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TAME-Esterase and Oxidative Stress Contribute to Dysmetabolic Syndrome in Dyslipidaemia

[1]A.H. Subratty, [2]L.H. Semfa and [2]M.D. Manraj
[1]Department of Health Sciences,
[2]SSR Resource Centre, Faculty of Science, University of Mauritius,
Reduit, Mauritius

Abstract: In this study, the level of oxidative stress and TAME-esterase activity in dyslipidaemic patients were studied. Fasting levels of total peroxides, triglycerides, total cholesterol, HDL-cholesterol, fasting and 2 h post load glucose were determined using commercially available kits among 110 individuals (mean age 47.5±8.5 years) subdivided into 3 groups namely: Optimal LDL (LDL<3.4 mmol L^{-1}), Borderline LDL (3.4 = LDL = 4.2 mmol L^{-1}) and High LDL (LDL>4.2 mmol L^{-1}). TAME-esterase activity was assessed using a simple colorimetric micromethod. Data was analysed using Epi-Info statistical software. Analysis of data showed no association between TAME-esterase activity, total peroxides and dyslipidaemia. TAME-esterase activity was higher in patients with abnormal glucose metabolism as compared to normoglycaemic subjects (p<0.05). It was concluded that TAME-esterase and hence the kinin-kallikrein system may play an important role in insulin resistance and endothelial dysfunction.

Key words: TAME-esterase, total peroxides, oxidative stress, endothelial dysfunction insulin resistance

INTRODUCTION

Oxidative stress occurs when there is a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences, which may lead to potential tissue damage (Ishibashi, 2006). Experimental findings showed that high levels of cholesterol led to increased production of reactive oxygen species (superoxide) within the endothelial microenvironment (Reilly *et al.*, 2007). Enzymes proposed to be involved in increased superoxide production include xanthine oxidase, NO synthase and NAD(P)H oxidase. Superoxide rapidly reacts with NO to form peroxynitrite (ONO_2^-), a highly reactive intermediate with cytotoxic properties (Vargas *et al.*, 2007). The resulting oxidative stress plays a key role in the initiation of endothelial injury and perpetuation of the atherosclerotic process (Mather *et al.*, 2001). Enhanced oxidative stress may directly induce endothelial dysfunction by decreased synthesis or release of NO by endothelial cells and by inactivating NO with superoxide in subendothelial space.

Oxygen free radicals not only bind to and inactivate NO; they also cause oxidation of lipoproteins in the subintimal spaces. Accumulative evidence suggests that oxidative modification of lipoproteins, in particular LDL, is one of the most important and critical events in the pathogenesis of atherosclerosis and endothelial dysfunction (Warnholtz *et al.*, 2001). Studies from numerous laboratories have shown that small, dense LDL is more susceptible to oxidation and predominance of these small, dense LDL molecules correlates with greater risk of coronary artery disease (Bays, 2003). Dyslipidaemia commonly occurs as part of a cluster of cardiovascular risk factors, which includes

Corresponding Author: A.H. Subratty, Department of Health Sciences, Faculty of Science, University of Mauritius, Reduit, Mauritius

hypertension, insulin resistance, obesity and impaired homeostasis (NCEP, 2001) These risk factors are collectively known as the metabolic syndrome and are common in type 2 diabetic population and are undoubtedly responsible for much of the excess cardiovascular mortality observed (Yuan *et al.*, 2007). Diabetic dyslipidaemia is commonly associated with increased triglycerides, decreased HDL-cholesterol and increased small, dense LDL subparticles thus contributing towards endothelium dysfunction occurs in response to cardiovascular risk factors.

Literature supports the paradigm of endothelial dysfunction as the common link between risk factors and atherosclerotic burden (Herman, 2006). Experimental and clinical studies have shown that hyperlipidaemia impairs endothelial function and promotes atherosclerosis (Warnholtz *et al.*, 2001). In cholesterol-fed animals, endothelial dependent relaxation was found to be impaired and could be restored with cholesterol lowering therapy (Kamata *et al.*, 1996). In humans, hypercholesterolaemia was associated with impaired endothelial dependent relaxation, both in atherosclerotic coronary arteries and in angiographically smooth coronary arteries (kamata *et al.*, 1996). The deleterious effects of cholesterol on endothelial dependent relaxation are probably related to a direct relation between cholesterol and bioavailability of NO. Flavahan (1992) showed that diminished bioavailability of NO caused by hypercholesterolaemia did not result from impairment in the synthesis or release of NO, but most probably resulted from an impairment of a signal transduction process in the endothelium.

