting glucose levels is 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 [4,5].

However, fat deposits can release triglycerides and free fatty acids into the blood causing hyperlipidaemia, which is a significant factor for atherosclerosis. Meanwhile, studies have shown that continuous deposits of triglycerides can result to non-alcoholic fatty liver disease (NAFLD) [6,7]. NAFLD represents a wide spectrum of disorders, the hallmark of which is hepatic steatosis. NAFLD was considered a benign condition but is now increasingly recognised as a major cause of liver-related morbidity and mortality [7]. Although the exact physiopathology of NAFLD is not fully understood [6,8], it was described as a two step model. The first step is supposed to be the increase of free fatty acids in hepatocytes, which results in a decrease of β-oxidation, which aggravates accumulation of fatty acids and insulin resistance. The second step includes all mechanisms contributing to the generation of proinflammatory cytokines, oxidative species and thereby enhances lipid peroxidation of the hepatocyte membranes [6,9] and the development of inflammation and fibrosis [10]. The present study aimed to evaluate tumor necrosis factor alpha (TNF-α) and plasma glucose level changes due to dietary fat and β-carotene in Wistar rats.

2. MATERIALS AND METHODS

2.1 Preparation of the Extract

Fresh carrots were purchased from Meat Market in Abakaliki, Ebonyi State, Nigeria. The carrots were shade dried for three weeks and were grounded into powder. The pulverized carrots were wrapped in Whatman filter paper and placed into the Soxhlet extractor chamber. Then, 250 ml of N-Hexane was added into the Soxhlet flask and placed on a heating mantle. The solvent was heated at 50°C, the Soxhlet extractor condenses the sample in the filter paper and the content of the carrots was extracted until clear solvent came out of the extraction chamber. The extract was concentrated using Water bath at 50°C and was then stored in the refrigerator. The beta-carotene was used at 300 and 150 mg/kg body weight according to the methods of Wardi et al. [11] and Zahra et al. [12].

2.2 Animal Procurement

Thirty (30) Male Wistar rats of the average weight of 71.05 g were procured from and 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 weeks. Ethical approval was sort and obtained from the Federal University Ndufu Alike Ikwo Ethics and Animal Handling Committee with approval FUNAI/ETHAHCOM 2017/21.

2.3 High Fat Diet Preparation

Cow fat was purchased from Meat Market in Abakaliki, Ebonyi State, Nigeria. The fat was dissolved by heating, collected in metal containers and stored in the refrigerator. High Fat Diets were prepared by mixing 60% of cow fat and 40% of normal rat chow as described by Xiao et al. (2013) and then was stored in the refrigerator.

2.4 Animal Experimentation

The rats used for the study 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 300mg/kg body weight (bwt) of β-Carotene daily for 14 weeks. Group D received High-fat diet (HFD) daily for 12 weeks and then 300 mg/kg bwt of β-Carotene daily for 2 weeks. Group E received 300 mg/kg bwt 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 bwt of β-Carotene daily for 2 weeks as shown in Table 1.

2.5 Biochemical Study

After 14 weeks, the animals were starved for 24 hours and then sacrificed using cervical dislocation method. The rats were dissected, and cardiac puncture collected blood samples, centrifuged and the sera were used for the assay of blood glucose and TNF-α. The blood glucose measurements were determined by the glucose oxidase method using commercial kits from Randox Diagnostic Solutions and tumor necrosis factor-alpha (TNF-α) was assayed using ELISA kits (Alpco Diagnostics, Salem, USA), according to the manufacturer's instruction. This assay employed antibody specific for rat TNF-alpha coated on a 96-well plate.

2.6 Data Analysis

Data obtained were expressed as mean ± SD. The level of homogeneity among the groups was tested using one way Analysis of Variance (ANOVA). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test. A value of $p < 0.05$ was considered significant using Statistical Package for Social Sciences (version 20.0).

