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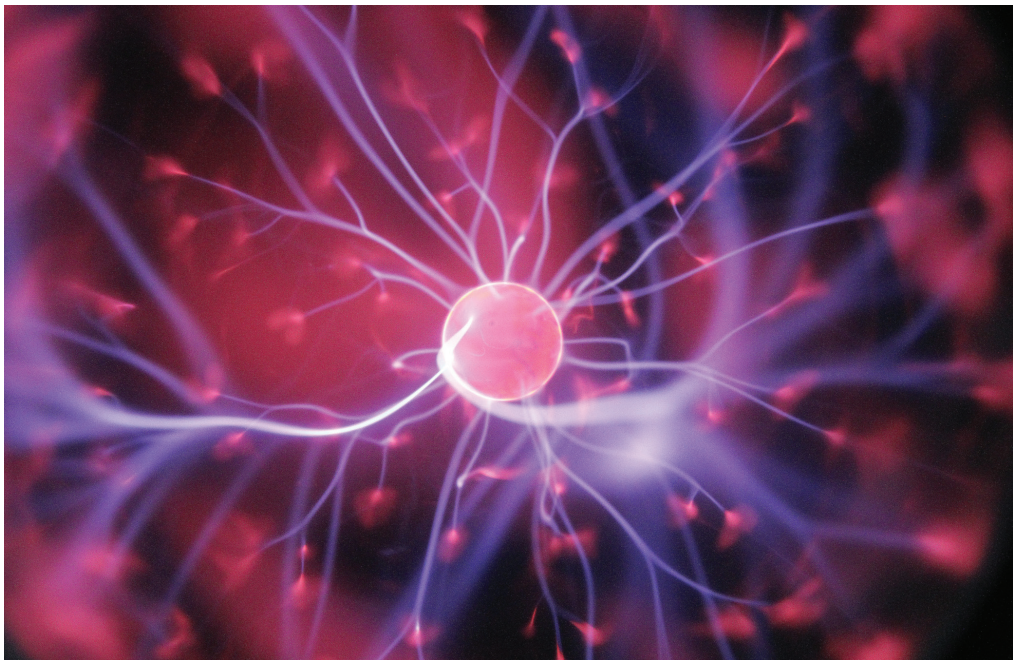

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From the Editor's Desk

This issue of the journal deals with a variety of topics, from the risk of falls in diabetic people to endothelial dysfunction and tirofiban use in percutaneous transluminal coronary angioplasty.

Hlayisi and colleagues (page 48) studied the risk of falls in young to middle-aged patients with diabetes compared with non-diabetic subjects. They demonstrated a high risk of falls in the diabetics, which was exacerbated by symptoms such as peripheral neuropathy and visual difficulties. Longer duration of diabetes as well as uncontrolled glycaemic status also increased the risk of falling. They highlight the need to assess fall risk in young to middle-aged patients with diabetes and suggest early intervention and rehabilitation, as well as health education to prevent falls. While fall risk in older patients is well described,[1] these authors point to a younger group of patients that is also at risk. Interventions such as balance training[2] and the use of video games[3] have been tried.

Previous reports have suggested an association between hypothyroidism and macrovascular complications. Johnson and Rayner (page 53) studied microvascular complications in patients with type 2 diabetes and hypothyroidism. They found no clear association between microvascular disease and hypothyroidism but found a doubling of cardiovascular disease risk. This finding is consistent with other studies.[4] The mechanism involved may be due to the increase in insulin resistance found in hypothyroidism[5] and its cardiovascular sequelae.

In the Ellisras Longitudinal Study 2017, Mogale *et al.* (page 57) found an association between major types of serum advanced glycation end-products (AGEs) and endothelial dysfunction in black patients with type 2 diabetes. AGEs are associated with vascular dysfunction and accelerated atherosclerosis in several ways,[6] with effects on collagen links and nitric oxide levels, and with cellular receptor activation. This causes extensive vascular disease and understanding the mechanisms involved may help to identify therapeutic targets to reduce this vascular risk in patients with diabetes.

Diets rich in *n*-6 polyunsaturated fatty acids (PUFAs) and saturated fatty acids have been associated with increased risk of obesity and the metabolic syndrome, whereas diets high in *n*-3 long-chain PUFAs are associated with lower risk. Ojwang and co-workers (page 62) investigated the association of dietary fatty acids and plasma phospholipid fatty acid patterns with measures of adiposity and the metabolic syndrome. They identified certain fatty acid patterns that provided possible protective associations with adiposity and the metabolic syndrome in a South African setting, whereas other fatty acid patterns were associated with adiposity and the metabolic syndrome. With rapid urbanisation and changing diets, both fat content and fat percentage of the diet are having an impact on the health of populations.[7] Plasma free fatty acids may contribute to cardiovascular disease by disturbing insulin sensitivity and may induce low-grade inflammation.[8]

As part of the Ellisras Longitudinal Study 2017, Chungag et al. (page 72) evaluated the prevalence of hypertension in a group of 10- to 14-year-old girls and boys to determine the association between blood pressure and measures of adiposity. Levels of hypertension and pre-hypertension were 20 and 12%, respectively, and systolic blood pressure was associated with increasing levels of adiposity. This highlights the importance of weight-control strategies for the prevention of hypertension in adolescents. Not only is there a physical risk to health, but psychosocial factors also come into play. Weight-control strategies in this age group are well described,[9] but have proved difficult to implement on a population level. Conservative weight-loss measures have had only a modest effect and some studies suggest a better outcome with early bariatric surgery.[10]

Ghonim and colleagues (page 76) evaluated the safety and effectiveness of intracoronary versus intravenous administration of a thrombolytic, tirofiban, in diabetic patients with acute ST-segment elevation myocardial infarction, during primary percutaneous coronary intervention. There was benefit in the intracoronary administration in several outcome measures, when compared to intravenous administration. The major adverse cardiac events were similar in both groups. Tirofiban (Aggrastat®) is a GPIIa/IIIb antagonist that blocks platelet aggregation.[11]

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High risk of falls in young to middle-aged adults (20–55 years) with diabetes

VERA-GENEVEY HLAYISI, CHRISTINE ROGERS, LEBOGANG RAMMA

Abstract

Background: **Falls, leading to accidents or unintentional injury, are the second most prominent cause of death worldwide, and over 80% of fall-related fatalities occur in low-income countries. Few investigations in South Africa have focused on fall risk, specifically among young to middle-aged adults (younger than 55 years of age) with diabetes.**

Aims: **This study aimed to determine fall risk in young to middle-aged adults (20–55 years of age) with diabetes and compare it to those without diabetes. A secondary aim was to determine the association between fall risk and characteristics of diabetes, including type, duration (in years), control (glycaemic status), age, gender and diabetes co-morbidities (peripheral neuropathy and vision difficulties).**

Methods: **A cross-sectional, matched-groups design with a cohort (individuals with diabetes) and control (non-diabetic) group was utilised. Assessments carried out in both groups included: the Dynamic Gait Index (DGI), the Modified Clinical Test of Sensory Integration (M-CTSIB), the Diabetic Neuropathy Symptoms (DNS) score and a visual acuity screen. Data were analysed using both descriptive and inferential statistical tests.**

Results: **A total of 192 participants between 20 and 55 years of age were assessed, 110 in the cohort and 82 in the control. The DGI showed 22% of the cohort participants were at risk of falling compared to only 1% of those in the control group. The M-CTSIB showed more than half (56%) of the cohort participants were at risk of falling and only up to 21% in the control group. The difference in fall-risk findings between the two groups for both DGI and M-CTSIB was statistically significant ($p < 0.001$). As expected, in both the DGI and M-CTSIB assessments, in the cohort group, those with symptoms of peripheral neuropathy and visual difficulties showed an even higher prevalence of fall risk compared to those without. In the cohort group, longer diabetes duration and uncontrolled glycaemic status correlated with increased risk of falling.**

Conclusion: **This study determined in that young to middle-aged South African adults with diabetes were at a higher risk of falling when compared to those without diabetes. These findings highlight the need to assess fall risk in young to middle-aged patients with diabetes to enable early identification and appropriate rehabilitation. Furthermore, prevention of falls through health education and balance screening in patients with diabetes may be a feasible strategy to minimise the negative impact of falls and injuries in a developing country such as South Africa.**

Keywords: diabetes, fall risk, middle age, adults, fall prevention, rehabilitation

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S Afr J Diabetes Vasc Dis 2019; **16**: 48–52

Introduction

Falls leading to accident or unintentional injury are the second most prominent cause of death worldwide.[1] More than 80% of fall-related injuries and fatalities occur in low- and middle-income countries, therefore the prevention of falls is a major social and public health concern for countries such as South Africa.[1] Falls are not only associated with mortality, but are a concern, particularly in resource-challenged settings such as South Africa, where rehabilitation services including audiology and physiotherapy are regarded as not widely available in most health centres, especially the public sector. The number of fall-related injuries is predicted to double by 2030 due to the increased risk of falls.[1-3]

There are hundreds of risk factors for falls and the intrinsic risk factors are related to individuals' health profiles. Some of the most important include: age, gender, balance and gait deficits, medication, as well as limited vision.[1] There is also evidence in the literature establishing diabetes as one of the possible contributors to an increased risk of falling.[4-6]

Diabetes is a metabolic disorder characterised by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action or both.[7] Diabetes may contribute to increased risk of falls as it is related to decreased sensorimotor function, musculoskeletal/neuromuscular deficits, foot and body pain, pharmacological complications and other co-morbidities.[4-6]

It is estimated that 422 million people worldwide above 18 years of age have diabetes and this number is projected to reach 642 million by 2040.[8] The high prevalence of diabetes and its projected increase may imply an increase in the number of individuals at risk of falls.[4,5] Falls in patients with diabetes may lead to physical disabilities, affect the quality of life, and lead to an increase in costs to the health system as a result of the increase in the number of fall-related injuries and hospitalisations, as well as rehabilitation.[7]

Several studies have reported on increased fall risk in patients with diabetes.[3,4,6] However, some of these studies have several methodological limitations that make it difficult to draw a clear association between fall risk and diabetes.[6] Some of the noteworthy

methodological limitations include choice of participants in terms of age, and the use of insensitive outcome measures. For example, studies investigate fall risk mainly in older adults with diabetes and therefore age-related deterioration is a confounder.[3] In other studies, there is a lack of interrogation of how multiple sensory deficits (neuropathy and retinopathy), which are highly prevalent in diabetes patients, impact on fall risk.[4] And lastly, there is limited research from developing countries such as South Africa, where there is a high prevalence of diabetes and socio-economic factors that further increase the risk of fall-related injuries.[7,8]

It is important to evaluate and diagnose risk for falls early, to enable prevention and rehabilitation of injury and associated debilitating effects thereof. This study therefore aimed to add to the knowledge base in fall-risk identification and prevention in young to middle-aged adults with diabetes in a low- to middle-income country such as South Africa.

Methods

The study aims were to determine (1) the fall risk in young to middle-aged adults (20–55 years of age) with diabetes and compare to those without diabetes; (2) the associations between fall risk and characteristics of diabetes including type, duration (in years), control (glycaemic status in g/mol documented in participants' files), age, gender and diabetes co-morbidities (peripheral neuropathy and vision difficulties).

The study utilised an observational, cross-sectional, matched-groups design with a cohort (patients with diabetes) and control (volunteers without diabetes) group of participants. The sample size required for this study was determined using a G-power analysis calculator with a power of 0.95 and an error probability of 0.05, and the targeted sample size for this study was 222 individuals; 111 participants per group.[9]

All participants (cohort and controls) were selected to participate in this study based on the following inclusion criteria: clinically confirmed diagnosis of diabetes of either type (cohort group); and above the age of 18 and below 55 years. The upper limit of 55 years was selected in order to avoid the impact of age-related deterioration (> 55 years).[10]

The exclusion criteria (established through case history) were: (1) no prior use of ototoxic drugs to avoid vestibulotoxicity, which may influence fall risk.[11] A history of all medication taken was documented in the participants' files. (2) No history of head injury, radiotherapy to the head or ear surgery, as these may influence fall risk;[12] (3) no clinical diagnoses or reports of neurological impairments that may influence fall-risk findings, such as multiple sclerosis, cerebrovascular accidents (ischaemic and haemorrhagic strokes), Parkinson's disease and ataxia.[13]

Participants were recruited from a primary healthcare clinic in Limpopo province, Polokwane, which has the highest poverty rate in comparison to other South African provinces; 78.9% of the population live below the national poverty line and it accounts for 2.8% of the national diabetes prevalence numbers in people aged 25 years and older. Sampling was carried out using purposive and convenience sampling for the cohort and control group, respectively. Participants in both groups were matched for age and gender to allow for comparison between the two groups.

Ethics clearance was obtained from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (HREC134/2015) prior to the commencement of the study. This study adhered to the ethical principles outlined in the Declaration of Helsinki (2013) throughout, which included transparency and integrity of data.[14]

Data were obtained through a participant case-history interview, a medical-folder review (to obtain information related to participants' diabetes type and status), as well as assessment for fall risk through static and dynamic balance tests with the Dynamic Gait Index (DGI) and the Modified Clinical Test of Sensory Integration (M-CTSIB).[15-17] Participants were screened for possible diabetes complications, including peripheral neuropathy, with the diabetic neuropathy symptoms (DNS) score, and vision screening was done with the Snellen E visual screening chart.[18,19.]

In this study there were two main data-collection tools used to assess static and dynamic balance to quantify fall risk as the main variable of interest using the DGI and M-CTSIB assessments.[15-17] The DGI is commonly used clinically and in research, and can predict dynamic balance disorders and fall risk. It is reported to have adequate discriminative ability with 0.84 for sensitivity and 0.89 for specificity.[16] The M-CTSIB assesses balance function in terms of sensory integration and static balance with a 0.99 test–retest reliability and 0.68–1.0 inter-rater reliability.[17] Both the DGI and M-CTSIB were administered as described in the literature to ensure instrument validity.[15,17]

Statistical analysis

Proportions (%) were used to report participant and diabetes characteristics, information on diabetes complications, and fall-risk prevalence data from both DGI and M-CTSIB assessments. Pearson's correlation coefficient determined the strength of associations between the presence of fall risk and characteristics of diabetes (control and duration), patient age and presence of co-morbidities. Independent *t*-tests were used to determine significance of differences in prevalence between the participant groups ($\alpha = 0.05$).

Results

A total of 192 individuals participated in this study; 110 in the cohort (participants with diabetes) and 82 in the control (without diabetes) group. There were similar distributions of gender in both groups, with the following age distributions for each group: cohort: median 46 years, range 20–55; control: median 43 years, range 21–55. Overall, the majority (49%) of participants were 49 years or younger. The difference in distribution of the ages between the cohort and control groups was not statistically significant across all age bands.

The majority (92%) of the cohort participants presented with type 2 diabetes and only 8% had type 1 diabetes. Most (74%) participants had an uncontrolled glycaemic status (diabetes control, defined as 7 g/mol, was determined by a medical doctor and documented in the patient file). The duration of disease ranged from a month up to 33 years post-diabetes diagnosis, with most living with diabetes for less than five years. In terms of complications, more than half of the participants in the cohort group had a possible complication with their diabetes, where 51% screened positive for diabetic neuropathy, while 56% failed the vision screening.

The DGI assessment consisted of eight tasks with varying demands, with each item scored on a four-level ordinal scale, with a maximum possible score of 24. A score of 19 or less indicated decreased dynamic balance and therefore an increased risk of falling.[15,16]

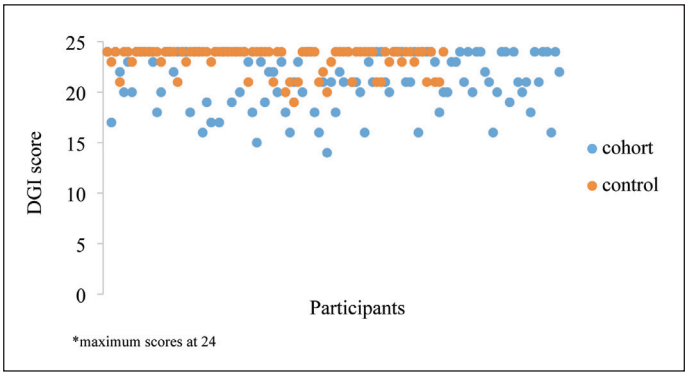

Fig. 1. DGI score distribution.

The results showed that 22% (n = 24) of the participants with diabetes were at risk of falling (score < 19 of 24), compared to only 1% (n = 1) in the control group. These findings show that according to the DGI, one in five young to middle-aged adults with diabetes were at risk of falling. The distribution of the DGI scores of the cohort and control is illustrated in Fig. 1 and indicates that the cohort group scored lower than the control group. The difference in DGI score distribution between the groups was statistically significant [t (166) = –6.14, p < 0.001].

The correlation between DGI scores (fall risk) and participants' age demonstrated weak inverse correlations between DGI scores and age (r = –0.29 and r = –0.37) for the cohort and control groups, respectively. These correlation findings indicate that with an increase in age there was a decrease in DGI score and therefore a higher risk of falls.

Specific to the cohort group (paticipants with diabetes), the correlation of the DGI scores with diabetes duration (years post diagnosis) and control (glycaemic status) was also investigated and findings showed a weak inverse correlation with diabetes duration (r = –0.13) and diabetes control (r = –0.23). These findings indicate that a longer diabetes duration in years and a higher glycaemic status (uncontrolled diabetes) was correlated with a decrease in DGI score and therefore a higher risk of falls.

The M-CTSIB assessment was carried out under four conditions, which were eyes open and closed, on foam and on a firm surface. The use of foam disrupts proprioceptive cues, adding to the challenge experienced by the vestibular system. Participants were timed under each condition for their ability to maintain static balance for the standard 30 seconds, and the total score was out of 120 seconds.[14]

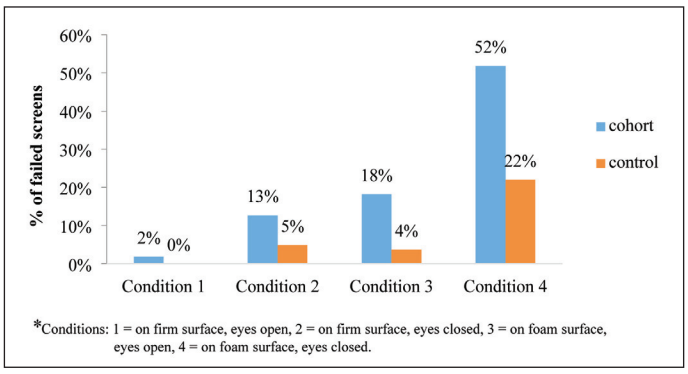

Fig. 2. M-CTSIB conditions.

Assessment findings showed 56 and 21% of the cohort and control participants, respectively, failed to maintain static balance for 30 seconds across all conditions and were at risk of falling. M-CTSIB findings therefore showed that one in two young to middle-aged adults with diabtes were at risk of falling, compared to one in five in those without diabetes.

Participants at risk of falling in both groups mostly had difficulties in condition three and four of the M-CTSIB screening, where balance needs to be maintained with eyes closed and on the foam, which demands more input from the vestibular system (Fig. 2). The difference in fall risk between the groups was statistically significant [t (149) = –6.13, p < 0.001].

The correlation between M-CTSIB scores and participants' age indicated weak inverse correlations, with r = –0.29 and r = –0.14 for cohort and control groups, respectively. This indicated that increasing age was related to a lower M-CTSIB score and a higher fall risk. Specific to the cohort group, a weak negative correlation with diabetes duration (r = –0.20) and diabetes control (r = –0.06) was found, indicating that with longer diabetes duration (in years) and a higher glycaemic status, a lower M-CTSIB score was obtained, indicating a higher fall risk.

Participants with diabetes and possible complications presented with a statistically significantly higher risk of falls compared to those with diabetes and no complications (Table 1).

Discussion

This study aimed to determine fall risk in paticipants with diabetes and compare that with a matched group of participants without diabetes. Twenty-two per cent of the participants with diabetes in this study were at risk of falling, based on their DGI scores. Similar findings were reported in a previous study,[20] establishing that 38% of their participants with diabetes, on symptom reports, experienced instability when walking. The current study findings cannot ascribe diabetes as the cause of the increased risk of falls, however other authors explain that fall risk in patients with diabetes may be due to pharmacological complications and peripheral neuropathy.[6,19] Our study also established that in participants with diabetes who screened positive for diabetic peripheral neuropathy (n = 56), there was more than a six-times higher fall risk, at 39%, compared to those who screened negative (n = 54) at 6%.

The current study therefore established that young to middle-aged South African adults (20–55 years) with diabetes were at risk of falls. Furthermore, those with diabetes and co-morbidities were at an even higher risk.

