CONSUMPTION OF FRUITS AND VEGETABLES DECREASED PLASMA HOMOCYSTEINE IN POSTMENOPAUSAL WOMEN

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ABSTRACT

The major consequences of menopause are related primarily to estrogen reduction and this could lead to the accumulation of homocysteine in the vascular endothelium. Vitamins B_{12}, B_6, and folate are required for homocysteine metabolism and deficiency of these could lead to increased plasma homocysteine. It could be speculated that daily consumption of these vitamin nutrients may prevent early CVD events in part in postmenopausal women. This study was designed to examine the relationship of frequent consumption of fruits and vegetables on anthropometric indices, plasma homocysteine and estradiol in healthy postmenopausal women. One hundred and fifty healthy participants consisting of a hundred postmenopausal (60.2±10.5)years and fifty premenopausal women (44.3±6.2) years were recruited into this study. Anthropometric indices and percentage body fat measurements were determined using standard procedures. Plasma total homocysteine (tHcy) and estradiol were estimated by Enzyme-Linked immunosorbent assay (ELISA).There was a significant increase in plasma tHcy ($p<0.000$) and decrease in estradiol ($p<0.000$) compared to the control values. The anthropometric indices and %body fat measurement also showed significant changes ($p<0.005$). Notable significant variations in tHcy were obtained within the socioeconomic classes, the high-income group exhibited the lowest value ($p<0.047$). Graded decreases in tHcy were observed with the consumption of fruits and vegetables with the daily consumers showing the lowest tHcy level ($p<0.034$). Plasma tHcy was inversely correlation with estradiol ($r= -0.433$, $p<0.000$). The duration of menopause exerted significant changes in all the measured biochemical parameters. The changes in plasma tHcy and estradiol levels varied within the socioeconomic classes, frequency of fruits and vegetable consumption as well as the duration of menopause. The results provide evidence that daily consumption of fruits and vegetables could be beneficial in postmenopausal State.

Key Words: Postmenopausal women, cardiovascular disease, homocysteine, Estrogen, fruits, vegetables

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Introduction

Available evidence has suggested that cardiovascular disease (CVD) increases after menopause (Regensteiner 2015). The major consequences of menopause are related to estrogen reduction *(*Lee et al., 2006). Estrogen, a cardioprotective agent is known to modulate methionine metabolism by interfering with the transsulfuration pathway (Dimitrova *et al,* 2002). Reduced estrogen in the postmenopausal state could lead to the accumulation of homocysteine an intermediate product of methionine metabolism in the vascular endothelium and the production of homocysteine thiolactone. Homocysteine promotes atherosclerosis by different mechanisms (Qiang et al., 2012). These mechanisms are oxidation of Hcy giving rise to superoxide anion radical and hydrogen peroxide, and as well as increased oxidative stress (Tsutsumi et al., 2008) and ultimately incorporation of Hcy thiolactone formed by methionyl-tRNA synthesis into proteins by N-homocyteinylation (Ferretti *et al.,* 2010). Deficiencies of vitamins $B_{12}$and folatecould lead to varying degrees of disturbed Hcy metabolism and invariably to elevated plasma total Hcy level.

Since homocysteine metabolism is influenced by vitamins B_{12}, B_6 and folate status, a dietary deficiency of these vitamins leads to the

accumulation of homocysteine and invariably the production of Hcy thiolactone. Homocysteine thiolactone free radicalsare notably injurious to the body and could also accumulate. Due to the loss of the protective function of estrogen on the cardiovascular physiology, postmenopausal women could be exposed to high plasma hcy concentrations and this in effect will become deleterious to the cardiovascular system (Baszczuk and Kopczynski, 2014)

Available evidence has shown that altered metabolism of homocysteine is involved in the oxidative process occurring in atherogenesis (Shenov *et al.*, 2014). Increased dietary consumption of vitamins B_{12}, B_6 as well as folate could reduce plasma homocysteine and invariably reduce the risk of CVD (Curro et al, 2014).

