Cardiovascular Topics

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 (C16:1n-7) level in plasma phospholipids was positively associated with BMI in men and women,[13,17] and higher levels of plasma C16:1n-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 (C20:3n-6) in overweight and obese individuals.[13] Positive associations of serum phospholipid C20:3n-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 multi-country 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 thin-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 (C18:1n-7t), rumenic acid (C18:2n-7tt), stearidonic acid (C18:4n-3) and eicosatrienoic (C20:3n-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-sn-glycerol-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. Non-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 model$_1$). This model was further adjusted for lifestyle

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.

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 of adiposity ($p < 0.0001$). The dietary intake data

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 (C16:1n-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 (C18:1n-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 (C18:1n-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 (C18:1n9t)	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 (C20:1n-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 (C22:1n-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 (C24:1n-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 (c18:3n-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 (C20:5n-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 (C22:5n-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 (C22:6n-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 (C18:2n-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 (C18:3n-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 (C20:2n-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 (C20:3n-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 (C20:4n-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-n6)	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 (C22:4n-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 (C22:5n-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 (C20:3n-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.

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 C20:3n-6 were significantly higher in overweight men than in lean men, whereas C16:0, C18:3n-6 and MUFAs, C16:1n-7, *cis*-vaccenic acid (C18:1n-7), oleic acid (C18:1n-9) and gondoic acid (C20:1n-9) were higher in lean than in overweight

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

	Dietary fatty acid patterns[a]	
Dietary fatty acids	*Non-marine* ($\lambda_1 = 60\%$)[b]	*Marine* ($\lambda_2 = 29\%$)
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 (C16:1n-7)	0.89	0.28
Oleic acid (C18:1n-9)	0.95	0.23
n-3 fatty acids		
α-Linolenic acid (C18:3n-3)	0.89	0.22
Eicosapentaenoic acid (C20:5n-3)	0.21	0.96
Docosahexaenoic acid (C22:6n-3)	0.23	0.96
n-6 fatty acids		
Linoleic acid (C18:2n-6)	0.80	0.31
Arachidonic acid (C20:4n-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* ($\lambda 1 = 24\%$)	*n-3 VLC-PUFA* ($\lambda 2 = 11\%$)	*High-LA* ($\lambda 3 = 11\%$)	*n-6 VLC-PUFA* ($\lambda 4 = 10\%$)	*n-9 LC-MUFA* ($\lambda 5 = 10\%$)	*n-3 EFA* ($\lambda 6 = 7\%$)
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 (C16:1n-7)	–0.84	–0.13	–0.25	0.08	-0.12	0.23
Cis-vaccenic acid (C18:1n-7)	–0.73	0.10	0.16	0.08	0.40	–0.20
Oleic acid (C18:1n-9)	–0.82	–0.15	–0.26	0.11	-0.01	0.35
Elaidic acid (C18:1n9t)	0.44	–0.58	–0.25	–0.04	0.09	–0.02
Gondoic acid (C20:1n-9)	–0.17	–0.07	0.52	–0.05	0.54	–0.13
Erucic acid (C22:1n-9)	0.35	–0.01	0.05	0.22	0.42	–0.05
Nervonic acid (C24:1n-9)	0.16	0.09	0.07	–0.01	0.85	–0.04
n-3 fatty acids						
α-linolenic acid (C18:3n-3)	–0.23	0.02	0.28	–0.15	–0.09	0.71
Eicosapentaenoic acid (C20:5n-3)	–0.37	0.57	–0.25	–0.32	–0.01	0.42
Docosapentaenoic acid (C22:5n-3)	–0.08	0.71	–0.01	0.12	0.15	0.27
Docosahexaenoic acid (C22:6n-3)	0.05	0.79	0.25	–0.08	0.08	–0.24
n-6 and n-9 fatty acids						
Linoleic acid (C18:2n-6)	0.08	0.31	0.80	–0.10	0.05	0.16
γ-linolenic acid (C18:3n-6)	–0.41	–0.08	–0.17	0.27	–0.37	0.49
Eicosadienoic acid (C20:2n-6)	0.09	0.06	0.82	0.30	0.15	0.13
Dihomo-γ-linolenic acid (C20:3n-6)	0.00	0.29	0.14	0.65	–0.06	0.30
Arachidonic acid (C20:4n-6)	0.04	0.66	0.12	0.42	0.24	–0.31
Docosadienoic acid (C22:2-n6)	0.44	0.08	0.47	–0.06	0.45	–0.24
Adrenic acid (C22:4n-6)	–0.13	0.02	0.03	0.83	0.08	0.01
Osbond acid (C22:5n-6)	–0.12	–0.04	–0.09	0.82	0.01	–0.32
Mead acid (C20:3n-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*	
	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*	
	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.