Table 1. Experimental Protocol and Treatment

Gp	Status	Rats	Treatment duration
A	Negative Control	5	Normal rat chow and 5 ml/kg body weight of water daily for 14 weeks
B	Fat-Diet alone	5	High Fat Diet Daily for 14 weeks
C	β-Carotene alone	5	300 mg/kg body weight of β-Carotene daily for 14 weeks and normal rat chow
D	Fat-Diet before High dose β-Carotene	5	Fat Diet and 300 mg/kg body weight of β-Carotene daily for 14 weeks
E	High dose β-Carotene before Fat-Diet	5	300 mg/kg body weight of β-Carotene and Fat Diet daily for 14 weeks
F	Fat-Diet before Low dose β-Carotene	5	Fat Diet and 150 mg/kg body weight of β-Carotene daily for 14 weeks

3. RESULTS

The result of the biochemical studies showed a decrease in plasma glucose level in Groups D and C when compared to Groups A, B and F. The result also showed that the TNF-α was higher in Group B when compared with the other groups and lower in Group E when compared to the treatment groups. These results showed that the serum levels of Plasma Glucose, as well as the levels of the adipocytokine marker (TNF-α), were significantly increased in the animals in Group B when compared with the animals in the Control Group (Group A) ($P<0.05$). However,

serum levels of TNF-α were significantly decreased (P<0.05) in the animals treated with β-Carotene (Group D) and in animals in Group F when compared with the animals in Group B (P<0.05) as shown in Tables 2, 3 and 4. The result showed there was no significant increase in the parameters in Group C when compared with the animals in the Control Group (Group A) while there was no significant difference in the parameters in the animals in Group E when compared with the animals in Group D.

Table 2. The effect of HFD and βC on the plasma glucose, adipocytokine markers

Group	Plasma Glucose (g/dl)	TNF-α (mg/ml)
A	247.37±1.36	5.30 ± 0.20
B	255.27 ±1.46	6.17 ± 0.08
C	221.87 ± 1.88	5.37 ± 0.08
D	236.87 ±9.25	5.9 1± 0.84
E	228.20 ± 4.33	5.17 ± 0.84
F	252.89 ± 4.60	5.76 ± 0.77

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

Table 3. t-test TNF-α

Variable (Groups)	Mean ± SD	P-value	Remarks
A-B	5.30 ± 0.20 6.17 ± 0.08	0.0001	Significant
A-C	5.30 ± 0.20 5.37 ± 0.08	0.0001	Significant
B-D	6.17 ± 0.08 5.9 1± 0.84	0.0001	Significant
B-E	6.17 ± 0.08 5.17 ± 0.84	0.0001	Significant
B-F	6.17 ± 0.08 5.76 ± 0.77	0.0001	Significant

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

4. DISCUSSION

The results of the present study have shown significant increase in serum Plasma Glucose as well as tumor necrosis factor alpha (TNF-α) levels in the group of animals fed with dietary fats for 14 weeks. However, the elevated serum lipid profiles and the adipocytokine marker (TNF-α) were reversed upon the treatment with beta-carotene for 2 weeks. This was in accordance with the study by Chung et al. [13], who observed that most of the carotenoids appeared to be inversely correlated to fat mass, suggesting that during obesity, carotenoids are sequestered in adipose tissues, decreasing their plasma concentrations.

Table 4. t-test analysis showing blood glucose

Variable (Groups)	Mean ± SD	P-value	Remarks
A-B	28.18±1.19 13.79±0.94	0.0001	Significant
A-C	28.18±1.19 32.78±1.52	0.0001	Significant
B-D	13.79±0.94 20.94±0.91	0.0001	Significant
B-E	13.79±0.94 22.46±0.61	0.0001	Significant
B-F	13.79±0.94 11.58±0.83	0.0001	Significant

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

Moreover, Van Helden et al. [14] have shown that anti-obesity effect of β-Carotene has been demonstrated to be linked to its pro-vitamin A effects which is more of the reduction in the oxidative stress induced activities. Furthermore, the findings of the present study have showed that there was a significant increase in the plasma glucose levels when animals in the Control Group were compared to animals in Group C, suggesting the unfolded protein response and the disruption of the endoplasmic reticulum homeostasis [6]. These results were in agreement with the results of Ozcan et al. [15]; Nakatani et al. [16]; Ozawa et al. [17] and Milanski et al. [18]. The significant difference was also noticed when group A was compared with Group B, Group A with C, Group B with D, B with E and finally B with F for the variable adipocytokine markers (TNF-α) levels in animals fed with dietary fats for 14 weeks. This is in agreement with Milanski et al. [18], who observed that the Consumption of High-Fat diets for 14 weeks led to a significant increase in the expression of the inflammatory cytokines TNF- α. Morin et al. [19] and Hariri & Thibault [5] had observed that adipose tissue-derived tumor necrosis factor-alpha (AT-TNF) has been associated with genetic models of insulin resistance and obesity. Presently it is unknown if the secreted AT-TNF protein is bioactive or whether it can be increased by environmentally induced obesity [12,19]. Thus, data generated from the present study suggested that high fat diets and obesity can influence TNF bioactivity and secretion but in a fat specific manner [19, 7]. High levels of adipose tissue-derived tumor necrosis factor-alpha (AT-TNF) mRNA and protein have previously been associated with genetic models of obesity and insulin resistance and as such there are endogenous TNF

inhibitors which is hitherto unknown and can lead to the AT-TNF activity increase [5,19,20,21]. The resultant effect of this inhibition is to increase the activity of the adipose tissue tumor necrosis which can lead to severe consequences such obesity, uncontrollable blood glucose increase and chronic heart diseases [5,22].

