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Effect of Glycaemic Control on Serum Retinol and Beta Carotene Levels in Type 2 Diabetics in Calabar, Nigeria

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ABSTRACT

The study aimed to determine the effect of glycaemic control on serum retinol and beta-carotene levels in diabetics and non-diabetics. Fasting blood glucose (FBS), glycated haemoglobin (HbA1c), serum retinol and beta-carotene levels were estimated using colorimetric methods in 100 diabetic subjects and 65 age-matched non-diabetic controls. The body mass index (BMI) of all subjects was determined. The mean FBS, HbA1c, retinol and beta-carotene levels were significantly ($p<0.05$) higher in diabetics than in non-diabetic subjects. No significant ($p>0.05$) difference was observed in the serum retinol and beta-carotene levels in diabetics with poor glycaemic control and those with good control. A significant negative correlation ($p<0.01$, $r = -0.314$) was observed between HbA1c and serum retinol levels of the diabetic population. Age, sex, BMI and duration of diabetes do not have any significant ($p>0.05$) effect on the serum retinol and beta-carotene levels in all the subjects. Diabetics have higher levels of retinol and beta-carotene perhaps as a result of high dietary intake. This study found that glycaemic control and duration of diabetes do not seem to affect the metabolism of vitamin A.

INTRODUCTION

Vitamins are organic nutrients that are required in small quantities for a variety of biochemical functions. The body does not generally synthesise them, hence their absence or relative deficiency in the diet leads to characteristic deficiency states and diseases (Mayes, 2000). Vitamin A (retinol) and its provitamin beta-carotene are essential for maintenance of sight and antioxidant activity against free radicals in the body. Free radicals have been implicated in the aetiogenesis of most degenerative diseases including diabetes mellitus (Arora and Gores, 1995). Oxidative stress impairs insulin action by changing the physical state of the plasma membrane of target cells for insulin action (Vessby, 2000). The role of beta-carotene and vitamin A in glucose metabolism still remain unclear because of conflicting reports. Their role in carbohydrate metabolism has been attributed to the antioxidant properties of beta-carotene (Mayes, 2000). Some studies have reported that free radicals cause disruption in insulin action and mitigate glucose intolerant state, but administration of vitamin A alleviates the situation (Ford *et al.*, 1999). Some enzymes such as glucokinase, phosphofructokinase, and phosphoenol pyruvate, which are involved in glucose metabolism, have been reported to be influenced at the gene

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level by retinoic acid (Palmer and Paulson, 1997). Facchini *et al.* (1996) reported improvements in blood glucose levels in diabetics taking diet supplements rich in vitamin A. These suggest that vitamin A and beta-carotene may be associated with insulin secretion and carbohydrate metabolism. Poor glycaemic control and protein glycosylation have been implicated in the development of the various diabetic complications and disordered metabolism of nutrients in diabetes.

This work therefore assesses the effect of glycaemic control on serum levels of vitamin A and beta-carotene in diabetics and non-diabetics to determine the status of these antioxidant vitamins in diabetes.

MATERIALS AND METHODS

Study design

The study was carried out in Calabar, Cross River State, Nigeria to determine the serum beta carotene and retinol levels in type 2 diabetics attending the diabetic clinic of the University of Calabar Teaching Hospital (UCTH), and Non-Diabetic subjects selected from apparently healthy individuals attending the out-patient clinic of UCTH, as well as the effects of glycaemic control on the serum levels of these vitamins. The objectives of the study were explained to the volunteers before recruitment into the study. The ethics committee of the UCTH approved the study protocol. Subjects were selected based on the following criteria; age range between 45 and 75 years at the time of study, known type 2 diabetic patient for the past one year, and non-diabetic according to the World Health Organisation diagnostic criteria for diabetes (WHO, 1999). Subjects who were pregnant or hypertensive were excluded from the study. Diabetes in this study was defined based on laboratory findings as fasting plasma glucose levels greater than 7.00mmol/l in two or more occasions or 2-hour postprandial plasma glucose levels greater than 11.10mmol/l in two or more occasions (WHO, 1999). Diabetes duration was defined as time since diagnosis of type 2 diabetes.

