



EVALUATION OF PLASMA GLUCOSE, INSULIN AND GLYCATED HAEMOGLOBIN LEVELS AMONG MALE DAILY BREAD CONSUMERS

Clinical Biochemistry

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ABSTRACT

Bread is highly nutritious and consumed in many ways by most. This study was carried out to determine the effect of daily bread consumption on serum insulin, glycated hemoglobin, and plasma glucose level among male daily bread consumers. Thirty male students were randomly selected and fed with bread for 21 days. Their pre and post-blood samples were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) method for insulin and glycated hemoglobin whereas glucose estimation was done using the glucose oxidase method. Pre and post Body Mass Index (BMI) were also determined. Results showed that there was a significant decrease in the BMI of the participants in daily post bread eaters (21.80 ± 2.21) compared to baseline (20.04 ± 2.36) ($p < 0.05$) while there were no significant differences in the mean levels of fasting plasma glucose, glycated hemoglobin, and insulin in daily post bread eaters (4.94 ± 0.24 , 5.88 ± 0.63 , and 6.43 ± 3.22 respectively) ($p > 0.05$). Likewise, no significant association existed between the parameters studied. Therefore no significant alteration was observed in the parameters studied except for the decreased BMI observed after bread consumption among the participants which could be attributed to the increase in academic stress activities experienced by participants as the studied participants were students at examination during the post sample collection.

KEYWORDS

Bread, plasma glucose, glycated hemoglobin, insulin, diabetes mellitus.

INTRODUCTION

Glucose, a fundamental source of cellular energy, is released by the breakdown of endogenous glycogen stores that are primarily located in the liver. Glucose is also released indirectly in the muscle through intermediary metabolites. These whole-body energy stores are replenished from dietary glucose, which, after being digested and absorbed across the gut wall, is distributed among the various tissues of the body [1]. Although glucose is required by all cells, its main consumer is the brain in the fasting or "postabsorptive" phase, which accounts for approximately 50% of the body's glucose use. Another 25% of glucose disposal occurs in the splanchnic area (liver and gastrointestinal tissue), and the remaining 25% takes place in insulin-dependent tissues, including muscle and adipose tissue [2]. Approximately 85% of endogenous glucose production is derived from the liver, with glycogenolysis (conversion of glycogen to glucose) and gluconeogenesis (glucose formation) contributing equally to the basal rate of hepatic glucose production. The remaining ~15% of glucose is produced by the kidneys [2]. Glucagon, which also plays a central role in glucose homeostasis, is produced in response to low normal glucose levels or hypoglycemia and acts to increase glucose levels by accelerating glycogenolysis and promoting gluconeogenesis. After a glucose-containing meal, however, glucagon secretion is inhibited by hyperinsulinemia, which contributes to the

suppression of hepatic glucose production and maintenance of normal postprandial glucose tolerance [3]. The hormone amylin contributes to the reduction in postprandial glucagon, as well as modest slowing of gastric emptying.

Bread is highly nutritious and eaten in one form or another by nearly every person on earth. It is the most commonly grown grain worldwide with approximately 750 million metric tons produced annually [4] and it makes up approximately 10% of the adult caloric intake [5]. Bread is solid foam; typical bread has a crust with the characteristic golden brown color and white crumbs. Bread has a short life due to its chemical composition and moisture content compared to other baked products. Nutritionally, bread is categorized as a source of grains in the food pyramid and is a good source of carbohydrates and nutrients such as magnesium, iron, selenium, B vitamins, and dietary fiber [6]. Several ingredients are used for bread making with the mandatory ingredients including flour, water, yeast, and salt. Other optional ingredients such as sugar, fat, milk, emulsifier, fruits, flavors, etc may be added [7].