Table 1. Diabetes complications and fall risk

Participants with diabetes	Number	Fall risk with DGI	Statistical difference in DGI scores (α = 0.05)	Fall risk with M-CTSIB	Statistical difference in M-CTSIB scores (α = 0.05)
Positive for neuropathy	56	22	0.3	40	0.9
Positive for visual difficulties	62	15		50	
Negative for diabetic neuropathy	54	3		21	
Negative for visual difficulties	48	9		25	

It is essential to note the risk of falls in patients with diabetes, especially those with complications such as peripheral neuropathy, as it predisposes them to fall-related injuries. Falls are ranked among the 20 most expensive conditions to treat in general, considering hospitalisation, possible need for surgery, and thereafter implications for rehabilitation.[3] Therefore, in a low socio-economic context such as South Africa, prevention of falls through health education and balance screening in patients with diabetes may be a feasible strategy to minimise further strain on the healthcare system.[5]

It was also found with the M-CTSIB in this study that more than half (56%) of the participants with diabetes could not maintain postural stability, especially under condition four (eyes closed on foam). Postural instability and therefore a risk of falling in patients with diabetes may be explained by the impact the disease has on the eyes, ears and legs, which are integral in maintaining proprioception and avoiding falls.[13]

Supporting the notion that complications of diabetes, such as peripheral neuropathy and reduced visual acuity increase fall risk, 87% of participants with both conditions were unable to maintain postural stability and therefore were at risk of falling. Fall risk in patients with diabetes with sensory impairments such as peripheral neuropathy is attributed to the lack of precise proprioceptive response (sensory ataxia) from the lower limbs.[21] It is also worth noting that in another study,[15] (n = 1 662) visual impairment was reported to impact on the vestibulo-ocular reflex, an important system that maintains balance and prevents falls. Consequently, this study highlights that there is a crucial need to investigate fall risk in order to prevent fall-related injuries, especially in patients with diabetes and accompanying sensory impairments, such as low vision.[5,22]

Of particular interest is the age of the participants (20–55 years). Findings support other work that suggests that fall risk should be explored in ever-younger populations, and challenges beliefs that falls are a problem primarily of older adulthood. Multiple sensory impairments, especially in an economically active age range, can result in significant negative impacts on occupational productivity, as well as cognitive and functional decline.[23,24]

This study represents one of the first studies that investigated fall risk among young to middle-aged patients with diabetes in South Africa. Its strength lies in its design, a matched control group to ensure that the influence of confounding variables was minimised. Our study design is in contrast to most of the previous studies, which employed mostly retrospective reviews of records without control groups. Also, participants were carefully selected to ensure that only individuals under 55 years were selected, to minimise the impact of age-related deterioration confounding findings.

However, the study also had some limitations. Because of its descriptive nature, causal relationships could not be inferred. This study investigated mainly fall risk and did not include a quality-of-life aspect that may have enabled the researchers to have more insight with regard to the impact of diabetes-related fall risk particular to the age group included. Furthermore, history of previous falls, which is in itself an important risk factor for future falls, was not investigated.

One key clinical implication emerging from the current study is that, in patients with diabetes, fall risk should be assessed and rehabilitated early.[24] Future research could add to the knowledge base with studies focusing on incidence of fall risk and other balance dysfunctions in patients with diabetes, through longitudinal, prospective research, effective methods of fall prevention and rehabilitation, and the impact of diabetes-related fall risk on quality of life.[25]

Conclusion

Overall, participants with diabetes presented with a statistically significantly higher risk of falls compared to those without diabetes. Furthermore, those with possible complications (neuropathy and vision difficulties), uncontrolled diabetes and longer diabetes duration showed an even higher risk of falling. Therefore, the study findings highlight the need to assess for fall risk in young to middle-aged (20–55 years) patients with diabetes to enable early identification and appropriate rehabilitation.

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Reduced carbs help type 2 diabetes patients regulate blood sugar levels

Patients with type 2 diabetes improve their ability to regulate blood sugar levels if they eat food with a reduced carbohydrate content and an increased share of protein and fat. This is shown by a small recent study conducted at Bispebjerg Hospital in collaboration with, among other partners, Aarhus University and the Department of Nutrition, Exercise and Sports at the University of Copenhagen. The findings are contrary to the conventional dietary recommendations for type 2 diabetics.

Nutritional therapy is important to treat type 2 diabetes optimally, but the recommendations are unclear. According to the Danish Health Authority, up to 85% of newly diagnosed patients with type 2 diabetes are overweight, and they are typically advised to follow a diet focused on weight loss: containing less calories than they burn, a low fat content and a high content of carbohydrates with a low 'glycaemic index' (which indicates how quickly a food affects blood sugar levels).

A central aspect in the treatment of type 2 diabetes is the patient's ability to regulate their blood sugar levels, and new research now indicates that a diet with a reduced carbohydrate content and an increased share of protein and fat improves the patient's ability to regulate his or her blood sugar levels compared with the conventional dietary recommendations. In addition, it reduces liver fat content and also has a beneficial effect on fat metabolism in type 2 diabetes patients.

'The purpose of our study was to investigate the effects of the diet without "interference" from a weight loss. For that reason, the patients were asked to maintain their weight. Our study confirms the assumption that a diet with a reduced carbohydrate content can improve patients' ability to regulate their blood sugar levels – without the patients concurrently losing weight,' explains senior consultant, Dr Thure Krarup, from the Department of Endocrinology at Bispebjerg Hospital. He continues: 'Our findings are important, because we've removed weight loss from the equation. Previous studies have provided contradictory conclusions, and weight loss has complicated interpretations in a number of these studies.'

A diet with a reduced carbohydrate content, a high protein content and a moderately increased fat content reduces liver fat content. A diet with a reduced carbohydrate content may be beneficial to patients with type 2 diabetes – even if it does not lead to weight loss. The study forms part of CutDM, which, supported by a grant of DKK 4m from Arla Food for Health, examined whether a diet with reduced carbohydrate content and increased protein and fat content improved type 2 patients' blood sugar regulation.

Twenty-eight patients with type 2 diabetes participated in the study over a total period of 12 weeks. For six weeks, the patients were given a conventional diabetes diet with a high carbohydrate content, and, for the other six weeks, they were given a diet with a reduced carbohydrate content, high protein content and moderately increased fat content. The patients were given the diet types in random order.

Based on the growing body of evidence, we might rethink the dietary recommendations for patients with type 2 diabetes, stresses Thure Krarup. 'The study shows that by reducing the share of carbohydrates in the diet and increasing the share of protein and fat, you can both treat high blood sugar and reduce liver fat content. Further intensive research is needed in order to optimise our dietary recommendations for patients with type 2 diabetes,' says Thure Krarup, stressing that the findings should be confirmed in large-scale, long-term, controlled trials.

A diet with a reduced carbohydrate content, high protein content and moderately increased fat content improves glycaemic control (the ability to regulate blood sugar) by reducing blood sugar after meals and 'long-term blood sugar' (measured by HbA_{1c} level, which is a blood test used to measure the average blood sugar level over approximately the past two months).

Source: Medical Brief 2019

A cross-sectional cohort study with microvascular complications in patients with type 2 diabetes with and without hypothyroidism

LOUISE JOHNSON, BRIAN RAYNER

Abstract

Objectives: **Previous reports have suggested an association between hypothyroidism and macrovascular complications in type 2 diabetes (T2DM) but the association with microvascular complications is not well documented. This study aimed to determine whether there were significant differences in these complications in patients with T2DM with and without hypothyroidism.**

Methods: **This was a retrospective, cross-sectional, case–control study from a single centre specialising in diabetes in South Africa. T2DM was defined by American Diabetes Association criteria. The cases were all patients treated for hypothyroidism and the controls were clinically and biochemically confirmed euthyroid, who were under follow up between 1 January and 1 July 2016. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (eGFR) of < 60 ml/min, determined by the CKD-epidemiology collaboration equation (CKD-EPI) and/or albumin/creatinine ratio > 3 mg/mmol. Diabetic retinopathy (DR) was defined as the presence of aneurysms, bleeds, exudates and new vessel formation on the retina examined by an ophthalmologist. Diabetic peripheral neuropathy (DPN) was defined as the presence of symptoms, loss of 128-Hz sensation and abnormal 10-gm monofilament. Cardiovascular disease (CVD) was defined as the presence of major adverse cardiovascular events (MACE).**

Results: **There were 148 cases and 162 controls. Compared to the controls, the cases were older (65.6 vs 59.4 years, $p < 0.00001$), more likely to be female (67.6 vs 39.5%, $p < 0.0001$) and white (89.2 vs 79.6%, $p = 0.02$), have a lower HbA_{1c} level (7.5 vs 8.2%, $p = 0.0001$), eGFR (64.4 vs 72.7 ml/min, $p = 0.0006$) and triglyceride level (2.18 vs 2.55 mmol/l, $p = 0.04$), have a higher high-density lipoprotein cholesterol level (1.13 vs 1.02 mmol/l, $p = 0.001$), a longer duration of diabetes (14.8 vs 11.6 years, $p = 0.001$) and using fewer antidiabetic agents (1.82 vs 2.19, $p = 0.001$). There was a higher prevalence of CKD (44 vs 57.8%, $p = 0.03$) and CVD (59.3 vs 45.3, $p = 0.06$), and a trend towards higher DR (66.7 vs 47.6, $p = 0.09$). There was no difference in body mass index, hypertension, low-density lipoprotein cholesterol level (all patients received statin therapy), DPN and amputations. After adjusting for confounding factors, there was no association between CKD and DR, and hypothyroidism, but the trend to association with CVD persisted (OR 1.97. $p = 0.07$).**

Conclusions: **Hypothyroidism in T2DM was not associated with microvascular disease after adjusting for confounding factors. There was a nearly two-fold risk of CVD. The study is limited by the retrospective and observational design.**

Keywords: hypothyroidism, type 2 diabetes, microvascular and macrovascular complications

The National Health and Nutritional Examination Survey III showed a prevalence of overt and subclinical hypothyroidism (SCH) of 0.3 and 4.3%, respectively.[1] Insulin resistance, type 2 diabetes mellitus (T2DM) and hypothyroidism are reported to occur more commonly than by chance, although the exact aetiology is uncertain.[2] Both T2DM and hypothyroidism are associated with cardiovascular disease (CVD), often through complex mechanisms, and the concurrence of hypothyroidism and diabetes may further amplify endothelial dysfunction, insulin resistance, poorer diabetic control and microvascular complications.[3]

In a systematic review and meta-analysis of SCH in T2DM, the prevalence was 10.2%, and T2DM was associated with a 1.93-fold increase in risk for SCH. Furthermore, SCH was associated with an overall odds ratio of 1.74 for diabetic nephropathy, 1.42 for diabetic retinopathy (DR), 1.85 for peripheral arterial disease, and 1.87 for diabetic peripheral neuropathy (DPN).[4] However data from individual studies have not always been consistent with these associations.[5,6]

Given the paucity of data and the contradictory findings of studies, we aimed to investigate the association of T2DM and hypothyroidism with micro- and macrovascular complications in South Africa.

Methods

This was a retrospective, observational, cross-sectional study of patients with T2DM performed in a large private practice specialising in diabetes. The cases were all patients treated for hypothyroidism including those with SCH, and the controls were clinically and biochemically confirmed euthyroid under follow up between 1 January and 1 July 2016. The aim of the study was to compare the prevalence and severity of micro- and macrovascular complications, and indices of glycaemic control between the cases and controls.

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Diabetic kidney disease (DKD) was defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min determined by the CKD-epidemiology collaboration equation (CKD-EPI) and/or a urine albumin/creatinine ratio (ACR) > 3 mg/mmol in the absence of other causes of kidney disease. DR was defined as the presence of aneurysms, bleeds, exudates and new vessel formation on retinal examined by an ophthalmologist; DPN as the presence of symptoms, loss of 128-Hz sensation and/ or abnormal 10-gm monofilament; and CVD as the presence of major adverse cardiovascular events (MACE: coronary angioplasty, stent, coronary artery bypass grafting, myocardial infarction or cerebrovascular accident) and amputation (surgical removal of any part of a lower limb due to diabetic causes).

SCH was defined as a thyroid stimulating hormone (TSH) level > 4 mIU/l with a normal T4 and T3 level, and overt hypothyroidism as a T4 less than the normal range (7.6–16.1 pmol/l) and TSH > 4 mIU/l. Thyroxine was given to all cases to maintain the T4 and TSH level within the normal range. Diabetes was defined according to the American Diabetes Association criteria.[7] Cases were excluded if they were receiving amiodarone or lithium, or had had previous thyroid surgery or ablation therapy.

The study was approved by the University of Cape Town, Research Ethics Committee (331/2017).

Statistical analysis

Descriptive statistics were used to summarise total cohort characteristics. For purposes of analysis, the cohort was divided into black (Africans) and non-black (whites and other race groups). Median with interquartile range was used to summarise continuous variables, and frequency and percentages were used to summarise categorical variables. Differences in continuous variables between cases and control patients were compared using a Wilcoxon rank sum test, while categorical variables were compared using Pearson chi-squared test or Fisher's exact test.

Logistic regression was used to determine associations, magnitude and direction between the dichotomous T2DM outcomes (DKD, CVD, DPN and retinopathy) and hypothyroidism, adjusted for a priori selection of confounders and covariates. Highly skewed continuous variables were log transformed prior to entering into the model. Linear regression was used to assess associations between eGFR and a priori selection of covariates. Goodness-of-fit and influential observations were assessed after fitting each model. All analyses were performed using Stata software (Version 14.2, Stat Corp, College Station, TX).

Results

We identified 310 subjects, of whom 162 were controls and 148 were cases. All the hypothyroid cases were receiving thyroxine. The overall demographics of the population are shown in Table 1. The ethnic breakdown was predominantly white (84%), black (13%) and other races (3%), and hypertension was present in 83% of the hypothyroid group and 79% of the controls.

There were significant differences in the baseline characteristics between the two groups. There were more females in the hypothyroid group (60.8 vs 39.2%, $p = 0.001$) and fewer blacks (10.8 vs 21.4%, $p = 0.021$) compared to controls. In addition the mean age of the patients with hypothyroidism and duration of diabetes was 65 vs 58 years ($p < 0.001$) and 13 vs 10 years ($p = 0.001$), respectively. T4 levels were slightly higher in the cases (12 vs 13.1 pmol/l, $p = 0.004$), but there was no difference in TSH level.

In respect of diabetic control, the cases had better glycated haemoglobin (HbA_{1c}) levels (6.9 vs 8%, $p < 0.001$) and used fewer hypoglycaemic medications ($p = 0.001$) (Table 2). There were differences in use of hypoglycaemic agents with more patients in the control group receiving dipeptidyl peptidase-4 (DPP4) inhibitors (40.1 vs 26.4%, $p = 0.04$), incretin mimetics (GLP agonists) (13 vs 6.1%, $p = 0.01$), and a trend towards more insulin use (51.9 vs 41.9. $p = 0.08$). There were no significant differences in the use of metformin and sulphonylureas.

Regarding components of the metabolic syndrome, waist circumference was not available, but in the cases, high-density lipoprotein (HDL) cholesterol was significantly higher (1.1 vs

Table 1. Demographics of the total group, cases and controls.

Variable	Total group (*n* = 310)	Controls (*n* = 162)	Cases (*n* = 148)	*p*-value
Age (years)	62 (54–71)	58 (52–67)	65 (58–75)	< 0.001
Gender, male (%)	146 (47.1)	98 (60.5)	48 (32.4)	< 0.001
Race, non-black (%)	261 (84.2)	129 (79.6)	132 (89.2)	0.021
Duration of T2DM (years)	11 (7–18)	10 (5–16)	13 (9–19)	0.001
BMI (kg/m^2)	34 (30–41)	34 (29–40)	34 (30–41)	0.370
HbA_{1c} (%)	7.4 (6.3–9.1)	8.0 (6.7–9.6)	6.9 (6.1–8.7)	< 0.001
TSH (mIU/ml)	1.6 (1.0–2.5)	1.6 (1.2–2.2)	1.6 (0.8–3.1)	0.973
T4 (pmol/l)	12.3 (11.–15)	12.0 (10–13)	13.1 (11–16.5)	0.004
Total cholesterol (mmol/l)	4.5 (3.7–5.4)	4.5 (3.7–5.4)	4.4 (3.7–5.3)	0.776
Triglycerides (mmol/l)	2.0 (1.3–2.8)	2.1 (1.4–3.2)	1.9 (1.3–2.6)	0.034
HDL-C (mmol/l)	1.0 (0.9–1.2)	1 (0.8–1.1)	1.1 (0.9–1.3)	0.001
LDL-C (mmol/l)	2.6 (2.0–3.2)	2.7 (1.9–3.3)	2.5 (2.0–3.2)	0.766
eGFR (ml/mim)	71 (55–88)	75 (58–90)	66 (52–82)	0.001
Urine ACR (mgm/mmol)	1.8 (0.7–4.9)	1.6 (0.7–5.2)	2.0 (0.8–4.9)	0.717

Table 2. Use of hypoglycaemic drugs in total group, cases and controls

No. of hypoglycaemic drugs, *n* (%)	Overall	Controls	Cases	*p*-value
1	101 (32.6)	38 (23.5)	63 (42.6)	0.001
2	122 (39.4)	66 (40.7)	56 (37.8)	
3	67 (21.6)	46 (28.4)	21 (14.2)	
4	20 (6.5)	12 (7.4)	8 (5.4)	
Metformin, *n* (%)	254 (81.9)	135 (83.3)	119 (80.4)	0.503
Sulphonylurea, *n* (%)	93 (30.0)	52 (32.1)	41 (27.7)	0.399
GLP agonist, *n* (%)	30 (9.7)	21 (13.0)	9 (6.1)	0.041
DPP4 inhibitor, *n* (%)	104 (33.6)	65 (40.1)	39 (26.4)	0.01
Insulin, *n* (%)	146 (47.1)	84 (51.9)	62 (41.9)	0.08

Table 3. Micro- and macrovascular outcomes between cases and controls on univariate analysis

Outcome (*n*, %)	Total group *n* = 310	Controls *n* = 162	Cases *n* = 148	*p*-value
Amputation,	8 (2.6)	5 (3.1)	3 (2.0)	0.725
CKD (*n* = 297)	90 (30.3)	38 (24.7)	52 (36.4)	0.029
CVD	54 (17.4)	22 (13.6)	32 (21.6)	0.062
Neuropathy	148 (47.7)	79 (48.8)	69 (46.6)	0.706
Retinopathy (*n* = 75)	33/75 (44.0)	11/33 (33.3)	22/42 (52.4)	0.099

Table 4. Associations between DKD and patient characteristics on uni- and multivariate analysis

Variable	Univariate OR (95% CI)	*p*-value	Multivariate OR (95% CI)	*p*-value
Age, 5-year increase	1.57 (1.37–1.79)	< 0.001	1.63 (1.39–1.91)	< 0.001
Gender, female	1.05 (0.64–1.72)	0.854	0.77 (0.43–1.38)	0.382
Race, non-black	1.16 (0.58–2.33)	0.668	0.67 (0.30–1.48)	0.322
Hypothyroid	1.74 (1.06–2.88)	0.029	1.25 (0.68–2.29)	0.467
HBA_{1c}	0.91 (0.32–2.58)	0.854	2.07 (0.60–7.19)	0.250
Duration of T2DM	1.60 (1.14–2.24)	0.007	0.90 (0.60–1.33)	0.592

1 mmol/l, $p = 0.001$) and triglyceride levels were significantly lower (1.9 vs 2.1 mmol/l, $p = 0.034$). There was no difference in low-density lipoprotein (LDL) cholesterol, but all subjects received statin therapy unless contra-indicated.

Microvascular complications tended to occur more frequently in the hypothyroid group. The eGFR was significantly lower in the cases (66 vs 75 ml/min, $p = 0.001$), but there was no difference in urine ACR. On univariate analysis (Table 3), the odds ratio for DKD was 1.74 ($p = 0.029$), DPN was 0.92 ($p = 0.7$) and DR was 2.2 ($p = 0.1$). Because of baseline differences between the groups, a multivariate analysis was performed to adjust for confounders (Tables 4–6). The differences between the groups were no longer significant. The most important predictor of microvascular complications was a five-year increase in age, especially for DKD (OR 1.63, $p < 0.001$) and DPN (OR 1.19, $p = 0.008$).

There was a trend towards a higher incidence of CVD in the hypothyroid group (OR 1.76, p = 0.06) that was still present in the multivariate analysis (OR 1.97, $p = 0.07$) (Table 7). The most important predictor of CVD was age, and female gender appeared protective. Amputations due to diabetes were 3.1% in the controls and 3.0% in the cases ($p = 0.725$)

Discussion

This was a cross-sectional, retrospective study comparing primarily micro- and macrovascular complications in type 2 diabetes subjects with and without hypothyroidism. It was done in a single-physician practice in South Africa in a predominantly white population, and results must be interpreted in this context.

The major findings of the study were the following: hypothyroid subjects were significantly more likely to be female, older, with a longer duration of diabetes and less likely to be black; diabetic control, defined by HbA_{1c} level, was better in hypothyroid subjects than in the controls despite less use of hypoglycaemic agents; hypothyroid subjects had higher HDL cholesterol and lower triglyceride levels; lower eGFR and greater prevalence of CKD; there was a trend to increased DR with no differences in amputations or DPN; and a trend to increased CV events. However after adjustment for baseline differences, the association of hypothyroidism with DKD and DR was no longer apparent using multivariate analysis. However the trend for CVD remained.

