Evaluation of Essential Fatty Acids, Folic Acid And Vitamin B_{12} in Type 2 Diabetes Mellitus

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ABSTRACT: Recent evidence has linked low plasma folic acid and vitamin B_{12} concentrations with endothelial dysfunction, often found in type 2 diabetes mellitus. But no available study has associated decreased level of essential fatty acid with type 2 diabetes mellitus in Nigerians. This study was designed to evaluate plasma levels of essential fatty acids, folic acid and vitamin B_{12} in type 2 diabetes mellitus patients. Sixty eight type 2 diabetes mellitus patients with a mean age of 63.48 ± 10.91 years were recruited. Twenty six apparently healthy volunteers with a mean age of 38.32 ± 12.26 years were included as controls. Anthropometric indices and biochemical parameters were determined using standard techniques. The results showed significant decreases in plasma linoleic acid, arachidonic acid, folic acid and vitamin B_{12} ($p < 0.05$) compared with the control values. On the other hand, there were significant increases in fasting plasma glucose, body mass index ($p < 0.001$) and waist circumference ($p < 0.05$) compared with the control values. Significant correlation was obtained between arachidonic acid and folic acid levels ($p < 0.05$). The main findings from this study were decreased plasma linoleic acid, arachidonic acid, and folic acid and vitamin B_{12} in association with increased WC and BMI in type 2 diabetic patients. Thus providing evidence that decreased levels of essential fatty acids, folic acid and vitamin B_{12} are associated with type 2 diabetes mellitus in Nigerians.

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INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by increase in the blood glucose along with alterations in carbohydrate, fat and protein metabolism, associated with defects in insulin secretion and or insulin action or both (Andreoli et al, 1990; Lebovitz, 1994). This metabolic disease is one of the most common endocrine disorders affecting almost 6% of the world's population (Adeghate et al, 2006). The disease may be a consequence of nature and nurture. The presence of genetic factor (nature) and environmental cause (nurture); faulty dietary intake in excess, poverty, malnutrition, improper concepts of hygiene and health and sedentary lifestyle may lead to increased incidence of the condition (Enase, et al 1992).

The prevalence of type 2 Diabetes mellitus is increasing at epidemic proportions worldwide and recently it has become a major global health issue in economically developed countries as well as in countries undergoing rapid economic expansion (King et al, 1998). In Nigeria however, available evidence has shown that there is an increase in the prevalence of diabetes mellitus, especially of type 2 (Bamgboye, 2010). The relative importance of impaired insulin release and insulin resistance in the pathogenesis of type 2 diabetes mellitus has been evaluated in several studies (Chen *et al*, 1995; Haffner *et al*, 1995; Weyer *et al*, 1999). Study by Vessby (2000) indicated that reduced levels of linoleic acid and reduced activity of delta-5-desaturase in humans are associated with insulin resistance in type 2 diabetes mellitus. The link between intake of linoleic in the diet, delta-5-desaturase activity and insulin resistance-type 2 diabetes mellitus is important for the optimal function of cell. Reduced level of vitamin B_{12} as reported in several studies (Filoussi et al , 2003, Pflipsen, et al, 2009). is a potential risk factor for development of neuropathy in diabetic patients particularly when certain medication for the treatment of DM is associated with vitamin B_{12} deficiency (Filoussi et al, 2003, Pflipsen, et al, 2009). Cardiovascular complications often coexist deficiency of vitamin B12 and or folate (Weiss et al, 2003).

This study was design to determine the levels of omega-3 and omega-6 fatty acids, vitamin B_{12} and folic acid in type 2 diabetic patients and apparently healthy subjects.

MATERIALS AND METHODS

Subjects: The subjects for the study consisted of 68 patients (31 males, 37 females) with type 2 diabetes mellitus as diagnosed by the attending Consultant Physician based on clinical and laboratory findings. They were selected at Obafemi Awolowo University Teaching Hospital Nigeria. The subjects were aged between 40 and 84 years; the males had a mean age of 63.3 ± 11.4 years. The females had a mean age of 63.7 ± 10.8 years. An informed consent was obtained from each of the patients to participate in the study. Twenty six (26) apparently healthy volunteers were recruited as control subjects. Ethical approval was obtained from the Medical Ethical Committee of the Obafemi Awolowo University Teaching Hospital.

