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Influence of Oxidative Stress, Skeletal Muscle Mass and Obesity on Type 2 Diabetes Mellitus among Patients in Kumasi Metropolis

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ABSTRACT

The pathogenesis of type 2 diabetes is a complex phenomenon. Many research works have implicated obesity and dyslipidaemia as possible causes of type 2 diabetes mellitus. The aim of this study was to determine the association between BMI, oxidative stress and skeletal muscle mass in the aetiology of type 2 diabetes. The study was conducted at the Komfo Anokye Teaching Hospital, Kumasi Ghana. The study involved 120 diabetic subjects and 80 non diabetics as control, matching age and sex with the diabetics. Anthropometric parameters measured include height, weight, waist circumference, hip circumference, thigh circumference, plasma glucose was determined by the enzymatic method and plasma creatinine concentration by the Jaffe reaction using creatinine reagent. Urine sugar was estimated using a urine test strip. Malonyldialdehyde level in serum was estimated spectrophotometrically according to the Buege and Aust method. The body mass index was significantly higher in the diabetics than the controls (26.45±6.49 and 22.13±3.30 kg m^{-2}) ($p<0.001$), respectively. Fasting blood glucose levels were obviously higher in the diabetics than in the non-diabetics (9.925±0.544 and 5.448±0.88 mmol L^{-1}) ($p<0.0015$). Serum MDA concentration was significantly higher in the diabetics than the controls (0.29±0.03-0.23±0.02 μmol L^{-1} ($p<0.0502$) while Serum creatinine of the diabetics was non significantly lower than that of the controls (98.70±53.94-101.9±34.00μmol L^{-1} ($p<0.3677$). Overweight, skeletal muscle and oxidative stress may play a significant role in the aetiology of type 2 diabetes or at least aggravate the diabetes.

Key words: Body mass index, fat distribution, thigh circumference, waist circumference

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterised by excessive urine excretion and a fasting blood sugar in excess of 7.0 mmol L^{-1} or random blood sugar in excess of 11.1 mmol L^{-1} as defined by the World Health Organisation (Wild *et al*., 2004). The pathogenesis of type 2 diabetes involves two main abnormalities: Insulin resistance and inadequate insulin secretion from pancreatic β-cells. There is a strong genetic influence on susceptibility of type 2 diabetes. However, the susceptibility genes that predispose 1 to type 2 diabetes have not been identified due to their polygenic nature. In pre-type 2 diabetes, there is initially high production of insulin but organ tissues fail to respond adequately to the insulin. The hyperinsulinaemia associated with insulin resistance is a consequence of both an increase in insulin secretion and a reduction in insulin clearance rates. In the short term the pre-diabetic is able to maintain normal plasma glucose despite a continued decline in insulin sensitivity. However, as the insulin

resistance gradually increases, the pancreas is less able to compensate by increasing insulin production (Purrello and Rabuazzo, 2000). Progressive hyperglycaemia eventually gives rise to overt diabetes. In the long term, the β-cells are not able to secrete enough insulin to overcome resistance and chronic hyperglycaemia develops which further impairs insulin secretion and action (glucotoxicity). There is an increase in lipid metabolism to compensate for the declining glucose metabolism and the rising free fatty acids gives rise to 'lipotoxicity' which results in further damage to β-cells (Arner, 2002).

Obesity has been identified as a predisposing factor of type 2 diabetes (Kahn *et al*., 2006; McTernan *et al*., 2002). In predisposed people, type 2 diabetes commonly arises with progressive obesity especially visceral obesity (Kolaczynski, 2000). Type 2 diabetes is associated with insulin resistance. The pathological mechanisms involve in insulin resistance are not well understood. However, a number of cellular and molecular metabolic abnomalities have been proposed in this condition which includes insulin receptor defects, defective receptor signalling pathway, defective glucose mobilization and utilization and free fatty acid dysregulation. The development of obesity is characterized by an imbalance between energy intake and energy expenditure. Obesity results from increased energy intake and reduced energy expenditure (Simoneau *et al*., 1999). Obesity and insulin resistance are well established risk factors for type 2 diabetes mellitus (Haffner *et al*., 1990; Hofso *et al*., 2009).

