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Body mass index (BMI) and total plasma homocysteine concentration in the obese subjects: The possible diabetes mellitus and essential hypertention link

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Abstract
Background: Hyperhomocysteinemia is associated with type-2 diabetic mellitus, obesity and essential hypertension among others. Obesity, which is a known risk factor for type-2 diabetes mellitus and hypertension/cardiovascular heart diseases is classified based on BMI. Plasma concentration of homocysteine is determined by food intake, rate of body metabolism and renal excretion rate of homocysteine.
Aim: Since obesity is linked with hyperhomocysteinemia, this study was therefore designed to evaluate the plasma total homocysteine means in the different BMI groups in this region and evaluate the correlations of homocysteine with C- reactive protein and vitamin B12.
Methods: 120 subjects were grouped according to their BMI that is below 25, from 25-29.9, from 30-34.9, from 35-39.9 and then those with BMI of 40 and above. A questionnaire was used to extract information from these volunteers who signed the consent form. Blood was taken from each subjects into plain bottles and the serum separated into another plain container. This was analyzed for plasma homocysteine, C reactive protein and vitamin B12 and the results grouped based on BMI. The mean values of these parameters for each BMI group was calculated and compared.
Results: There was a steady increase of plasma homocysteine mean and C reactive proteins mean as the BMI increases while the B12 mean decreases as the BMI increases. The correlation between plasma homocysteine level and B12 was negative.
Conclusion: Plasma total homocysteine mean values were found to increase as the BMI increases. Therefore, a maintenance of plasma homocysteine level close to the value of the mean concentration of 6.6nmol/l seen in the normal weight BMI group in this environment may slow down the development of type-2 diabetic mellitus and essential hypertension in the obese.

Keywords: Plasma total homocysteine, hyperhomocysteinemia, type-2 diabetic mellitus and essential hypertension

Introduction
Hyperhomocysteinemia can be defined as a condition were excess homocysteine levels is found in blood of an individual [1]. This hyperhomocysteinemic state has been implicated in a lot of conditions like obesity [2], diabetic mellitus [3], cardiovascular diseases [4], depression [5], and even in patients with bone disorders [6]. Type 2 diabetic mellitus and essential hypertension are linked to a common denominator, which is obesity. Studies have shown an increase plasma total homocysteine level with obesity, type 2 diabetes mellitus and essential hypertension [7]. The correlation between diabetes and homocysteine has conflicting reports [8], this has become a very important study especially in areas where the incidence of obesity, type-2 diabetics and hypertension/cardiovascular diseases are high. Hyperhomocysteinemia is common to all the above conditions (hypertension, obesity and type 2 diabetes) and patients with such hypertension, obesity and type 2 diabetes has been found to have reduced blood concentration of antioxidants [9]. Port Harcourt and rivers state has one of the highest prevalence of diabetes, hypertension and obesity in Nigeria [10, 11] and since plasma total homocysteine is associated with all, this study is therefore necessary to see if the effect of obesity on plasma total homocysteine level is steadily increased with increase BMI in this region and also affirm the correlation of plasma homocysteine level with C-reactive protein

and vitamin B.

Materials and Methods

This is a cross sectional study design where 100 apparently healthy non-hypertensive, non-diabetic and non-alcoholic subjects who had no arthritis or hypothyroidism were selected after the importance of the study was explained to them. Those who gave their consent by signing the consent form were selected for this study. A self-administered questionnaire was used to exact information about subject's biodata and medical history. I was available to offer help to subjects who had difficulties filling both the consent form and the questionnaire.

The height of subjects were measured using a height meter. This was done with the subject standing with feet together and without shoes or headgear, back and heels against a vertically ruled bar to which a movable horizontal bar was applied to the vertex of the subject's head and measurement taken in meters [12]. Weight was measured using a weighing scale (Harson). Subjects were requested to wear light cloths and then stand erect on the scale. The weight was measured in kilograms. The BMI was calculated by dividing weight (kg) by the height in meters squared [13].