Diabetes patients are increasing by population growth, aging, urbanization, increasing prevalence of obesity, and physical inactivity [8]. Lipid profile abnormality, increasing blood glucose and glycated hemoglobin (HbA1c), and obesity are the most risk factors for diabetes

and lead to complications of this disease [6]. An important factor to control and manage diabetes is dietary habits and nutrition. A novel approach in dietary habits is the glycemic index (GI) and glycemic load (GL). Studies have shown that soluble and non-soluble fiber and a low GI diet can control glycemic response [7]. Refined carbohydrates and grains without bran have higher GL and GI than whole grains and harm health. According to findings, soluble dietary fibers reduce postprandial glucose, but the efficacy of whole grains on chronic diseases such as diabetes and ischemic heart disease (IHD) and mortality is controversial. Bread is the most important source of a carbohydrate diet. Since bread is low cost and is the most of Nigeria's diet and approximately more people consume it on all days of the week, investigation of the effect of bread on glycemic response is important. Findings of the consumption of both whole and refined grains in recent studies are inconsistent. HbA1c is formed by irreversible nonenzymatic glycosylation of hemoglobin (Hb) exposed to glucose in the bloodstream [8]. Specifically, through the Maillard reaction, glucose attaches to the NH₂-terminal valine of the β -chain of Hb [9]. HbA1c concentration in the blood depends on two parameters: the life span of red blood cells (RBCs) (~120 days) and the glucose concentration in the blood [9]. Several organizations around the world have recommended that diabetes mellitus can be diagnosed using one of the following tests: glycatedhaemoglobinA1c (HbA1c), fasting plasma glucose (FPG), or oral glucose tolerance test (OGTT). The advantages of using HbA1c for the diagnosis of diabetes include its robust stability at ambient temperature, and the ability to use non-fasting, random blood samples, allowing this test to be performed at any time of the day [10].

In Nigeria, bread is believed to be the second most consumed food after rice [1]. Due to frequent lectures and the large curriculum of the medical school, most students find it difficult to cook daily and thus resort to consuming fast food, especially bread as a result of its affordability and availability. Bread when consumed is broken down into simple sugars which provide energy for the body that is required for vital functions of the body. The carbohydrate contained in the bread breaks down into sugar quickly which then floods into the bloodstream, raising the blood glucose level. When it happens, it triggers the body to release insulin to mop it up. Excessive release of insulin into the bloodstream can make the cells insulin resistant, making it difficult to control sugar levels thereby resulting in increased serum insulin level, glycosylation of hemoglobin, hyperglycemia, diabetes mellitus, weight gain, and increased LDL among others [11]. Diabetes mellitus, cardiovascular diseases, and hypercholesterolemia, among other diseases, are prevalent diseases in this present day and they pose serious challenges to human health [12,13]. Bread has been among the most consumed food in Nigeria contain mostly carbohydrate which is readily broken down into glucose making blood sugar level increase rapidly leading to a condition known as hyperglycemia which characterizes diabetes mellitus. Insulin and glucagon are the regulatory hormones for maintaining glucose levels in the blood and therefore, evaluation of insulin and glycated hemoglobin will help to give insight into specific health education needed to prevent the occurrence of these diseases as a result of daily consumption of bread.

MATERIALS AND METHODS

Study site

This research was carried out in the College of Health Sciences, Nnamdi Azikiwe University, Okofia, Nnewi Campus, Anambra State.

Research Design

This is an experimental study that was designed to evaluate the serum levels of insulin and glycated hemoglobin in response to changes in plasma glucose levels following daily bread consumption among male students of Nnamdi Azikiwe University, Nnewi Campus, Nigeria. A total of thirty (30) healthy male students of College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi campus who were aged between 18 and 30 years were randomly recruited to serve as both test and control groups. Participants (students of the College of Health Sciences) were properly informed about the study and only those who give their consent were recruited for the study. Thereafter, 6mls each of baseline samples (after an overnight fast) was collected from the participants on day 0 as control samples, and levels of glucose, insulin, and glycated hemoglobin were evaluated. Subsequently, in addition to their normal diet, each of the subjects was given 100g of Yale bread for 21 days. After an overnight fast, 6mls of post-research (test) samples were collected on the 22nd day and the

levels of glucose, insulin, and glycated hemoglobin were re-evaluated.

Inclusion Criteria

This study was limited only to healthy adult male students of Nnamdi Azikiwe University, Nnewi Campus.

Exclusion Criteria

Individuals with diabetes mellitus, smokers, alcoholics, individuals on diabetic drugs as well as female students were excluded from the present study.