The type and size of the skeletal muscle is also another predisposing factor of type 2 diabetes. Skeletal muscle, containing creatine is the most important site of insulin resistance and accounts for approximately 90% of overall glucose disposal (Ferrannini *et al*., 1985). The amount of creatine per unit of skeletal muscle is stable and in normal renal function, plasma creatinine, a direct metabolic product of creatine's concentration is therefore a direct reflection of skeletal mass (Bousnes and Taussky, 1945). Low serum creatinine level depicts a low skeletal mass and is associated with a high risk of type 2 diabetes in non obese men (Harita *et al*., 2009) probably because of the decreased ability of glucose and lipid disposal. Skeletal muscle in obese individual and in mice have been shown to have reduced number or defective mitochondria and therefore, reduced skeletal muscle utilization of glucose and lipids (Kelley *et al*., 2002; Bonnard *et al*., 2008) partly responsible for the observed insulin resistance in obese subjects.

Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems involving increased production of free radical or decreased activity of antioxidant scavenging effect or both (Mullarkey *et al*., 1990). Mechanisms of oxidative stress in the pathogenesis of diabetes involves not only the oxygen free-radical generation but also non-enzymatic glycation of proteins, auto-oxidation of glucose, limiting glutathione metabolism that requires the utilization of NADH during auto-oxidation of glucose, decrease in antioxidant enzymes and activity, lipid peroxide formation and reduce ascorbic acid levels (Mullarkey *et al*., 1990). Also, metabolic stress during changes in energy metabolism, changes in sorbitol pathway activity, changes in the level of inflammatory factors and the damage of tissue from hypoxia and ischemic reperfusion injury (Baynes, 1991) all worsen the oxidative stress.

The generation of malondialdehyde (MDA), a Thiobarbituric Acid Reacting Substance (TBARS) (Gupta and Chari, 2006) is as a result of damage of cellular membrane polyunsaturated fatty acids. Plasma concentrations of lipid hydroperoxides, TBARS, isoprostanes, protein carbonyls, methionine sulfoxidation, tyrosine products, 8-hydroxy-2'-deoxyguanosine (8-OHdG), leukocyte DNA-MDA adducts and DNA-strand breaks have been investigated to ascertain oxidative stress (Kadiiska *et al*., 2005). However, the detection of increased levels of oxidation products in tissues

though is not, sufficient to implicate oxidative stress in the pathology unless the damage can be precisely related to the development of pathology and until it can be proved that inhibition of oxidative damage prevents or retards the disease process.

This work is therefore focused on the determination of the association between oxidative stress marker, obesity and skeletal muscle mass to the development or progression of type 2 diabetes in a section of the Ghanaian population. There are still conflicting reports on the role of obesity and skeletal muscle mass on the aetiology of type 2 diabetes that is ethnicity related. For instance reports show low serum creatinine level to be associated with a high risk of type 2 diabetes in non obese middle-aged Japanese men (Harita *et al*., 2009). In another study in Japan, whole-body skeletal muscle mass was found not associated with either glucose tolerance or insulin sensitivity in overweight and obese men and women (Kuk *et al*., 2008) contrary to findings in Caucasian and Mexican Americans where obesity and insulin resistance are well established risk factors for type 2 diabetes mellitus (Hofso *et al*., 2009; Resnick *et al*., 2003).

MATERIALS AND METHODS

A cross sectional study conducted on diabetes mellitus patients who patronize the Komfo Anokye Teaching Hospital (KATH) in the Kumasi metropolis (Ghana) and are on some form of drug medication. The study involved 120 sequentially enrolled diagnosed type II diabetics aged between 25-70 years who reported at the diabetic clinic and 80 non-diabetic healthy volunteers (some of who accompanied their patients) marching age to sex of the diabetic patients were selected for the negative controls. All procedures were approved by the Committee on Human Research Publication and Ethics of School of Medical Sciences, KNUST (CHRPE/Student/113/09). A written informed consent form was completed by all the participants who were recruited into the study after the study was explained in a language they understood.