Anthropometric Measurement: The weights in kilogrammes of all subjects were determined using the standard measuring scale. Heights in metres were taken with the subjects in a standing position, without foot wears. The subjects were made to stand against the wall with the hind soles of their feet touching the wall. The heights were directly read off using a ruler placed on the heads.Waist circumference in centimetres was taken as the minimum circumference between the coastal margins and the iliac Crest, measured in the horizontal plane with subjects standing.

Systolic and diastolic blood pressures in millimetres of mercury were measured using accosson sphygmomanometer.

Blood Sample Collection

Blood samples were drawn in the morning after an overnight fast of about 10-14 hours into Sodium Fluoride Oxalate bottles (2mg/ml) and Potassium-Ethylenediaminetetraacetic acid (kEDTA)-1mg/ml. The plasma were collected within 2 hours after centrifugation and stored at -20ºC before analysis.

Methods of Analysis*:*

The plasma glucose concentration was estimated using the Glucose Oxidase method obtained from Randox Laboratories in the United Kingdom.

Essential fatty acids were estimated by high performance liquid chromatography (HPLC). Using glucose oxidase method(Plasm glucose was estimated Accuracy and precision of biochemical tests were monitored by including commercially prepared quality control sera with each batch of test assay.

Statistical Analysis

All results were subjected to statistical analysis using SPSS version 14 and these were expressed as means ± standard error of means. Student t-test was used for statistical comparisons of two means and the differences were regarded as significant at $p < 0.05$. Post Hoc analysis was performed. Pearson's correlation coefficient was used to assess the relationship between parameters.

RESULTS

Table 1: Shows the biophysical and biochemical parameters of all subjects. There were significant increases in fasting plasma glucose, BMI, systolic and diastolic blood pressure ($p<0.001$) as well as waist circumference (WC) ($p<0.05$), when compared with the corresponding control values. There were significant decreases in plasma linoleic acid, arachidonic acid, folic acid and vitamin B_{12} ($p<0.001$), when compared with the corresponding control values. The patients were slightly older than the control subjects ($p<0.001$).

Table 2: Shows the biophysical and biochemical parameters in type 2 diabetic males and control males. There were significant increases in fasting plasma glucose, body mass index ($p < 0.05$), systolic blood pressure and diastolic blood pressure ($p < 0.001$) in type 2 diabetic males, when compared with the corresponding control males. On the other hand, significant decreases were obtained in plasma linoleic acid, vitamin B_{12} ($p < 0.001$), arachidonic acid and folic acid ($p < 0.05$) in type 2 diabetic males when compared with control male values. There was no significant difference in waist circumference of type 2 diabetic males compared with the male controls.

Table 3: Shows the biophysical and biochemical parameters in type 2 diabetic females and control females. There were significant increases in fasting plasma glucose, body mass index ($p < 0.05$), systolic blood pressure and diastolic blood pressure ($p < 0.001$) in type 2 diabetic females, when compared with the control females. On the other hand, there were significant decreases in linoleic acid, arachidonic acid, vitamin B_{12} ($p < 0.001$) and folic acid ($p < 0.05$) in type 2 diabetic females, compared with the corresponding control female values. There was no significant difference in waist circumference.

Table 4: Shows Pearson's correlation coefficient of all parameters in type 2 diabetic subjects.

There was a significant correlation between age and fasting plasma glucose ($r = -0.342$, $p < 0.01$); arachidonic acid ($r = -0.254$, $p < 0.05$) and systolic blood pressore ($r = 0.268$, $p < 0.05$). Diastolic blood pressure was significantly correlated with folic acid ($r = -0.293$, $p < 0.05$). Systolic blood pressure was significantly correlated with diastolic blood pressure ($r = 0.279$, $p < 0.05$). Body mass index was significantly correlated with waist circumference ($r = 0.282$, $p < 0.05$) and diastolic blood pressure ($r = 0.269$, $p < 0.05$). Arachidonic acid was significantly correlated with folic acid ($r = 0.293$, $p < 0.05$) and waist circuference ($r = -0.243$, $p < 0.05$). Waist circuference was significantly correlated with arachidonic acid ($r = -0.243$, $p < 0.05$). Vitamin B_{12} was not corrlated with any of the measured parameters.