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 (C24:1n-9), docosahexaenoic acid (C22:6n-3), C20:3n-6, arachidonic acid (C20:4n-6) and docosadienoic acid (C22:2-n6) were higher in overweight women than in their lean counterparts. Similar MUFAs, i.e. C16:1n-7, C18:1n-7, C18:1n-9 and C20:1n-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.

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 (C18:3n-3) and n-6 FAs, and therefore was named the 'non-marine' FA pattern. The second pattern was named the 'marine' FA pattern because it was characterised by high positive loadings of eicosapentaenoic acid (C20:5n-3) and C22:6n-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 C16:1n-7, C18:1n-7 and C18:1n-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 (C22:5n-3), C22:6n-3 and C20:5n-3, as well as C20:4n-6. The third pattern presented the highest positive loadings of C18:2n-6 and eicosadienoic acid (C20:2n-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 (C22:4n-6), C22:2-n6 and C20:3n-6. The fifth pattern extracted was named the 'n-9 LC-MUFA' pattern and presented with positive loadings of C24:1n-9 and gondoic acid (C20:1n-9). The sixth and last pattern had a positive loading of one FA, i.e. C18:3n-3, and we named it 'n-3 EFA' pattern.

Dietary FA patterns were weakly associated with measured outcomes (Table 4). The non-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 non-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 non-marine FA pattern showed marginal and positive associations with WC, but not with other

measures of adiposity or the MetS. The non-marine FA pattern had positive loadings of FAs from SFAs, MUFAs and PUFAs, specifically from two SFAs (C16:0 and C18:0), two MUFAs (C16:1n-7, C18:1n-9) and two PUFAs (C18:2n-6 and C18:3n-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 C18:1n-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 C20:5n-3, C22:5n-3 and C22:6n-3, as well as the n-6 PUFA arachidonic acid (C20:4n-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 C20:5n-3, and estimated delta 5 desaturase activity and negative loading of C20:3n-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 C22:6n-3 and C20:5n-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 C18:3n-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 C20:4n-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 C20:4n-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, C20:3n-6, C22:4n-6 and osbond acid (C22:5n-6) and was positively associated with the MetS. Mayneris-Perxachs *et al.*[18] also reported a positive association between plasma phospholipid C20:3n-6 PUFAs and the MetS among older adults in Spain.

Higher concentrations of plasma phospholipid C20:3n-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 C20:3n-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 C20:3n-6 with the MetS requires further investigation.[18]

The fifth pattern, n-9 LC-MUFA, loaded positively with C20:1n-9 and C24:1n-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 C20:1n-9 were significantly higher in lean men and women compared to their overweight counterparts, whereas C24:1n-9 was higher in overweight compared to lean women only. Nervonic acid (C24:1n-9) and C20:1n-9 are both products

of endogenous metabolism by elongation from oleic acid,[55] but plasma C24:1n-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 C18:3n-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 C18:3n-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] Alpha-linolenic acid (C18:3n-3) is an essential FA and a precursor from which n-3 LC-PUFAs are synthesised. Increased consumption of C18:3n-3-rich foods elevates its tissue levels as well as levels of C22:6n-3 and C20:5n-3 in the liver lipids.[58] Alpha-linolenic acid can be beneficial to health. Firstly, C18:3n-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, C18:3n-3 competes for the same metabolic enzymes, as does C18:2n-6, and increased dietary intake may be a worthy approach to decrease elongation of n-6 FAs leading to reduced plasma C20:4n-6 levels and increased plasma levels of C22:6n-3 and C20:5n-3.[58] As C18:3n-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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