Original Research Article

EVALUATION OF INSULIN, MALONDIALDEHYDE AND BLOOD PRESSURE IN MALE OBESE INDIVIDUALS IN NNEWI AND SUBSEQUENT EFFECT OF GREEN TEA SUPPLEMENTATION.

ABSTRACT

Background: Obesity is a major public health issue worldwide, contributing to increased cardiovascular diseases, diabetes, insulin resistance and oxidative stress. This is due to sedentary lifestyles; poor dieting and low consumption of antioxidant supplement (example green tea). The objective of this study was to evaluate the level of fasting blood sugar, insulin, insulin resistance blood pressure and MDA in obese subjects and subsequent effect of green tea at 6weeks and 12weeks supplementation.

Method: This was a cross sectional and interventional study. In the cross sectional study, 88 obese subjects (46 class I and 42 class II obese) and 50 normal weight subjects (control) were recruited. In the interventional study, 20 male obese subjects were randomly selected and were given 200ml of commercially prepared green tea. Fasting blood samples were collected before the intervention (baseline), at 6weeks and 12weeks of intervention and were later analyzed by standard method Enzyme Linked immunoassay and colorimeteric method. It was analysed statistically using SPSS version 23.0.

Results: There were significant increases in the mean levels of HOMA-IR, systolic and diastolic blood pressures, fasting plasma glucose and insulin in obese subjects (class II and class I obese) when compared with control group ($P<0.05$), likewise in Class II obese when compared with Class I obese ($P<0.05$) while in the case of MDA, there was a significant increase only in Class II obese subjects when compared with the normal weight subjects ($P<0.05$). Green tea supplementation significantly reduced the mean level of MDA, fasting plasma glucose, weight, HOMA-IR and blood pressure at 12weeks of intervention while only Insulin and waist circumference were significantly reduced at 6weeks and 12weeks of intervention.

Conclusion: In conclusion, obesity is the major cause of diabetes, high blood pressure and insulin resistance. Green tea could be beneficial to diabetic patients and obese hypertensives. Green tea compounds- phytochemicals could be beneficial as one of the components of their diet.

Keywords: Obesity, green tea, blood pressure, insulin resistance, oxidative stress

INTRODUCTION

Obesity is a medical condition in which excess body fat accumulates to the extent that it may have a negative effect on health. Recent studies have shown that obesity-

associated risk factors depend not on excess body weight *per se*, but rather on the regional distribution of the excess body fat. In light of this, it is now well recognized that abdominal fat is a significant risk factor for obesity-associated diseases; in fact, visceral fat accumulation stimulates pro-oxidant and pro-inflammatory states [1]. In obesity, modulation of metabolic pathways plays critical roles in the pathogenesis of many diseases [2]. There is a strong positive association between obesity and type II diabetes, dyslipidaemia, cardiovascular disease and hypertension [3]. Hence, creation of appropriate strategies to reduce weight, insulin resistance, oxidative stress and to increase total antioxidant capacity in obese, have remain the focus of this study.

Recent studies on humans show that green tea has many health benefits including reduced risk of cardiovascular disease and some cancers, anti-effects on blood pressure, weight loss, antiviral and antibacterial activities, anti-mutagenic, anti-inflammatory and reduce insulin resistance [4]. Green tea contain appreciable amounts of phytochemicals especially catechins that further comprised of different chemical moieties that include epigallocatechin-3-gallate (EGCG), epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC). Amongst these, EGCG is present in higher amounts and considered to be an effective antioxidant. In the recent era, diet based therapy has been revitalized globally and people are adopting the approach of using natural products as an intervention against various ailments [5]. Keeping in view the health challenges associated with obesity, limited research and controversial findings on effect of green tea on ameliorating blood pressures, oxidative stress and diabetes, this study aims at evaluating these parameters in obesity and subsequent effect of green tea supplementation.