Table 1 biochemical and biophysical parameter of all Subjects (Mean + SEM)

Variables	Patients (n =70)	Controls (n = 30)	t-value	p-value
Age (Yrs)	63.50 ± 1.33	38.42 ± 2.34	9.65	p<0.001
BMI (kg/m2)	29.45 ± 0.66	24.39 ± 0.76	4.35	p<0.001
WC (cm)	91.69 ± 1.95	84.08 ± 1.78	2.28	p<0.05
Syst BP (mmHg)	155.93 ± 2.58	115.38 ± 2.00	9.29	p<0.001
Diast BP (mmHg)	96.54 ± 1.83	73.00 ± 0.81	7.81	p<0.001
FPG (mmol/L	7.98 ± 0.48	4.73 ± 0.18	4.12	p<0.001
LA (µg/L)	105.03 ± 1.63	162.42 ± 7.54	-10.95	p<0.001
AA (µg/L)	59.70 ± 0.85	68.49 ± 1.02	-5.77	p<0.001
Folic Acid (µg/L)	52.65 ± 0.98	59.34 ± 1.09	-3.88	p<0.001
Vit B_{12} (µg/L)	48.47 ± 0.74	58.48 ± 1.51	-6.61	p<0.001

BMI = Body Mass Index

WC = Waist Circumference

Syst BP = Systolic Blood Pressure

Diast BP = Diastolic Blood Pressure

FPG = Fasting Plasma Glucose

LA = Linoleic Acid

AA = Arachidonic Acid

Vit B_{12} = Vitamin B_{12}.

Table 2 : Biophysical and Biochemical parameters in type 2 diabetic males and control males (Mean + SEM)

Variables	Type 2 Diabetic Males n = 32	Controls Males n = 10	t-value	p-value
Age (Yrs)	63.26 ± 2.05	38.00 ± 4.15	5.74	p<0.001
BMI (kg/m2)	28.17 ± 0.90	22.16 ± 0.76	3.49	p<0.05
WC (cm)	90.68 ± 2.63	81.78 ± 1.21	1.79	NS
Syst (mmHg)	161.19 ± 3.92	112.44 ± 3.40	6.45	p<0.001
Diast (mmHg)	95.26 ± 2.60	72.22 ± 1.10	4.7	p<0.001
FPG (mmol/L	8.70 ± 0.82	4.84 ± 0.32	2.51	p<0.05
LA (µg/L)	106.50 ± 2.39	168.51 ± 12.90	-7.68	p<0.001
AA (µg/L)	60.22 ± 1.47	67.81 ± 1.36	-2.68	p<0.05
Folic Acid (µg/L)	53.64 ± 1.36	59.95 ± 2.07	-2.27	p<0.05
Vit B_{12} (µg/L)	47.72 ± 1.16	59.28 ± 0.97	-5.17	p<0.001

BMI = Body Mass Index

WC = Waist Circumference

Syst BP = Systolic Blood Pressure

Diast BP = Diastolic Blood Pressure

FPG = Fasting Plasma Glucose

LA = Linoleic Acid

AA = Arachidonic Acid

Vit B_{12} = Vitamin B_{12}

Table 3: Biophysical and Biochemical Parameters in type 2 diabetic females and control females (Mean + SEM)