Sample collection: After carefully selecting a satisfactory vein in the *Cubital fossa* for venipuncture (the median cubital vein), the site was disinfected with 70% alcohol with a cotton swab. With a tourniquet in place, about 3 mL of blood was taken from each subject after an overnight fast of about 8-12 h. The blood was then dispensed into labeled plain BD vacutainer®, tubes and fluoride oxalate coated tubes for fasting blood glucose (Becton Dickenson, Plymouth, UK) which was immediately analyzed. After clotting, blood sample in the plain tubes were centrifuged at 3000 g for 3 min and the serum stored at-20°C until ready for analysis. Early morning urine was also collected in a clean wide mouth bottle and screwed capped, for urine sugar and was immediately analyzed.

Anthropometric measurement: Body weights were measured (to the nearest 0.5 kg), with the subject standing on a weighing scale (wearing light clothing) after the weighing scale was adjusted to 0 kg and calibrated using known weights. Heights were measured (to the nearest 1.0 cm). Measurements of the thighs circumference was taken from the middle point between the inguinal fold and the proximal border of patella. The waist measurements were taken from the middle point between the iliac crest and the last rib, as recommended by the World Health Organization (WHO, 1995). Measurements were made twice to the nearest centimeter and the mean used for subsequent analysis. All measurements were read in centimeters (cm) but the height was converted to meters. BMIs were then calculated as weight in kilograms divided by the height in meter squared.

Biochemical assay: Plasma glucose was determined enzymatically on an automated machine (Roche 9180 Electrolyte Analyzer (AVL Medical Instruments, AG and Switzerland) with specific reagent kit designed for the equipment and creatinine by the Jaffe reaction using Creatinine reagent (ELITech®). Urine sugar was estimated using a urine test strip. Presence or absence of glucose in the urine was indicated by a colour change of the urine strip.

Malondialdehyde (MDA): The malonyldialdehyde level in serum was estimated spectrophotometrically according to (Buege and Aust, 1978). About 0.2 mL^{-1} aliquot of serum was added to 1.0 mL^{-1} of 0.375% thiobarbituric acid solution in 0.25 M HCl and mixed with 4.0 mL^{-1} of 15% trichloroacetic acid. After incubation at 100°C for 15 min, the samples were cooled, centrifuged and the supernatants evaluated spectrophotometrically at 535 nm against a reference blank. The MDA was determined by using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} and results were expressed in μmol L^{-1}.

Data analysis and statistics: Results were expressed as Means±SEM. Data were analysed by one-way ANOVA followed by the Bonferroni test for multiple comparison using Graph Pad Prism version 4 (Graph Pad Software, San Diego California). Unpaired Student t-tests were used to assess for significance. Statistical significance was set at $p\leq0.05$ for the various parameters in the study.

RESULTS

Hip circumference, height, weight were not significantly different between the diabetic and the control. However, the waist circumference, WHR and BMI of diabetics were significantly ($p<0.001$) higher in the diabetics than that of the controls whereas the thigh circumference was significantly lower in the diabetics.

Serum creatinnine level, though lower in the diabetics but was not statistically significantly different from the controls. Fasting blood glucose and MDA were significantly higher in diabetics, whilst 6.9% of the diabetics had very high amount of sugar in the urine (pos+++), 2.5% of the diabetics and controls had trace amount but a large percentage of the controls (95%) had no urine sugar.

Creatinnine levels were lower in the obese diabetics and underweight diabetics though not statistically significant but the trend indicates a reduced muscle mass in the obese and underweight subjects. MDA was significantly higher in the diabetics than in the normal or underweight, indicating an increased expression of oxidative stress in obesity. Similarly, plasma glucose was significantly higher in the obese diabetics than the normal or overweight. Even in the nondiabetics, glucose in the obese was nonsignificantlyl higher than in the normal weight and underweight. The trend further strengthens the metabolic dysregulatory effect of obesity.