It has been shown that obesity is associated with an overexpansion of adipose tissue, along with increases in blood pressure, glycemia, inflammation, and thrombosis [22,23,24]. Research to develop nutritional interventions to prevent or treat obesity and its associated diseases is greatly needed and as such previously, researches had demonstrated the ability of eicosapentaenoic acid (EPA) to prevent high-fat (HF) diet-induced obesity, insulin resistance, and inflammation in mice [22,23,24].

Research has shown that mature rats had more fat mass when compared to the young rats but the whole body insulin resistance as estimated by fasting plasma insulin level was similar to those of young rats [6,20]. Obesity has been associated with many pathological conditions and as such the tumor Necrosis Factor-α (TNF-α) which is one of the key mediators of inflammation involved in the obesity-related insulin resistance development [7,19]. The present report has shown to have a relationship between inflammatory changes and the body weight, with particular reference to TNF-α. Genetic polymorphisms and epigenetic factors, such as diet, could affect TNF-α activity and as such the TNF-α is associated with obesity, anorexia and cachexia [7,25]. Despite the role of TNF-α in obesity-related diseases anti-TNF-α antibody therapy is associated with an increase in adiposity and as such the results suggest that inflammation is more likely a consequence rather than a cause of obesity [3,25]. The adipose tissue TNF protein levels were shown to be higher in young rats, suggesting that these animals may secrete an inhibitor that reduces AT-TNF activity while AT-TNF activity was increased in mature animals in relation to adipose cell size. Other research has shown that inflammation and body weight, with particular reference to TNF-α. Genetic polymorphisms and epigenetic factors, such as diet, could affect TNF-α activity [25,26].

The results had suggested that inflammation is more likely a consequence rather than a cause of obesity and that obesity is now considered as a state of chronic low-grade inflammation as shown by Moussavi et al. [26] and Peluso et al. [27]. Adipose tissue, apart from its classical role as an energy storage depot, is also a major endocrine organ secreting many factors, whose local and circulating levels are affected by the degree of adiposity. Obesity leads to infiltration of the expanded adipose tissue by macrophages and increased levels in proinflammatory cytokines [3,21]. The first indication for increased cytokine release in obesity was provided by the identification of increased expression of TNF-alpha, a proinflammatory cytokine, in the adipose tissue of obese mice [21,27]. TNF-alpha is expressed in and secreted by adipose tissue, its levels correlating with the degree of adiposity and the associated insulin resistance [3,28]. Thus, it can be considered that obesity corresponds to a sub-clinical inflammatory condition that promotes the production of pro-inflammatory factors involved in the pathogenesis of insulin resistance.

5. CONCLUSION

It has been shown that beta-carotene was effective in reducing the levels of plasma glucose level and level of the TNF-α induced by high dietary fat consumption in the experimental rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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. Javier A, Carmen PR. Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: A systematic review. British Journal of Nutrition. 2012;107:8-22.
2. Wolfram G, Bechthold A, Boeing H, Ellinger S, Hauner H, Kroke A, et al. Evidence-based guideline of the German Nutrition Society: Fat intake and prevention

of selected nutrition-related diseases. Annals of Nutrition and Metabolism. 2015; 67(3):141-204.