Ethical Consideration

The ethical approval for this research was obtained from the ethical committee of Nnamdi Azikiwe College of Health Sciences, Nnewi Campus.

Methods of Laboratory Analysis

All the reagents and kits were obtained commercially and the manufacturer's standard operating procedures were strictly observed.

Determination of insulin and glycated hemoglobin level

A Sandwich ELISA assay was used for the determination of insulin^[14] and glycated hemoglobin (HbA1c)^[15] levels respectively.

Determination of plasma glucose (FBS)

Plasma glucose level was determined using the glucose oxidase method as described by^[16].

Statistical Analysis

Statistical package for social science (SPSS) version 22.0 was used for the analysis of the data obtained. Data collected were subjected to statistical analysis using student t-test and Pearson correlation. Values were deemed significant at $p < 0.05$.

RESULTS

Table 1 shows the Levels of body mass index (BMI), Fasting Plasma Glucose (FPG), Glycated Hemoglobin (HbA1C), and Insulin in Pre and Post Daily Bread Intake. The mean BMI level of the study participants in post 21day daily bread eaters was significantly decreased (21.80 ± 2.21) compared with the baseline (22.04 ± 2.36) ($p < 0.05$) while there was no significant difference in the mean level of fasting plasma glucose, glycated hemoglobin, and insulin in daily post bread eaters respectively ($p > 0.05$).

Also, Table 2 showed that there was no significant association between the parameters studied respectively [$p > 0.05$].

Table 1: Levels of Body Mass Index (BMI), Fasting Plasma Glucose (FPG), Glycated Hemoglobin (HbA1C), And Insulin in Pre and Post Daily Bread Intake.

Parameters	Pre	Post	t-test	p-value
BMI (Kg/m ²)	22.04±2.36	21.80±2.21	3.215	0.003*
FPG (mmol/L)	4.88±0.23	4.94±0.24	-1.853	0.074
HbA1C (%)	5.92±0.77	5.88±0.63	0.364	0.719
Insulin (ng/ml)	6.58±3.64	6.43±3.22	0.713	0.482

*Statistically significant at $p < 0.05$.

Table 2: Levels of Association between Glucose, GlycatedHaemoglobin (HbA1c), Insulin, and Body Mass Index (BMI).

Parameters		Glucose	HbA1C	Insulin	BMI
Glucose	R	1	-0.081	-0.165	-0.067
	p-value	-	0.671	0.383	0.726
HbA1C	R	-0.081	1	0.179	-0.124
	p-value	0.671	-	0.343	0.513
Insulin	R	-0.165	0.179	1	0.242
	p-value	0.383	0.343	-	0.198
BMI	R	-0.067	-0.124	0.242	1
	p-value	0.726	0.513	0.198	-

DISCUSSION

Diabetes mellitus (DM) is a chronic metabolic disease resulting from the diminished or absent secretion of insulin or due to reduced tissue

sensitivity to insulin. It has a global health burden, especially in developing countries like Nigeria [13]. Diabetes is a global endemic with rapidly increasing prevalence in developing countries such as Nigeria and Type 2 diabetes mellitus is one of the leading causes of preventable death in the world, with stroke, myocardial infarction, and other cardiovascular diseases being the most common causes of death for adults with diabetes [17], with its main pathophysiological features been impaired insulin secretion and increased insulin resistance [18]. Many factors like less glycemic control, smoking, high blood pressure, elevated cholesterol levels, obesity, and lack of regular exercise are risk factors that accelerate the deleterious effects of diabetes [19]. Diabetes mellitus enhanced by modifiable factors such as behavioural risk factors; tobacco use; unhealthy diet and obesity, physical inactivity and harmful use of alcohol [20]. The present study evaluated the plasma glucose, insulin, and glycated hemoglobin levels among male daily bread consumers. In this study, there was a significant decrease in the BMI of the participants in daily post bread eaters compared with baseline. It may be the impact of academic stress on the participants undergoing examination. There are many mechanisms in which stress can lead to changes in body weight thus giving rise to a lower BMI. It could result from changes or alterations in health behaviors [21,22]