Table 5. Associations between DPN and patient characteristics on uni- and multivariate analysis

Variable	Univariate OR (95% CI)	*p*-value	Multivariate OR (95% CI)	*p*-value
Age, 5-year increase	1.21 (1.10–1.34)	< 0.001	1.19 (1.05–1.35)	0.008
Gender, female	1.10 (0.70–1.71)	0.698	1.07 (0.65–1.77)	0.789
Race, non-black	2.11 (1.11–4.02)	0.023	1.66 (0.83–3.31)	0.152
Hypothyroid	0.92 (0.59–1.43)	0.706	0.63 (0.37–1.08)	0.094
CKD present	1.76 (1.06–2.90)	0.028	1.22 (0.70–2.16)	0.486
HBA_{1c}	1.37 (0.54–3.52)	0.507	1.47 (0.51–4.23)	0.480
Duration of DM	1.63 (1.21–2.20)	0.001	1.37 (0.97–1.93)	0.075

Table 6. Associations between DR and patient characteristics on uni- and multivariate analysis

Variable	Univariate ($n = 75$) OR (95% CI)	*p*-value	Multivariate ($n = 75$) OR (95% CI)	*p*-value
Age, 5-year increase	1.26 (1.02–1.54)	0.029	1.19 (0.89–1.60)	0.235
Hypothyroid	2.2 (0.86–5.65)	0.102	1.61 (0.55–4.68)	0.386
CKD present	1.59 (0.62–4.06)	0.334	0.89 (0.29–2.75)	0.838
HBA_{1c}	5.67 (0.64–50.13)	0.118	6.52 (0.56–75.22)	0.133
Duration of DM	1.98 (0.97–4.07)	0.061	1.24 (0.52–2.99)	0.624

Table 7. Associations between CVD and patient characteristics on uni- and multivariate analysis

Variable	Univariate ($n = 75$) OR (95% CI)	*p*-value	Multivariate ($n = 75$) OR (95% CI)	*p*-value
Age, 5-year increase	1.35 (1.18–1.56)	< 0.001	1.41 (1.17–1.70)	< 0.001
Gender, female	0.25 (0.13–0.48)	< 0.001	0.16 (0.07–0.36)	< 0.001
Race, non-black	1.10 (0.48–2.49)	0.826	0.63 (0.24–1.62)	0.336
Obese	0.74 (0.39–1.42)	0.371	0.87 (0.41–1.84)	0.713
Hypothyroid	1.76 (0.97–3.19)	0.064	1.97 (0.94–4.13)	0.073
HBA_{1c}	0.90 (0.26–3.13)	0.872	1.48 (0.33–6.71)	0.613
HDL-C	0.43 (0.15–1.27)	0.127	0.63 (0.18–2.23)	0.472
Duration of T2DM	1.70 (1.11–2.60)	0.014	1.19 (0.71–2.01)	0.512

The increased prevalence of hypothyroidism in women in this study probably reflects underlying gender differences, previously reported.[1,8] Similarly, the increased age in the cases probably reflects the higher prevalence in older people.[8] The lower prevalence of hypothyroidism in black people was also reported in previous studies,[1] but our results need to be interpreted with caution in view of the skewed nature of the population.

Hypothyroidism has previously been reported in a systematic review and meta-analysis to be associated with microvascular complications that included DR, DKD and DPN. Although on univariate analysis, an association with DKD was demonstrated, this was not confirmed on multivariate analysis. The most important predictor of microvascular complications in our study was found to be increasing age, especially in relation to DKD and DPN. Unfortunately only 24.2% of our subjects went for formal retinal examination and this could have masked an association between DR due to a type 1 statistical error.

The relationship between hypothyroidism, including SCH and CVD, has been well established,[9] and treatment with thyroxine may reduce this risk.[10] In our study there was a trend for increased CVD in both univariate and multivariate analysis. The reason for not showing a clear association with CVD was probably mitigated by the fact that all the cases received thyroxine and statins to control LDL cholesterol. There was no difference in LDL cholesterol between the cases and controls as a result. Raised LDL cholesterol due to hypothyroidism is probably one of the major mechanisms for CVD.

An interesting finding in this study was that hypothyroid cases had improved glycaemic control, used less hypoglycaemic medication, and had higher HDL cholesterol and lower triglyceride levels. This is suggestive of reduced insulin resistance, which is contrary to reports in the literature.[11] It is possible that because all cases received thyroxine to control T4 and TSH levels, there was reversal of the insulin resistance that contributed to developing T2DM. Improvement in insulin resistance with thyroxine has been reported in experimental models and humans.[12,13]

The major limitation of this study was that it was a singlecentre study, and sample size was not calculated. The negative findings may be due to inadequate statistical power of the study. Although we attempted to control for confounders, this does not completely negate the effect of confounders on the micro- and macrovascular outcomes. Furthermore, the patients with hypothyroidism were adequately treated and therefore biochemically euthyroid, thus negating the potential negative micro- and macrovascular consequences of hypothyroidism. The retinopathy group had a limited sample size due to many subjects not attending their ophthalmological examination. This limits the conclusions regarding the association of hypothyroidism and DR.

Conclusions

In this retrospective, observational study, a link between hypothyroidism and SCH and diabetic microvascular complications was not found, but there was a nearly two-fold risk for CVD. Cases also demonstrated improved glycaemic control despite fewer antidiabetic drugs, and indirect evidence for less insulin resistance than the controls with T2DM. These findings warrant further study for confirmation.

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Ellisras Longitudinal Study 2017: elevated serum levels of carboxymethyl-lysine, an advanced glycation end-product, are associated with higher odds of developing endothelial dysfunction in black South African patients with type 2 diabetes mellitus (ELS 29)

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Abstract

This case–control study investigated the association between major types of serum advanced glycation end-products (AGEs) and selected serum/plasma markers of endothelial dysfunction in black patients with type 2 diabetes mellitus at Dr George Mukhari Academic Hospital. Serum AGEs were measured using either enzyme-linked immunosorbent assay (ELISA) or spectrofluoremetry. Serum markers of endothelial dysfunction were measured using either ELISA or calometry. The correlation and associations between major types of serum AGEs and markers of endothelial dysfunction were investigated using the Spearman correlation coefficient and bivariate logistic regression analysis, respectively. Although both serum total immunogenic AGEs and serum carboxymethyl-lysine (CML) were moderately and negatively associated with endothelial dysfunction, only serum CML was significantly associated with a higher odds for the development of endothelial dysfunction (low nitric oxide levels) in our diabetic subjects. It can therefore be concluded from this study that high serum levels of CML may predispose to endothelial dysfunction in black South Africans with type 2 diabetes.

Keywords: serum AGEs, endothelial dysfunction, markers of endothelial dysfunction, black South Africans, type 2 diabetes mellitus

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Clinical and research-based evidence indicates that both type 1 and type 2 diabetes mellitus are associated with long-term microvascular complications (nephropathy, retinopathy and neuropathy) and macrovascular complications (myocardial infarction and cerebrovascular accident).[1,2] Available evidence also suggests that the pathogenesis of these vascular complications of diabetes involve endothelial activation or dysfunction.[3] Endothelial dysfunction, defined as impaired biosynthesis of endothelium-derived nitric oxide (NO) or its reduced bioavailability, is an established mediator of the atherosclerotic process.[3,4] Indeed, most of the traditional and emerging cardiovascular risk factors are known to promote the development and progression of vascular atherosclerosis through their deleterious effect on the endothelium.[4] The development of endothelial dysfunction in diabetes mellitus is attributable, among other factors, to the formation and action of advanced glycation end-products (AGEs).[5,6]

AGEs are a heterogeneous group of compounds formed by the non-enzymatic reaction between reducing sugars such as glucose and proteins, nucleic acids and lipids.[5] The formation of AGEs is reported to be enhanced by both chronic hyperglycaemia and oxidative stress, two conditions that are closely associated with diabetes mellitus.[2,6]

Available evidence also suggests that in diabetes mellitus, AGEs may promote endothelial dysfunction via a variety of mechanisms. Firstly, collagen cross-linked AGEs in the vascular wall may trap and quench NO on its way from the endothelium to the smooth muscle layer to stimulate their relaxation.[7] Secondly, the interaction of certain serum AGEs with the receptor for advanced glycation end-products (RAGE) on vascular endothelial cells results in the activation and translocation of nuclear factor kappa B (NF-κB) into the nucleus.[8] Once in the nucleus, NF-κB up-regulates several genes whose protein and peptide products are involved in the activation of the endothelium or endothelial dysfunction.[1,7] Thirdly, serum AGE/RAGE interaction on the vascular endothelium may result in deactivation of the enzyme, endothelial nitric oxide synthase (eNOS), which synthesises NO in the endothelium.[9] Fourthly, the superoxide anion (O_2^-) generated during the formation of AGEs may react with NO to form the peroxy-nitrite ion $(ONOO)^-$, thereby reducing the bioavailability of NO.[9,10] Lastly, AGEs may impair Ca^{2+} signalling in endothelial cells, thereby interfering with several endothelial cell processes, including the biosynthesis of NO.[11]

Racial/ethnic disparities in endothelial dysfunction have been observed in a number of studies. For example, African-Americans

are reported to have reduced NO bioavailability compared to their Caucasian counterparts.[12,13] Also, Tibetan type 2 diabetes patients are reported to have less NO levels than their Chinese Han counterparts.[14] On the other hand, research evidence has also shown that both tissue and serum AGE levels may be influenced by genetics.[12,15] Taken together, this information suggests that the association between serum (and tissue) AGE levels and endothelial dysfunction may be influenced by the genetic make-up and ethnicity/race of an individual. However, with the exception of a single study that investigated the association between serum AGE levels and endothelial dysfunction among Chinese type 2 diabetes patients,[16] there is no other information in the literature regarding the association between serum levels of AGEs and endothelial dysfunction. In particular, no study has ever been conducted to investigate the association between serum AGE levels and endothelial dysfunction among type 2 diabetes patients of black African descent. Therefore, the aim of this study was to investigate the association between the different types of serum AGEs and circulating markers of endothelial dysfunction among black South African patients with type 2 diabetes mellitus.

Methods

A random sample of 138 black type 2 diabetes patients attending the diabetes clinic of Dr George Mukhari Academic Hospital (DGMAH) for medical review, and a convenient sample of 81 age-matched non-diabetic control subjects were recruited into this study. The control subjects were recruited mainly from the orthopaedic wards of DGMAH. Controls were included in the study if they had fasting blood glucose level of < 6.1 mmol/l. Both type 2 diabetes patients and control subjects were excluded from the study if they had any sign of renal impairment, history or evidence of any of the factors known to affect endothelial dysfunction, such as the traditional cardiovascular risk factors, uncontrolled hypertension, dyslipidaemia, cigarette smoking and obesity.

All type 2 diabetes patients and control subjects gave their informed consent after the purpose of the study and their rights were clearly explained to them. The study was conducted in accordance with the requirements of the research and ethics committee of the University of Limpopo (MREC/P/2013/PG).

After an overnight fast, venous blood samples for measurement of levels of the different types of serum AGEs, urea and electrolytes, as well as selected circulating markers of endothelial dysfunction were collected from all participants into blood collection tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). The samples were left to clot for 30 min and then centrifuged at 4 000 rpm for 15 min at 4°C. Aliquots of the resultant serum samples were then stored at –80°C until analysed. For blood glucose and glycated haemoglobin (HbA_{1c}) measurements, blood samples were collected into citrate and EDTA blood tubes, respectively.

Serum total immunogenic AGEs (TIAGEs), Nε-carboxymethyllysine (CML) and Nε-carboxyethyl-lysine (CEL) were measured using STA-317, STA-316 and STA-300 OxiselectTM ELISA kits, respectively, (2BScientific, Upper Heyford, UK), according to the manufacturer's instructions. Fluorescent serum AGEs (FAGEs) were measured according to the method described by Munch *et al*.[17] In brief, 20 µl of serum was diluted to a volume of 10 ml with 20 mM phosphate buffered saline, pH 7.4. Fluorescence of the diluted sample was then measured spectrofluorometrically (excitation at 370 nm and emission at 440 nm) using a GloMaxR multidetection spectrofluorometer (Promega Corp, Madison, WI, USA). Fluorescent readings were expressed as arbitrary units (emission intensity/excitation intensity).

Plasminogen activator inhibitor-1 (PAI-1) was measured using ELISA kits purchased from Cell Biolabs, and NO and endothelin-1 (ET-1) were measured using colorimetric and immunometric kits, respectively, purchased from Cayman Chemical's ACE. Fasting blood glucose levels were measured using a commercially available glucose oxidase-based kit adapted to the Beckman Coulter® UniCell DXC 800 Synchron® Clinical System available in the National Laboratory Health Services (NLHS) laboratory at the DGMAH. HbA_{1c} level was measured using the immune chemiluminescent assay kit adapted to the Abbot Architect system Ci 8200 in the NLHS laboratory at DGMAH, in accordance with the manufacturer's instructions.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (Version 23.0), SPSS Inc, Chicago, IL, USA. Continuous variables are expressed as mean ± standard deviation (SD) while categorical variables are expressed as percentages. Means of the experimental and control groups were compared using the student's *t*-test, and $p < 0.05$ was regarded as statistically significant differences between the groups. Bivariate logistic regression and the Spearman rank correlation coefficient were used to determine the association and correlation between the major types of serum AGEs and circulating markers of endothelial dysfunction, respectively. Significance level was set at $p < 0.05$.

Results

Table 1 shows the demographic, clinical and laboratory characteristics of the type 2 diabetes patients and the non-diabetic controls. With the exception of the fasting blood glucose and HbA_{1c} levels, there were no significant differences in any other demographic, clinical or laboratory parameters between the diabetic and the non-diabetic groups.

Table 1. Demographic, clinical and laboratory characteristics of the study subjects

Characteristics	Type 2 diabetes group (*n* = 120) mean ± SD	Non-diabetic control group (*n* = 83) mean ± SD	*p*-value
Gender			
Male, *n* (%)	49 (41)	36 (44)	0.512
Female,*n* (%)	71 (59)	47 (56)	0.734
Age (years)	56.9 ± 9.4	51.1 ± 9.8	0.152
FBG (mmol/l)	11.6 ± 3.3	5.2 ± 6.3	0.012*
HbA_{1c} (%)	9.7 ± 1.2	6.1 ± 2.6	0.037*
HbA_{1c} (mmol/mol)	81 ± 0.99	43 ± 5	0.037*
BMI (kg/m²)	26.6 ± 4.7	25.8 ± 5.5	0.081
TC (mmol/l)	4.20 ± 1.80	4.03 ± 0.95	0.174
LDL (mmol/l)	2.3 ± 0.15	2.1 ± 0.2	0.511
TG (mmol/l)	1.2 ± 0.5	1.32 ± 0.4	0.712
SBP (mmHg)	127 ± 10.9	1 28 ± 8.7	0.141
DBP (mmHg)	81 ± 10.8	82 ± 8.4	0.091
Urea (mmol/l)	6.0 ± 2.5	5.6 ± 1.3	0.452
Creatinine (µmol/l)	94 ± 55.9	86.4 ± 21.1	0.318

FBG: fasting blood glucose; HbA_{1c}: glycated haemoglobin; BMI: body mass index; TC: total cholesterol; LDL: low-density lipoprotein; TG: triglycerides; SBP: systolic blood pressure; DBP: diastolic blood pressure; GFR: glumerular filtration rate.

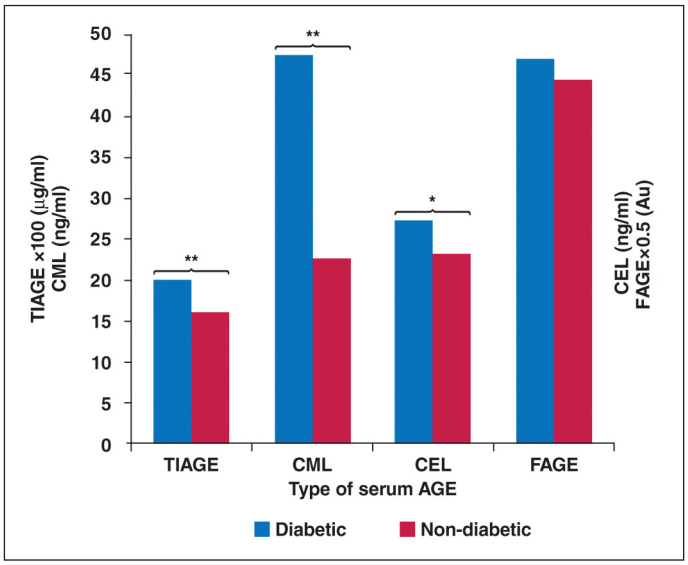

Fig. 1. Comparison of median levels of total immunogenic AGEs (TIAGEs), Nε-carboxymethyl-lysine (CML), Nε-carboxyethyl-lysine (CEL) and fluorescent AGEs (FAGEs) between the type 2 diabetes and non-diabetic control groups. *Significant at $p < 0.01$, **significant at $p < 0.001$.

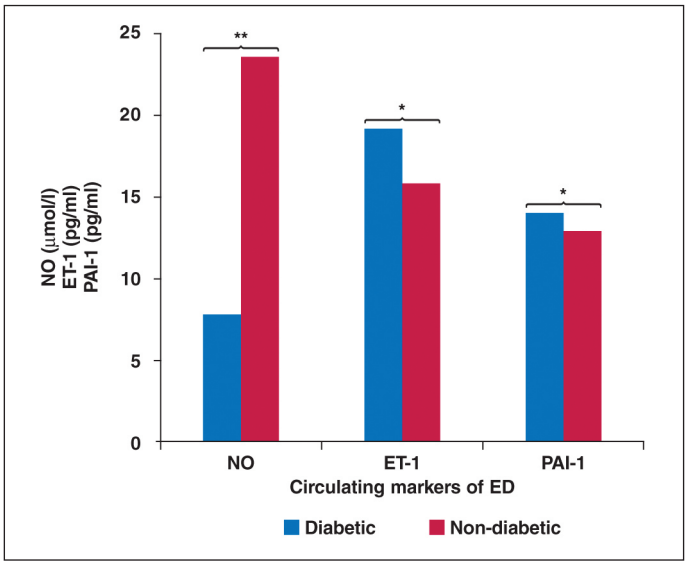

Fig. 2. Comparison of mean serum levels of nitric oxide (NO), endothelin-1 (ET-1) and plasminogen activator inhibitor (PAI-1) between the type 2 diabetes and non-diabetic control groups. *Significant at $p < 0.05$; **significant at $p < 0.01$.

As shown in Fig. 1, the mean serum levels of TIAGEs, CML and CEL were significantly higher in the diabetic than the non-diabetic group ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively). On the other hand, there was no significant difference between serum FAGE levels of the diabetic and non-diabetic groups.

As shown in Fig. 2, the mean NO serum level of the diabetic patients was significantly lower than that of the non-diabetic control group ($p < 0.001$). On the other hand, the mean serum ET-1 and PAI-1 levels of the diabetic group were significantly higher than those of the control group ($p < 0.05$) (Fig. 2).

Gender and age of the study subjects, as well as the different types of serum AGEs (TIAGEs, CML, CEL and FAGEs) measured in the diabetic group were correlated with the corresponding selected circulating markers of endothelial dysfunction (NO, ET-1 and PAI-1) using the Spearman rank correlation coefficient (rs).

Results shown in Table 2 suggest a significant weak negative correlation ($p < 0.05$) between the age of the study subject and serum levels of NO, as well as a significant moderate negative correlation between serum TIAGE and NO levels ($p < 0.05$), and between serum CML and NO levels ($p < 0.05$) (Table 2). Table 2 also shows a significant weak positive correlation between serum TIAGE and ET-1 levels ($p < 0.05$), as well as between serum CML and ET-1 levels ($p < 0.05$).

Table 2. Correlations between age of the study subjects, gender, different types of serum AGEs and selected circulating markers of endothelial dysfunction

	Serum NO (pmol/l)		Serum ET-1 (ng/ml)		Serum PAI-1 (ng/ml)	
Parameters	r_s	***p*-value**	r_s	***p*-value**	r_s	***p*-value**
Age	–0.236*	0.031	0.149	0.302	0.080	0.582
Gender	0.191	0.896	0.048	0.741	–0.230	0.109
TIAGEs (µg/ml)	–0.382*	0.026	0.279*	0.012	–0.185	0.108
CML (ng/ml)	–0.412*	0.011	0.281*	0.021	–0.228	0.112
CEL (ng/ml)	–0.015	0.920	0.150	0.297	–0.145	0.758
FAGEs (Au)	0.050	0.722	–0.036	0.802	0.175	0.224

rs: Spearman rank correlation coefficient; TIAGEs: total immunogenic advanced glycation end-products; CML: Nε-carboxymethyl-lysine; CEL: Nε-carboxyethyl-lysine; FAGEs: fluorescent advanced glycation end-products; Au: arbitrary units; *Correlation is significant at $p < 0.05$ level.

Table 3. Bivariate logistic analysis of the association between gender, age and the major types of serum AGEs with endothelial dysfunction (less than the first quartile of NO levels)

Parameters	COR	95% CI	*p*-value
Age	0.600	1.372–2.62	0.460
Gender	1.040	0.996–1.12	0.296
TIAGEs (µg/ml)	0.348	0.014–8.916	0.523
CML (ng/ml)	1.910	0.655–0.893	0.013*
CEL (ng/ml)	1.172	0.963–1.638	0.112
FAGEs (Au)	0.991	0.882–1.038	0.141

COR: crude odds ratio; CI: confidence interval; TIAGEs: total immunogenic advanced glycation end-products; CML: Nε-carboxymethyl-lysine; CEL: Nε-carboxyethyl-lysine; FAGEs: fluorescent advanced glycation end-products; *Significant at $p < 0.05$.

Bivariate logistic regression analysis of the association between age and gender of the diabetic subjects, as well as serum levels of the major types of serum AGEs with endothelial dysfunction (serum NO levels less than the first quartile) revealed that only higher serum levels of CML were significantly associated with higher crude odds of endothelial dysfunction [COR (95% CI), 1.910 (0.655–0.893) ($p < 0.05$) (Table 3).

Discussion

As expected, serum levels of TIAGEs, CML and CEL were found to be significantly higher in the diabetic patient group compared with the non-diabetic control group. However, serum FAGE levels of diabetic patients were not significantly different from those of non-diabetic controls. This observation might be attributed to the nature of the control group used in the study.

High serum FAGE levels, in particular high serum levels of pentosidine, the most abundant fluorescent AGE in plasma and tissues, have been associated with the development and progression of osteoporosis in diabetic and non-diabetic menopausal women.[18,19] Whether the high level of pentosidine observed in the cited studies was the cause or product of osteoporosis is currently not clear. It is possible that our patient control group, which was recruited from orthopaedic wards at DGMAH, may have included non-diabetic postmenopausal women with osteoporosis-related fractures. While this likelihood was not verified in the current study, it might explain the observed high levels of FAGE in the non-diabetic control group.