According to the response-to-injury hypothesis, hyperlipidaemia may be injurious to the endothelium. Another possible explanation for the association between cholesterol and endothelial dependent relaxation is increased oxidative stress. Even before the appearance of any ultrastructural change in the vessel wall, chronic elevations in serum cholesterol are often associated with an impaired endothelium dependent vasodilation. This early hallmark of endothelial dysfunction is thought to result from an imbalance between the influence of vasodilators and vasoconstrictors involved in the regulation of vascular tone (Frisbee *et al.*, 2007).

N-α-tosyl L-arginine methyl ester (TAME)-esterase has been reported to be a component of the kinin-kallikrein system, involved in the sequence of events leading to the activation of the system (Subratty and Gunny, 2004).

A study carried out on Type 2 diabetic patients showed that TAME-esterase activity was significantly elevated in diabetic patients. Furthermore this was associated with increased triglycerides and total cholesterol levels, thereby suggesting a possible contribution of TAME-esterase in a furthering of diabetic dyslipidaemia leading to endothelial dysfunction (Subratty *et al.*, 2003). Results so far tend to show that the enzyme TAME-esterase could be a biological marker associated with endothelial dysfunction and the onset of vascular diseases such as hypertension, cardiovascular disease (Subratty and Gunny, 2005).

This study was therefore undertaken to determine the level of oxidative stress and TAME-esterase activity in dyslipidaemic patients and to evaluate possible associations between total peroxides level as marker of oxidative stress, TAME-esterase activity and lipid disorders.

MATERIALS AND METHODS

A cross-sectional case control study design was used to explore possible association between oxidative stress, TAME-esterase activity and dyslipidaemia. Participants were recruited from existing population and family databases available at the SSR Centre for Medical Studies and Research, University of Mauritius. Participants were selected on the basis of information available from their files. Non-smokers, normotensive and subjects presenting with a plasma LDL-cholesterol >160 mg dL^{-1} (>4.2 mmol L^{-1}) constituted the dyslipidaemic group. However the participants forming part of the control group were also non-smokers, normotensive but with a plasma LDL-cholesterol <130 mg dL^{-1} (<3.4 mmol L^{-1}). Each participant gave a signed informed consent form upon joining this study.

Sampling

A total of 20 mL of venous blood was collected for biochemical assays, which also included TAME-esterase and oxidative stress assays. Oral Glucose Tolerance Test (OGTT) was carried out for individuals who were not known to be diabetic in order to evaluate the glucose metabolism status. However, before glucose was given to the participant, a urinanalysis was performed using Boerhinger Mannheim Uristik strips to detect presence of any glycosuria, which may be indicative of an unknown diabetic status. OGTT was carried out on the same day. After the initial blood collection in a fasting state, the participant was given 75 mL of glucose dissolved in 250 mL of water. Venous blood was collected in fluoride oxalate tubes 2 h after ingestion and plasma glucose was determined on this sample using commercially available glucose oxidase kits.

Anthropological Measurements

Height was measured to the nearest centimetre (cm), with the individual standing without shoes, feet close to a wall where a standiometer was fixed. Subjects were weighed without shoes on a calibrated electronic scale (EXCELL MHW platform scale). Weight was recorded to the nearest kilogram (kg). Body Mass Index (BMI) was calculated as weight in kg/height in metres and given in kg m^{-2}. Waist and hip circumferences were measured with a measuring tape, to the nearest centimetre. Waist-Hip ratios (WHR) were calculated as the ratio waist/hip. Blood pressure measurements were performed in a sitting position, after 15 min rest, using a standard mercury sphygmomanometer (ACCOSON, England), with the average of 2 measurements of systolic and diastolic BP recorded from both arms.

A standard questionnaire was used to interview participants on their past medical history and present treatment. Participants were also asked about their behavioural habits such as smoking, physical exercise and alcohol consumption.

Determination of Plasma Glucose and Lipids

Plasma glucose, total cholesterol, triglycerides and HDL-cholesterol were determined by enzymatic colorimetric assays using a multiparametric autoanalyser, the COBAS Mira Plus (Roche Diagnostics Systems). The reagents used were purchased from RANDOX Laboratories. Multiparametric calibrator and quality control sera (normal and abnormal) were included for each assay.

For HDL-cholesterol determination, a direct method was used that did not require precipitation of the non-HDL cholesterol (chylomicrons, VLDL and LDL). LDL cholesterol was calculated in mmol L^{-1} using the Friedewald formula (Friedewald *et al.*, 1972).