Subjects

A total of one hundred and sixty five subjects were randomly selected for the study. One hundred (56 males and 44 females) diabetic subjects participated as test subjects whereas sixty-five (36 males and 29 females) apparently healthy individuals participated as control subjects. All subjects were advised to report to the clinic in the fasting state on the morning of the day for sample collection.

Blood pressure

The systolic and diastolic blood pressures of subjects were taken at 3 intervals one-month prior to sample collection. This is to rule out hypertension. The pressure was also taken on the day of the test in a seated relaxed position.

Anthropometric data

Body weight and height were measured while subjects wore light indoor clothing but no shoes. This was used to calculate the body mass index (BMI), which is used as a measure of relative body weight. Normal body weight was defined as BMI between 18-25 kg/m^2 while generalised obesity was defined as BMI >30 kg/m^2 (WHO, 2000).

Glycaemic Control

Measurement of glycated haemoglobin was used as index of glycaemic control. Good glycaemic control was defined as HbA1c <7.0% while poor glycaemic control was defined as HbA1c >8.0% (ADA, 2002)

Dietary Data

Information on frequency of consumption of common vitamin A-rich foods by subjects was obtained using a structured food consumption questionnaire. Food and fruits rich in vitamin A and beta-carotene commonly seen in our locality include: green leafy vegetables, carrots, pumpkins, papayas, mangoes, oranges, cucumbers, eggs, milk and meat.

Other Data

Data on the sight/vision of subjects recruited for the study were obtained from their medical history. A structured questionnaire was used to obtain data on age, marital status, occupation, physical activity, alcohol consumption, smoking habits and medication.

Sample Collection

Fasting venous blood samples were collected from each subject for fasting plasma glucose, glycated haemoglobin and serum retinol and beta-carotene estimation.

Methods

Fasting plasma glucose was estimated using the glucose oxidase method of Barham and Trinder (1972), glycated haemoglobin was estimated using the column chromatography method with cation exchange resin of Trivelli *et al.* (1971), while serum retinol and beta carotene were estimated using the Carprice colorimetric method.

Statistical analysis

Results are presented as mean ± S D. The significance of difference between groups was tested using the t test analysis. A two-sided p value <0.05 was considered statistically significant for t- test. T test analysis was used to test for significance of difference in these instances; FPG, HbaA1c, systolic and diastolic blood pressure, retinol and beta carotene levels in diabetic and non-diabetic subjects, retinol and beta carotene levels in good and poor glycaemic control states, in the young (40-55) and elderly (>55), and in male and female subjects of the study.

The variation of serum retinol and beta-carotene level with duration of diabetes, and BMI was determined using a one-way analysis of variance (ANOVA). P value < 0.05 was also considered significant for the ANOVA.

Associations between variables were determined using the pearsons correlation analysis on Microsoft Excel and SPSS software 10.0 version (California Inc.). A two-sided p value <0.01 was considered statistically significant for the correlation analysis.

RESULTS

Table 1 shows the baseline characteristics of the study population. In the diabetic population, 63% were females while 37% were males. 6% of the population were single, 70% were married, 16% were widowed whereas 8% were divorced. In terms of occupation, 45% were civil servants, 30% were businessmen, 15% were labourers whereas 10% were unemployed. 60% went about their daily business by trekking or use of public transport, 8% were chauffeur driven, 12% were self-driven whereas 20% used other means of transportation. For social habits such as alcohol intake and smoking, 37% were moderate drinkers, 53% were non-drinkers, 91% were non-smokers while 9% were moderate smokers. In terms of hypoglycaemic agents used by diabetics, 30% used biguanides, 45% used sulphonylureas, 25% made use of insulin, 80% took vitamin supplements whereas 100% took anti-malarial drugs. In terms of vision,