Previous studies reported in the literature have used circulating levels of NO, ET-1 and PAI-1, among others, as surrogate markers of endothelial dysfunction in vivo.[3,4,20] According to these previous studies, serum levels of NO and its metabolites are expected to be decreased, while serum levels of both ET-1 and PAI-1 are expected to be increased in conditions associated with endothelial dysfunction, such as type 2 diabetes mellitus. Therefore the findings of significantly reduced NO levels and significantly higher serum levels of both ET-1 and PAI-1 are in perfect agreement with the results of these previous studies. However, these findings should be interpreted with caution, since these circulating markers of endothelial dysfunction may come from sources other than the vascular endothelium.[4,20]

The observation in this study that serum NO levels were negatively and significantly correlated with the age of the study subject is in agreement with the well-documented observation that endothelial function decreases with advanced age.[21,22] The findings that serum levels of both TIAGEs and CML were negatively and significantly correlated with serum NO levels and positively and significantly correlated with serum levels of ET-1 were also not unexpected, since high levels of some serum AGEs are known to promote endothelial dysfunction through their interaction with RAGE on the surface of the vascular endothelial cell.1 The finding that serum CML level was the only parameter in this study that was significantly associated with increased odds of developing endothelial dysfunction suggests that serum CML is the major type of serum AGEs that interacts with RAGE to promote endothelial dysfunction.

Limitations

There are several limitations that should be taken into consideration when interpreting results of this study. Firstly, the sample size was small and study subjects were recruited from a single health institution, therefore the findings could not be generalised beyond the study samples. Secondly, the study was cross-sectional and therefore cause and effect relationships could not be inferred from the results. Thirdly, the possible confounding effect of exogenous dietary and smoking-related AGEs on serum AGE levels was not addressed. Fourthly, the control group selected for this study might have confounded the results, particularly those of the FAGEs. Fifthly, we did not concurrently measure serum AGE levels and circulating markers of endothelial dysfunction of other South African race groups for comparison purposes.

Despite these limitations, we believe that the results of this study are of great interest in that they are the first to describe the status of serum AGE levels among black South African patients with type 2 diabetes, as well as the association between serum AGE levels and endothelial dysfunction in black South African patients with type 2 diabetes mellitus.

Conclusions

The results of this study showed that serum AGE levels were significantly higher in type 2 diabetes patients than in non-diabetic black South Africans, and with the exception of CEL were not influenced by gender. In addition, serum FAGE levels appeared to be positively associated with increasing age of the subjects in the non-diabetic controls, but not in in the diabetic subjects. Furthermore, the findings of this part of the thesis showed that serum TIAGEs, CML, CEL, ET-1 and PAI-1 levels were significantly elevated, whereas serum levels of NO were significantly reduced in black South African patients with type 2 diabetes compared to those in non-diabetic control subjects. Moreover, the findings indicated that serum TIAGE and CML levels, but not CEL and FAGE levels were correlated with endothelial dysfunction in black South African patients with type 2 diabetes mellitus. However, only serum CML levels were associated with a higher odds of developing endothelial dysfunction in these black South African type 2 diabetes patients.

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Being too fat or too thin 'can cost four years of life'

Being overweight or underweight, as measured by the body mass index (BMI), could knock four years of life expectancy, a five-year UK population cohort study of nearly two million people found.

Researchers found that, from the age of 40 years, people at the higher end of the healthy BMI range had the lowest risk of dying from disease. But people at the top and bottom ends of the BMI risked having shorter lives.

BMI is calculated by dividing an adult's weight by the square of their height. A 'healthy' BMI score ranges from 18.5 to 25 kg/m^2. According to the report, most doctors say it is the best method they have of working out whether someone is obese because it is accurate and simple to measure.

The study showed that life expectancy for obese men and women was 4.2 and 3.5 years shorter respectively than people in the entire healthy BMI weight range. The difference for underweight men and women was 4.3 (men) and 4.5 (women) years.

The report says BMI was associated with all causes of death categories, except transport-related accidents, including cancer, cardiovascular diseases and respiratory diseases. However, not everybody in the healthy category is at the lowest risk of disease, according to report author Dr Krishnan Bhaskaran at the London School of Hygiene & Tropical Medicine.

He is quoted in the report as saying: 'For most causes of death we found that there was an "optimal" BMI level, with risk of death increasing both below and above that level. At BMIs below 21 kg/m^2, we observed more deaths from most causes, compared with the optimum BMI levels. However, this might partly reflect the fact that low body weight can be a marker of underlying ill-health. For most causes of death, the bigger the weight difference, the bigger the association we observed with mortality risk. So a weight difference of half a stone would make a relatively small (but real) difference; we could detect these small effects because this was a very large study.'

Some experts have questioned whether BMI is an accurate way of analysing a person's health. However, the report says, Dr Katarina Kos, senior lecturer in diabetes and obesity at the University of Exeter, believes it is. 'For the majority of people, BMI is a good measure.'

Kos said the study did not contain any surprises but added that overweight people who could lower their BMI may reap the health benefits. 'We know from the diabetes remission data how low-calorie diets and weight loss can improve diabetes, for example,' she said. 'And we know weight loss can also help in improving risk so that would also then improve mortality rates.'

The study suggested that a higher BMI in older people may not be as dangerous, because a bit of extra weight was 'protective' for them. But Kos, who worked on a study on this topic in 60 to 69-year-olds last year, disagreed with the findings. Her study on what is known as the obesity risk paradox, did not 'support acceptance' of the theory.

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Plasma phospholipid fatty acid patterns are associated with adiposity and the metabolic syndrome in black South Africans: a cross-sectional study

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Abstract

Background: **Diets rich in *n*-6 polyunsaturated fatty acids (PUFAs) and saturated fatty acids (SFA) have been associated with increased risk of obesity and the metabolic syndrome (MetS), but the evidence is inconsistent, whereas diets high in *n*-3 long-chain (LC)-PUFAs are associated with lower risk. There is limited information about the association of plasma phospholipid fatty acids (FAs) with obesity and the MetS among black South Africans.**

Objective: **To investigate the association of dietary FAs and plasma phospholipid FA patterns, respectively, with measures of adiposity (body mass index, waist circumference, waist-to height ratio) and the MetS in black South Africans.**

Methods: **Factor analysis was used to identify FA patterns from 11 dietary FAs and 26 individual plasma phospholipid FAs. Cross-sectional association of the identified patterns with measures of adiposity and the MetS was investigated. A random sample of 711 black South African adults aged 30 to 70 years (273 men, 438 women) from the North West Province was selected from the South African leg of the Prospective Urban and Rural Epidemiology (PURE) study. Sequential regression models adjusted for confounders were applied to investigate the association between dietary FAs and plasma phospholipid FA patterns with measures of adiposity and the MetS.**

Results: **Two patterns were derived from dietary FAs and six patterns from plasma phospholipid FAs that explained the cumulative variance of 89 and 73%, respectively. The association of FA patterns with adiposity and the MetS was weaker for dietary FA patterns than for plasma phospholipid FA patterns. The plasma phospholipid FA pattern with high loadings of saturated FAs (high-Satfat) and another with high loadings of *n*-3 very-long-chain PUFAs (*n*-3 VLC-PUFAs) were positively associated with measures of adiposity and the MetS, while patterns with positive loadings of LC monounsaturated fatty acids (*n*-9 LC-MUFA) and a positive loading of *n*-3 essential FAs (*n*-3 EFA) showed inverse associations with the MetS and some measures of adiposity.**

Conclusions: **The *n*-9 LC-MUFA and *n*-3 EFA patterns seemed to provide possible protective associations with adiposity and the MetS, whereas the high-Satfat and *n*-3 VLC-PUFA patterns were associated with adiposity and the MetS in our study participants. The results are reflective of the metabolic difference between overweight and obese compared to lean individuals.**

Keywords: phospholipid fatty acid patterns, dietary fatty acid patterns, adiposity, metabolic syndrome, waist:height ratio

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South Africa is currently experiencing rapid nutritional, economic, demographic and epidemiological transitions with likely consequences for lifestyle and health.[1] The prevalence of overweight and obesity in South Africa in 2012 was 31% in men and 64% for women.[2] This increased in 2016 to 68% in women but remained the same for men.[3] Abdominal obesity among black South African women is particularly associated with elevated blood pressure (BP), lower high-density lipoprotein cholesterol, higher serum triglycerides, and elevated fasting plasma glucose, indicative of insulin resistance.[4] Unhealthy diet is a major risk factor associated with the rising prevalence of obesity and the metabolic syndrome (MetS).[5,6]

Fat intake among the black urban population of South Africa has increased from 16.4 to 26.2% of total energy over the past 50 years.[7] The transition from more traditional to Western diets, characterised by an increase in *n*-6 polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), industrial trans fatty acids (FAs),[8] as well as a decrease in *n*-3 PUFA intake, is also prevalent in this population.[9] Diets high in percentage energy from animal protein and total fat intake may increase the risk of non-communicable diseases in rural and urban black South Africans,[10] and this may be related to meat intake, which is a major source of both MUFAs and SFAs in South Africans.[11] By contrast with this, however, a study that investigated dietary intake of carbohydrate and SFAs in 18 countries undergoing rapid nutritional transition documented that SFA intake was associated with lower risk of mortality.[12]

Studies investigating circulating FAs have also reported some conflicting results. A recent study examined the relationship between body mass index (BMI) and plasma phospholipid FA composition in men aged between 48 and 65 years and reported higher plasma phospholipid levels of palmitic (C16:0) and stearic acid (C18:0) in obese individuals.[13] Furthermore, plasma concentrations of C16:0 were positively associated with risk for total mortality in men and women in a prospective study in the USA.[14] SFAs, myristic acid (C14:0), C16:0 and C18:0 in plasma were positively associated with the MetS, while longer-chain SFAs, and arachidic (C20:0), behenic (C22:0) and lignoceric acid (C24:0) were inversely associated in men and women from Taiwan.15 Another study also reported lower levels of plasma C22:0 and C24:0 in the MetS participants.[16] Palmitoleic acid (C16:1*n*-7) level in plasma phospholipids was positively associated with BMI in men and women,[13,17] and higher levels of plasma C16:1*n*-7 were associated with multiple metabolic risk factors in men and women.[18,19]

In different populations, total *n*-3 FAs in plasma were associated with lower BMI, waist circumference (WC) and hip circumference[20] and inversely associated with the MetS,[21] while omega-6 PUFA have been associated with obesity and the MetS. Pickens and associates reported higher plasma phospholipid levels of dihomo-γ-linolenic acid (C20:3*n*-6) in overweight and obese individuals.[13] Positive associations of serum phospholipid C20:3*n*-6 with BMI, as well as total *n*-6 PUFAs with waist:hip ratio were also documented in a study of Mexican women.[17] Some studies also report positive associations of specific plasma phospholipids *n*-6 PUFAs with metabolic risk,[18,22] while other studies report inverse associations of total *n*-6 PUFAs in erythrocytes and serum, respectively, with the MetS.[23,24] Due to inconsistent results in different studies relating to circulating *n*-6 PUFAs, further research to understand their role in association with obesity and the MetS is highly recommended.[25]

Since people consume food rather than individual nutrients, it is difficult to isolate the individual nutrients in the diet and link them to disease and health.[26] Therefore, the analysis of food intakes into patterns derived from various combinations of nutrients or foods has developed as a preferred alternative to investigating associations between nutrients and diseases.[27] Several studies have applied factor and cluster analysis to derive patterns from food and tissues in investigating the association of these patterns with health and diseases.[28]

FA patterns from adipose tissue and plasma have been employed to describe associations of FAs with obesity[29] and the MetS.[22,30] Despite the extensive use of plasma FAs in research, there is limited epidemiological research on the use of both dietary and circulating FA patterns in association with obesity and the MetS in black populations in Africa. To address the key gaps in the current knowledge, the aim of this study was to investigate the associations of dietary and plasma phospholipid FA patterns with adiposity measures [BMI, waist circumference (WC) and waist-to-height ratio (WHtR)] and the MetS in a selected group of black South African adults. This study was based on a random sub-sample of 711 participants selected from the South African site (North West Province) of the multicountry Prospective Urban and Rural Epidemiological (PURE) study. This study made use of cross-sectional data collected at baseline during the months of August to November 2005.

Methods

A sub-sample of 711 black South African participants were randomly selected from 2 010 adults recruited at baseline (in 2005) from urban (1 004) and rural (1 006) households in the North West Province to assess dietary FA intake and plasma phospholipid FA status. Those included were apparently healthy subjects older than 30 years at baseline, with no reported diseases of lifestyle, tuberculosis or HIV, and used chronic medication for diabetes and hypertension only.

Ethical approval for the South African PURE study was obtained from the Ethics Committee of North-West University (Ethics number 04M10). Participants provided written informed consent and participation was voluntarily.

Transportation was provided for the study subjects to reach the data-collection centres in both rural and urban areas. During face-to-face interviews by trained fieldworkers, each participant completed questionnaires in his or her preferred language (Afrikaans, Setswana or English). The questionnaires included demographic,[31] physical activity[32] and quantitative food-frequency questions (QFFQ),[33,34] and made use of, among others, validated food photo-books to estimate portion sizes.[35] Reproducibility[33] and details of dietary assessments have been published elsewhere.[10]

Dietary macronutrients and FAs were calculated using the South African Medical Research Council food composition tables.[36] Twenty-eight dietary FAs were included initially, but FAs that had a daily median intake of less than 0.10 mg were excluded. A total of 11 dietary FAs were used to derive FA patterns for investigation in this study.

Anthropometric measurements were performed by trained research assistants according to standards prescribed by the International Society for the Advancement of Kinanthropometry.[37] A portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan) was used to measure weight. Height was measured using a calibrated stadiometer (Seca, Hamburg, Germany). Waist and hip circumferences were recorded using steel tapes (Lufkin, Apex, NC, USA). BMI and WHtR were calculated using weight (kg)/height (m^2) and waist (cm)/height (cm) formulas, respectively. Blood pressure (mmHg) was measured in duplicate (five minutes apart) on the right upper arm. Appropriately sized cuffs were used for obese subjects.

Fasting blood samples were collected from the antecubital vein with a sterile winged infusion set with minimal stasis. The samples were collected and plasma and serum were prepared and aliquoted by a registered nurse and then stored at –80°C in the urban areas. In rural areas, the samples were stored at –18°C for up to five days, where after it was transported to the laboratory facility and stored at –80°C until analysed.

Fasting plasma glucose concentration was determined by the hexokinase method using the Synchron® system (Beckman Coulter Co, Fullerton, CA, USA). The sequential multiple analyser computer (SMAC) using the Konelab™ auto-analyser (Thermo Fisher Scientific Oy, Vantaa, Finland) performed quantitative determinations of high-density lipoprotein cholesterol (HDL-C), triglycerides and total cholesterol (TC). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation.[38]

EDTA plasma samples were thawed and extracted with chloroform:methanol (2:1 v/v) according to the modified Folch method.[39] The plasma phospholipid FA fraction was isolated by thi*n*-layer chromatography from the extracted lipids.[40] Subsequently, the phospholipid FA fraction was transmethylated to FA methyl esters and analysed by quadrupole gas chromatography electron ionisation mass spectrometry by means of an Agilent Technologies 7890 A GC system, as described by Baumgartner *et al*.[40]

Thirty-two FAs were measured in fasted plasma samples from 711 participants. Six FAs, i.e. pentadecanoic acid (C15:0), margaric acid (C17:0), trans vaccenic acid (C18:1*n*-7t), rumenic acid (C18:2*n*-7tt), stearidonic acid (C18:4*n*-3) and eicosatrienoic (C20:3*n*-3) were below the limit of quantification and therefore not included. The remaining 26 plasma phospholipid FAs were quantified and expressed as a percentage of total FAs. Quality of data was assured with a separate calibration for each FA, monitoring of internal standard (1,2-diheptadecanoyl-snglycerol-3 phosphorylchloline, Matreya, Pennsylvania, USA) and Levey Jennings graphs for a pooled plasma control analysed with each batch.

The MetS was defined according to recommendations by the Joint Interim Statement of six international associations as the presence of three or more of the following: (1) fasting plasma glucose levels ≥ 5.6 mmol/l or the use of oral hypoglycaemic medication; (2) serum triglycerides ≥ 1.7 mmol/l; (3) serum HDL ≤ 1.0 mmol/l for men and ≤ 1.3 mmol/l for women; (4) BP ≥ 130/85 mmHg or the use of BP medication; and (5) WC of ≥ 94 cm for men and ≥ 80 cm for women.[41]

Statistical analysis

Continuous variables were described as medians and interquartile ranges if data deviated from the normal distribution according to the Kolmogorov–Smirnov test, whereas categorical variables were presented as percentages. No*n*-normally distributed data were log transformed before inclusion in regression models. Participants' characteristics were compared by gender and BMI categories using the Mann–Whitney *U*-test or chi-squared test for continuous and categorical variables, respectively. Differences between individual FAs and ratios by BMI and gender groups were tested with the Mann–Whitney test. A BMI < 25 kg/m^2 was considered as underweight and/or normal-weight or lean, whereas BMI ≥ 25 kg/m^2 was considered overweight and/or obese. The effect size of the differences between groups was calculated using the Man–Whitney *U*-value and sample size of the groups.[42]

Principal-component-based varimax factor analysis of the correlation matrix was used to define dietary FA (based on the QFFQ) and plasma phospholipid FA patterns. The identification and naming of 11 dietary FAs and 26 plasma phospholipid FAs used in this study are based on relevant literature and the levels of specific FAs observed in our population.[43] The number of factors to retain was established by the Kaiser criterion (eigenvalues > 1) and scree-plot visual inspection. Loadings with absolute values > 0.5 were considered as relevant for the contribution to each FA pattern. The associations between FA patterns and outcomes were evaluated by sequential regression models, logistic regression for the dichotomous outcome (MetS), and generalised linear models for continuous outcomes (WC, BMI and WHtR).

The first step of the sequential modelling analyses was based on models that contained only dietary FAs or plasma phospholipid FA patterns and was referred to as a crude model. The crude model was then adjusted for gender and age (adjusted model1). This model was further adjusted for lifestyle confounders, including the level of education, physical activity, alcohol and total energy intake, and self-reported smoking status, creating a fully adjusted model. We further adjusted this model for contraceptives (adjusted for in plasma phospholipid FA pattern models only) and dietary factors, including total fats, carbohydrates, dietary fibre and energy from added sugar as individual confounders and as combined covariates.

Model fitting was evaluated using the adjusted *R*-square for linear regression and maximum re-scaled R-square statistic for logistic regression. Linear regression results are presented as standardised β and 95% confidence intervals (CI) with their significance levels, and odds ratio and 95% CI with significance levels for logistic regression. All analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA)[44] and $p < 0.05$ was considered significant.

Results

The baseline characteristics of the 711 participants are shown in Table 1. The majority were women (61.6%) and the median age was comparable between men and women. Men had higher HDL levels and were more likely to smoke. By contrast, the women had higher serum triglycerides, as well as higher levels of measures

Table 1. Demographics, health and dietary intake data of an apparently healthy cohort of 711 black South African adults participating in the PURE study

Variables	Men (*n* = 273) Median (Q_1, Q_3)[b]	Women (*n* = 438) Median (Q_1, Q_3)[b]	*p*-value[c]
Demographics			
Age (years)	52 (46, 60)	52 (45, 59)	0.80
Education (educated), *n* (%)	155 (57.6)	263 (62.2)	0.22
Tobacco use (current smoker), *n* (%)	163 (59.7)	205 (46.8)	0.0008
Alcohol (g/week)	6.4 (0, 24.9)	0 (0, 3.9)	< 0.0001
Physical activity index	2.8 (2.5, 3.1)	2.8 (2.5, 3.3)	0.71
Waist circumference (cm)	75.4 (69.7, 82.4)	82.0 (71.7, 92.6)	< 0.0001
Waist-to-height ratio	0.45 (0.4, 0.5)	0.52 (0.5, 0.6)	< 0.0001
Body mass index (kg/m^2)	20.0 (18.1, 23.2)	26.0 (21.8, 31.9)	< 0.0001
Systolic blood pressure (mmHg)	135 (121, 152)	132 (118, 150)	0.06
Diastolic blood pressure (mmHg)	88 (78, 98)	88 (70, 97)	0.84
Fasting glucose (mmol/l)	4.8 (4.3, 5.4)	4.9 (4.3, 5.4)	0.53
Total cholesterol (mmol/l)	5.0 (4.1, 6.0)	5.1 (4.4, 6.2)	0.35
High-density lipoprotein cholesterol (mmol/l)	1.54 (1.2, 2.1)	1.5 (1.2, 1.8)	0.04
Low-density lipoprotein cholesterol (mmol/l)	3.1 (2.3, 4.0)	3.4 (2.7, 4.2)	0.06
Triglycerides (mmol/l)	1.0 (0.8, 1.5)	1.2 (0.9, 1.8)	0.002
Dietary intake[a]			
Total energy (kcal/day)	1874 (1377, 2612)	1628 (1189, 2212)	0.001
Total carbohydrate (g/day)	285.4 (199, 378)	248.8 (180.6, 325.1)	0.01
Total fibre (g/day)	14.8 (25, 30)	17.9 (12.7, 25.2)	0.004
Total protein (g/day)	55.0 (38, 75.7)	46.2 (33.1, 65.0)	< 0.0001
Total fat (g/day)	45.3 (28.5, 63.7)	40.5 (26.3, 64.4)	0.10
Total saturated fatty acids (g/day)	10.5 (6.5, 15.7)	9.5 (5.6, 16.6)	0.13
Total mono-unsaturated fatty acids (g/day)	11.4 (6.8, 17.8)	10.4 (6.0, 18.3)	0.14
Total polyunsaturated fatty acids (g/day)	13.6 (8.8, 19.6)	13.1 (7.5, 20.0)	0.47
Total *n*-3 polyunsaturated fatty acids (g/day)	0.4 (0.2, 0.6)	0.33 (0.20, 0.5)	0.15
Total *n*-6 polyunsaturated fatty acids (g/day)	13.3 (8.8, 19.2)	12.9 (7.3, 19.6)	0.55

[a]Baseline demographic details of participants.
[b]Data are presented as median (interquartile range): Q_1, lower interquartile range; Q_3, upper interquartile range.
[c]Significance levels of differences in parameters between men and women, based on Mann–Whitney and chi-squared tests for continuous and categorical variables, respectively.

of adiposity ($p < 0.0001$). The dietary intake data revealed that men had higher total energy, total carbohydrate and total protein intakes, whereas the women, on the other hand, had higher total dietary fibre intake.