Fasting blood sugar was negatively correlated with BMI and WC but significantly with BMI. BMI was positively significantly correlated with WC and negatively with MDA and creatinnine and WC was negatively correlated with MDA and positively correlated with creatinnine.

DISCUSSION

The anthropometry of the subjects suggests that obesity and fat distribution may be the key factors of diabetes in the subjects. The Waist Circumference (WC), Waist-to-Hip Ratio (WHR) and

Table 1: Anthropometric measurements of diabetics and non diabetics

Parameters	Diabetics	Control	p-value
Waist circumference (cm)	91.78±11.12	84.33±6.86	0.0001
Hip circumference (cm)	100.52±10.60	112.18±12.72	0.1636
WHR	0.91±0.02	0.75±0.04	0.0001
Height (m)	1.64±0.12	1.64±0.01	0.4059
Weight (kg)	73.47±17.34	71.64±19.92	0.2002
Body mass index (BMI) (kg m^{-2})	26.45±0.73	22.13±0.54	0.0001
Thigh circumference (cm)	57.15±0.93	60.71±0.71	0.0021

$p<0.05$ is significant

Table 2: Biochemical characteristics of the diabetics and non diabetics

Parameters	Diabetics	Control	p-value
Creatinnine (μmol L^{-1})	98.70±6.03	101.90±5.38	0.3677
FBS (mmol L^{-1})	9.925±0.54	5.448±0.88	0.0037
MDA (μmol L^{-1})	0.29±0.025	0.23±0.02	0.0502
Urine sugar			
Neg	58(72.50)	38(95.0)	0.0033
Trace	2(2.50)	1(2.50)	1
Pos(+)	5(6.25)	1(2.50)	0.6623
Pos(++)	7(8.75)	0	0.0938
Pos(+++)	4(6.90)	0	0.2999
Pos(++++)	4(6.90)	0	0.2999

Creatinine: Serum creatinine concentration FBS: Fasting blood sugar MDA: Malondialdehyde Neg: Negative, Pos: Positive

Body Mass Index (BMI) (Table 1) were statistically significantly higher ($p<0.001$) in the diabetics than the controls. High WC and WHR are the makers of visceral fat accumulation. It has been established that obesity contributes to the pathogenesis of insulin resistance, dyslipidaemia and a higher prevalence of type 2 diabetes. The accumulation of visceral fat is particularly assumed to play an important role in the etiology of the disease (Bjorntorp, 1999; Despres *et al*., 1995). Several current studies agree that waist circumference is probably a better indicator of visceral fat than is WHR. Indeed, several studies found waist circumference to be a better marker of visceral fat and to correlate more strongly with cardiovascular risk factors than WHR (Pouliot *et al*., 1994; Dobbelsteyn *et al*., 2001). However, the WHR has been a well tested risk factor for type 2 diabetes mellitus (Seidell *et al*., 1997).

In this study there was no significant difference in the weights of the diabetic and the control. However, it has been shown that in obese individual, with type 2 diabetes, reduction in weight, may lead to improved insulin resistance, lipid profile and glycaemic control (Wing *et al*., 1987).

Serum creatinnine was lower in the diabetic (Table 2), obese and underweight subjects (Table 3) compared to the control. Even though this was not statistically significant, there was a trend and the tendency for poor glucose utilization with smaller muscle mass. Plasma glucose was correspondingly higher in the diabetic, obese and underweight. This assertion is supported by the negative correlation between FBS and BMI as well as WC (Table 4) but positively associated with creatinnine. Skeletal muscle mass contains 98% of total creatine and in a homeostatic state, plasma creatinine concentration is a direct reflection of skeletal mass (Bousnes and Taussky, 1945). Skeletal muscle is the main tissue that accounts for approximately 90% of overall glucose disposal (Ferrannini *et al*., 1985). It was not therefore surprising that, low serum creatinine level was found