3. Huang XF, Xin X, McLennan P, Storlien L. Role of fat amount and type in ameliorating diet-induced obesity: Insights at the level of hypothalamic arcuate nucleus leptin receptor, neuropeptide Y and pro-opiomelanocortin mRNA expression. Diabetes, Obesity and Metabolism. 2004; *6*(1):35-44.
4. Buettner R, Scholmerich J, Bollheimer LC. High fat diets: Modelling the metabolic disorders in human obesity in rodents. Obesity. 2007;15:798-808.
5. Hariri N, Thibault L. High-fat diet-induced obesity in animal models. Nutrition Research Reviews. 2010;23(2):270-299.
6. Ichihara S, Yamada Y. Genetic factors for human obesity. Cellular and Molecular life Sciences. 2008;65(7-8):1086-1098.
7. 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.
8. Kaser S, Ebenbichler CF, Tilg H. Pharmacological and non-pharmacological treatment of non-alcoholic fatty liver disease. International Journal of Clinical Practice. 2010;64:968–983.
9. 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.
10. Day CP, James OF. Steatohepatitis: A tale of two “hits”? Gastroenterology. 1998; 114:842–845.
11. Wardi J, Reifen R, Aeed H, Zadel L, Avni Y, Bruck R. Beta-Carotene Attenuates Experimentally Induced Liver Cirrhosis in Rats. IMAJ. 2001;3:151-154.
12. Zahra N, Alim-Un-Nisa, Arshad F, Malik MS, Kalim I, Hina S, et al. Comparative Study of Beta-Carotene Determination by various Methods: A Review. Bio Bulletin. 2016;2(1):96-106.
13. Chung HK, Kang B, Lee JH, Shim JY, Park S, Lee SH, et al. Increased arterial stiffness is associated with reduced plasma levels of beta-carotene in treated hypertensive patients with type 2 diabetes mellitus, Nutrition, Metabolism and Cardiovascular Disease. 2009;6:e9–e11.
14. Van Helden YG, Godschalk RW, Swarts HJ, Hollman PC, van Schooten FJ, Keijer J. Beta-carotene affects gene expression in lungs of male and female Bcmo1 mice in opposite directions. Cellular and Molecular Life Sciences. 2011;68:489–504.
15. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004;306:457–461
16. Nakatani Y, Kaneto H, Kawamori D, Yoshiuchi K, Hatazaki M, Matsuoka TA, et al. Involvement of endoplasmic reticulum stress in insulin resistance and diabetes. Journal of Biological Chemistry. 2005; 280:847–851.
17. Ozawa K, Miyazaki M, Matsuhisa M, Takano K., Nakatani Y, Hatazaki M, et al. The endoplasmic reticulum improves insulin resistance in type 2 diabetes. Diabetes. 2005;54:657–663.
18. Milanski M, Degasperi G, Coope A, Morari, J, Dennis R, Mltsukumo D, et al. Saturated fatty acid produces an inflammatory response predominantly through the activation of TLR4 signalling in the hypothalamus: implication for pathogenesis of obesity. The Journal of Neuroscience. 2009;29(2):359-70.
19. Morin CL, Eckel RH, Marcel T, Pagliassotti MJ. High fat diets elevate adipose tissue-derived tumor necrosis factor-alpha activity. Endocrinology. 1997;138(11): 4665-71.
20. Morin CL, Pagliassotti MJ, Windmiller D, Eckel RH. Adipose tissue-derived tumor necrosis factor-alpha activity is elevated in older rats. J Gerontol A Biol Sci Med Sci. 1997;52(4):B190-5.
21. Ghibaudi L, Cook J, Farley C, van Heek M, Hwa JJ. Fat intake affects adiposity, comorbidity factors, and energy metabolism of sprague-dawley rats. Obesity Research. 2002;10:956–63.
22. Sullivan EM, Pennington ER, Sparagna GC, Torres MJ, Neufer PD, Harris M. et al. Docosahexaenoic acid lowers cardiac mitochondrial enzyme activity by replacing linoleic acid in the phospholipidome. J Biol Chem. 2018;12;293(2):466-483.
23. Jayarathne S, Koboziev I, Park OH, Oldewage-Theron W, Shen CL, Moustaid-Moussa N. Anti-Inflammatory and Anti-Obesity Properties of Food Bioactive

Components: Effects on Adipose Tissue. Prev Nutr Food Sci. 2017;22(4): 251-262.

24. Holt PR, Alemán JO, Walker JM, Jiang CS, Liang Y, de Rosa JC, et al. Docosahexaenoic acid supplementation is not anti-inflammatory in adipose tissue of healthy obese postmenopausal women. Int J Nutr. 2017;1(4):31-49.
25. Tzanavari T, Giannogonas P, Karalis KP. TNF-alpha and obesity. Curr Dir Autoimmun. 2010;11:145-56.
26. Moussavi N, Gavino V, Receveur, O. Could the quality of dietary fat, and not just its quantity, be related to the risk of obesity? Obesity. 2008;16(1):7-15.
27. Peluso I, Palmery M. The relationship between body weight and inflammation: Lesson from anti-TNF-α antibody therapy. Hum Immunol. 2016;77(1):47-53.
28. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, et al. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2010;17(1):4-12.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25346