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Original article

Blood indices of omega-3 and omega-6 polyunsaturated fatty acids are altered in hyperglycemia

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ABSTRACT

Polyunsaturated fatty acids (PUFAs) may favorably influence the risk and clinical course of diabetes mellitus (DM). In particular, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA) alleviate oxidative injury and insulin resistance characteristic of DM. Uncertainty still remains, however, as to the composition and proportions of blood PUFAs in relation to fasting blood glucose levels. This study, thus, aims to examine the patterns of blood PUFA indices in normoglycemic (NG) and hyperglycemic (HG) Saudi subjects. Age, gender, FA profiles, and laboratory records of 143 subjects collected from September 2014 to March 2018 were retrospectively analyzed. Means, prevalence rates, associations, risk measures, and the diagnostic accuracy of PUFAs were determined. HG subjects had significantly lower AA (0.70%, 95% CI: 0.59–0.80% *vs* 0.46%, 95% CI: 0.38–0.53%) and higher EPA/AA ratio (0.36, 95% CI: 0.30–0.42 *vs* 0.69, 95% CI: 0.61–0.77). Gender-wise comparisons revealed that ω-6/ω-3 ratio was the only PUFA index significantly elevated in HG males (0.36, 95% CI: 0.26–0.45 *vs* 5.68, 95% CI: 4.98–6.38) while both DHA (2.91%, 95% CI: 2.54–3.29% *vs* 3.37%, 95% CI: 3.13–3.60%) and ω-3 index (3.1%, 95% CI: 2.70–3.49% *vs* 3.63%, 95% CI: 3.38–3.88%) were significantly elevated in HG females. Furthermore, reduced AA and elevated EPA/AA ratio were more prevalent in HG subjects (26.53 *vs* 28.72 and 30.61 *vs* 38.29, respectively) and exhibited the highest diagnostic accuracy for HG among all PUFA indices. Altogether, our study revealed that distinct, gender-specific blood PUFA indices are differentially regulated in HG subjects which may be valuable for DM management.

1. Introduction

Fatty acids (FAs) are long-chain hydrocarbons (4–36 carbons) with a terminal carboxyl group. Based on the presence of double bonds, FAs are either saturated or unsaturated. FAs exist in an unbound form (free FAs) or esterified to glycerol to form triglycerides which facilitates their long-term storage and transport between tissues. FAs are also esterified to glycerol to form phospholipids which are major constituents of cell membranes and act as mediators of signal transduction, saltatory nerve conduction, and cell death (Dowhan 2017, de Carvalho and Caramujo 2018, Alsughayyir et al., 2022). Moreover, FAs are used to synthesize eicosanoids which have varied physiological functions. For instance, prostaglandins are formed from modified prostanoic acid, and are essential for smooth muscle contraction, gastric secretion, renal electrolyte balance, and inflammatory response. Thromboxanes, which contain a six-membered, oxygen-containing ring in prostanoic acid, are found in activated platelets and act as vasoconstrictors, while leukotrienes, derivatives of hydroperoxyeicosatetraenoic acids with three conjugated double bonds, promote chemotaxis, inflammation, and allergic reactions (Calder 2020).

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Polyunsaturated fatty acids (PUFAs) are essential, long-chain FAs obtained from fish, seeds, vegetables, eggs, and dairy products. Based on the location of the first double bond counting from the methyl end of the aliphatic hydrocarbon chain, PUFAs are classified into omega-3 (ω-3 or n-3) and omega-6 (ω-6 or n-6) types. Through cascades of saturation and elongation reactions, alpha-linolenic acid (ALA; 18:3ω-3) is metabolized to eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3), while linoleic acid (LA; 18:2ω-6) serves as the precursor to arachidonic acid (AA; 20:4ω6) (Simopoulos 2016).

Evidence from preclinical and epidemiological studies suggests that PUFAs regulate key metabolic processes that are perturbed in dysglycemia characteristic of diabetes mellitus (DM). In particular, modulation of inflammatory, oxidative, and insulinotropic mediators by PUFAs may favorably or unfavorably contribute to vascular injury, endothelial and β-cell dysfunction, and insulin resistance (Poreba et al., 2018). For instance, EPA and DHA both increase and decrease inflammatory leukotrienes and thromboxanes (Simopoulos 2008), and elevated ω-6/ω-3 ratio due to increased n-6 PUFAs precipitates inflammation and thrombosis, further aggravating atherosclerotic lesions (Kromhout and de Goede 2014).

Although increased fish consumption is related to a diminished DM risk, the clinical outcome of cardiovascular complications in dysglycemic patients was independent of n-3 PUFAs (Siscovick et al., 2017). Notably, the composition and proportion of individual blood FAs were found in one report to be related to DM risk and complications (Salas-Salvado et al., 2011), but ambiguity still remains with regard to PUFAs and their clinical value in DM prevention and management (Hill et al., 2007, Simopoulos 2014). This study thus aims to investigate alterations in blood PUFA indices and their diagnostic accuracy relative to glycemic control in Saudi subjects.