2. METHODS

2.1 Study Design: The research was carried out at Nnewi, Anambra state, Nigeria and biochemical analyses were performed at Nnamdi Azikiwe Teaching Hospital (NAUTH) Nnewi, Anambra State, Eastern Nigeria. This hospital was chosen because they have competent personnel (Medical laboratory scientist) and equipment. In the cross sectional study, 88 obese subjects (46 class I and 42 class II obese) and 50 normal weight subjects (control) were recruited. In interventional study, 20 male obese subjects were randomly selected and were given green tea.

Source of green tea: The green tea was obtained from Lipton Company (Unilever Ghana Ltd (GH) and was of the same brand and batch number 16252 with NAFDAC Reg. NO: B1-8866. Phytochemical analysis on the green tea was performed on a BUCK M910 gas chromatography equipped with a flame ionization detector according to Kelly D; Nelson R; [6] to note the concentration of active ingredients (phenol) present.

Preparation of green tea: Two (2) green tea bags, each weighing 1.6g were dissolved in 200ml of boiled water and this was left to dissolve for 5mins before consumption. The green tea was taken once daily for 12weeks (3months).

2.2 Inclusion criteria and Exclusion criteria:

Subjects recruited were between the ages of 29 and 47years with body mass index of 30 - 35 Kg/m^2 (for Class I obese), 35 – 40 kg/m^2 (class II obese) and 19-24.9 Kg/m^2 for non-obese (controls). Apparently healthy individuals who were not on any medications for diabetes, hypertension and other CVD were recruited. Subjects on alcohol, cigarette, children, adolescents, morbid obese (BMI above $41Kg/m^2$), bedridden, physically challenged, and subjects above 50 years were excluded from the study.

2.3 Ethical approval and informed consent:

Ethical approval was sought and obtained from the Research Ethics Committee of the Nnamdi Azikiwe University Teaching hospital (RECNAUTH) Nnewi, Anambra state with reference NAUTH/CS/66/VOL10/2017/010. The participants were informed about the study designs and their written informed consent was obtained before they were recruited.

2.4 Data Collection Procedure: Subjects who indicated interest in the study, following discussion at business areas, churches, offices, recreation outlets, and restaurant were given detailed designed questionnaire to fill.

2.5 Anthropometric measurements: The weights of the subjects were evaluated with scale (Gulfex Medical and Scientific, England)). The subjects' heights were recorded in meters using a height scale calibrated in centimeters. As a measure of generalized obesity, each adult participant's BMI was computed by dividing the weight in kilograms, by the square of the height in meters (kg/m^2). To determine abdominal obesity, measurement of the waist circumference (WC) was taken using a stretch-resistant tape (HTS, China). Blood pressure (BP) systolic and diastolic pressure readings were taken from the participant's left arm using sphygmomanometer (Omron Medical, United Kingdom). The reading was taken in the morning to the nearest mmHg.

2.6 Sample collection, Storage and Analysis

5mls of blood sample was collected from fasting subjects between 8 and 10am using standard procedure as described by Lewis *et al*., (2006). 1ml of whole blood was dispensed into fluoride oxalate bottle and the plasma separated for glucose analysis while the remaining 4ml of whole blood was dispensed into plain bottle and allowed to clot, retracted and spun at 3000RPM for 10minutes after which the serum was separated into two aliquots and stored. Plasma glucose was analyzed immediately while serum if not assayed immediately was stored at -20°C not more than 2weeks before analyses. For the cross-sectional study, one

point blood sample was collected from each participant both for normal weight and test subjects while in intervention study, three point sample was collected from each test subject: baseline, 6weeks and 12weeks following green tea supplementation. Glucose was assayed colorimetrically using Glucose oxidase method of Trinder, (1969) [8]. MDA level was determined by the colorimetric method of Gutteridge and Wilkins, [9]. The serum insulin level was estimated based on solid phase enzyme linked immunosorbent assay (ELISA) method using ACUBIND kit and mindray (MR- 96A) ELISA machine. Insulin Resistance (IR), was assessed by homoeostasis model assessment–insulin-resistance index (HOMA–IR), according to the following formulas: 'fasting insulin value (mU/L) × fasting blood sugar level (mmol/L) / 22.5' [10], values exceeding 2.25 would denote insulin resistance. Quality control was ensured by using pooled control sera from apparently healthy individual and commercially purchased control (Randox (USA) Control level 1.