Table 1. Baseline Characteristics of the study population

	Diabetics N = 100 (%)	*Non-diabetics N = 65 (%)*
Age (years)	51±9.00	51.20±12.26
Females	63	53.80
Males	37.00	46.20
BMI (kg/m^2)	24.39±3.69	23.02±4.1
Marital Status		
Single	6	8
Married	70	42
Widowed	16	7
Divorced	8	8
Occupation		
Civil Servant	45	30
Businessmen	30	20
Labourer	15	10
Unemployed	10	5
Physical Activity		
Trekking/Public Transport	60	35
Chauffeur driven	8	5
Self driven	12	10
Others	20	10
Alcohol consumption		
Moderate drinkers	37	40
Non drinkers	53	25
Smoking habit		
Non smokers	91	50
Moderate smokers	9	15
Vision		
Normal sight	52	60
Short sighted	18	2
Long sighted	10	3
Blurred vision	20	-
Medication		
Biguanides	30	-
Sulphonylureas	45	-
Insulin	25	-
Vitamin supplements	80	30
Anti malaria drugs	100	70

52% had normal sight, 18% were short sighted, 10% were long sighted whereas 20% suffered from blurred vision. 69% have been suffering from diabetes for less than 5 years, 18% have had it between 6 and 10 years whereas 13% have had it for more than ten years.

In the non-diabetic population, 53.80% were females, 46.2% were males, 12.3% were single, 64.62% were married, 10.76% were widowed whereas 12.3% were divorced. In terms of occupation,

46.14% were civil servants, 30.76% were businessmen, 15.38% were labourers and 7.69% were unemployed. 53.83% of the non-diabetics went about their daily activities by trekking or public transport, 7.69% were chauffer driven, 15.38% were self-driven whereas 15.38% fell into other categories of transportation. For social habits such as alcohol intake and smoking, 61.52% were moderate drinkers, 38.45% were non-drinkers and 76.90% were non-smokers whereas 23.07% were moderate smokers. In terms of medication, 30% took vitamin supplements whereas 70% took anti malaria drugs. 60% had normal vision, 2% were short sighted, 3% were long sighted and none had blurred vision.

Table 2 shows the frequency of consumption of common vitamin A-rich foods in the diabetic and non-diabetic populations of the study. In the diabetic population, those that consumed green leafy vegetables, carrots, pumpkins, papayas, mangoes, oranges, cucumbers, eggs, milk, meat and carbohydrate-rich foods more than once per week were 98%, 61.9%, 98%, 76%, 69%, 87%, 92%, 64%, 88%, 77% and 95% respectively, while those that consumed these foods for less than 3 times per month were 2%, 39.10%, 2%, 24%, 31%, 13%, 8%, 36%, 12%, 23% and 5% respectively. In the non-diabetic population, those that consumed green leafy vegetables, carrots, pumpkins, papayas, mangoes, oranges, cucumbers, eggs, milk, meat and carbohydrate-rich foods more than once per week were 98%, 30%, 94%, 33%, 44%, 51%, 62%, 71%, 89%, 85% and 99% respectively, while those that consumed these foods for less than 3 times per month were 2%, 70%, 6%, 67%, 56%, 49%, 38%, 29%, 11%, 15% and 1% respectively.