There is a paucity of information on the relationship of frequent consumption of fruits and vegetables on plasma homocysteine in postmenopausal women in this environment where most of the diet is carbohydrate-based. It could be speculated that daily consumption of these vitamin nutrients in postmenopausal women will prevent early CVD events.

The present study was designed to assess the relationship of frequent consumption of fruits and vegetables on plasma homocysteine, estradiol and anthropometric indices inhealthy postmenopausal women in Remo Ogun state Nigeria.

MATERIALS AND METHODS

One hundred and fifty healthy non-smoking participants were recruited into this study. These consisted of one hundred postmenopausal (60.2±10.5)years and fifty premenopausal women (44.3±6.2)years. The Babcock University Health Research Ethics Committee, Ileshan Ogun state gave approval for this study. All participants gave Written / oral informed consent. Structured questionnaire administered to each participant indicating the educational level, occupation, and income (earning) as described in an earlier study (Ebesunun *et al,* 2008)was adopted in assessing socio-economic status and stratification into low, middle and income groups. The questionnaire also includes social-demographic and dietary characteristics.

Women who were on lipid-lowering drugs, hormones therapy users, hypertensive, diabetics, and thyroid dysfunctions were excluded from this study.

Procedures

Measurements of Anthropometric indices and Percentage Body Fat

The anthropometric indices were determined using standard methods. The height was taken using stadiometer with the participant's head in Frankfort plane. https://healthabc.nia.nih.gov/sites/default/files/Y8_Height_0.pdfad

Waist circumference (WC) was measured with a non- stretchable tape by locating the top of the iliac crest and measuring in a horizontal plane around the abdomen immediately above the level of the iliac crest.

Hip circumference (HC) was determined with a non- stretchable tape at the maximum perimeter of the hip in centimeter.

Waist to hip ratio (WHR) was calculated.Omron body fat monitor BF 400 was used to measure the body weight, body mass index, and body fat percentage. The BF 400 estimates the body fat percentage by the Bioelectrical Impedance (BI) method and displays the body fat mass as the percentage of body weight. The age, gender and height of participant were inputted into the machine. Participants were asked to stand on the scale, placing the feet on the electrodes and ensuring even distribution of weight on the scale. The body fat mass and body fat percentage were calculated by a formula that includes five factors: electrical resistance, height, weight, age, and gender as contained in the manufacturer's manual.

Blood pressure measurements were taken using. OMRON® (Digital Automatic Blood Pressure Monitor DABPM) with appropriate size cuffs. The participants sat quietly and rested for 10 minutes with the legs uncrossed. The right arm of the participants were placed on the table with the palm facing upward. The cuff was placed above the elbow aligning the mark ART on the cuff with the brachial artery. The cuff was wrapped snugly onto the arm and securely fastened with the Velcro. The BP monitor was turned on and the blood pressure measurement was taken.

Blood sample collection

Blood samples were taken after an overnight fast of 10-14 hours. Samples were collected into plain, K3EDTA and fluoride oxalate bottles and

transported in a cold chain box to the laboratory for separation. The separated plasma was aliquot into labeled plain bottle and stored at -20 0C awaiting analysis Plasma tHcy was estimated using reagent kit purchased from ElabScience Biotechnology Wuhan PRC and the plasma estradiol was estimated using reagent kit purchasedfrom Teco Diagnostic Anaheim USA. The method was based on enzyme-linked immunosorbent assay (ELISA). Quality control samples were included in every assay to check the coefficient of variation.

Data Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 21. Student's *t-test* was used to compare differences between two means and the differences were regarded as significant at $p < 0.05$. The relationship between variables was assessed using Pearson's correlation coefficient. Graphs were plotted using Excel**.**

Results

The results are presented in tables and figures.