From each selected patient, 3mls of blood was drawn into plain bottles which was left to stand for about 2 hours to allow the blood to clot and the serum separated into another plain container and labeled accordingly. The separated serum was racked and batch-analyzed for plasma homocysteine, C-reactive protein and vitamin B-12 the same day using the Human Homocysteine ELISA Kit manufactured by Bioassay Technology Laboratory, Immunoturbidimetric method manufactured by Fortress and 'vitamin B-12 ELISA' manufactured by 'Calbiotech' respectively.

The BMI was then calculated by dividing weight in kilograms of each patient over their height in meters square. Subjects were grouped according to their BMIs and the mean plasma total homocysteine, C - reactive protein and vitamin B12 calculated for the total number of subjects and for each BMI group. The correlation of plasma total homocysteine with patient's vitamin B-12 and C-reactive protein were evaluated. Each BMI group's plasma total homocysteine mean were compared.

Table 1: Mean of homocysteine, C- reactive protein and Vit B12 for total subjects

N = 120	Homocysteinenmol/ml	C-reactive protein	Vit B12 (pg/ml)
Mean	8.0	5.6	349.6
Standard Error	0.5	0.2	11.7
Standard Deviation	4.5	1.8	98.2
Minimum	4.8	2	96
Maximum	32.7	12	537

Table 2: BMI groups and their mean homocysteine, Vit B12 and CRP

BMI	N	Homocysteinenmol/ml	Vit B12 Pg/ml	C Reactive Protein
<25	21	6.22 ±0.71	300.63 ±45.3	4.8 ±1.4
25 – 30	24	7.20 ±1.08	283.54 ±59.2	5.5 ±1.0
30 – 35	25	8.38 ±7.32	256.64 ±36.8	5.8 ±1.5
35 – 40	27	8.83 ±4.12	239.40±83.6	5.8 ±2.1
>40	23	10.24 ±0.71	206.40 ±85.0	6.2 ±1.8

N= number of subjects, CRP= C-reactive protein

Table 3: Comparison of the means of the parameters by BMI class

	Normal	Obese	Overweight	ANOVA
C-reactive protein	300.6 ±45.2	231.9 ±78.5	283.5 ±59.2	0.0001*
Homocysteine	6.2 ±0.7	8.6 ±4.1	8.3 ±2.3	0.1882**
Vitamin B12	402.1 ±61.3	318.0 ±103.8	384.3 ±80.2	0.0041*

*Difference is statistically significant (p< 0.05)

**Difference is not statistically significant (p >0.05)

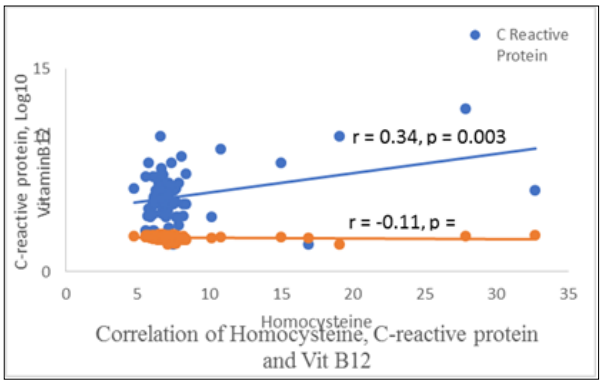

Fig 1: Correlation of Homocysteine, CRP and VIT B12.

As homocysteine increases, C-reactive protein (CRP) tends to increase (positive correlation)
As homocysteine increases, Vitamin B12 tends to reduce (negative correlation)