2.7 Statistical analyses

Statistical analyses were performed using statistical package for social sciences (SPSS) software version 23.0 software. The variables were expressed as mean ± SD. A preliminary comparison of differences between obese Class I, Class II, and non-obese (control), was assessed using Analysis of Variance (ANOVA) while Post Hoc was used for inter-group variability. Paired t-test was used to assess the mean difference between two related variables and level of significant was considered at $P<0.05$.

3.0 RESULTS

3.1 Anthropometric measurement in Obese (Class II and Class I) and normal weight groups (control)

The test groups were age matched with the control group, therefore there was no significant difference in the mean age across the groups ($P>0.05$). (Class II; 38.2±5.26, Class I;

38.95±5.69, normal weight group (control) 36.6 ±5.1). There were significant increases in the mean values of weight, waist circumference, W/H ratio, height, BMI in obese subjects (class II and class I obese) when compared with control group (P<0.05), likewise in Class II obese when compared with Class I obese (P<0.05) except height which did not decrease significantly.

Table 1 Anthropometric measurement in Obese (Class II and Class I) and non-obese groups (control) MEAN ± SD

PARAMETER	CLASSII Obesity	CLASS I Obesity	CONTROL (normal weight)	Fvalue	Pvalue	**POST HOC** B/C	A/C	A/B
AGE (yrs.)	38.2±5.26	38.95±5.69	36.6 ±5.1	2.6	0.075	0.087	0.435	1.000
HEIGHT (m)	1.7 ± 0.07	1.72 ±.071	1.75 ±.07	7.6	.001*	.045*	.000*	0.624
WEIGHT(kg)	109±8.35	97 ±8.45	69.7 ±4.6	423.6	.000*	.000*	.001*	.000*
BMI (kg/m^2)	38.2±1.06	32.9 ±1.06	22.7 ±1.1	2542	.000*	.000*	.000*	.000*
WAIST(cm)	114± 7.28	106 ±5.1	85 ± 9.0	206	.000*	.000*	.000*	.000*
HIP (cm)	111.9±9.7	111.8 ±4.6	96 ± 9.7	59.5	0.000*	0.000*	0.000*	1.000
W/H RATIO	1.03±.059	0.95 ±.05	0.89 ±.07	59.5	0.000*	0.000*	0.000*	0.000*

*KEY : A represents class ii obesity, B represents class i obesity, C represents control. BMI = Body mass index, Key * = Results compared are significantly different at P-value < 0.05 (P < 0.05).*

In Table 2, the mean levels of fasting plasma glucose, insulin, HOMA-IR, systolic and diastolic blood pressure increased significantly in obese group (class II and class I) when compared with their control likewise in Class II obese when compared with Class I obese

(P<0.05) while in case of MDA, significant increase was found only in Class II obese subjects when compared with the normal weight subjects.

Table 2 Mean Fasting plasma glucose, fasting blood insulin, HOMA-IR and blood pressure in obese subject (Class II and Class I) and non-obese groups (control) MEAN ± SD