Table 2 shows that total intakes of fat, SFAs and MUFAs were significantly higher in overweight/obese women compared to lean women. Plasma phospholipid FAs within SFA, MUFA and PUFA classes differed across BMI categories and gender. In men, the plasma levels of SFAs, C18:0, C20:0, C22:0, C24:0 and *n*-6 PUFA C20:3*n*-6 were significantly higher in overweight men than in lean men, whereas C16:0, C18:3*n*-6 and MUFAs, C16:1*n*-7, cis-vaccenic acid (C18:1*n*-7), oleic acid (C18:1*n*-9) and gondoic acid (C20:1*n*-9) were higher in

Table 2. Dietary intake of fats, individual fatty acids and plasma phospholipid fatty acid profile by BMI categories and gender in 711 black South African adults

	Men (*n* = 273)				Women (*n* = 438)			
Variables	**BMI[a] < 25 kg/m² (*n* = 233)**	**BMI ≥ 25 kg/m² (*n* = 40) Median (Q_1, Q_3)**	***p*-value**	**Effect size**	**BMI ≥ 25 kg/m² (*n* = 191) Median (Q_1, Q_3)**	**BMI ≥ 25 kg/m² (*n* = 247) Median (Q_1, Q_3)**	***p*-value**	**Effect size**
Total energy, dietary fat intake								
Total energy (kcal/day)	1856 (1374, 2599)	2052 (1370, 2681)	0.48		1579 (1139, 2188)	1651 (123, 2224.)	0.50	
Total fat (g/day)	44.8 (28.5, 63.0)	46.8 (31.5, 66.9)	0.71		35.5 (22.8, 59.2)	44.9 (27.9, 68.0)	0.002	
Total saturated fatty acids (g/day)	10.4 (6.5, 15.4)	12.4 (8.1, 16.3)	0.71		8.4 (4.7, 15.5)	10.8 (6.2, 17.1)	0.003	
Total polyunsaturated fatty acids (g/day)	13.8 (8.8, 19.6)	12.8 (8.7, 18.7)	0.32		11.8 (7.0, 19.5)	13.5 (8.2, 20.9)	0.29	
Total *n*-3 PUFAs (g/day)	0.4 (0.2, 0.6)	0.34 (0.2, 0.6)	0.58		0.3 (0.2, 0.5)	0.4 (0.2, 0.5)	0.09	
Total *n*-6 PUFAs (g/day)	13.5 (8.7, 19.1)	12.4 (9.2, 19.8)	0.39		12.1 (6.9, 18.9)	13.4 (8.1, 20.2)	0.27	
n-6/*n*-3 ratio	33.1 (24.4, 152.4)	34.2 (22.5, 53.1)	0.84		37.3 (27.57,49.73)	36.8 (26.1, 51.9)	0.76	
Total mono-unsaturated fatty acids (g/day)	11.2 (6.8, 16.4)	13.4 (7.2, 19.7)	0.48		8.5 (5.0, 16.4)	12.0 (6.4, 19.4)	< 0.0001	
Plasma phospholipid fatty acids (% total FAs)[c]								
Saturated fatty acids								
Myristic acid (C14:0)	0.25 (0.2, 0.3)	0.25 (0.2, 0.3)	0.75		0.26 (0.2, 0.3)	0.27 (0.2, 0.3)	0.21	
Palmitic acid (C16:0)	27.6 (25.2, 29.5)	26.3 (24.6, 28.0)	0.002	0.37	26.7 (24.8, 29.2)	25.7 (24.0, 27.6)	0.21	
Stearic acid (C18:0)	14.0 (12.4, 15.5)	15.1 (14.4, 16.2)	< 0.0001	0.31	14.8 (13.5, 16.3)	15.5 (14.3, 17.0)	0.0004	0.38
Arachidic acid (C20:0)	0.27 (0.21, 0.34)	0.34 (0.27, 0.39)	0.02	0.33	0.30 (0.25, 0.37)	0.36 (0.30, 0.43)	< 0.0001	0.35
Behenic acid (C22:0)	0.94 (0.66, 1.17)	1.22 (0.97, 1.43)	0.0006	0.32	1.03 (0.78, 1.35)	1.27 (1.07, 1.54)	< 0.0001	0.35
Lignoceric acid (C24:0)	1.01 (0.81, 1.23)	1.13 (0.91, 1.36)	0.02	0.40	1.00 (0.81, 1.24)	1.07 (0.9, 1.27)	0.03	0.45
Mono-unsaturated fatty acids								
Palmitoleic acid (C16:1*n*-7)	0.88 (0.5, 1.6)	0.6 (0.4, 0.8)	0.0007	0.34	0.8 (0.5, 1.2)	0.6 (0.4, 0.8)	0.003	0.40
Cis-vaccenic acid (C18:1*n*-7)	1.46 (1.26, 1.69)	1.19 (1.01, 1.44)	0.002	0.30	1.41 (1.20, 1.65)	1.30 (1.12, 1.5)	0.03	0.41
Oleic acid (C18:1*n*-9)	10.0 (7.6, 13.0)	7.5 (6.2, 9.3)	0.0002	0.30	8.2 (7.0, 11.3)	7.16 (6.3, 8.3)	< 0.0001	0.35
Elaidic acid (C18:1*n*9*t*)	0.31 (0.20, 0.56)	0.27 (0.20, 0.5)	0.32		0.34 (0.21, 0.81)	0.32 (0.21, 0.61)	0.50	
Gondoic acid (C20:1*n*-9)	0.11 (0.09, 0.12)	0.10 (0.08, 0.11)	0.04	0.36	0.11 (0.09, 0.12)	0.10 (0.09, 0.12)	0.02	0.42
Erucic acid (C22:1*n*-9)	0.06 (0.04, 0.08)	0.07 (0.04, 0.09)	0.29		0.06 (0.05, 0.09)	0.07 (0.05, 0.09)	0.50	
Nervonic acid (C24:1*n*-9)	1.66 (1.44, 1.95)	1.61 (1.35, 1.95)	0.75		1.69 (1.42, 2.02)	1.88 (1.55, 2.21)	0.005	0.41
n-3 polyunsaturated fatty acids								
α-linolenic acid (c18:3*n*-3)	0.09 (0.07, 0.12)	0.08 (0.05, 0.10)	0.18		0.08 (0.06, 0.1)	0.1 (0.05, 0.1)	0.77	
Eicosapentaenoic acid (C20:5*n*-3)	0.6 (0.5, 0.9)	0.6 (0.5, 0.9)	0.32		0.6 (0.4, 0.9)	0.6 (0.4, 0.8)	0.92	
Docosapentaenoic acid (C22:5*n*-3)	0.5 (0.4, 0.7)	0.6 (0.4, 0.8)	0.51		0.6 (0.5, 1.0)	0.5 (0.4, 0.8)	0.92	
Docosahexaenoic acid (C22:6*n*-3)	3.8 (3.0, 4.8)	4.29 (3.2, 5.6)	0.09		4.4 (3.5, 5.5)	5.0 (4.05, 5.8)	0.0008	0.39
n-6 and *n*-9 polyunsaturated fatty acids								
Linoleic acid (C18:2*n*-6)	16.1 (13.6, 18.5)	16.4 (14.1, 18.6)	0.71		15.9 (12.8, 18.4)	16.0 (13.8, 18.7)	0.50	
γ-linolenic acid (C18:3*n*-6)	0.13 (0.1, 0.2)	0.10 (0.07, 0.14)	0.04	0.39	0.1 (0.1, 0.2)	0.10 (0.07, 0.13)	0.07	
Eicosadienoic acid (C20:2*n*-6)	0.34 (0.29, 0.39)	0.35 (0.29, 0.4)	0.71		0.36 (0.31, 0.42)	0.38 (0.33, 0.44)	0.07	
Dihomo-γ-linolenic acid (C20:3*n*-6)	2.7 (2.4, 3.3)	3.1 (2.7, 3.9)	0.006	0.32	2.8 (2.4, 3.2)	3.1 (2.72, 3.61)	< 0.0001	0.35
Arachidonic (C20:4*n*-6)	12.9 (11.5, 14. 3)	14.1 (12.6, 15.7)	0.08		13.5 (12.0, 15.0)	14.2 (12.7, 15.6)	0.007	0.44
Docosadienoic acid (C22:2-*n*6)	0.018 (0.013, 0.023)	0.018 (0.014, 0.022)	0.75		0.02 (0.014, 0.027)	0.025 (0.018, 0.032)	< 0.0001	0.37
Adrenic acid (C22:4*n*-6)	0.6 (0.5, 0.7)	0.6 (0.5, 0.7)	0.51		0.6 (0.5, 0.7)	0.6 (0.5, 0.7)	0.29	
Osbond acid (C22:5*n*-6)	1.4 (1.2, 1.6)	1.4 (1.1, 1.7)	0.71		1.4 (1.2, 1.6)	1.4 (1.1, 1.6)	0.15	
Mead acid (C20:3*n*-9)	0.3 (0.2, 0.4)	0.2 (0.1, 0.3)	0.09		0.2 (0.1, 0.3)	0.17 (0.1, 0.3)	0.0003	0.41

[a]BMI, body mass index; PUFA, polyunsaturated fatty acids.
[b]Median (Q_1, Q_3) = differences between FAs across BMI and gender were tested by independent *t*-test according to BMI < 25 and BMI ≥ 25 kg/m². Data are presented as median; Q_1: lower interquartile range, Q_3; upper interquartile range.
[c]Plasma phospholipid fatty acids (% total FAs) = fatty acids are expressed as a percentage (%) of total FA. Significance levels of differences in parameters between men and women based on Mann–Whitney tests.

lean than in overweight men. Although significant differences were observed for these FAs between overweight and lean groups in men, most had a small effect size of approximately 0.30 to 0.40.

Plasma levels of similar SFAs, i.e. C18:0, C20:0, C22:0 and C24:0, as well as nervonic acid (C24:1*n*-9), docosahexaenoic acid (C22:6*n*-3), C20:3*n*-6, arachidonic acid (C20:4*n*-6) and docosadienoic acid (C22:2-*n*6) were higher in overweight women than in their lean counterparts. Similar MUFAs, i.e. C16:1*n*-7, C18:1*n*-7, C18:1*n*-9 and C20:1*n*-9, but also mead acid, were higher in lean than in overweight women. In women, small effect sizes (~ 0.35–0.44) were found for most FAs, except for C24:0, which had a medium effect size of 0.45.

Table 3. Factor loadings for dietary and plasma phospholipid fatty acids

	Dietary fatty acid patterns[a]	
Dietary fatty acids	**Non-marine (λ1 = 60%)[b]**	**Marine (λ2 = 29%)**
Saturated fatty acids		
Myristic acid (C14:0)	0.78	0.10
Palmitic acid (C16:0)	0.97	0.22
Stearic acid (C18:0)	0.94	0.21
Behenic acid (C22:0)	0.67	0.29
Mono-unsaturated fatty acids		
Palmitoleic acid (C16:1*n*-7)	0.89	0.28
Oleic acid (C18:1*n*-9)	0.95	0.23
n-3 fatty acids		
α-Linolenic acid (C18:3*n*-3)	0.89	0.22
Eicosapentaenoic acid (C20:5*n*-3)	0.21	0.96
Docosahexaenoic acid (C22:6*n*-3)	0.23	0.96
n-6 fatty acids		
Linoleic acid (C18:2*n*-6)	0.80	0.31
Arachidonic acid (C20:4*n*-6)	0.70	0.48
The Kaiser's measure of sampling adequacy = 0.0.84		

	Plasma phospholipid fatty acid patterns[c]					
Plasma phospholipid fatty acids	**High-Satfat (λ1 = 24%)**	***n*-3 VLC-PUFA (λ2 = 11%)**	**High-LA (λ3 = 11%)**	***n*-6 VLC-PUFA (λ4 = 10%)**	***n*-9 LC-MUFA (λ5 = 10%)**	***n*-3 EFA (λ6 = 7%)**
Saturated fatty acids						
Myristic acid (C14:0)	–0.13	–0.28	–0.06	–0.06	–0.56	0.01
Palmitic acid (C16:0)	–0.10	–0.54	–0.47	–0.40	–0.34	–0.05
Stearic acid (C18:0)	0.80	–0.21	–0.04	0.08	–0.04	–0.16
Arachidic acid (C20:0)	0.86	–0.08	0.01	–0.04	0.36	–0.08
Behenic acid (C22:0)	0.92	–0.03	0.06	–0.07	0.24	–0.07
Lignoceric acid (C24:0)	0.81	–0.19	–0.01	–0.06	0.34	0.10
Mono-unsaturated fatty acids						
Palmitoleic acid (C16:1*n*-7)	–0.84	–0.13	–0.25	0.08	–0.12	0.23
Cis-vaccenic acid (C18:1*n*-7)	–0.73	0.10	0.16	0.08	0.40	–0.20
Oleic acid (C18:1*n*-9)	–0.82	–0.15	–0.26	0.11	–0.01	0.35
Elaidic acid (C18:1*n*9t)	0.44	–0.58	–0.25	–0.04	0.09	–0.02
Gondoic acid (C20:1*n*-9)	–0.17	–0.07	0.52	–0.05	0.54	–0.13
Erucic acid (C22:1*n*-9)	0.35	–0.01	0.05	0.22	0.42	–0.05
Nervonic acid (C24:1*n*-9)	0.16	0.09	0.07	–0.01	0.85	–0.04
n-3 fatty acids						
α-linolenic acid (C18:3*n*-3)	–0.23	0.02	0.28	–0.15	–0.09	0.71
Eicosapentaenoic acid (C20:5*n*-3)	–0.37	0.57	–0.25	–0.32	–0.01	0.42
Docosapentaenoic acid (C22:5*n*-3)	–0.08	0.71	–0.01	0.12	0.15	0.27
Docosahexaenoic acid (C22:6*n*-3)	0.05	0.79	0.25	–0.08	0.08	–0.24
n-6 and n-9 fatty acids						
Linoleic acid (C18:2*n*-6)	0.08	0.31	0.80	–0.10	0.05	0.16
γ-linolenic acid (C18:3*n*-6)	–0.41	–0.08	–0.17	0.27	–0.37	0.49
Eicosadienoic acid (C20:2*n*-6)	0.09	0.06	0.82	0.30	0.15	0.13
Dihomo-γ-linolenic acid (C20:3*n*-6)	0.00	0.29	0.14	0.65	–0.06	0.30
Arachidonic acid (C20:4*n*-6)	0.04	0.66	0.12	0.42	0.24	–0.31
Docosadienoic acid (C22:2-*n*6)	0.44	0.08	0.47	–0.06	0.45	–0.24
Adrenic acid (C22:4*n*-6)	–0.13	0.02	0.03	0.83	0.08	0.01
Osbond acid (C22:5*n*-6)	–0.12	–0.04	–0.09	0.82	0.01	–0.32
Mead acid (C20:3*n*-9)	–0.58	–0.15	–0.53	0.32	0.05	0.28
The Kaiser's measure of sampling adequacy = 0.78						

[a]Fatty acid patterns derived from dietary fatty acids. [b]Variance explained by the single factor. [c]Fatty acid patterns derived from plasma phospholipids.
High-Satfat, saturated fatty acid pattern; *n*-3 VLC-PUFA, *n*-3 very-long-chain polyunsaturated fatty acid pattern; high-LA, high linoleic acid pattern; *n*-6 VLC-PUFA, *n*-6 very-long-chain polyunsaturated fatty acid pattern; *n*-9 LC-MUFA, *n*-9 long-chain mono-unsaturated fatty acid pattern; *n*-3 EFA, *n*-3 essential fatty acid pattern.

Table 4. Associations of dietary fatty acid patterns with adiposity and the MetS in 711 black South African adults in regression models

	Linear regression models						Logistic regression models	
	Body mass index		Waist circumference		Waist:height ratio		Metabolic syndrome	
Models	**β[a] (95% CI)[a]**	***p*-value**	**β (95% CI)**	***p*-value**	**β (95% CI)**	***p*-value**	**OR (95% CI)[b]**	***p*-value**
Crude model[c]								
Non-marine	0.05 (–0.02, 0.13)	0.15	0.07 (–0.004, 0.14)	0.07	0.06 (–0.02, 0.13)	0.14	1.13 (0.96, 1.32)	0.14
Marine	–0.015 (–0.09, 0.06)	0.69	0.007 (–0.07, 0.08)	0.85	0.004 (–0.07, 0.08)	0.92	0.99 (0.84, 1.16)	0.88
R^2 (%)		0.03		0.02		0.03		0. 43
Adjusted model$_1$[d]								
Non-marine	0.04 (–0.02, 0.11)	0.20	0.06 (–0.01, 0.13)	0.09	0.04 (–0.02, 0.11)	0.19	1.12 (0.95, 1.33)	0.19
Marine	–0.02 (–0.09, 0.05)	0.55	0.004 (–0.07, 0.08)	0.91	–0.0015 (–0.07, 0.07)	0.97	0.98 (0.82, 1.16)	0.78
R^2 (%)		20.48		6.35		17.25		17.69
Fully adjusted model[e]								
Non-marine	0.04 (–0.02, 0.11)	0.21	0.06 (–0.01, 0.13)	0.09	0.04 (–0.03, 0.11)	0.25	1.15 (0.96, 1.38)	0.12
Marine	–0.02 (–0.10, 0.04)	0.47	0.002 (–0.07, 0.07)	0.96	–0.005 (–0.07, 0.06)	0.88	0.94 (0.78, 1.14)	0.53
R^2 (%)		26.68		13.34		22.72		20.39

[a]Standardised betas and standardised 95% confidence intervals (CI). [b]OR, odds ratio and 95% CI. [c]Crude model; consisted of plasma phospholipid fatty acid patterns only. [d]Adjusted model$_1$; crude model and additionally adjusted for age and gender. [e]Fully adjusted model; adjusted model$_1$, additionally adjusted for lifestyle confounders (physical activity, self-reported smoking, total dietary energy and alcohol intake (Kcal) and level of education).