Determination of Serum Tame-Esterase Activity

Serum [TAME]-esterase activity was determined by a simple colorimetric micro method (Seigelman *et al.*, 1962). TAME was purchased from SIGMA chemical company (UK).

Determination of Total Peroxides

The Biomedica-Oxystat assay was used to measure the peroxide concentration in EDTA-plasma. The assay has a limit of detection of 7 µM L^{-1} with intra-assay and inter-assay coefficients of variation of 3.1 and 5.1%, respectively.

Quality Control

Normal and abnormal multiparametric sera (ABX Diagnostics N-control, P-control) were used as internal quality controls in glucose and lipids assays.

Statistical analyses were performed using the Epi-Info 6.4 (CDC, Atlanta, USA) statistical software. Comparisons between mean values in groups with normally distributed data were done by ANOVA. For data with skewed distribution or when variance between groups was not homogenous, Kruskal-Wallis tests were applied. Associations between the different variables were examined by determination of Pearson's correlation coefficient. Data are expressed as mean±SD for normally distributed data and as median (interquartile range) for skewed data. p-value = 0.05 was considered as significant.

RESULTS

A total of 110 subjects (58 men and 52 women), mean age 47.5±8.3 years, were studied, grouped according to their LDL-cholesterol level. Based on the LDL-cholesterol values, qualitative traits were assigned to individuals, using the NCEP ATP III (2001) criteria. The optimal LDL subgroup consisted of 65 individuals (34 men, 31 women), the borderline LDL subgroup consisted of 23 individuals (8 men, 15 women) and the high LDL subgroup consisted of 21 individuals (16 men, 5 women). Findings (Table 1) show that there was no significant age difference between the different subgroups. Anthropometric variables were similar in all subgroups. Fasting glucose was slightly higher in the high-LDL subgroup but this difference did not reach statistical significance (p = 0.167). Post-load glycaemia (2 h glucose) was significantly higher in the borderline-high LDL subgroup compared to optimal-LDL subgroup (p = 0.042). Subjects in the borderline-high LDL subgroup had significantly higher plasma triglycerides in comparison with those in the optimal subgroup (p = 0.005). Total cholesterol and LDL-cholesterol were significantly higher in borderline-high and high-LDL subgroups (p<0.001) as compared to the optimal subgroup. HDL-cholesterol was higher in borderline-high and high-LDL subgroups but did not reach statistical significance (borderline, p = 0.086; high, p = 0.107). No significant differences in serum TAME-esterase activity and total peroxides concentrations were observed between the different subgroups (p>0.05).

Variables that correlated with TAME-esterase activity in men were post-load glycaemia and total peroxides. In women, TAME-esterase activity was correlated with fasting glucose and a slight correlation was observed with post-load glycaemia. No correlation was observed with lipid values in men. However, TAME-esterase showed a positive correlation with total cholesterol and a slight correlation with triglycerides and LDL-cholesterol in women. No consistent correlation was found with anthropometric variables in both groups. TAME-esterase activity was slightly correlated with age in both men and women.

Table 1: Comparison of quantitative variables including physical and biochemical characteristics in different LDL subgroups

Parameters	Optimal LDL	Borderline LDL	High LDL	p-value
No (male/female)	65 (34/31)	23 (8/15)	21 (16/5)	
Age (years)	46.70±8.61	48.00±8.82	50.3 2±7.20	0.239
BMI (kg m^{-2})	24.50(22.90-27.30)	24.60 (21.10-28.90)	24.70 (22.80-33.10)	0.925
WHR	0.85±0.07	0.85±0.06	0.87±0.08	0.656
Fasting glucose (mmol L^{-1})	5.22 (4.83-5.81)	5.10 (4.79-5.67)	5.58 (4.91-5.88)	0.577
2 h glucose (mmol L^{-1})	6.17 (5.29-7.26)	7.00 (6.03-8.94)[a]	6.63 (5.99-7.10)	0.075
Triglycerides (mmol L^{-1})	1.12 (0.87-1.44)	1.64 (1.23-2.05)[b]	1.44 (1.06-1.83)	0.01
Total cholesterol (mmol L^{-1})	4.48±0.65	5.84±0.31[c]	6.90±0.57[c]	<0.001
HDL-cholesterol (mmol L^{-1})	1.16 (1.04-1.42)	1.35 (1.15-1.48)	1.30 (1.23-1.37)	0.107
LDL-cholesterol (mmol L^{-1})	2.73 (2.36-3.04)	3.72 (3.59-3.97)[c]	4.74 (4.55-5.18)[c]	<0.001
TAME-esterase (μM mL^{-1} h^{-1})	12.10 (9.40-18.50)	12.90(6.90-23.90)	13.40 (7.70-31.10)	0.825
Total peroxides* (μM L^{-1})	296.4 (180.2-449.6)	319.0 (182.8-482.7)	227.9 (178.0-361.5)	0.636