Table 3: Comparison between the BMI (kg m^{-2}) and other biochemical parameters

Parameter	Underweight	Normal	Obese	p-value
Creatinine (µmol L^{-1})				
Diabetics	95.93±36.42	97.40±53.43	92.50±10.97	0.9383
Non diabetics	98.50±44.10	102.70±33.49	102.50±11.97	0.3979
MDA (µmmol L^{-1})				
Diabetics	0.28±0.06	0.30±0.22	0.38±0.06	0.04925
Non diabetics	0.21±0.09	0.22±0.16	0.26±0.09	0.2318
FBS (mmol L^{-1})				
Diabetics	8.17±1.13	7.18±1.79	11.17±1.13	0.1150
Non diabetics	4.63±1.31	4.39±2.27	5.63±1.31	0.6099

Creatinine: Serum creatinine concentration FBS: Fasting blood sugar MDA: Malondialdehyde

Table 4: Pearson correlation coefficient of the biochemical and anthropometric parameters of the diabetics

Parameters	FBS	BMI	WC	MDA	CREAT
FBS		-0.234*	-0.084	0.089	0.100
BMI			0.498**	-0.123	-0.045
WC				-0.059	0.092
MDA					0.002

FBS: Fasting blood sugar, MDA: Malondialdehyde, BMI: Body mass index, WC: Waste circumference, CREAT: Serum creatinine concentration *p<0.05, **p<0.01, FBS: Fasting blood sugar, MDA: Malondialdehyde

to be associated with the diabetics underweight, normal weight and obese as compared to the nondiabetic. Even though this was not again statistically significant, there is a tendency that creatinine could be a risk factor for the pathogenesis of type 2 diabetes (Harita *et al*., 2009).

Oxidative stress may be a common pathway linking several mechanisms for the pathogenesis of diabetes. Mechanisms that contribute to increased oxidative stress in diabetes may include nonenzymatic glycosylation (glycation), autoxidative glycosylation and possibly metabolic stress resulting from changes in energy metabolism and the sorbitol pathway activity, as well as the level of inflammatory mediators and the levels of antioxidant defenses and the extent of damage of surrounding tissues as a result of hypoxia and ischemic injury (Ceriello and Motz, 2004; Baynes, 1991; Robertson, 2004).

In this study, MDA, the marker of oxidative stress was significantly higher in the diabetic underweight, normal weight and obese subjects than the non diabetic subjects (Table 3). Malondialdehyde concentration, a lipid peroxidation product is higher in patients with newly diagnosed type 2 diabetes mellitus (Armstrong *et al*., 1996) and in poorly controlled type 2 diabetic patients (Peuchant *et al*., 1997). Changes in lipid metabolism occur in diabetes, particularly in patients with vascular complications (Sato *et al*., 1979) probably due to increased dependence on fatty acid as an alternative energy source (Mooradian, 2009). It is a common phenomenon in poorly controlled diabetes. In glucose deficiency, cells utilize triglycerides from the adipose tissue as a source of energy (Goldberg, 2001). Structural metabolic changes are oxidative and oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with the development of vascular diseases and diabetes. The assessment of the effect of lipid peroxidation in diabetics is handicapped by the many and complex products formed (Esterbauer *et al*., 1987) and limitations in the methods for assaying the levels and products of lipid peroxidation (Gutteridge and Halliwell, 1990). Although, there are several reports of increased peroxidation of lipids in plasma lipoproteins,

in erythrocyte membrane proteins and in various tissues in diabetes, the relative significance of enzymatic against nonenzymatic products of the lipid peroxidation in diabetes is still contested (Godin and Wohaieb, 1988).

CONCLUSION

BMI, WC and WHR were significantly increased in the diabetic subjects. BMI and WC were negatively correlated to blood glucose whereas MDA and creatinnie were positively correlated to plasma glucose. Plasma creatinine was poorly correlated to fasting blood glucose because the subjects were not obese. MDA was significantly increased in the diabetics but weakly correlated to blood glucose. Even though MDA was not significantly correlated to the blood glucose, the significantly high level and the trend suggest it may be involved in the etiology of type 2 diabetes mellitus.

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