						POST HOC		
PARAMETER	Class II Obesity	CLASS I Obesity	Normal Weight	Fvalue	Pvalue	BvsC	AvsC	BvsC
SBP (mm/Hg)	136.9±8.0	130.9±14	123.7±7.8	20.5	.000*	0.012*	0.000*	0.026*
FPG (mmol/L)	6.04 ±.77	5.59 ±.88	5.12 ±.74	16	0.000*	0.035*	0.000*	0.012*
INSULIN (µIU/ml)	7.7 ±2.6	6.3 ±2.2	4.7 ±1.4	26	0.001*	0.019*	0.000*	0.014*
HOMA-IR	2.1 ±.75	1.6 ±.61	1.05 ±.32	39.5	0.000*	0.001*	0.000*	0.000*
DBP(mm/Hg)	95.2±7.1	88 ±9.8	82.9 ±8.9	23	0.000*	0.001*	.000*	0.012*
MDA(nmol/ml)	3.94±1.27	3.72 ±.91	3.30 ±.87	5.1	.007*	0.122	.008*	0.985

Key * = Results compared are significantly different at P-value < 0.05 (P < 0.05). *KEY : A represents class ii obesity, B represents class i obesity, C represents control.* ***FPG****= Fasting Plasma Glucose,* ***HOMA-IR*** *Homeostatic Model Assessment-Insulin Resistance,* ***MDA*** *malondealdehyde,* ***SBP****, systolic Blood Pressure,* ***DBP*** *Diastolic Blood Pressure.*

In Table 3**,** significant weight loss was observed only after 12 weeks of green tea supplementation when compared with baseline and also at 12weeks when compared with 6weeks of supplementation(P<0.05) unlike waist circumference which reduced significantly

after 6 and 12wks intervention when compared with baseline (P <0.05). Furthermore at 12weeks supplementation, there were significant decreases in systolic blood pressure, fasting plasma glucose, fasting blood insulin, MDA, homeostatic model assessment - Insulin resistance (HOMA-IR) (P <0.05) when compared with their baseline values, however FPG, HOMA-IR, MDA and systolic pressure did not significantly decrease after the first 6weeks of intervention (P>0.05)

Table 3 Mean level of blood pressure, Fasting Blood Glucose, Fasting Blood Insulin, MDA and HOMA-IR at different stages of green tea supplementation

PARAMETERS	**BASELINE**	**6WEEKS**	**12WEEK**	**POST HOC**		
N= 20	(A)	(B)	(C)	A vs B	A vs C	B vs C
MDA (nmol/L)	3.95±.66	3.83±.97	3.31±.88	0.693	0.018*	0.071
Waist circu. (cm)	112.6±8.9	112.2±9.2	111.9±9.5	0.016*	0.006*	0.297
SBP (mm/Hg)	133.6±8.8	133.5±8.9	132.9±8.6	0.614	0.031*	0.017*
DBP (mm/Hg)	93.5±7.6	93.35±7.84	93.1±7.85	0.614	0.104	0.204
FPG (mmol/L)	5.6±.83	5.4±.77	5.3±.75	0.089	0.003*	0.031*
FBI (uIU/L)	7.9±1.2	6.0±1.6	5.14±.99	0.000*	0.001*	0.065
HOMA-IR	2.0±.74	2.2±.61	1.6±.47	0.316	0.039*	0.006*

Key * = Results compared are significantly different at P-value < 0.05 (P < 0.05).

FPG*= Fasting Plasma Glucose,* ***HOMA-IR*** *Homeostatic Model Assessment-Insulin Resistance,* ***MDA*** *malondialdehyde,* ***SBP****, systolic Blood Pressure,* ***DBP*** *Diastolic Blood Pressure*

Disscussion

Obesity is becoming one of the most prevalent health concerns among all populations and age groups worldwide. It results to a significant increase in mortality and morbidity related to coronary heart diseases, diabetes type 2, metabolic syndrome, stroke, oxidative stress and cancers [11].