Previous studies have shown that consumption of bread is associated with higher BMI (Asiki *et al.*, 2018). Men consumed three slices of bread per day on average slightly higher than women who consumed about two slices per day. A cohort study in Spain showed an association between weight gain and consumption of white bread but not with wholemeal bread [23]. The present study revealed no significant alteration in mean plasma glucose level in daily post-bread eaters compared with the baseline value obtained. It contradicts the report of [24] whose results indicated that consumption of Jizi439 steamed bread resulted in significantly lower plasma glucose in both healthy and diabetic subjects [25]. Also, [26] found that higher total bread intake and total bread-rice intake were associated with FBG in type 2 diabetic patients but disagrees with the present finding. The current result; may be a result of disparity in the composition make-up of the bread utilized for the study or differences in the duration of the study. In the same vein, the mean glycated hemoglobin level did not differ significantly between post-daily bread eaters and baseline values obtained. Also, there was no significant difference in the mean insulin level in daily post-bread eaters compared with baseline [27], documented significant reductions in postprandial glucose and insulin level in contrast with the current report and suggested, that the consumption of whole-grain foods is able to improve acutely the postprandial glucose and insulin homeostasis compared to similar refined foods in healthy subjects [27]. Further, [27] and colleagues found a positive association between white bread consumption and HbA1C levels [27]. It may be attributable to the disparity in the quantity and nature of bread administered to the participants.

CONCLUSION

The present study revealed a significant decrease in the mean BMI with no statistically significant alterations in mean plasma glucose, HbA1C and insulin levels after bread consumption compared with baseline values. However, decreased BMI observed after bread consumption among the participants could be attributed to academic stress activities as the studied participants were students at examination during the post sample collection while other findings regarding HbA1C and insulin levels may be a result of the disparity in the nature of bread administered, quantity or duration of the study. Further studies with a longer time frame and a larger sample size may be studied to unravel the mechanism responsible for the present findings.