Table 5. Associations of plasma phospholipid fatty acid patterns with adiposity and the MetS in 711 black South African adults in regression models

	Linear regression models						Logistic regression models	
	Body mass index		Waist circumference		Waist:height ratio		Metabolic syndrome	
Models	**β[a] (95% CI)[a]**	***p*-value**	**β (95% CI)**	***p*-value**	**β (95% CI)**	***p*-value**	**Exponent of β (95% CI)**	***p*-value**
Crude model[b]								
High-Satfat	0.37 (0.31, 0.44)	< 0.0001	0.28 (0.21, 0.35)	< 0.0001	0.31 (0.24, 0.38)	< 0.0001	1.62 (1.34, 1.96)	< 0.0001
n-3 VLC-PUFA	0.21 (0.15, 0.28)	< 0.0001	0.22 (0.15, 0.28)	< 0.0001	0.22 (0.15, 0.28.)	< 0.0001	1.76 (1.44, 2.15)	< 0.0001
High-LA	0.04 (–0.02, 0.11)	0.20.	–0.05 (–0.12, 0.02)	0.16	–0.04 (–0.10, 0.03)	0.29	1.09 (0.92, 1.30)	0.33
n-6 VLC-PUFA	0.05 (–0.0, 0.12)	0.13	0.06 (–0.005, 0.13)	0.07	0.07 (0.001, 0.14)	0.045	1.28 (1.08, 1.53)	0.006
n-9 LC-MUFA	–0.05 (–0.11, 0.02)	0.17	–0.10 (–0.16, –0.03)	0.007	–0.09 (–0.16, –0.02)	0.009	0.63 (0.52, 0.75)	< 0.0001
n-3 EFA	–0.12 (–0.18, –0.05)	0.0007	–0.06 (–0.13, 0.006)	0.07	–0.09 (–0.16, –0.02)	0.009	0.81 (0.68, 0.96)	0.02
R^2 (%)		19.72		13.87		15.62		18.30
Adjusted model$_1$[d]								
High-Satfat	0.29 (0.22, 0.35)	< 0.0001	0.25 (0.18, 0.32)	< 0.0001	0.23 (0.16, 0.30)	< 0.0001	1.44 (1.17, 1.76)	0.0004
n-3 VLC-PUFA	0.18 (0.12, 0.24)	< 0.0001	0.20 (0.13, 0.27)	< 0.0001	0.18 (0.12, 0.24)	< 0.0001	1.70 (1.37, 2.10)	< 0.0001
High-LA	0.03 (–0.04, 0.09)	0.43	–0.04 (–0.11, 0.03)	0.26	–0.04 (–0.11, 0.02)	0.21	1.10 (0.89, 1.30)	0.43
n-6 VLC-PUFA	0.029 (–0.03, 0.09)	0.36	0.05 (–0.016, 0.12)	0.13	0.05 (–0.02, 0.11)	0.16	1.26 (1.04, 1.51)	0.02
n-9 LC-MUFA	–0.04 (–0.10, 0.02)	0.20	–0.10 (–0.16, –0.03)	0.005	–0.09 (–0.15, –0.02)	0.007	0.61 (0.5, 0.73)	< 0.0001
n-3 EFA	–0.06 (–0.13, 0.001)	0.05.	–0.060 (–0.13, 0.010)	0.09	–0.05 (–0.12, 0.011)	0.10	0.84 (0.69, 1.01)	0.07
R^2 (%)		30.78		16.73		25.76		29.74
Fully adjusted model[e]								
High-Satfat	0.27 (0.20, 0.34)	< 0.0001	0.22 (0.15, 0.30)	< 0.0001	0.20 (0.13, 0.27)	< 0.0001	1.54 (1.21, 1.95)	0.0004
n-3 VLC-PUFA	0.14 (0.08, 0.20)	< 0.0001	0.16 (0.087, 0.23)	< 0.0001	0.15 (0.08, 0.21)	< 0.0001	1.72 (1.38, 2.16)	< 0.0001
High-LA	–0.004 (–0.070, 0.06)	0.90	–0.06 (–0.13, 0.01)	0.11	–0.06 (–0.13, 0.01)	0.07	1.14 (0.93, 1.4)	0.22
n-6 VLC-PUFA	0.029 (–0.04, 0.09)	0.37	0.05 (–0.02, 0.12)	0.14	0.05 (–0.02, 0.11)	0.15	1.25 (1.02, 1.54)	0.03
n-9 LC-MUFA	0.002 (–0.06, 0.07)	0.957	–0.06 (–0.13, 0.02)	0.13	–0.05 (–0.11, 0.02)	0.17	0.61 (0.50, 0.75)	< 0.0001
n-3 EFA	–0.06 (–0.13, 0.005)	0.07	–0.06 (–0.13, 0.014)	0.12	–0.05 (–0.12, 0.02)	0.17	0.81 (0.66, 0.99)	0.04
R^2 (%)		33.69		20.38		28.38		31.09

[a]Standardised betas and standardised 95% confidence intervals (CI). [b]Crude model; consisted of plasma phospholipid fatty acid patterns only. [c]Adjusted model$_1$; crude model and additionally adjusted for age and gender. [d]Adjusted model$_2$; adjusted model$_1$, additionally adjusted for lifestyle confounders (physical activity, self-reported smoking, total dietary energy and alcohol intake (KJ) and level of education). [e]Fully adjusted model; adjusted model$_1$, additionally adjusted for contraceptive use.High-Satfat, saturated fatty acid pattern; *n*-3 VLC-PUFA, *n*-3 very-long-chain polyunsaturated fatty acid pattern; high-LA, high linoleic acid pattern; *n*-6 VLC-PUFA, *n*-6 very-long-chain polyunsaturated fatty acid pattern; *n*-9 LC-MUFA, long-chain mono-unsaturated fatty acid pattern; *n*-3 EFA, *n*-3 essential fatty acid pattern.

The factor analysis identified two dietary FA and six plasma phospholipid FA patterns according to the Kaiser criterion and scree-plot visual inspection. Results are shown in Table 3. Eleven dietary FAs and 26 phospholipid FAs were entered into the analysis. The factors generated explained 89% of the cumulative variance in dietary FA patterns and 73% in plasma phospholipid FA patterns. The Kaiser's measure of sampling adequacy was 0.84 and 0.78 for the dietary FA and plasma phospholipid FA patterns, respectively. Loadings with absolute values higher than 0.5 were considered relevant for the contribution to each FA pattern. The patterns are characterised and named according to the highest loadings of the specific FAs present in a given pattern.

Among dietary FAs, the first extracted pattern presented with high positive loadings of saturated FAs, MUFAs, α-linolenic acid (C18:3*n*-3) and *n*-6 FAs, and therefore was named the 'no*n*-marine' FA pattern. The second pattern was named the 'marine' FA pattern because it was characterised by high positive loadings of eicosapentaenoic acid (C20:5*n*-3) and C22:6*n*-3.

The six plasma phospholipid FA patterns are discussed in the order in which they were derived. The first pattern presented with positive loadings of LC-SFAs, C18:0, C20:0, C22:0 and C24:0 and very high negative loadings of C16:1*n*-7, C18:1*n*-7 and C18:1*n*-9; we named it the 'high-Satfat' pattern. The second pattern was named '*n*-3 VLC-PUFA' and presented with high positive loadings of docosapentaenoic acid (C22:5*n*-3), C22:6*n*-3 and C20:5*n*-3, as well as C20:4*n*-6. The third pattern presented the highest positive loadings of C18:2*n*-6 and eicosadienoic acid (C20:2*n*-6) and was named accordingly as the 'high-LA' pattern. The fourth pattern was named '*n*-6 VLC-PUFA' since it was characterised with high positive loadings of adrenic acid (C22:4*n*-6), C22:2-*n*6 and C20:3*n*-6. The fifth pattern extracted was named the '*n*-9 LC-MUFA' pattern and presented with positive loadings of C24:1*n*-9 and gondoic acid (C20:1*n*-9). The sixth and last pattern had a positive loading of one FA, i.e. C18:3*n*-3, and we named it '*n*-3 EFA' pattern.

Dietary FA patterns were weakly associated with measured outcomes (Table 4). The no*n*-marine FA pattern showed marginal positive associations with WC in the crude model and the association remained marginal after adjusting for age and gender (β = 0.06, 95% CI = –0.01–0.13, $p = 0.09$). The association was lost after adjustment for lifestyle variables and energy intake. On the other hand, we did not find any associations with the marine FA pattern (Table 4). Neither pattern revealed any association with BMI, WHtR or the MetS. Further adjustment to the regressions for total fat, fibre, carbohydrates and added sugar did not result in any significant associations. The variables in the adjusted models explained 0.02 to 27% of the variation in measures of adiposity and 0.4 to 20% of the variation in the MetS.

Plasma phospholipid FA patterns resulted in stronger associations with measures of adiposity and the MetS (Table 5). The high-Satfat and *n*-3 VLC-PUFA patterns were positively associated with all measures of adiposity and the MetS. The associations remained significant in the fully adjusted model. The omega-6 VLC-PUFA pattern showed marginal and positive associations with WC and WHtR in the crude model, but associations were lost after further adjustments. This pattern also showed higher odds for having the MetS and remained significantly associated in the fully adjusted model (odds ratio, OR = 1.25, 95% CI = 1.02–1.54, $p = 0.03$).

The *n*-9 LC-MUFA pattern was inversely associated with WC and WHtR in the crude model as well as after adjustment for age and gender. The associations were, however, lost after adjustments for lifestyle variables and energy intake. This pattern also showed lower odds for having the MetS and remained significantly associated in the fully adjusted model (OR = 0.61, 95% CI = 0.50–0.75, $p \leq 0.0001$).

The omega-3 EFA pattern showed an inverse association with BMI, WC and WHtR, but in the fully adjusted model marginal significance remained for BMI only. This pattern also showed lower odds for having the MetS and remained significantly associated in the fully adjusted model (OR = 0.81, 95% CI = 0.66–0.99, $p = 0.04$). The variables in all the adjusted models explained 14 to 34% of the variation in measures of adiposity, and 18 to 31% of the variation in the MetS.

We further adjusted all regression models for use of contraceptives and intakes of total fat, fibre, carbohydrates, and energy from added sugar in association with plasma phospholipid FAs. Additional adjustment for these variables did not result in different associations with anthropometric indices. The association between high-LA pattern and the MetS remained marginally significant after adjusting for additional variables, whereas the associations with the *n*-6 VLC-PUFA and *n*-3 EFA patterns were lost.

Discussion

The results of this study add new information about identified FA patterns both in diet and plasma phospholipids among a selected group of black South Africans from the North West Province. We identified for the first time two dietary FA patterns and six plasma phospholipid FA patterns (Table 3) by means of factor analysis in this group of black adults. The dietary no*n*-marine FA pattern showed a weak positive association with WC, whereas the marine pattern did not show any associations with outcomes measured.

On the other hand, two plasma phospholipid FA patterns (high-Satfat and *n*-3 VLC-PUFA) were positively associated with all measures of adiposity and the MetS. The omega-6 VLC-PUFA pattern showed a positive association with the MetS, but not with measures of adiposity. The *n*-9 LC-MUFA and the *n*-3 EFA patterns showed an inverse association with the MetS in fully adjusted models and tended to be negatively associated with some measures of adiposity. The high-LA pattern was neither associated with measures of adiposity nor the MetS. Our findings indicate that dietary FA patterns were weakly associated, whereas plasma phospholipid FA patterns were more strongly associated with measures of adiposity and the MetS.

Previous studies have reported FA patterns, derived from different components of blood and tissue in association with obesity[29] and the MetS,[22,30] but not with dietary patterns. These patterns were generated by varying numbers of FAs ranging from nine to 34 FAs,[22,29,30] and some included estimated desaturase activities,[30] by means of use of factor[29,30] and cluster[22] analysis. Consequently, these derived patterns differed from that obtained in our study.

A dietary pattern, consisting of SFAs, PUFAs, MUFAs and other nutrients, was not associated with obesity among Iranian adults.[45] On the contrary, a multiracial study in the USA reported a positive association of intakes of total fat, total saturated fat, LC-SFAs, myristic acid (C14:0), C16:0 and C18:0, and MUFAs with BMI.[46] Furthermore, a study investigating the association of dietary patterns with the MetS concluded that a pattern high in meat products was associated with a higher prevalence of the MetS.[47]

In our study, the dietary no*n*-marine FA pattern showed marginal and positive associations with WC, but not with other measures of adiposity or the MetS. The no*n*-marine FA pattern had positive loadings of FAs from SFAs, MUFAs and PUFAs, specifically from two SFAs (C16:0 and C18:0), two MUFAs (C16:1*n*-7, C18:1*n*-9) and two PUFAs (C18:2*n*-6 and C18:3*n*-3). The dietary marine FA pattern showed no association with outcomes measured.

Our results are in agreement with a study in the USA that also found no associations of *n*-3 LC-PUFAs with BMI due to low intakes of these FAs in their participants.46 In our study and the study in the USA, lower intakes of *n*-3 PUFA compared to the FAO/WHO recommendation of 0.25–2 g/day were found.[48] Under-reporting of dietary intake may significantly influence nutrient pattern investigation and association with disease,[49] however, in the PURE

study, over- and under-reporters of dietary intake (subjects with reported energy intakes ≥ 30 000 or ≤ 3 000 KJ) were excluded prior to analyses.[50] Apart from the marine FA pattern, we did not derive other clear dietary FA patterns, likely due to the homogenous nature of food intake in this group of adults. Therefore, factor analysis may not be the most appropriate method to investigate dietary FAs in this population and the associations observed should be interpreted with caution.

The first plasma phospholipid FA pattern, high-Satfat, was positively associated with all measures of adiposity and the MetS. This pattern had high positive loadings of SFAs C18:0, C20:0, C22:0 and C24:0, as well as negative loadings of MUFAs. In our study, the plasma phospholipid levels of these saturated FAs were also higher in overweight men and women compared to their leaner counterparts, although effect sizes tended to be small.

Plasma phospholipid VLC-SFAs, such as C20:0, C22:0 and C24:0 have previously been reported to be inversely associated with the MetS among adults in Taiwan.[15] In a study in Japan, serum VLC-SFAs were also inversely associated with the MetS and positively associated with HDL-C.[16] The authors concluded that these VLC-SFAs may be indicative of healthier metabolic health.[15,16] Li and colleagues[22] derived a cluster that consisted of the same VLC-SFAs mentioned above. This cluster was also associated with the healthier metabolic profile,[22] but was not identical to the high-Satfat pattern identified in this current study, as it did not have negative loadings of MUFAs.

High intakes of MUFAs are generally considered the driving force behind the protective effect of the Mediterranean diet on cardiovascular diseases.[51] The combined presence of high loadings of some SFAs, particularly C18:0 and low loadings on MUFAs may therefore explain the association with obesity and the MetS found in our study. Plasma C18:0 levels were higher and plasma C18:1*n*-9 levels were lower in the overweight/obese groups than among their leaner counterparts in the current study, and the same FAs had positive and negative loadings, respectively, in the high-Satfat pattern. These two FAs made up a considerable proportion of the FAs in the plasma phospholipid profile and may be the driving force behind the positive association of the high-Satfat pattern with all measures of adiposity and the MetS in the current study.

The second pattern, *n*-3 VLC-PUFA, had high positive loadings of C20:5*n*-3, C22:5*n*-3 and C22:6*n*-3, as well as the *n*-6 PUFA arachidonic acid (C20:4*n*-6). This pattern was positively associated with all measures of adiposity and the MetS. In line with our findings, an *n*-3 FA pattern (with positive loading of C20:5*n*-3, and estimated delta 5 desaturase activity and negative loading of C20:3*n*-6) in the study by Warensjo *et al*.[30] predicted the development of the MetS in Swedish men, independent of lifestyle factors. The main difference between our study and that of Warensjo and colleagues[30] is that they included estimated desaturase activity in their patterns and measured FAs in serum.

Omega-3 PUFAs, especially C22:6*n*-3 and C20:5*n*-3, have multiple beneficial effects and are generally inversely associated with obesity and related risk factors, as detailed in a recent review.[52] Other studies have also reported the inverse association of circulating *n*-3 PUFAs with measures of adiposity and the MetS.[20,21] It should be kept in mind that the PURE-SA study population reported very low intakes of *n*-3 FAs; however, despite these low intakes, their plasma levels were considered sufficient.[43] Continuous low intake of *n*-3 LC-PUFAs, as reported in the present study, can result in up-regulation of the endogenous synthesis of *n*-3 LC-PUFAs from C18:3*n*-3. The possibility therefore exists that this upregulated conversion is a response to the cardiovascular risk milieu, reflecting reverse causality, rather than being the other way around. Further research is needed to elucidate the endogenous conversion of dietary *n*-3 PUFAs in black African populations.

It is also possible that the positive association of this pattern with adiposity and the MetS could have been driven by the C20:4*n*-6, which formed part of this pattern. Omega-3 and *n*-6 FAs compete for incorporation into target tissues and metabolism by common enzymes, which may lead to opposing health effects.[53] The eicosanoid metabolic products from C20:4*n*-6 promote inflammatory responses. There is some evidence that a higher ratio of *n*-6 PUFAs to *n*-3 PUFAs is associated with a higher prevalence of obesity and the MetS.[54]

The fourth plasma phospholipid pattern, *n*-6 VLC-PUFA, had positive loadings of *n*-6 VLC-PUFAS, C20:3*n*-6, C22:4*n*-6 and osbond acid (C22:5*n*-6) and was positively associated with the MetS. Mayneris-Perxachs *et al*.18 also reported a positive association between plasma phospholipid C20:3*n*-6 PUFAs and the MetS among older adults in Spain. Higher concentrations of plasma phospholipid C20:3*n*-6 were observed in both overweight men and women compared to their leaner counterparts in our study, but the *n*-6 VLC-PUFA pattern was not associated with BMI in the fully adjusted model. Plasma phospholipid levels of C20:3*n*-6 were also positively associated with BMI in participants from the USA and Mexico.[13,17] There was, however, also a longitudinal study that found higher total circulating *n*-6 PUFAs, in particular linoleic acid and arachidonic acid, to be protective of risk factors for the MetS, including both systolic and diastolic BP and plasma triglycerides in men,[24] indicating that different *n*-6 FAs showed opposite associations with the MetS. The association of C20:3*n*-6 with the MetS requires further investigation.[18]

The fifth pattern, *n*-9 LC-MUFA, loaded positively with C20:1*n*-9 and C24:1*n*-9, and negatively with myristic acid (C14:0). This pattern showed an inverse association with WC and WHtR, but lost association when adjusted for lifestyle variables and energy intake. However, lower odds for having the MetS remained after adjustment for covariates. In our study, levels of C20:1*n*-9 were significantly higher in lean men and women compared to their overweight counterparts, whereas C24:1*n*-9 was higher in overweight compared to lean women only. Nervonic acid (C24:1*n*-9) and C20:1*n*-9 are both products of endogenous metabolism by elongation from oleic acid,55 but plasma C24:1*n*-9 may also be related to fish intake.[56] Since fish consumption was very low in our study population, this pattern could therefore reflect an upregulated metabolism of oleic acid in our lean study participants.

The sixth pattern, *n*-3 EFA, was positively loaded with C18:3*n*-3 and tended to be inversely associated with all measures of adiposity and showed lower odds for the MetS. This is in agreement with a study that found C18:3*n*-3 in serum cholesteryl esters to be inversely associated with abdominal obesity in a recent cross-sectional study of 60-year-old men and women.[57] Alphalinolenic acid (C18:3*n*-3) is an essential FA and a precursor from which *n*-3 LC-PUFAs are synthesised. Increased consumption of C18:3*n*-3-rich foods elevates its tissue levels as well as levels of C22:6*n*-3 and C20:5*n*-3 in the liver lipids.[58] Alpha-linolenic acid can be beneficial to health. Firstly, C18:3*n*-3 intake was associated with a moderately lower risk of cardiovascular disease in randomised, controlled studies as outlined in reviews.[59,60] Secondly, as explained above, C18:3*n*-3 competes for the same metabolic enzymes, as does C18:2*n*-6, and increased

dietary intake may be a worthy approach to decrease elongation of *n*-6 FAs leading to reduced plasma C20:4*n*-6 levels and increased plasma levels of C22:6*n*-3 and C20:5*n*-3.[58] As C18:3*n*-3 is an essential FA, this pattern, identified in our study participants, is probably related to food intake and therefore indicative of a higher intake of vegetable oils, legumes, nuts and seeds.[61]

Strengths and limitations

A rigorous methodological approach of sequential regression modelling enabled us to investigate the associations between dietary FA and plasma phospholipid FA patterns, respectively, and measures of adiposity and the MetS. Another strength of our study is the use of both dietary FA and plasma phospholipid FA patterns,[27] which is a preferred method to investigate the association between diet and diseases.[27]

Our work is not free of limitations. Firstly, inaccuracies associated with collecting dietary intake data may have influenced the dietary FA results; however, in our population, fieldworkers collecting dietary data were intensively trained and supervised, and both under- and over-reporters of dietary intake were excluded.[50] In addition, repeatability of the QFFQ was also demonstrated.[10] Secondly, the cross-sectional design does not account for possible reverse causation between measures of adiposity and dietary FA intake or plasma phospholipid FA concentration, nor can causality be inferred. Thirdly, a possible limitation of the study is incomplete information on FA composition in the food composition databases. This limitation was compensated for by our study design that also considered plasma phospholipid FAs. Fourthly, we assessed the associations with indirect measurements of adiposity, including BMI, WHtR and WC, as secondary markers of total and central adiposity, whereas imaging methods would better differentiate between lean and fat mass.

Conclusion

To our knowledge, this is the first study to investigate and document novel data on dietary FA and plasma phospholipid FA patterns and their association with measures of adiposity and the MetS in a selected group of black South African adults. This study presents evidence that although marginal association was found with dietary FA patterns, some circulating plasma phospholipid FA patterns were more strongly and significantly associated with BMI, WC, WHtR and the MetS. The high-Satfat and *n*-3 VLC-PUFA patterns were positively associated with adiposity and the MetS, whereas the *n*-9 LC-MUFA and *n*-3 EFA patterns were inversely associated with adiposity. These patterns may suggest possible differences in FA metabolism between lean and overweight/obese individuals. It should also be considered that, in a study population with low-fat intakes, such as the PURE participants, plasma FA levels may reflect endogenous FA generation rather than dietary intakes, which could result in different findings than those reported in other studies from affluent communities.

Our results are not sufficiently conclusive to make recommendations on dietary FA intakes in this population. Further prospective cohort studies that explain possible differences in characteristics of FA metabolism among black South African men and women are needed. More studies that apply the use of dietary FA and plasma or tissue FA patterns are required to determine whether the results from the current study can be generalised to the black population of African descent.

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Ellisras Longitudinal Study 2017: association of hypertension with increasing levels of adiposity in 10- to 14-year-old boys and girls in the Eastern Cape (ELS 31)

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Abstract

Objectives: **Previous studies suggest a strong relationship between obesity and hypertension. This study aimed at evaluating the prevalence of hypertension and pre-hypertension in 10- to 14-year-old boys and girls in the Eastern Cape Province of South Africa and to determine the association between blood pressure parameters and selected measures of adiposity.**

Methods: **A cross-sectional, school-based study of 540 10- to 14-year-old children from seven schools in the Eastern Cape Province was carried out. Anthropometry and blood pressure parameters were determined.**

Results: **All measures of adiposity and blood pressure were significantly higher in the girls ($p < 0.05$). The prevalence of hypertension and pre-hypertension was over 20 and 12%, respectively. Systolic blood pressure and pulse pressure were associated ($r > 0.27$; $p < 0.05$) with increasing levels of adiposity.**

Conclusion: **This study highlights the importance of weight-control strategies for the prevention of hypertension in these adolescents and later on in life.**

Keywords: adolescent, school, hypertension, pre-hypertension, adiposity, obesity

An increasing number of studies are reporting hypertension and pre-hypertension in the paediatric population.[1-3] Once considered rare or secondary only to known causes, essential hypertension is now a reality among children.[4] Lifestyle risk factors for hypertension are generally very subtle to find since most children in this phase of life do not smoke or drink and are mostly active.