Table 3 shows the mean age, BMI, systolic and diastolic blood pressure, fasting plasma glucose, glycated haemoglobin, beta-carotene and retinol levels in diabetics and non-diabetic subjects. The mean FPG, HbA1c, b-carotene and retinol levels were significantly ($p<0.05$) higher in diabetics than in non-diabetics. No significant ($p>0.05$) differences were observed in

Table 2. Frequency of consumption of common vitamin A-rich foods

Food type	*Frequency*[1]				*Availability*[2]
	Diabetics		*Non-diabetics*		
	≥ 1 time/week	*≤ 3 times/mo*	*≥ 1 time/week*	*≤ 3 times/mo*	
Green leafy vegetables	98	2	98	2	12
Carrots	62	39	30	70	12
Pumpkin	98	2	94	6	12
Papaya	76	24	33	67	12
Mango	69	31	44	56	3
Orange	87	13	51	49	6
Cucumber	92	8	62	38	6
Egg	64	36	71	29	12
Milk	88	12	89	11	12
Meat	77	23	85	15	12
Carbohydrates	95	5	99	1	12

[1] Values are percentages
[2] Values are median number of months per year

Table 3. Mean age, BMI, blood pressure, fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), beta-carotene and retinol levels in diabetics and non-diabetics

Subjects	*Age (years)*	*BMI (kg/m^2)*	*Blood pressure*		*FPG (mmol/l) (mmHg)*	*HbA1c (%)*	*β-carotene (μg/dl)*	*Retinol (μg/dl)*
			Diastolic	*Systolic (mmHg)*				
Diabetics n = 100	51.34 ±9.00	24.39 ±3.69	77.00 ±9.30	122.10 ±14.30	8.00 ±3.63	8.27 ±1.18	70.10 ±24.10	46.98 ±13.60
Non Diabetics n = 65	51.20 ±12.26	23.02 ±4.19	76.10 ±7.35	120.5 ±10.50	4.07 ±0.61	6.60 ±1.28	53.80 ±14.09	41.40 ±15.08
P value	p>0.05	p>0.05	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05	p<0.05

the ages, BMI, and systolic and diastolic blood pressures of both groups.

Table 4 shows the serum retinol and b-carotene levels in different states of glycaemic control. No significant (p>0.05) differences were observed in the serum retinol and beta-carotene levels in diabetics with good glycaemic control and those with poor glycaemic control.

Tables 5 and 6 show the influence of duration of diabetes and BMI respectively on serum beta carotene and retinol levels in the diabetic population, whereas Tables 7 and 8 show the influence of sex and age respectively on serum beta carotene and retinol levels of all subjects of the study. No significant variations (p>0.05) were observed in the serum retinol and beta carotene levels with duration of diabetes (>5 years, 6-10 years & >10 years) and BMI (BMI ranges: 15-25 kg/m^2, 25-30 kg/m^2 & >30 kg/m^2). No age or sex related differences were observed in serum retinol and beta carotene levels in all populations of the study.

Figure 1 shows the correlation plot of HbA1c and serum retinol levels in the diabetic population of the study. A significant negative correlation (p<0.01, r = -0.314) was observed between HbA1c and serum retinol levels.

DISCUSSION

Vitamin A (retinol) and its provitamin beta-carotene play an essential role in the pancreatic secretions of insulin and glucagon that regulate glucose metabolism and gluconeogenesis in the body. The actual status of these vitamins in diabetes is still controversial because of inconsistent and conflicting results. Some studies reported lower serum retinol concentration in type 1 and type 2 diabetes (Harvivi *et al.*, 1991; Martinoli *et al.*, 1993; Olmedilla *et al.*, 1997), some reported higher serum vitamin A levels in patients with impaired glucose tolerance and type 2 diabetes (Sasaki *et al.*, 1995; Tavriduo, 1997), others observed no significant differences in their levels between diabetics and non-diabetics (Basuldo *et al.*, 1997) whereas some established an inverse association between serum carotenoids and type 2 diabetes (Coyne *et al.*, 2005). These controversies have been attributed to nutrition, environmental factors and the presence of other ailments.