Significant increases were obtained in the body weight and BMI ($p<0.005$), % body fat ($p<0.007$), waist circumference ($p<0.031$), hip circumference ($p<0.005$) and diastolic blood pressure ($p<0.005$) when compared with the corresponding control values. Statistical increase was observed in plasma tHcy ($p<0.000$).The plasma estradiol ($p<0.000$) was significantly reduced compared with the control value (table1).

A statistically significant decrease was noted in plasma tHcy ($p<0.034$) based on the frequency of consumption of fruits and vegetables with daily consumers showing markedly reduced level as shown in figure1.

Plasma homocysteine, estradiol, and duration of menopause were significantly lower ($p<0.05$) within the groups. Graded increases in plasma tHcy were obtained in postmenopausal women based on the duration of menopause while graded decreases were observed in the plasma estradiol (table2).

Plasma tHcy correlated inversely with estradiol ($r= -0.433$, $p<0.000$) (table3).

A progressive significant decrease was obtained in plasma tHcy ($p<0.047$) based on socio-economic class with the high-income group showing the lowest value. The plasma estradiol also varied with socio-economic class with the highest value obtained in the high-income group (table 4 and figure 2) respectively.

Table 1: Anthropometric indices, blood pressure, % body fat, estradiol and homocysteine in all subjects (mean±SD)

Variables	Postmenopausal n=100	Premenopausal n=50	t-value	p-value
Weight (Kg)	72.0±20.6	62.9±15.4	2.912	0.005*
Height (m)	1.61±0.06	1.61±0.12	0.425	0.691
BMI (Kg/m^2)	28.2±7.9	24.44±5.45	2.932	0.005*
% Body Fat	30.5±10.0	26.31±8.04	2.443	0.007*
WC (cm)	94.4±19.4	88.40±13.79	2.223	0.031*
HC (cm)	101.9±19.9	93.27±15.46	2.877	0.005*
WHR	0.95±0.47	0.93±0.06	1.885	0.061
SBP (mmHg)	123.78±11.30	124.52±14.74	-0.282	0.779
DBP (mmHg)	79.64±6.99	74.94±9.07	-2.092	0.005*
Estradiol(pg/ml)	17.8±10.00	66.6±32.71	13.730	0.000*
tHcy(µmol/L)	10.1 ± 1.31	8.3±0.55	-4.407	0.000*

P = level of significance, n = number of subjects, SD= Standard deviation, BMI=Body mass index, WC= Waist circumference, HC= Hip circumference, WHR=Waist Hip Ratio, SBP = Systolic Blood Pressure, DBP= Diastolic Blood Pressure, tHcy= Total homocysteine

Table 2: Homocysteine, estradiol and duration of menopause (years)

Variables	1-10yr n=48	11-20yr n=32	21-30yr n=20	F value	p value
Estradiol(pg/ml)	19.33±8.78	15.89±12.29	12.02±10.00	62.100	0.000*
tHcy (µmol/L)	9.17±1.40	10.18±1.22	10.97±1.10	6.875	0.000*

P = level of significance, n = number of subjects, SD= Standard deviation, tHcy=Total Homocysteine, yr= years

Table 3: Pearson's correlation coefficient of plasma homocyteine with etsradiol

	r value	p value
Estradiol (pg/ml)	-0.433	0.000**

Table 4: Plasma homocysteine and estradiol based on socio-economic classes (Mean±SD)

Variables	Low Income n = 50	Middle Income n = 41	High Income n = 9	F value	p value
Estradiol (pg/ml)	17.39±9.79	17.88±10.96	19.53±7.18	0.175	0.840
tHcy (µmol/L)	11.11±1.35	10.11±1.14	9.97±1.16	3.161	0.047*

p = level of significance, n = number of subjects, SD= Standard deviation, tHcy=Total Homocysteine