Hypertension in children has been associated with family history and low birth weight.[5] However, as in the adult population, the prevalence of obesity and overweight have reached pandemic levels in children in rural and urban communities in developing and industrialised countries.[6] Consequently, complications of overweight and obesity such as hypertension and diabetes have also become commonplace in children.[7]

Indeed, studies in the USA suggest that blood pressure increases correlate with body mass index in children and adolescents.[8] Kemp et al.[9] showed an eight and 20% prevalence of pre-hypertension and hypertension, respectively, in grade 1 children in a rural South African community. Furthermore, we previously showed that the prevalences of pre-hypertension and hypertension in adolescents in Mthatha were 13.6 and 22% in males and 16.5 and 20.9% in females, respectively.[3]

Although studies have demonstrated hypertension in children, it remains under-diagnosed or not diagnosed at all since blood pressure measurement is not routine in paediatric patients. Importantly, adult criteria for the diagnosis of hypertension are often applied to children and adolescents. Consensus guidelines for defining pre-hypertension and hypertension in children require the systolic and diastolic blood pressure values to be converted to percentiles for age, gender and height,[10] which is often a challenge for the already overworked physicians in developing countries. This has therefore led to the perception that children do not suffer from hypertension, with the consequent under-diagnosis of the problem.[11] Nevertheless hypertension in childhood had been shown to track to adulthood, when it progresses to established hypertension.[12]

Overweight and obesity in childhood are risk factors for hypertension in children.[13] Overweight and obesity, as expressed by various measurements of adiposity, have shown relationships with hypertension. In this study we explored the impact of increasing adiposity on blood pressure and consequently hypertension.

Methods

A cross-sectional study was carried out in seven selected middle schools in the Eastern Cape Province, South Africa, from May to September 2016. Data were collected once during this period from participating children. Ten- to 14-year-old boys and girls were recruited into the study in order to determine gender differences on adiposity and blood pressure.

Ethical clearance was obtained from the University of Fort Hare (CH1011SCHU01) and consent was obtained from parents and children involved in the study and from the schools' authorities. All consenting children who were not pregnant or lactating or suffering from any debilitating condition were included in the study. Data were collected on the school premises where the children were comfortable and had a sense of security. Boys and girls were required to rest in a seated position for 10 minutes, after which their right upper arms were fitted with appropriate arm-size cuffs and blood pressure was measured at three-minute intervals

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using the Omron (Hem 7120) automated blood pressure machine. The mean of three recordings of systolic (SBP) and diastolic blood pressure (DBP) and heart rate (HR) were computed. SBP and DBP were converted to percentiles for age, gender and height for each child, based on the Paediatric Task Force standards.[14]

Both waist (WC) and hip circumference (HC) were measured using the World Health Organisation guidelines.[15] Participating children were requested to stand upright with feet together and arms hanging freely at the sides. WC was measured at the smallest circumference of the waistline with a non-stretch tape. Boys and girls were requested to dress lightly on days of data collection. HC was measured at the largest circumference around the greater trochanter of the femur.[15]

Height was measured using a stadiometer. Boys and girls were requested to take off their shoes and to step on the stadiometer platform with feet together and close to the stadiometer rod. The movable bar was lowered to just touch the head. Height was read off to the nearest cm.

Personal data such as height, age and gender were entered into the Omron body composition monitor (BF511). Then each child was requested to step onto the electrode pads of the body composition monitor and hold the arm piece tightly in both hands, with arms held out at right angles to the body, until the equipment stopped scanning. The equipment displayed weight, body mass index (BMI) and total fat mass (TFM). BMI was converted to percentiles for age and gender.

Statistical analysis

Data were analysed using Stata version 14. Data were checked for normality, and differences between the means of normally distributed data were assessed using the *t*-test or ANOVA with Dunnet's test, while the Kruskal–Wallis test with Friedman's post hoc test was used for skewed data. Spearman's correlation coefficient (*r*) was used to determine the relationships between blood pressure parameters and selected measures of adiposity.

Adiposity was categorised as lean with BMI < 85th percentile or ≤ 75th percentile for WC, HC or TFM for gender, and overweight/obesity as BMI ≥ 85th percentile or > 75th percentile of WC, HC and TFM for gender. Fisher's exact test was used to determine the relative risk for hypertension associated with overweight/obesity as determined for the four selected measures of adiposity. Statistical significance was set at $p \leq 0.05$.

Table 1. Characteristics of the learners by gender

Characteristics	Boys	Girls
Number	250	290
Age (years)	11.9 ± 0.6	11.9 ± 0.5
BMI (kg/m²)	18.9 ± 0.2	20.2 ± 0.3*
WC (cm)	65.4 ± 0.7	69.2 ± 0.7**
TFM (%)	22.5 ± 0.01	24.1 ± 0.01**
HC (cm)	80.1 ± 0.6	85.6 ± 0.7**
WHtR	0.44 ± 0.01	0.46 ± 0.00**
SBP (mm Hg)	110.1 ± 0.7	112.7 ± 0.6*
DBP (mm Hg)	70.6 ± 0.5	73.1 ± 0.4*
PP (mm Hg)	39.5 ± 0.5	39.5 ± 0.0.4

Calculated percentages were cohort specific.
BMI: body mass index, WC: waist circumference, TFM: total fat mass, HC: hip circumference; WHtR: waist-to-height ratio, SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure. *$p < 0.05$, **$p < 0.01$.

Results

A total of 540 10- to 14-year-old boys and girls were recruited into this study. Male and female participants were of similar ages. Females had significantly ($p < 0.05$) higher BMI, WC, HC, TFM, waist-to-height ratio (WHtR), SBP and DBP (Table 1). On the other hand pulse pressure (PP) was similar for males and females.

The prevalence of overweight was 10.9% in the total cohort and was higher in the girls (13.5%) compared to boys (8.0%). The prevalence of obesity was 14.0% in the total cohort, 12.8% in the boys and 15.2% in the girls (Table 2). Similarly, the prevalence of pre-hypertension and hypertension were higher in girls compared to boys.

In order to better understand the relationship between blood pressure and measures of adiposity (BMI, WC, TFM, WHtR), Spearman's rank correlations were performed. Pairwise correlations between SBP, DBP, PP, BMI, WC, TFM and WHtR were positive in both boys and girls. BMI, WC, TFM and WHtR correlated modestly with SBP and PP in females (Table 3). Only BMI had a weak correlation with SBP and PP in males. On the other hand there was no correlation between DBP and all measures of adiposity in boys or girls.

In order to determine the effect of selected measures of adiposity on blood pressure values, boys and girls were classified according to their adiposity (BMI, WC, TFM, WHtR) quartiles. SBP, DB and PP [PP = (SBP – DBP)] for each quartile were computed and the prevalence of hypertension and pre-hypertension in each quartile was determined. SBP, DBP and PP increased progressively from the first quartile (lowest adiposity) to the fourth quartile (highest adiposity). The prevalence of hypertension and pre-hypertension were highest in the fourth quartile for all measures of adiposity. The first quartiles for all measures of adiposity had

Table 2. Prevalence of overweight, obesity, pre-hypertension and hypertension

Variables	Overweight	Obesity	Pre-hypertension	Hypertension
Total cohort, *n* (%)	59 (10.9)	76 (14.0)	66 (12.2)	112 (20.7)
Boys, *n* (%)	20 (8.0)	32 (12.8)	28 (11.2)	39 (15.6)
Girls, *n* (%)	39 (13.5)	44 (15.2)	45 (15.5)	76 (26.2)

Table 3. Spearman's rank correlation coefficients between blood pressure parameters and selected measures of adiposity

	Spearman's rank correlation coefficients			
Variables	BMI (kg/m²)	WC (cm)	TFM (%)	WHtR
SBP (mmHg)				
Boys	0.24*	0.12	0.10	0.11
Girls	0.39*	0.37*	0.33*	0.27*
DBP (mmHg)				
Boys	0.07	0.09	0.07	0.09
Girls	0.08	0.07	0.08	0.06
PP (mmHg)				
Boys	0.22	0.05	0.05	0.05
Girls	0.41*	0.38*	0.35*	0.32*

BMI: body mass index, WC: waist circumference, TFM: total fat mass, WHtR: waist-to-height ratio, SBP: systolic blood pressure; DBP: diastolic blood pressure, PP: pulse pressure. *$p < 0.05$.

Table 4. Components of blood pressure in the four quartiles of BMI, WC, TFM and WHtR

Parameters	1st quartile	2nd quartile	3rd quartile	4th quartile	*p*-value
BMI					
SBP (mmHg)	107.1 ± 1.5	110.4 ± 0.5	113.9 ± 1.0**	117.3 ± 1.1**##	0.0001
DBP (mmHg)	69.1 ± 1.1	71.8 ± 0.4	73.4 ± 0.8*	73.3 ± 0.9*	0.03
HR (beats/min)	87.9 ± 1.9	87.3 ± 0.7	84.9 ± 1.6	89.3 ± 1.3	0.14
PP (mmHg)	38.1 ± 1.3	38.7 ± 0.4	40.4 ± 0.9##	44.1 ± 0.9##	0.0001
HT, *n* (%)	3 (8.8)	59 (16.1)	14 (23.7)	13 (16.5)	
preHT, *n* (%)	9 (26.5)	125 (34.2)	23 (39.0)	34 (44.7)	
TFM					
SBP (mmHg)	108.8 ± 0.9	110.2 ± 0.9	112.2 ± 0.9**	114.9 ± 0.8**##	0.0001
DBP (mmHg)	70.6 ± 0.7	71.7 ± 0.7	72.4 ± 0.9	73.0 ± 0.6	0.14
HR (beats/min)	87.5 ± 1.1	86.1 ± 1.1	88.5 ± 1.2	87.7 ± 1.0	0.50
PP (mmHg)	38.2 ± 0.7	38.5 ± 0.7	39.8 ± 0.7	42.0 ± 0.7**##	0.0001
HT, *n* (%)	17 (12.8)	20 (14.8)	23 (11.8)	28 (20.1)	
preHT, *n* (%)	37 (27.8)	46 (34.1)	48 (24.6)	58 (43.6)	
WC					
SBP (mmHg)	108.1 ± 0.8	109.8 ± 0.9	112.3 ± 1.1**	115.8 ± 0.7**##	0.0001
DBP (mmHg)	70.9 ± 0.6	71.2 ± 0.7	71.7 ± 0.7*	73.7 ± 0.6*	0.05
HR (beats/min)	86.8 ± 1.1	87.6 ± 1.1	87.1 ± 1.2	89.3 ± 1.3	0.78
PP (mmHg)	37.2 ± 0.6	38.6 ± 0.7	40.7 ± 0.9##	42.1 ± 0.6##	0.001
HT, *n* (%)	25 (16.7)	27 (18.9)	19 (20.4)	46 (29.7)	
preHT, *n* (%)	49 (32.9)	44 (30.8)	31 (33.3)	69 (44.5)	
WHtR					
SBP (mmHg)	109.0 ± 0.9	111.1 ± 0.9	110.6 ± 0.9	115.4 ± 0.8***##$$$	0.0001
DBP (mmHg)	71.0 ± 0.7	72.3 ± 0.7	71.0 ± 0.6	73.3 ± 0.6*$	0.05
HR (beats/min)	86.9 ± 1.1	87.1 ± 1.2	88.1 ± 1.1	87.3 ± 1.0	0.75
PP (mmHg)	37.9 ± 0.6	38.8 ± 0.7	39.6 ± 0.7	42.1±0.7***##$	0.0001
HT, *n* (%)	19 (14.1)	26 (19.3)	28 (20.6)	40 (30.1)	
preHT, *n* (%)	37 (27.4)	54 (40)	42 (30.9)	59 (44.4)	

BMI: body mass index, WC: waist circumference, TFM: total fat mass, WHtR: waist-to-height ratio, HT: hypertension; preHT: pre-hypertension, SBP: systolic blood pressure; DBP: diastolic blood pressure, HR: heart rate, PP: pulse pressure.
Compared to first quartile ($p < 0.05$, **$p < 0.01$, ***$p < 0.001$), #compared to second quartile (#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$) and $comparing quartile 4 to quartile 3 ($p < 0.05$, $$$p < 0.01$; $$$$p < 0.001$).

the lowest levels of SBP, DBP, HR and PP. It also had the lowest prevalence of hypertension and pre-hypertension (Table 4).

Boys and girls were separated into lean and overweight/obese groups using selected measures of adiposity. BMI < 85th percentile was classified as lean and BMI ≥ 85th percentile as overweight/obese. WC, TFM and WHtR were separated into two groups: ≤ 75th percentile for gender was classified as lean while > 75th percentile was classified as overweight/obese. A greater WC conferred a 1.7-times greater risk of developing hypertension ($p = 0.008$) in the cohort. The relative risk of having hypertension conferred by high BMI, WC, WHtR and TFM was absent in boys but weak and not significant in girls (Table 5).

Discussion

In this study we showed that the prevalence of overweight and obesity in 10- to 14-year-old children in the Eastern Cape was over 10 and 14%, respectively, while the prevalence of pre-hypertension and hypertension were 12 and 20%, respectively. Gender-specific analysis showed that the girls were more obese and also had a higher prevalence of hypertension and pre-hypertension. Although the relative risk of having hypertension with increasing adiposity was small, children whose BMI, WC, TFM and WHtR were higher than the third quartile had significantly ($p < 0.05$) higher blood pressure than those in the lower quartiles.

Using all four selected measures of adiposity (BMI, WC, TFM and WHtR) our study showed that girls were larger and had a higher prevalence of overweight and obesity. Participants in this study were 10 to 14 years old, which is a period of much hormonal activity. Puberty begins in girls from eight to 12 years old, while in boys it begins from nine to 14 years old. This period in girls

Table 5. Relative risk of having hypertension with high measures of adiposity

Variables	Relative risk		
	Cohort	Males	Females
BMI	1.04	1.05	1.28
95% CI	0.544–1.975	0.652–1.716	0.974–1.675
p-value	0.86	0.862	0.090
WC	1.71	1.203	1.328
95% CI	1.284–2.279	0.774–1.870	1.003–1.758
p-value	0.0008	0.418	0.06
TFM	1.42	0.859	1.384
95% CI	0.891–2.00	0.539–1.370	0.877–1.558
p-value	0.183	0.542	0.189
WHtR	1.27	1.245	1.26
95% CI	0.766–2.119	0.790–1.961	0.956–1.671
p-value	0.351	0.385	0.133

BMI: body mass index, WC: waist circumference, TFM: total fat mass, WHtR: waist-to-height ratio, CI: confidence interval.

corresponds with an increase in BMI, and changes in body fat composition and distribution, while in boys it is a period of fat loss and muscle development,[16] thus explaining the differences in BMI, WC, TFM and WHtR between boys and girls.

Several studies have shown an association between BMI and blood pressure.[17,18] A Brazilian study showed that overweight and obese children had a 3.6-fold greater risk of having higher SBP and 2.7-times increased risk for higher DBP.[19] Both SBP and DBP as well as PP were higher in the girls than boys. The growth spurt of puberty, which is often accompanied by a rise in blood pressure,[20] occurs earlier in girls than in boys, thus explaining the higher blood pressure in girls.

The current study showed a difference in the prevalence of hypertension and pre-hypertension when overweight/obesity was classified as the fourth quartile of WC, TFM and WHtR. An increased WC is a known risk for metabolic diseases in both children[21] and adults. We have previously shown that BMI, TFM, WC and WHtR correlated similarly with SBP and PP in females, although these relationships were different in males. These results further strengthen the suggestion that the 10- to 14-year-old girls involved in the study were mostly pubertal and therefore increased adiposity contributed to higher blood pressure in the girls. Furthermore, another study showed that children who had low BMI but high WC were at great risk of developing hypertension.[22,18]

Our results show that children with WC and HC greater than the 75th percentile had an increased relative risk (1.7 and 1.5, respectively) of being hypertensive. This finding was confirmed by the fact that both pre-hypertension and hypertension were over 1.5 times more prevalent in 10- to 14-year-old girls compared to age-matched boys. On the other hand, higher BMI did not confer a significant risk of higher BP. However when subjects were separated into quartiles for BMI, WC, TFM and WHtR, it was noted that SPB, DBP and PP were significantly higher in boys and girls in the fourth quartile, indicating that adiposity contributes to blood pressure levels.

Although the fourth quartile of all measures of adiposity had significantly higher SBP, DBP and PP, only WC conferred a significantly ($p < 0.001$) greater risk (1.7 times) for hypertension. This finding is in agreement with Dong *et al.*,[17] who highlighted the importance of increased WC on the risk of hypertension in children. The retrospective study of data from the NHANES study showed that WC was associated with higher blood pressure in children and adolescents.[23]

Conclusion

This study demonstrates that the prevalence of hypertension and pre-hypertension was higher in 10- to 14-year-old girls than boys. The relative risk of having hypertension in this study cohort was greater in children who had larger WC. The linear relationship between blood pressure and BMI, WC, TFM and WHtR in children was weak. However, SBP, DBP, PP and mean arterial pressure increased with increasing quartiles of BMI, WC, TFM and WHtR. Consequently, the greatest prevalence of hypertension and pre-hypertension was in overweight and obese children, therefore confirming the role of increasing levels of adiposity in the prevalence of hypertension and pre-hypertension in 10- to 14-year-old children in the Eastern Cape.

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Clinical outcome of intracoronary versus intravenous high-dose bolus administration of tirofiban in diabetic patients undergoing primary percutaneous coronary intervention

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Abstract

Background: **Previous trials remain inconsistent regarding the advantages and hazards related to intracoronary (IC) compared with intravenous (IV) administration of thrombolytics. We aimed to evaluate the safety and effectiveness of IC versus IV tirofiban administration in diabetic patients (DM) with acute ST-segment elevation myocardial infarction (STEMI) during primary percutaneous coronary intervention (PCI)**

Methods: **This trial included 95 patients who were randomised to high-dose bolus plus a maintenance dose of tirofiban administered either IV or IC. The groups were compared for the incidence of composite major adverse cardiac events (MACE) at 30 days. Levels of cardiac markers were recorded pre- and post-intervention for myocardial perfusion.**

Results: **The MACE were not different between the groups, but post-procedure myocardial blush grade (MBG) 3 and thrombolysis in myocardial infarction (TIMI) 3 flow were significant in the IC group (p = 0.45, 0.21, respectively), favouring the IC strategy. Peak values of both creatine kinase-muscle/brain (CK-MB) and high-sensitivity troponin T (hs-TnT) were significantly lower in the IC group (155.68 ± 121, 4291 ± 334 ng/dl) versus the IV group (192.4 ± 86, 5342 ± 286 ng/dl) (p = 0.021, p = 0.035, respectively). The peak value was significantly lower in the IC group than the IV group in terms of ST-segment resolution and 30-day left ventricular ejection fraction (LVEF) (p = 0.016 and 0.023, respectively).**

Conclusion: **Thirty days post PCI, IC tirofiban was more efficient in ameliorating blood flow in the coronary arteries and myocardial tissue perfusion in DM patients after STEMI despite bleeding events, and MACE rates showed no significant difference between the groups. The IC group showed better improvement in LVEF.**

Keywords: diabetes mellitus, STEMI, intracoronary tirofiban, primary coronary intervention

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Impaired glucose metabolism accelerates the risk of arteriosclerosis and 80% of patients with diabetes mellitus (DM) die from cardiovascular diseases.[1] Previous trials have demonstrated a positive correlation between hyperglycaemia and the occurrence of heart failure, arrhythmia and other complications. Moreover, hyperglycaemia significantly increased the mortality rate of patients with diabetes complicated by myocardial infarction (MI).[2]

Acute occlusion of the major epicardial coronary artery usually leads to acute ST-segment elevation myocardial infarction (STEMI). Successful recanalisation and patency of the occluded vessels with percutaneous coronary intervention (PCI) or fibrinolytics diminishes the infarction size, saves the function of the ventricle and decreases morbidity and mortality rates.[3,4]

Several consequences, such as no reflow and slow flow, associated with more major adverse cardiac events (MACE), complications and high mortality rates have been observed in patients with DM complicated by acute MI (AMI) and undergoing primary PCI.[5,6] Platelet aggregation into the distal microvasculature or thrombus embolisation immediately after successful intervention impairs microvascular flow. Administration of glycoprotein IIb/IIIa inhibitors (GPI) and many catheter-based strategies have been attempted to overcome this phenomenon.[7,8]

American guidelines recommend tirofiban during PCI in patients with STEMI for high burden of thrombus or patients who received inadequate loading of P2Y12 inhibitors, and in patients with non-ST-elevation acute coronary syndrome (NSTE-ACS) and high risk.[9,10] European guidelines recommend tirofiban use in PCI for bailout situations if there is angiographic evidence of massive thrombus, slow or no reflow, or thrombotic complications.[11,12]

This trial attempted to assess whether intracoronary (IC) administration of high-dose bolus plus a maintenance-dose infusion of tirofiban would lead to better efficacy and safety and enhance clinical outcomes better than the standard intravenous (IV) bolus-plus-infusion regimen during PCI for diabetic patients with acute STEMI.

Methods

The study evaluated 95 consecutive diabetic patients undergoing primary PCI for STEMI. Patients were recruited to receive 25 µg/kg tirofiban bolus plus a maintenance dose of 0.15 µg/kg/min infusion either IV (group A: n = 50) or IC (group B: n = 45) for 24 hours.
We included adult patients between 18 and 75 years with a clinical presentation of STEMI and specific ECG criteria in the form of ST-segment elevation ≥ 1 mm in two or more contiguous leads, except V2 and V3 had to be ≥ 1.5 mm in females, ST-segment elevation ≥ 2.5 mm in males less than 40 years or ≥ 2 mm in males more than 40 years, or the presence of new-onset or presumed new left bundle branch block.[13]

The institutional ethics committee approved the study and all patients signed informed consent.