This present study shows that obesity significantly increases fasting plasma glucose, insulin, MDA, blood pressure and it causes insulin resistance ($P < 0.05$). In comparing class II with Class I obese, there were significant increases in FPG, Insulin, HOMA-IR, systolic and diastolic blood pressure ($P<0.05$) except MDA which did not differ significantly ($P>0.05$). This findings are in line with those of Gurung *et al*, [12] which also showed significant increased levels of fasting serum insulin and insulin resistance in obese group when compared with their controls. This indicates that class II obese groups are at the highest risk of developing atherosclerosis, hypertension and diabetes mellitus. This increase might be as a result of the link between obesity and impaired serum glycemic levels resulting from different cellular mechanisms including alterations of insulin signaling, changes in glucose transport, pancreatic β cell dysfunction, as well as enhanced oxidative stress (OS) and inflammation [13]. Obese individuals have demonstrated markers indicative of oxidative stress, including elevated measures of reactive oxygen species (ROS) [14] and diminished antioxidant defense, which is associated with lower antioxidant enzymes as a result of increased free fatty acid which inhibits NADPH oxidase causing dysregulation of cytokines consequently leading to insulin resistance. Oxidative stress is associated with systemic inflammation, endothelial cell proliferation and apoptosis, and increased vasoconstriction, and thus a noteworthy contributing factor to endothelial dysfunction. [15]

In this study, significant weight loss was observed only after 12 weeks of green tea supplementation when compared with baseline value and also at 12weeks when compared with 6weeks of supplementation($P<0.05$), however waist circumference reduced significantly after 6 and 12wks of intervention when compared with the baseline value ($P <0.05$). This is in line with an intervention study by Suzuk *et al.* [16] which revealed that subjects with high catechin intake had lower body weights, BMI, abdominal circumference and total abdominal fat area, after 12 weeks than those of the placebo group. In contrast, supplementation with 300 mg/d of EGCG for 12 weeks according to Mielgo-Ayuso *et al,* [17] did not improve weight-loss. However, few others demonstrated that green tea has no effect on FPG [18]; there were no glucose or insulin-lowering effects after consumption of 300 mL of green tea or water [19]. Weight reduction by green tea observed in this study may be due to reduced rate of digestion and an increase in energy expenditure and fat oxidation through β-adrenoceptor activated thermogenesis of brown adipose tissue [20] and also due to inhibition of catechol-O-methyl transferase (COMT) enzyme by epigallocatechingallate (EGCG) of the green tea [21]. Furthermore at 12weeks supplementation in this work, there were significant decreases in systolic blood pressure, fasting plasma glucose, fasting blood insulin, MDA, homeostatic model assessment - Insulin resistance (HOMA-IR) ($P <0.05$) when compared with their baseline values, however FPG, HOMA-IR, MDA and systolic blood pressure did not decrease significantly after the first 6weeks of intervention.($P>0.05$) Furthermore, the diastolic blood pressure did not reduce significantly throughout the 12weeks of supplementation($P>0.05$). This finding is in line with the works done by other researchers [22] [23] and also with the work of Liu *et al*. [24] which also showed that green tea extract caused a significant decrease in homeostasis model assessment of insulin resistance index after 16 weeks of consumption. The anti-hyperglycemic effect of green tea as seen in this study might be as a result of the increase in insulin-stimulated glucose uptake, inhibition of

the intestinal GLUT system and decrease in expression of genes that control gluconeogenesis . Mozaffari-Khosravi *et al.* observed significant decrease in systolic and diastolic blood pressures on individuals who consumed three glasses of green tea daily for 4 weeks, however, in the present study green tea did not reduce the blood pressures at 6weeks of supplementation. This may be as a result of GT dosage, rate of consumption or the brand of tea used. The decreased blood pressure observed in this study from green tea consumption is probably because it regulates vascular homeostasis by influencing the production of angiotensin II, prostaglandins, endothelin-1 as well as vasodilating substances such as prostacyclin [25]

Conclusion

Obesity is the major cause of diabetes and insulin resistance. Green tea could be beneficial to diabetic patients and obese subjects. Green tea compounds- phytochemicals could be beneficial as one of the components of their diet.

Consent

All authors declare that 'written informed consent was obtained from the subjects and other approved parties for publication of this paper.

Ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee (the ethical review committee of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki "ethical

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