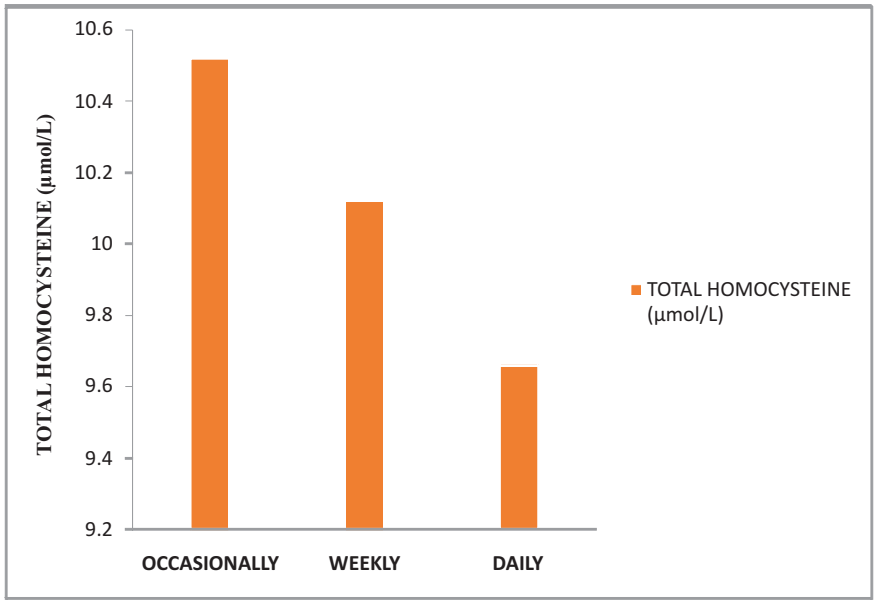

FIGURE1: PLASMA HOMOCYSTEINE, FRUITS AND VEGETABLES CONSUMPTION IN POSTMENOPAUSAL WOMEN

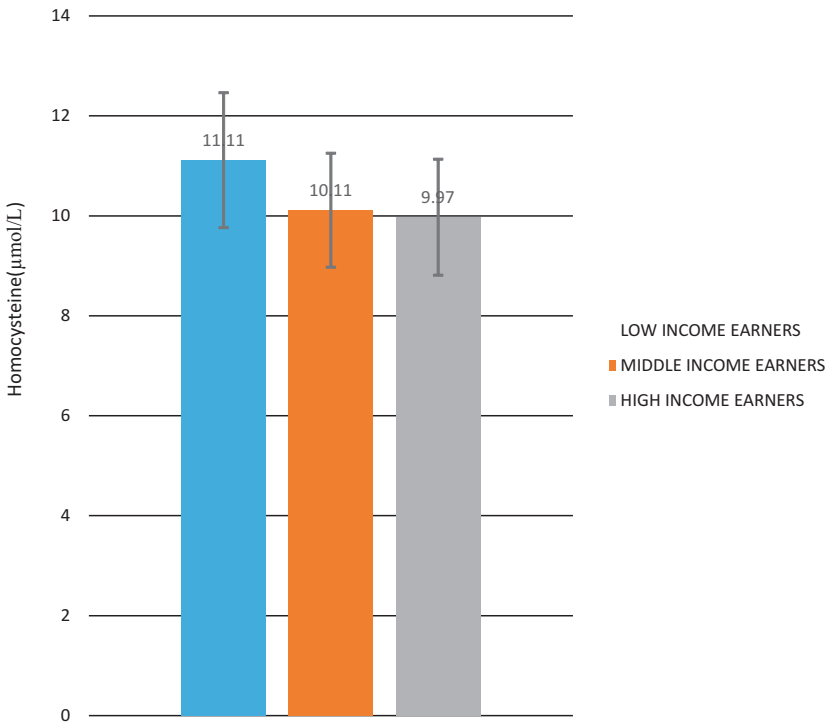

FIGURE2: TOTAL HOMOCYSTEINE AND SOCIO- ECONOMIC CLASSES IN POSTMENOPAUSAL WOMEN

DISCUSSION

Participants were healthy non-smoking Nigerian postmenopausal women of 60.2±10.5years and premenopausal women of 44.3±6.2years Anthropometric data were measured and dietary records were obtained using structured questionnaire. Remarkable increases in body weight, BMI, % BF, waist and hip circumferences, as well as diastolic blood pressure were obtained. These notable changes are associated with CVD as reported in recent finding (Salim *et al,*2020), this in part implicated waist to hip ratio as one of the most important indicators in cardiovascular disease development. The significant waist to hip ratio obtained in this study provides evidence that changes in body composition could be risk indicators for CVD.

The menopausal state could compromise many traditional CVD risk factors that include changes in body fat distribution. Body fat distribution pattern was suggested to be an indicator of the risk of developing premature CVD. (Canoy *et al.,* 2004). There were graded decreases in plasma estrogen (estradiol) concentration, this could interfere with the metabolism of homocysteine and invariably reduction in the transamination of homocysteine (Shah et al., 2006). Estrogen modulates thiolamino acid metabolism, particularly methionine metabolism. By interfering with the transsulfuration pathway, it diminishes homocysteine, and hence prevents its accumulation (Dimitrova *et al,* 2002). Accumulation of tHcy in the vascular endothelium leads to the production of homocysteine thiolactone a free-radical which could bind to apoprotein A1 and could result in its structural and functional modification (Dimitrova *et al,* 2002).

Plasma homocysteine levels were related to menopausal status in this study. Raise concentrations of homocysteine couldbea source of hydrogen peroxide, a harmful free radical, which could damage the endothelium. Asides genetic defects in the enzymes involved in homocysteine metabolism and nutritional deficiencies in vitamin cofactors, sex steroid hormones have been reported to modulate homocysteine levels (Aswathy et al, 2017). A similar finding of elevated tHcy in the postmenopausal women was observed in an earlier study (Mohamed et al, 2013).

Also, from this study, there were significant socioeconomic variations in tHcy with graded decreases from low income to high-income class,