Patients with marked uncontrolled hypertension (≥ 180/110 mmHg), rescue PCI and emergency coronary artery bypass grafting were excluded. Other exclusion criteria included patients presenting with cardiogenic shock, severe liver or kidney failure, bleeding diathesis, hypersensitivity or thrombocytopaenia with tirofiban, platelets < 150 000 cells/mm^3, active internal bleeding, history of ischaemic or haemorrhagic stroke within the last 30 days, atrioventricular malformation or aneurysm, neoplastic aortic dissection, acute pericarditis, haemorrhagic retinopathy and chronic haemodialysis.

Before the intervention all patients were treated with acetylsalicylic acid (300 mg) and clopidogrel (600 mg). After securing vascular access through the right femoral or radial arteries, a total of 70–100 IU/kg unfractionated heparin IV bolus was given, then an additional weight-adjusted unfractionated heparin was given to achieve approximately 250 seconds of activated clotting time (ACT).

In both groups, a bolus of 25 µg/kg of tirofiban was given immediately after the guidewire crossed the lesion successfully and antegrade flow was restored, aiming to secure maximum concentration of the drug at the culprit lesion site and distal microvascular bed. A bolus dose of tirofiban was given through the guiding catheter in the infarct-related artery (IRA) at 30 seconds in the IC group. Maintenance IV tirofiban of 0.15 µg/kg/min for 18 hours was started in both groups after the bolus dose. An aspiration thrombectomy catheter was used if necessary and, finally, a suitable drug-eluting stent (FDA approved) was employed in the IRA in all patients.

Acetylsalicylic acid, a P2Y12 inhibitor (clopidogrel 75 mg), a high-intensity statin, beta-blocker and an angiotensin converting enzyme inhibitor or angiotensin II receptor blocker were prescribed as per the guidelines. When the activated clotting time (ACT) was < 160 seconds and/or four hours after anticoagulation, the vascular sheath was removed by manual compression.

The time to reperfusion was recorded from the onset of chest pain until the visualisation of at least thrombolysis in myocardial infarction (TIMI) 2 flow in the IRA during PCI. Before and after coronary intervention, TIMI flow grades[14] and myocardial blush grade (MBG)[15] were evaluated blindly by two interventional cardiologists. For evaluation of left ventricular ejection fraction (LVEF), the biplane modified Simpson's method was used 48 hours after PCI and then again after 30 days.

The groups were compared for TIMI flow grades before and after the intervention, and MBG, maximum C-reactive protein (CRP) level, peak levels of both high-sensitivity troponin T (hs-TnT) and CK-MB, time to peak for hs-TnT and CK-MB, time to 50% ST resolution, and composite MACE rates at 30 days were recorded. Safety endpoints such as significant and minor bleeding and thrombocytopenia were noted.

According to the dye density, the MBG score was classified as grade 3 = normal myocardial contrast density compared to contrast density of a contra- or ipsilateral non-IRA, 2 = moderate myocardial blush where contrast density is less than that obtained from a contra- or ipsilateral non-IRA, 1 = minimal myocardial blush or contrast density, and grade 0 = no myocardial blush.[16]

MACE[17] included cardiovascular death, recurrent myocardial infarction, stent thrombosis or target vessel revascularisation in hospitalisation at one month. Thrombocytopenia was defined as platelet count < 100 000 cells/mm^3.[16] Intracranial haemorrhage and decrease in haemoglobin concentration ≥ 5 g/dl were considered as major bleeding. Minor bleeding was defined as 10 to 15% decrease in haematocrit, blood loss with 3 to 5 g/dl decrease in haemoglobin concentration, or ≥ 4 g/dl decrease in haemoglobin concentration with no observed blood loss.[18]

Statistical analysis

Patients' data were collected, revised and analysed using the statistical package for social sciences (SPSS) version 25.0 for windows (IBM Corp, Armonk, NY, USA). Data are presented as mean ± standard deviation (SD), frequency and percentage. Categorical variables were compared using the chi-squared ($\chi 2$) test. Continuous variables were compared with the Student's t-test (two-tailed) and one-way ANOVA test for parametric data with Bonferroni post hoc test to detect differences between subgroups. The level of significance was accepted if the p-value was < 0.05.

Results

The two groups showed no statistically significant differences in cardiovascular risk factors, baseline characteristics or medication (Table 1). The mean age was 58.5 ± 10.18 years in the IV group and 55.90 ± 11.66 years in the IC group. The groups showed no significant differences in baseline level of glycated haemoglobin (HbA_{1c}) (p = 0.08), onset-to-balloon and door-to-balloon times (p = 0.08, 0.3, respectively). Killip class frequency > 1 was 18% in group A (IV) and 24% in group B (IC) (p = 0.33) (Table 1).

Peak CK-MB value was significantly lower in the IC group than in the IV group (155.68 ± 121, 192.4 ± 86 U/l respectively) (p = 0.021). Peak hs-TnT value was significantly lower in the IC group than in the IV group (4291 ± 334, 5342 ± 286 ng/dl; p = 0.035). The percentage of patients with 50% resolution of ST-segment was significantly higher in the IC group than in the IV group (p = 0.016) (Fig. 1). The maximum CRP level, peak and time to peak of both CK-MB and hs-TnT showed statistically significant differences, as shown in Table 2. There was no significant difference in LVEF between the groups 48 hours after PCI (p = 0.632), but 30 days after PCI, the average LVEF in the IC group was higher than in the IV group (p = 0.023).

Angiographic characteristics of the two groups are presented in Table 3. Post-procedure TIMI 3 flow (Fig. 2) and MBG 3 were significant in the IC group (p = 0.045, 0.021, respectively). Comparison between the groups in terms of the culprit vessel affected and multivessel frequency showed no significant differences.

The incidence of MACE and major and minor bleeding during the hospital stay and at follow up are shown in Table 3. Only one

Table 1. Baseline characteristics of both groups

Parameters	Group A (IV) (*n* = 50)	Group B (IC) (*n* = 45)	t/χ^2	*p*-value
Age (mean ± SD)	58.56 ± 10.18	55.90 ± 11.66	0.72	0.41
Gender, *n* (%)				
Male	27 (54)	23 (51.1)	0.69	0.49
Female	23 (46)	22 (48.9)		
Body mass index (kg/m^2) (mean + SD)	26.1 ± 6.5	25.4 ± 8.2	0.1	0.78
Smoking, *n* (%)	34 (68)	31 (68.8)	0.69	0.48
Hypertension, *n* (%)	20 (40)	19 (42)	0.08	0.78
Family history of coronary artery disease, *n* (%)	9 (18)	7 (15.5)	0.61	0.54
Killip class > 1, *n* (%)	9 (18)	11 (24)	1.025	0.33
Aspirin, *n* (%)	49 (98)	43 (95.5)	0.05	0.87
Clopidogrel, *n* (%)	50 (100)	44 (97.7)	0.84	0.64
Beta-blockers, *n* (%)	41 (82)	39 (86.6)	0.06	0.85
ACEI or ARBs, *n* (%)	39 (78)	36 (80)	0.12	0.79
Statin, *n* (%)	44 (88)	39 (86.6)	0.15	0.73
Warfarin, *n* (%)	3 (6)	1 (2.2)	0.8	0.068
Onset-to-balloon time (min) (mean ± SD)	167 ± 12.4	151 ± 18.3	5.8	0.089
Door-to-balloon time (min) (mean ± SD)	46.8 ± 8.9	44 ± 7.6	1.72	0.38
Fasting glucose (mg/dl) (mean ± SD)	168 ± 29.8	192 ± 46.6	3.64	0.074
Glycated haemoglobin (HbA_{1c}) (mean ± SD)	7.8 ± 2.2	9 ± 1.3	3.1	0.087
Creatinine (mg/dl) (mean ± SD)	1.17 ± 0.41	1.08 ± 0.56	2.56	0.251
Low-density lipoprotein cholesterol (mg/dl) (mean ± SD)	132.6 ± 46	147.09 ± 51	2.79	0.091

ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin II receptor blocker.

patient developed major bleeding due to upper gastrointestinal bleeding. Five patients developed minor bleeding in group A (three patients developed access-site bleeding and two developed haematuria). In group B, one patient developed major bleeding in the lower gastrointestinal system and four developed haematuria.

Table 2. Comparison between the groups regarding cardiac biomarkers and left ventricular ejection fraction

Parameters	Group A (IV) (*n* = 50)	Group B (IC) (*n* = 45)	*t*	*p*-value
Peak CK-MB (U/l)	192.4 ± 86	155.68 ± 121	6.43	0.021*
Time to peak CK-MB (s)	12.9 ± 5.8	8.96 ± 3.2	11.4	0.001*
Peak hs-TnT (ng/dl)#	5342 ± 286	4291 ± 334	5.9	0.035*
Time to peak hs-TnT (s)	13.5 ± 3.1	9.24 ± 2.8	10.7	0.001*
50% ST-segment resolution (%)	56	77	7.6	0.016*
LVEF at 48 hours (%)	38.6 ± 5.3	41.5 ± 3.2	0.84	0.632
LVEF at 30 days (%)	42.6 ± 4.2	48.2 ± 6.1	6.23	0.023*
Maximum C-reactive protein level (ng/dl)	9.2 ± 2.3	5.7 ± 1.4	6.1	0.026*

#Normal high-sensitivity troponin level up to 14 ng/dl.
CK-MB: creatine kinase-muscle/brain; hs-TnT: high-sensitivity troponin T; LVEF: left ventricular ejection fraction.

Discussion

Diabetic patients usually have microangiopathy and microvascular dysfunction. After restoration of normal blood flow in the coronary arteries, there is still insufficient myocardial tissue reperfusion (i.e. no reflow and slow flow) in up to 30% of patients.[19,20] Higher incidence of re-infarction, heart failure, stroke and death was previously documented in diabetic than in non-diabetic patients.[21]

The main cause of slow flow and no reflow is thrombosis and microvascular embolisation. These microvascular complications are higher in AMI and primary PCI. Visible thrombus in coronary angiography can be removed by a suction catheter, but it was found that 61% of the thrombus was invisible in AMI.[22] Imperfect inhibition of platelet aggregation during PCI may increase the MACE. The use of adjuvant medical drugs such as GPIs considerably decrease the incidence of distal embolisation and thrombotic outcomes in STEMI patients.[23,24]

This study demonstrated that IC tirofiban administered for thrombotic complications or bail-out situations, in addition to loading oral antiplatelets in diabetic patients, was associated with

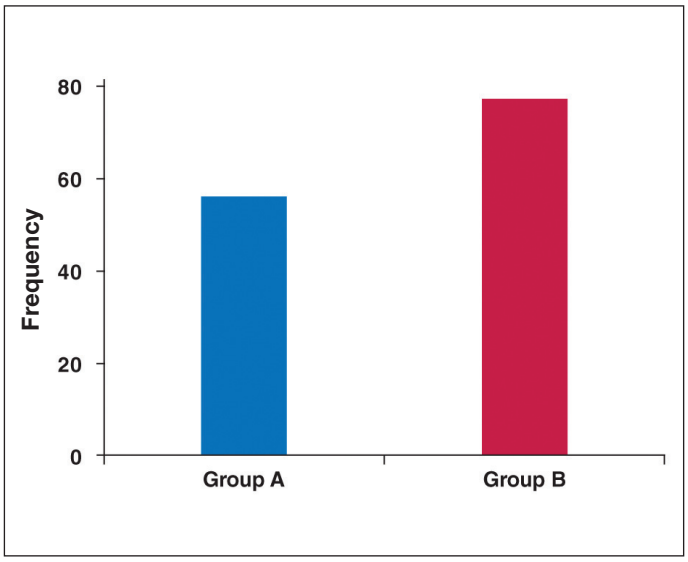

Fig. 1. Frequency of 50% ST-segment resolution in the groups.

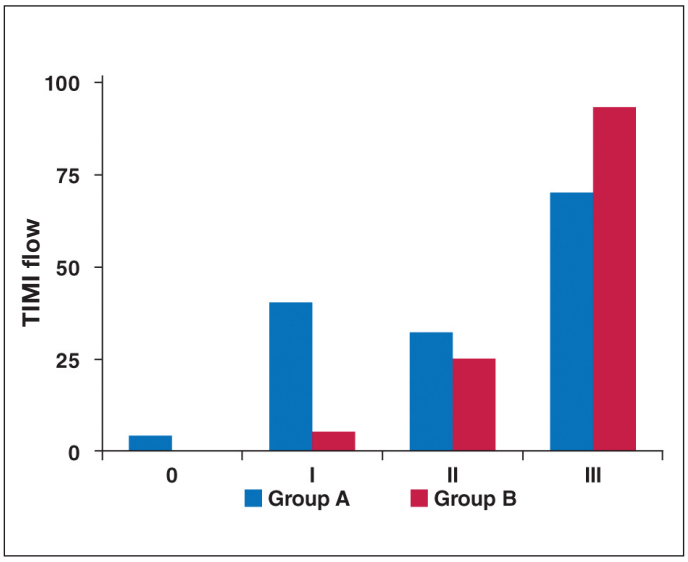

Fig. 2. Comparison of TIMI flow post intervention in the groups.

Table 3. Summary of angiographic characteristics, MACE and bleeding events in both groups

Parameters	Group A (IV) (*n* = 50)	Group B (IC) (*n* = 45)	$\chi^{2/t}$	*p*-value
TIMI 3 flow after procedure, *n* (%)	39 (78)	42 (93)	4.02	0.045*
MBG 3 after procedure	34 (68)	41 (82)	5.34	0.021*
Infarct-related vessel, *n* (%)				
Left anterior descending artery, *n* (%)	30 (60)	25 (55)	0.38	0.72
Circumflex artery, *n* (%)	7 (14)	5 (11.1)	0.072	0.91
Right coronary artery, *n* (%)	10 (20)	13 (28.8)	0.065	0.92
Triple vessels, *n* (%)	3 (6)	2 (4.4)	0.00	1.00
Balloon, *n* (%)	10 (20)	13 (28.8)		0.98
In-hospital MACE, *n* (%)				
In-hospital death, *n* (%)	2 (4)	1 (2.2)	0.00	1.00
In-hospital stroke, *n* (%)	0	0	0.00	1.00
In-hospital re-infarction, *n* (%)	1 (2)	0	0.05	0.993
In-hospital stent thrombosis, *n* (%)	1 (2)	0	0.05	0.993
In-hospital TVR, *n* (%)	0	0	0.00	1.00
1-month MACE, *n* (%)				
1-month death, *n* (%)	1 (2)	0		1.00
1-month stroke, *n* (%)	0	0	0.00	1.00
1-month re-infarction, *n* (%)	1 (2)	1 (2.2)	0.00	1.00
1-month stent thrombosis, *n* (%)	1 (2)	1 (2.2)	0.00	1.00
1-month TVR, *n* (%)	1 (2)	1 (2.2)	0.00	1.00
TIMI major bleeding, *n* (%)	1 (2)	1 (2.2)	0.00	1.00
TIMI minor bleeding, *n* (%)	5 (10)	4 (8.8)	0.02	0.95
Thrombocytopenia, *n* (%)	2 (4)	2 (4.4)	0.00	1.00

TIMI: thrombolysis in myocardial infarction; MBG: myocardial blush grade; MACE: major adverse cardiac events; TVR: target vessel restenosis.

greater reduction of peak hs-TnT, CK-MB levels and ST-segment resolution compared with IV tirofiban. Both regimens showed similar results for MACE and major and minor bleeding events during hospitalisation and after one month of follow up. The risk of bleeding did not appear to increase with IC administration of tirofiban.

Topol *et al.* showed that tirofiban in comparison with abciximab provided more platelet inhibition in diabetic patients during follow up and helped to prohibit PCI-related ischaemic and thrombotic complications.[25] The theory is to achieve a high drug concentration in the culprit epicardial vessel and small vasculature by administering IC tirofiban during PCI. Compared with IV delivery of tirofiban, IC delivery was associated with greater procedural success (e.g. TIMI grade 3 flow).[26]

Our findings revealed that no reflow and slow flow were effectively reduced and TIMI flow and MBG had better outcomes with IC injection of tirofiban. These results were in concordance with recent studies that proved that IC[27] and intralesional delivery of tirofiban through an aspiration catheter had better myocardial perfusion and fewer complications, even in complex PCI.[28]

Loss of endothelium-dependent vasodilation, inflammatory reaction and platelet-dependent micro-thrombosis are enhanced by hyperglycaemia, thereby aggravating the perfusion disturbance of coronary microcirculation.[29] The mortality rate was much higher in patients when MBG decreased to 0 to 1.[6,30]

To the best of our knowledge, this is the first study to demonstrate short-term outcomes and safety of IC injection of high-dose bolus tirofiban plus a maintenance IV, compared with IV tirofiban in diabetic patients with STEMI. We showed that IC tirofiban resulted in decreased inflammation in MI, which was evidenced by a significant reduction in peak CRP level. Previous studies have reported on the predictive value of CRP in determining the risk of future cardiovascular events.[31,32] Other studies have documented a post-procedure CRP rise in relation to myonecrosis.[33] The efficient inhibition of platelet aggregation by tirofiban led to inhibition of inflammatory mediators.[34]

In spite of no significant differences in bleeding events and MACE rates during the 30-day follow up after PCI, the IC tirofiban group showed an improvement in left ventricular function. However, we need large, long-term, multicentre, randomised trials to assess whether IC injection of tirofiban at the time of primary PCI improves clinical outcome in diabetic patients.

The results of this study have certain limitations. We used non-random selection of patients for IC tirofiban, the patient number was relatively small, and we evaluated IC tirofiban on STEMI but did not compare the effects in NSTE-ACS. Despite including elderly patients in the study, we did not compare major and minor bleeding incidence and platelet level reduction in different-aged populations. A possible improvement in clinical outcome could be observed with longer follow-up periods as left ventricular systolic function was improved.

Conclusion

IC tirofiban improved coronary blood flow and myocardial tissue perfusion effectively in diabetic STEMI patients during primary PCI. Improved LVEF was also observed 30 days post primary PCI. However, bleeding events and MACE rates showed no significant difference between the groups.

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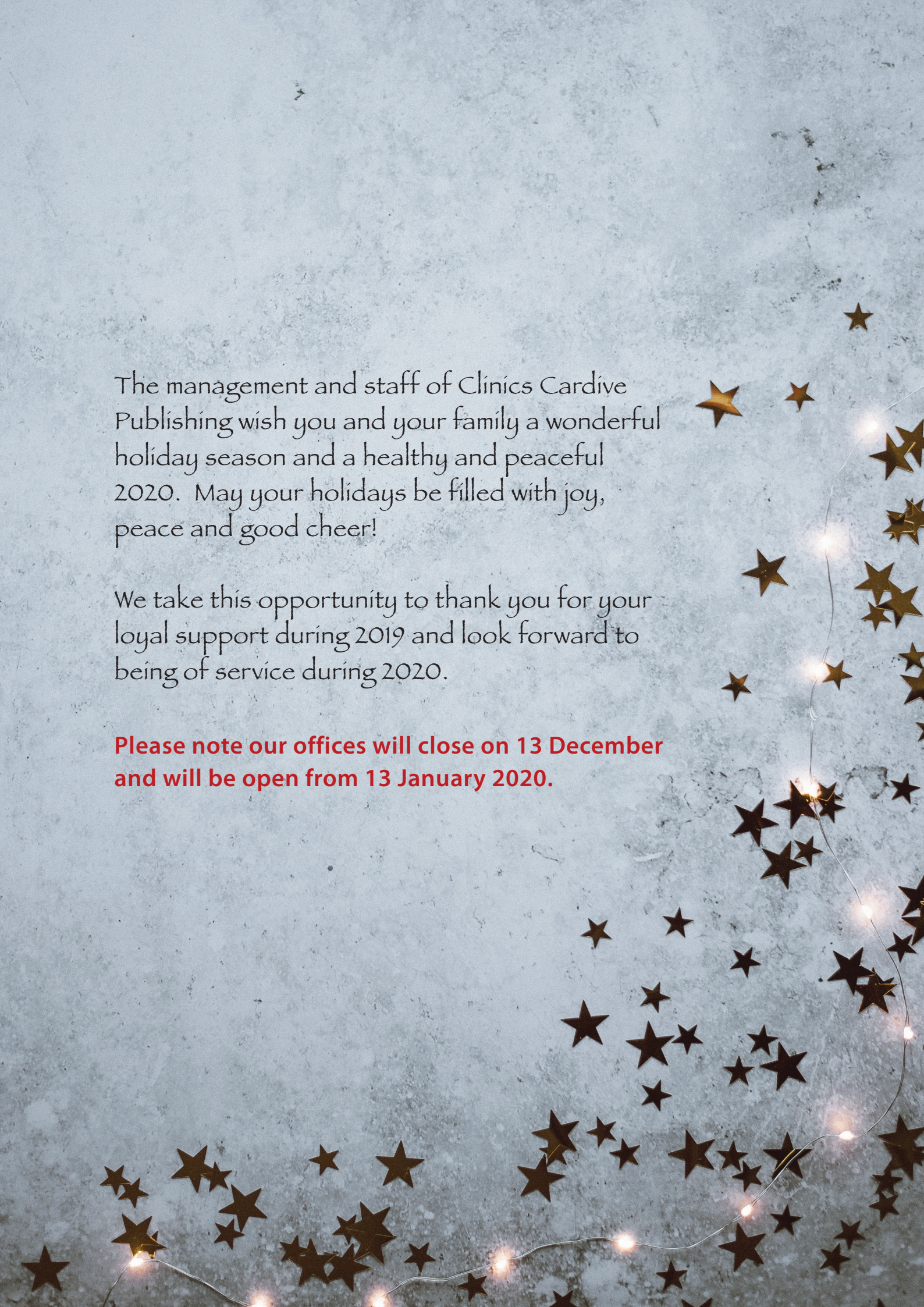

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