The beta-carotene and retinol levels were found to be significantly higher in diabetics than in the control subjects of the study. This may be attributed to the fact that the diabetics used in the study consumed a lot of vegetables rich in beta-carotene compared to the control group. Higher dietary vitamin A intake and

Table 4. Serum beta-carotene and retinol levels in different states of glycaemic control

Control state	*N*	*β-carotene (μg/dl)*	*Retinol (μg/dl)*
Good control			
HbA1c < 8.0%	35	73.09 ±28.27	51.90 ±9.40
Poor control			
HbA1c >8.0%	65	69.50 ±21.83	53.60 ±10.90
P value		p>0.05	p>0.05

Table 5. Influence of duration of diabetes on serum beta-carotene and retinol levels

Duration (Years)	*N*	*β-carotene (μg/dl)*	*Retinol (μg/dl)*
< 5	69	68.76 ± 20.75	49.60 ± 11.25
6 - 10	18	68.27 ± 30.90	43.97 ± 13.20
≥ 10	13	73.36 ± 23.80	43.00 ± 15.56
P - value		p>0.05	p>0.05

Table 6. Influence of BMI on serum beta-carotene and retinol levels

BMI (Range)	*N*	*β-carotene (μg/dl)*	*Retinol (μg/dl)*
Normal weight (18-25kg/m^2)	65	69.37 ± 26.76	48.60 ± 12.06
Pre-obese (25-30kg/m^2)	25	71.07 ± 23.16	47.24 ± 13.45
Obese (> 30kg/m^2)	10	67.97 ± 18.25	43.25 ± 12.46

Table 7. Influence of sex on serum beta-carotene and retinol levels

Sex	*N*	*β-carotene (μg/dl)*	*Retinol (μg/dl)*
Males	67	46.00 ± 12.17	62.70 ± 24.25
Females	98	47.78 ± 13.10	68.36 ± 21.04
P - value		p<0.05	p>0.05

Table 8. Influence of age on serum beta-carotene and retinol levels

Age (years)	*N*	*b-carotene (μg/dl)*	*Retinol (mg/dl)*
40-55	128	61.25 ± 21.58	56.70 ± 6.19
56-75	37	67.04 ± 23.27	45.89 ± 13.37
P - value		p>0.05	p>0.05