Variables	Type 2 Diabetic Females n = 38	Controls Females n = 20	t-value	p-value
Age (Yrs)	63.70 ± 1.78	38.65 ± 2.93	7.62	$p<0.001$
BMI (kg/m2)	30.51 ± 0.92	25.57 ± 0.98	3.27	$p<0.05$
WC (cm)	92.54 ± 2.86	85.29 ± 2.62	1.58	NS
Syst BP (mmHg)	151.51 ± 3.30	116.94 ± 2.46	6.7	$p<0.001$
Diast BP (mmHg)	97.62 ± 2.59	73.41 ± 1.11	6.2	$p<0.001$
FPG (mmol/L)	7.38 ± 0.56	4.66 ± 0.22	3.24	$p<0.05$
LA (µg/L)	103.80 ± 1.97	159.20 ± 9.48	-7.92	$p<0.001$
AA (µg/L)	59.25 ± 0.99	68.82 ± 1.41	-5.47	$p<0.001$
Folic Acid (µg/L)	51.82 ± 1.40	59.02 ± 1.30	-3.21	$p<0.05$
Vit B_{12} (µg/L)	49.09 ± 0.95	58.05 ± 2.27	-4.32	$p<0.001$

BMI = Body Mass Index

WC = Waist Circumference

Syst BP = Systolic Blood Pressure

Diast BP = Diastolic Blood Pressure

FPG = Fasting Plasma Glucose

LA = Linoleic Acid

AA = Arachidonic Acid

Vit B_{12} = Vitamin B_{12}

Table 4: Correlation Of All Parameters In The Type 2 Diabetic Patients

	Age (yrs)	BMI (kg/m2)	WC (cm)	Syst BP (mmHg)	Diast BP (mmHg)	FPG (mmol/L)	LA (µg/L)	AA (µg/L)	F A (µg/L)	Vit B_{12} (µg/L)
Age (yrs)				.269*		-.342**		-.254*		
BMI (kg/m2)			.282*		.269*					
WC (cm)		.282*						-.243*		
Syst BP (mmHg)	.269*				.279*					
Diast BP (mmHg)		.269*		.279*					-.293*	
FPG (mmol/L	-.342**									
LA (µg/L)										
AA (µg/L)	-.254*		-.243*						.293*	
Folic Acid (µg/L)					-.293*			.293*		
Vit B_{12} (µg/L)										

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

BMI = Body Mass Index

WC = Waist Circumference

Syst BP = Systolic Blood Pressure

Diast BP = Diastolic Blood Pressure

FPG = Fasting Plasma Glucose

LA = Linoleic Acid

AA = Arachidonic Acid

Vit B_{12} = Vitamin B_{12}

DISCUSSION

Sixty six percent of the type 2 diabetic patients had the disease for less than five years, while 33.9 percent had the disease for more than five years. 50.8 percent of the patients studied fell within the low-income earners, while 49.2 percent are within the middle class. 31.7 per cent of these subjects had no formal education, 19 per cent had only primary school education, 12.7 percent had secondary education and 36.5 percent had post-secondary education.

Findings from the present study showed that 78 per cent of the patients were on oral hypoglycaemic drugs, indicating that most of the patients had this disease for less than five years. 6.7 per cent were on insulin injection, while 15.3 percent were on both hypoglycaemic drugs and insulin injection.

Classifying these subjects using the World Health Organization (WHO) and the Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure guidelines, 47.1 percent of the patients were at stage 2 and these had systolic blood pressure ≥ 160 mmHg and diastolic blood pressure ≥ 100 mmHg. Thus suggesting that almost half of the patients may be predisposed to cardiovascular risk. Forty four percent of the patients were obese with BMI ≥ 30.0 kg/m^2. 16.1 percent of the male patients had waist circumference ≥ 102 cm, while 56.8 percent of the female patients had waist circumference ≥ 88 cm, indicating central obesity. Furthermore the result of this study shows that plasma linoleic acid concentration of the patients was significantly reduced. This finding is similar to an earlier report in which low concentration of plasma linoleic acid was obtained in type 2 diabetes mellitus patients (Hodge et al, 2007). Reduced levels of linoleic acid and reduced activity of delta-5-desaturase have been associated with insulin resistance in type 2 diabetes mellitus (Vessby, 2000). Increase intake of linoleic acid has been reported to improve insulin resistance in type 2 DM (Summers et al, 2002) and reduce the incidence of diabetes mellitus (Salmeron et al, 2001).