Results

Data was analyzed with the EPI info version 7 software (CDC USA). All data were presented in descriptive statistics (mean, frequency and percentage). ANOVA was used to compare the means across the BMI groups. Pearson's correlation was used to access the association of homocysteine, vitamin B-12 and C-reactive protein. All analysis was done at 95% confidence interval and the p-value of less than 0.05 was considered significant.
A total of 120 subjects were selected for this study and the mean age of the total subjects was 41.05 years. The minimum and maximum homocysteine, C-reactive protein and B12 values were 4.8 and 32.7nmol/l, 2 and 12nmol/l and 96 and 537nmol/l respectively (table 1). The total homocysteine mean value was 8.0nmol/l while that of the C-reactive protein was 5.6mg/l and the vitamin B12 was 349.6pg/ml (table1).
The subjects were divided into groups based on their BMI and this gave five groups. BMI below 25 had 21 subjects while BMI of 25 to 29.9 had about 24 subjects. Subjects with BMI 30 to 34.9 were about 25 subjects, 27 subjects had BMI between 35 and 39.9. 23 subjects had BMI above 40 (Table 2).
When the means of homocysteine, B12 and C-reactive proteins of each BMI group were calculated and subjects below 25 had a mean homocysteine value was 6.22nmol/l, while the mean B12 was 300.63 pg/ml and C-reactive protein was 4.8mg/l (table 2). For subjects with BMI between 25 and 29.9, the mean homocysteine value was 7.20nmol/l and 283.54pg/ml for the mean vitamin B12 while C-reactive protein had a mean of 5.5mg/l. The homocysteine mean value for subjects between 30 and 34.9 was 8.38nmol/l and 256.64 for vitamin B12 while C-reactive protein mean value was 5.8mg/l. Subjects with BMI between 35 and 39.9 had their homocysteine, vitamin B12 and C-reactive protein means as 8.83nmol/l, 239.40pg/ml and 5.8mg/l respectively. For those with BMI above 40, their homocysteine mean was 10.24nmol/l, vitamin B12 mean was 206.64pg/ml and a C-reactive protein mean of 6.2mg/l (Table 2).
The correlation of plasma homocysteine and C- reactive protein was positive as the C - reactive protein was found to increase as plasma homocysteine increases. While the correlation of plasma homocysteine and vitamin B12 was negative (Fig 1).

Discussion

Homocysteine is a non-proteingenic amino acid. It is synthesized from methionine by the removal of the c-methyl group at its terminal. Homocysteine is not obtained directly from diet but synthesized from methionine [1]. The amino acid cysteine is synthesized from homocysteine with the aid of the B complex vitamins. A high level of homocysteine in the blood (hyper-homocysteinemia) will therefore lead to reduced vitamin B complex in the blood as seen in this study [14]. This hyper-homocysteinemic state has been shown to induce inflammatory reaction which leads to endothelial cell injuries occurring in blood vessels and other organs [15]. This leads to inflammatory reactions and responses [16]. Studies have shown that factors like obesity, diabetics, C-reactive proteins, genetic and diet like vegetables and fruits rich in folic acid, vitamin B6 and B12 all affect the level of plasma total homocysteine [14]. C-reactive proteins have been shown to have a positive correlation with plasma homocysteine level [17] while folic acid, vitamin B6 and B12 have been shown to have a negative correlation with plasma total homocysteine [18]. In this study, the homocysteine mean was found to steadily increase with increase in BMI as shown in table 2. Same was the case of C-reactive protein that rose steadily with BMI except for the 30 to 34.9 group and the 35 to 40 group that had the same mean (5.8nmol/l). This finding affirms the fact that plasma total homocysteine value does not just increase with obesity but steadily increases with increase in the BMI. This is a very important finding as type-2 diabetes mellitus, essential hypertension and obesity have all been linked with hyperhomocysteinemia. It would therefore be necessary to evaluate hyperhomocysteinemia as a link factor in the obese, diabetic subjects and hypertension. The possibility that a reduction of blood homocysteine in the obese subjects may slow down the development of any of the obesity related disorders like type 2 diabetes and essential hypertension need to be evaluated and studies may have to be carried out to ascertain any link.
Any possible link may presently be poorly understood but increase in plasma homocysteine level has been associated with reduced levels of plasma antioxidants [19]. Studies have also linked reduced antioxidant levels to diseases like type-2 diabetics and essential hypertension among others [19]. For the type-2 diabetes, reduced antioxidants was associated with increased production of reactive oxygen which was linked to inducing insulin resistance and inflammatory condition [20, 21]. The insulin resistance may explain the association between hyperhomocystenemia and type 2 diabetes while the inflammatory reaction and subsequent endothelial injury may be the link of hyperhomocysteinemia and hypertension/cardiovascular diseases. To further buttress this point, increased plasma homocysteine levels have been found not to have a positive association with type-1 diabetics which is noninsulin dependent and also not associated with obesity [22, 23]. In this study, B12 which is a type of antioxidant was reduced with increase in homocysteine level and this was in keeping with other studies [24, 25, 26] and supports the oxidative stress hypothesis of high homocysteine level and increase risk of type-2 diabetics since increase plasma homocysteine reduces the concentration of plasma antioxidants.
The positive correlation of homocysteine level and C-reactive protein as seen in this study and in other studies[27] has been shown to be due to response to the inflammatory reaction induced by increased plasma homocysteine, since C-reactive proteins is a known marker of inflammation.
The observed steady increase of homocysteine mean as the BMI increases is an important finding in this study. Since hyperhomocysteinemia is find in the obese, the diabetic, the pre-diabetic and the hypertensive as well as causes a decrease in antioxidant level as seen in the correlation between homocysteine and B12, an antioxidant therapy for the obese, prediabetes and hypertensive may slow down or eliminate the progression of prediabetes to diabetes or even reduce the development of hypertension in diabetics and vice versa.