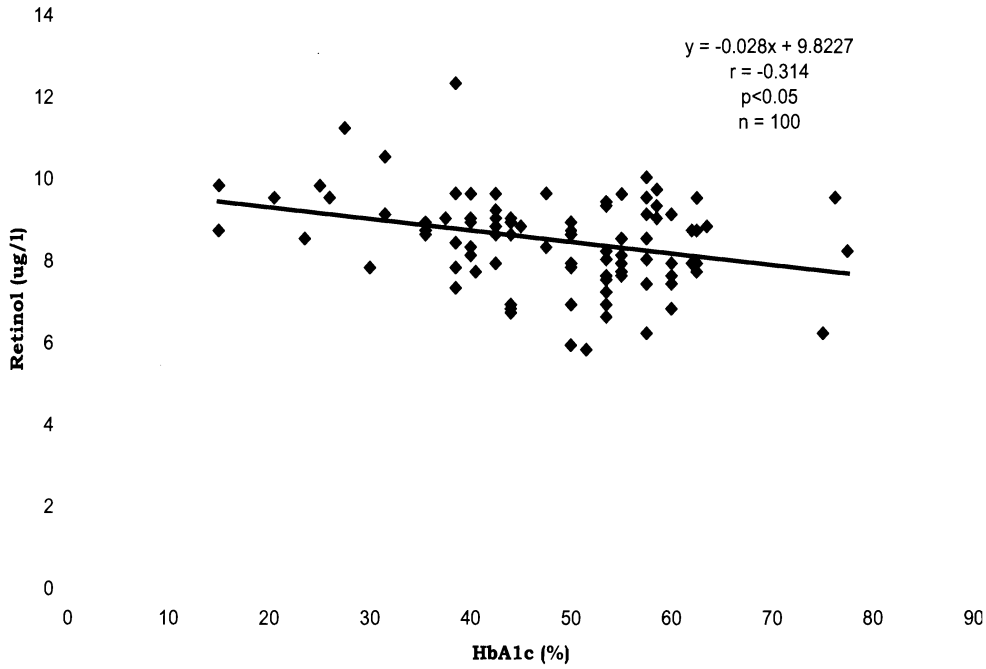

Figure 1. Correlation plot of HbA1c and Retinol in diabetics

Figure 1. Correlation plot of HbA1c and Retinol in diabetics

supplementation has been associated with higher serum levels (Comstock *et al.*, 1988; Ylonen *et al.*, 2003). A similar finding was made by Hozumi *et al.* (1998). Biological activities of carotenoids include induction of cell-to-cell communication (Stahl and Sies, 1997). Junctional communication of beta cell was shown to contribute to the control of insulin secretion and glucose tolerance (Charollais *et al.*, 2000). However, lower retinol and beta-carotene levels have been reported in diabetics independently by Baena *et al.* (2002) and Abahusain *et al.* (1999). An inverse relationship between plasma beta carotene levels and fasting plasma glucose have also been reported (Ylonen *et al.*, 2003). A significant negative correlation was observed between serum retinol levels and glycated haemoglobin levels of the diabetic population. This agrees with the findings of Hozumi *et al.* (1998) who reported a significant correlation between plasma beta-carotene levels and HbA1c and fructosamine in both diabetics and non-diabetics. However, no significant difference was observed in serum retinol and beta-carotene levels between diabetics with poor glycaemic control and those with good control. Two cross-sectional studies also failed to show a relation between beta-carotene intake and glycated haemoglobin (Boeing *et al.*, 2000; Sargeant *et al.*, 2000; Shoff *et al.*, 1993).

The duration of diabetes does not seem to have any effect on serum retinol and beta-carotene levels in this study. This may be due to the fact that 77% of diabetics used in the study have had the disease for less than five years. Olmedilla *et al.* (1994) also reported that the duration of diabetes does not have any effect on serum carotenoids levels except for lycopene.

Age, sex and body mass index (BMI) seem to exert no significant influence on serum retinol and beta-carotene levels in this study. However Olmedilla *et al.* (1997), observed higher retinol levels in males than in females in all populations as well as in the diabetic group. Both sexes have lower retinol concentrations than their sex-matched controls (Baena *et al.*, 2002). This was attributed to reduced mobilisation of vitamin A from the liver in diabetes (Basu & Basuldo, 1997). Higher concentrations of provitamin A have been reported in women than in men in all populations regardless of dietary habits (Abiaka *et al.*, 2002). No clear-cut association of retinol and beta-carotene was noted with age by Comstock *et al.* (1988). Contrary to our findings, plasma retinol levels have been reported to be significantly and independently reduced in younger subsets of diabetics as compared to the controls (Martinoli *et al.*, 1993). Obese people have also been reported to have lower retinol and beta-carotene concentrations that were 2-10% lower than in normal weight people (Neuhousar *et al.*, 2001). Plasma beta-carotene concentration was also reported to be inversely correlated with BMI in both males and females (Ylonen *et al.*, 2003). The reason for this remains speculative but it has been suggested that dietary differences (Strauss, 1999) and variability in body compartment size (Brady *et al.*, 1996) are likely explanations.

CONCLUSIONS

Results from this study have shown that diabetics have higher serum levels of retinol and beta-carotene that may be as a result of higher dietary intake as seen from the questionnaire. An inverse relationship exists between glycated haemoglobin and serum retinol levels in diabetics. Duration of diabetes and BMI do not seem to affect the serum retinol and beta-carotene levels. However, the essential roles of vitamin A in alleviating some of the complications of diabetes cannot be over-emphasised, diabetics should therefore be encouraged to consume more vegetables and vitamin A-fortified diets. This will go a long way in improving life expectancy and patients' outcome in diabetes.

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