Data are expressed as means±SD for normally distributed data and as median and range for skewed data. Differences between group means were tested using analysis of variance (ANOVA). *Determination of total peroxides was carried out on 84 individuals only (optimal LDL, n = 40; borderline LDL, n = 23; high LDL, n = 21). [a]p<0.05; [b]p<0.01; [c]p<0.001 v/s optimal LDL subgroup

TAME-esterase activity showed a weak positive correlation with total peroxide concentrations in men (p = 0.06). No correlation was found in women.

Total peroxides showed no consistent correlation with fasting glucose and 2 h glucose. Correlation with lipid values showed that total cholesterol and LDL-cholesterol were positively correlated in men whereas in women these two lipid variables were negatively correlated. As stated above, total peroxides were correlated with TAME-esterase in men but no correlation was found in women. WHR showed a consistent negative correlation in both groups. Total peroxides were negatively correlated with age in women only.

DISCUSSION

The aetiology of cardiovascular disease is complex and multifactorial, with numerous predisposing factors (Warnholtz *et al.*, 2001). Evidence increasingly suggests that various proatherogenic stimuli lead to increased production of reactive oxygen species within the endothelial microenvironment and the resulting oxidative stress plays a key role in mediating the pathologic manifestations of endothelial dysfunction associated with atherosclerosis (Cines *et al.*, 1998). All of the factors associated with metabolic syndrome are interrelated. Obesity and lack of exercise tend to lead to insulin resistance. Insulin resistance has a negative effect on lipid production, increasing very low-density lipoprotein and LDL-cholesterol and triglycerides levels in the bloodstream and decreasing HDL-cholesterol. This can lead to fatty plaque deposits in the arteries, which, over time, can lead to cardiovascular disease, blood clots and strokes. Insulin resistance also leads to increased insulin and glucose levels in the blood. Excess insulin increases sodium retention by the kidneys, which increases blood pressure and can lead to hypertension. Chronically elevated glucose levels in turn damage blood vessels and organs, such as the kidneys and may lead to diabetes.

In the present study, TAME-esterase activity was found to be significantly higher in patients with impaired glucose metabolism as compared to normoglycaemic individuals.

There is evidence reporting that activation of the kinin-kallikrein system can enhance insulin sensitivity (Uehara *et al.*, 1994). TAME-esterase is a component of the kallikrein family of proteases and one possible explanation to the increased TAME-esterase activity observed among our study population presenting with an abnormal glucose metabolism could be due to hyperactivation of the kinin-kallikrein. This response is an attempt to improve insulin sensitivity in individuals with insulin resistance syndrome. Various studies have shown that bradykinin, a kinin derived from the cleavage of high molecular weight kininogens by kallikreins, may have an effect in enhancing insulin action and insulin signalling at the skeletal muscle level, resulting in increase glucose uptake (Dietze *et al.*, 1996). Animal model studies as well as interventional studies involving individuals with insulin resistance syndrome showed that in presence of ACE inhibitors, there were sustained elevations of circulating bradykinin concentration and improved whole body insulin sensitivity (Dietze *et al.*, 1996). However, Dumke *et al.* (2002) have reported the possibility that kallikreins may influence post contraction insulin action via a bradykinin-independent mechanism. Kallikreins can act on proteins other than kininogens and it was postulated that through this catalytic activity, kallikreins might play a role in enhanced insulin action.

Our results suggest an increase in synthesis of kallikreins in patients with insulin resistance could be a compensatory mechanism towards enhancing insulin sensitivity and increase in glucose uptake. It is therefore proposed that TAME-esterase; a component of the kinin-kallikrein system may play an important role in insulin resistance and endothelial dysfunction. Insulin resistance *per se* plays an important role in the development of postprandial lipoprotein abnormalities observed in type 2-diabetes, even when fasting triglyceride levels are normal. A likely possible mechanism would thus seem to be a reduced inhibition of lipolysis (Watts and Playford, 1998).

In conclusion present study would thus tend to support the hypothesis that TAME-esterase activity may play a role during mechanisms leading to insulin resistance and endothelial dysfunction.

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