an indication that socioeconomic class could exert an effect on tHcy. A plausible explanation could be that the high-income class are likely more knowledgeable of the benefits of frequent consumption of fruits and vegetables, although affordability for the low income group could be a major factor if awareness on the importance of frequent consumption is bought to the fore.

The frequent consumption of fruits and vegetables was remarkably significant with the daily consumers showing the lowest plasma tHcy level, thus supporting the biological benefits of fruits and vegetables on homocysteine concentration, as these contain vitamin nutrients that are necessary for homocysteine metabolism.

Graded increases in tHcy were obtained in postmenopausal women based on the duration of menopause suggesting that the longer the duration, the greater they could be prone to the risk of CVD. Also. from this study, tHcy correlated inversely with estrdiol. Suggestingthat diminishing estradiol will lead to increase tHcy. A trend that could be deleterious to the body. The reason for this is however not clear.

In conclusion, this study has shown significant changes in estrogen, homocysteine, as well as body composition in postmenopausal women. These noted changes are possible risk factors for early cardiovascular events in postmenopausal individuals. The dietary supplementation of vitamin nutrients as reflected in the group with daily consumption of fruits and vegetables who had decreased tHcy will be of importance in ameliorating the effect of increased homocysteine. This could be exploited in the therapeutic management of the postmenopausal state. Socio-economic class clearly showed variations in plasma tHcy levels with high income class exhibiting the lowest level.

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Conflict of interest

No conflict of interest among the authors

Authors' Contribution

MOE, designed, supervised, wrote and edited manuscript for publication

AOL Was involved in the design, collection of data and analysis of the samples as well as writing up the manuscript for academic purpose

JKA, was involved in coordinating analysis of data and drafting of the manuscript

SMO - collection and analysis of samples as well as drafting

Consent for publication

Publication was approved by all the Authors

Availability of data

The data were readily obtained in Ileshan community in Remo local Governed of Ogun state

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