Available evidence revealed that higher serum linoleic acid levels have been associated with loweblood pressure (Grimsgaard *et al*, 1999), the reduced level in this study may have contributed in part to the high blood pressure obtaine in type 2DM. It could therefore be speculated that decreased lionleic acid could lead to high blood pressure..

Plasma arachidonic acid levels in type 2 DM were significantly lower. Available study (Broadhurst, 1997) reported that insulin stimulates desaturase enzyme system, which in turn influences the amount of polyunsaturated fatty acid available for membrane incorporation. Conversely the polyunsaturated fatty acid composition of membrane lipid influences the action of insulin (Broadhurst, 1997). In another study (Borkman et al, 1993) reported that the reaction catalyzed by delta-5-desaturase is directly related to the estimates of insulin sensitivity, raising the possibility that reduced delta-5-desaturase activity could contribute to impaired insulin action by decreasing the concentration of long chain polyunsaturated fatty acids in the membranes (Borkman *et al*, 1993). Arachidonic acid an important omega-6 polyunsaturated fatty acid serves as precursor for the synthesis of eicosanoids which are pro-inflammatory. This confers anti-hypertensive and cardio-protective properties. Reduction in plasma arachidonic acid may affect the production of epoxyeicotrienoic acid, which may lead to increase in premature cardiovascular risk in the patients. This study could not demonstrate whether the decreased in linoleic acid is as result of decreased delta -5- desaturase enzyme since this enzyme was not measured in this study. But it could be speculated otherwise that a decrease unsaturated membrane fatty acids and a reduced activity of delta-5-desaturase as demonstrated in earlier study (Vessby, 2000, Summers, 2002) could be contributory in part.

There was a considerable reduction in plasma folic acid in our patients and this reduction was positively correlation with decreased plasma arachidonic acid. Thus supporting an earlier study (Rao et al, 2006) which reported that reduced plasma folic acid is associated with decrease plasma concentrations of polyunsaturated fatty acids. Folic acid, because of its potential to enhance endothelial function in severe pathological conditions including type 2 DM and coronary artery disease, has gained considerable interest, a reduced level is suggesting that the patients are likely prone to impaired endothelial function.

Again several studies (Zhang et al, 2000, Weiss et al, 2003) have linked reduced plasma folic acid with hyperhomocysteinemia and this has been reported to have direct effect on endothelial function (Doshi et al, 2002). Hence reduced level of folic acid in all the patient could be a contributory factor to cardiovascular morbidity and mortality.

The decreased Vitamin B_{12} and folic acid obtained in this study suggest that the patients may have increased likelihood of developing premature CVD, since reduced plasma levels of both vitamins have been strongly associated with hyperhomocysteinaemia (Weiss et al, 2003) changes that has been closely associated with cardiovascular disease. Vitamin B_{12} is used to regenerate folate in the body, concomitant reduction in vitamin B_{12} will invariably lead to decreased folic acid such as obtained in this study, this could give rise to degenerative diseases and abnormal lipid accumulation. Whether reduced plasma vitamins B_{12} and folate are partly the cause of decreased plasma linoleic acid and arachidonic acid in these patients, could not be ascertain from the results of this study. However, without vitamin B_{12}, folate is trapped as 5-methyl folate, making folate unavailable for use (Vasudevan, and Sreekumari, 2007). Therefore low levels of vitamin B_{12} in the patients may lead to neurological disorders and hyperhomocysteinaemia, which could impair endothelial function. There is however an ongoing work on plasma homocysteine and type 2 DM.

CONCLUSION

The decreased levels of linoleic acid, arachidonic acid, folic acid and vitamin B_{12} obtained in this study indicated that type 2 diabetic patients are at increased risks of vascular and coronary artery disease. Therefore, dietary supplementation of these essential polyunsaturated fatty acids, folate and vitamin B_{12} may enhance wellbeing and reduce the propensity to type 2 diabetes mellitus complications.

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