Hypertension like diabetes has become a global health issue [27] and C-reactive protein which is an acute phase reactant has long been identified as a risk factor for cardiovascular diseases [28]. Increased plasma homocysteine which positively correlate with C reactive protein level has also been associated with atherosclerosis, thrombosis, endothelial cell damage and platelet activation which are all findings in cardiovascular diseases [29].

Therefore, increased C-reactive proteins and reduced antioxidants seen in subjects with increased plasma homocysteine level seen in the obesity, hypertension and diabetics may be a link as increased plasma homocysteine has been shown to cause a decrease plasma antioxidants but an increase plasma antioxidant have been associated with lowering blood pressure in patients with essential hypertension [30].

Since homocysteine levels increases steadily with BMI/obesity and increased homocysteine level is associated with reduced plasma level of antioxidants, then an increase in plasma homocysteine level and the subsequent reduction in antioxidants level seen in both the obese, the diabetic and the hypertensive may be the possible link and the reason why the obese develop type-2 diabetes mellitus and or essential hypertension. Further studies may therefore be necessary to evaluate if a reduction in the homocysteine level or an increase in the antioxidant level may slow down the incidence of type-2 diabetics and essential hypertension in the obese and why diabetics eventually becoming hypertensive subjects and Vis versa.

From this study, it may be difficult to say at what limit of plasma homocysteine concentration may need intervention measures to slow down or prevent the obese patient from developing type 2 diabetes mellitus and essential hypertension. The homocysteine mean of the normal weight subjects that is BMI less than 25, which was 6.22nmol/l in this study may be a guide and a first step in the right direction. Maintaining plasma homocysteine level at this concentration in the obese or prediabetic may be necessary to slow down or prevent their developing type 2 diabetes and essential hypertension in this region.

Some subjects irrespective of their BMI groups showed very high plasma homocysteine levels, way beyond the average mean of their groups. Though the number of such subjects was small, it was worthy of note and the enzyme 5-methylene tetra hydrofolate reductase deficiency (MTHFR) which has been associated with very high homocysteine level may be a likely explanation for these increase concentration of homocysteine [31]. Though no test was done in this study to evaluate this inherited disorders, this enzyme (MTHFR) deficiency could be a possible explanation for this very high plasma homocysteine level seen in few subjects This submission may not however be affirmative as the incidence and the prevalence of 5-methylene tetra hydrofolate reductase deficiency (MTHFR) in our environment has not been ascertained and therefore may be difficult to tell if this enzyme disorder exists in our environment in the first place.

Conclusion

From this study, the plasma homocysteine level was found to increase steadily with increase BMI and correlate negatively with vitamin B12. It may therefore be necessary to use the homocysteine mean of 6.0-6.5nmol/l found in the normal BMI population in this study (BMI below 25) as a guide or the cut off limit to control or reduce plasma homocysteine concentration. This intervention will increase subjects' antioxidant concentration which may probably reduce the incidence of type-2 diabetes mellitus and cardiovascular disease among obese/ pre-diabetic subjects in our environment.

Conflict of interest

All authors affirm that this study has no conflict of interest.

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