REFERENCES

- Ndichu J, Blohmke J, Kemp R, Adeoti J, Obayelu AE. The adoption of energy efficiency measures by firms in Africa: case studies of cassava processing in Nigeria and maize milling in Kenya. *Innov Dev* 2015, 5(2): 189-206.
- Akinwumi, A. Unleashing the potentials of agriculture for accelerated food security and economic growth in Nigeria. 63rd Foundation Day of the University of Ibadan, Nigeria, 2012.
- United States Department of Agriculture (2016). World Agricultural Supply and Demand Estimates. <https://www.usda.gov/oce/commodity/wasde/>.
- Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized nutrition by prediction of glycemic responses. *Cell* 2015; 163:1079-1094.
- Dietary Guidelines for Americans: U.S. Department of Agriculture, U.S. Department of Health and Human Services, 2010.
- Food and Agriculture Organization of the United Nations, Cassava for food and energy: (2008). Investing in cassava research and development could boost yields and industrial uses.
- Sacks DB. Hemoglobin A1c in diabetes: Panacea or pointless? *Diabetes* 2013; 62:41-43.
- Samuel R. The discovery of glycated hemoglobin: A major event in the study of nonenzymatic chemistry in biological systems. *Ann. N. Y. Acad. Sci.* 2005; 1043:9-19.
- Cohen RMF, Khera RS, Smith PK, Lindsell EP, Ciralo CJ, Palascak PJ, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. *Blood* 2008; 112:4284-4291.
- Sacks DB. A1C Versus Glucose Testing: A Comparison. *Diabetes Care* 2011; 34, 518-523.
- O'Connor, A. (2012). Bread consumption in the UK: what are the main attitudinal factors affecting current intake and its place in a healthy diet? *Br Nutr Foundation Nutr Bull* 2012; 37:368-379.
- Solomon M, Bamossy G, Askegaard S, Hogg MK. *Consumer Behaviour, A European Perspective*. 3rd Edition, Harlow, England, Pearson Education Limited, 2006.
- Ogbodo EC, Okafor CC, Ogah HGO, Ezeugwunne IP, Igwebuobi CF, Okezie AO, et al. Thyroid Hormone Profiling and Enzymatic Antioxidant Status in Diagnosis and Management of Type-II-Diabetes Mellitus: A Review of Literature. *World J Pharmaceut Life Sci* 2019; 5(12):06-21.
- Poovarasam S, Review on Analytical Methods for the Determination of Insulin. *Int. J Res Pharm Sci Int. J Pharm Sci Rev Res* 2021; 12(2):1428-1436
- R. A. Analike, A. C. Ihim, S. C. Iweanya, E. C Ogbodo, C. E. Onah, A. L. Asomugha, A. A. Amah, P. C. Obi, E. I. Nwobodo, C. O. Timothy (2019). Assessment of glycated haemoglobin, total protein and albumin levels in patients with type 2 diabetes mellitus visiting NAUTH, Nnewi. *Indian Journal of Pathology and Oncology*, 6(4):700-703
- Ihim, A.C., Analike, R. A., Chibuike, S., Ogbodo, E. C., Asomugha, A. L., Obi, P. C., Amah, A. K., Ugwu, M.C., Nwobodo, E. I. (2019). Evaluation of estimated glomerular filtration rate (eGFR), serum creatinine, urea and electrolytes profile levels in diabetic patients attending medical outpatient clinic in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. *Journal of Clinical Biochemistry and Research*, 6(2):217-221
- Newman JD, Schwartzbard AZ, Weintraub HS. Primary prevention of cardiovascular disease in diabetes mellitus. *J American Coll Cardiol* 2017; 70:883-893.
- Kaku K. Pathophysiology of Type 2 Diabetes and Its Treatment Policy. *Japan Med Assoc J* 2010; 53(1): 41-46.
- Elfaki ME, Raheem AM, Ahmed ES. Evaluation of Lipid Metabolism among Sudanese Patients with Type 2 Diabetes Mellitus. *Intern J Pure Appl Sci Technol* 2014; 23(1):28-33.
- WHO (2018). Non-communicable diseases (NCD) country profile. https://www.who.int/nmh/countries/nga_en.pdf?ua=1
- Tantawy SA, Abdul Rahman A, Abdul Ameer M. The relationship between the development of musculoskeletal disorders, body mass index, and academic stress in Bahraini University students. *Korean J Pain* 2017; 30(2):126-133.
- Kupeli N, Norton S, Chilcot J, Campbell IC, Schmidt UH, Troop NA. Affect systems, changes in body mass index, disordered eating and stress: an 18-month longitudinal study in women. *Health Psychol Behavioral Med* 2017; 5(1):214-228.
- Asiki G, Mohamed SF, Wambui D, Wainana C, Muthuri S, Ramsay M, Kyobutungi C. Sociodemographic and behavioural factors associated with body mass index among men and women in Nairobi slums: AWI-Gen Project. *Global Health Action* 2018; 11:1470738
- Bautista-Castano I, Sánchez-Villegas A, Estruch R, et al. Changes in bread consumption and 4-year changes in adiposity in Spanish subjects at high cardiovascular risk. *Br J Nutr*. 2013; 110:337-346.
- Su-Que L, Ya-Ning M, Xing-Pu L, Ye-Lun Z, Guang-Yao S, Hui-Juan M. Effect of consumption of micronutrient enriched wheat steamed bread on postprandial plasma glucose in healthy and type 2 diabetic subjects. *Nutr J* 2013; 12:64.
- Akhoundan M, Shadman Z, Jandaghi P, Aboeeraad M, Larijani B, Jamshidi Z, et al. The Association of Bread and Rice with Metabolic Factors in Type 2 Diabetic Patients. *PLoS ONE* 2016; 11(12): e0167921.
- Marventano S, Vetrani C, Vitale M, Godos J, Riccardi G, Grosso G. Whole Grain Intake and Glycaemic Control in Healthy Subjects: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients* 2017; 9:769
- Gholamreza A, Heidari-Beni M, Broujeni MB, Alireza E, Amini M, Reza G, et al. Effect of whole wheat bread and white bread consumption on pre-diabetes patient. *Pak J Med Sci* 2013; 29(1)Suppl:275-279.