2. Materials and methods

2.1. Study design

The study protocol was approved by the Biomedical Ethics Unit of Al-Borg Medical Laboratories (approval number: 07/21). Subject consent was waived as the study was retrospective in nature. Age, gender, and laboratory data for 143 subjects whose results included both fasting blood glucose (FBG) and PUFAs were collected from September 2014 to March 2018. Normoglycemia (NG) was set at a FBG of < 100 mg/dl while ≥ 100 mg/dl defined hyperglycemia (HG) in accordance with the ADA guidelines (Alfhili et al., 2022a). Characteristics of study subjects are presented in Table 1.

2.2. Determination of PUFA indices

Gas chromatography is the method of choice to detect and quantify separated chemical species in biological matrices (Iqbal et al., 2020, Yasien et al., 2022). Blood levels of PUFAs were determined as previously described (Donahue et al., 2009, Tan et al., 2012). EDTA-anticoagulated whole blood samples were centrifuged at 2,500 RPM for 15 min and the plasma and buffy coat were discarded. An aliquot of the packed red blood cells (RBCs) was nitrogen-dried at 45 °C and FAs were then extracted using isopropanol and hexane, *trans*-esterified with boron trifluoride in methanol by heating at 100 °C for 10 min, and finally analyzed for composition and proportion by Agilent 5890 Gas Chromatograph equipped with a flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). Using a standard mixture of methylated FAs (Supelco Inc., Bellefonte, PA, USA), peak retention times were determined for EPA ($C_{20}H_{30}O_2$), DHA ($C_{22}H_{32}O_2$), and AA ($C_{20}H_{32}O_2$), and the relative content of each, expressed as a percentage, was calculated by dividing the area under the peak by the total area of all FAs identified. This corresponds to the proportion of each FA relative to their total weight in membrane phospholipids. The ώ-3 index was calculated as the sum of EPA and DHA whereas the ώ-6/ώ-3 ratio was calculated by dividing all identified ώ-6 over ώ-3 FAs.

Table 1
Characteristics of study subjects.

Characteristic	NG (*n* = 49)	HG (*n* = 94)	*P* value
Age (years)	41.04 (36.64–45.44)	40.68 (37.56–43.80)	0.8937
Males (%)	44.89	29.78	–
Females (%)	55.11	70.21	–
Hematocrit (%)	40.06 (38.12–41.99)	42.21 (41.09–43.33)	***0.0416***
RBC count (x10^6/μL)	5.16 (4.94–5.37)	5.42 (5.29–5.55)	***0.0305***
Hemoglobin (g/dL)	13.92 (13.14–14.70)	14.78 (14.34–15.22)	***0.0398***
ESR (mm/h)	19.65 (12.27–27.03)	14.78 (11.56–17.99)	0.1617
WBC count (x10^6/μL)	5.58 (5.03–6.12)	6.55 (5.94–7.17)	***0.0404***
Platelet (x10^9/L)	271 (247.1–295.1)	252.5 (237.4–267.7)	0.1764
Sodium (mEq/L)	140.9 (140.3–141.5)	139.8 (139.2–140.4)	***0.0115***
Potassium (mEq/L)	4.56 (4.42–4.70)	4.54 (4.47–4.62)	0.7996
Calcium (mg/dL)	9.65 (9.55–9.76)	9.6 (9.52–9.68)	0.4265
Chloride (mEq/L)	102.4 (101.6–103.2)	100.4 (99.76–101.0)	***<0.0001***
TC (mg/dl)	186 (174.7–197.8)	195.4 (186.5–204.2)	0.2216
LDL-C (mg/dL)	122.6 (111.5–133.7)	127.3 (119.4–135.3)	0.4903
HDL-C (mg/dL)	48.33 (44.90–51.76)	44.27 (41.71–46.82)	0.0626
TG (mg/dL)	117.0 (100.6–133.4)	172.1 (153.8–190.4)	***0.0001***
ALT (U/L)	25.98 (16.87–35.09)	26.60 (21.84–31.35)	0.8949
AST (U/L)	21.80 (16.88–26.71)	20.53 (17.81–23.26)	0.6266
Creatinine (mg/dL)	1.11 (0.79–1.44)	0.80 (0.71–0.90)	***0.0218***
Urea (mg/dL)	32.85 (26.73–38.98)	32.67 (28.75–36.59)	0.9583
Uric acid (mg/dL)	5.12 (4.65–5.59)	4.91 (4.60–5.23)	0.4550
Microalbuminuria (mg/24 h)	97.69 (-5.32–200.7)	62.35 (28.72–95.98)	0.4126
Specific gravity	1.017 (1.015–1.019)	1.007 (0.98–1.03)	0.5069
TSH (mIU/L)	2.38 (1.55–3.22)	2.69 (1.91–3.47)	0.6227
Free T_4 (ng/dL)	1.03 (0.98–1.07)	1.05 (1.02–1.09)	0.3613
25-OH-D_3 (nmol/L)	14.46 (11.74–17.17)	12.82 (11.21–14.43)	0.2715

Results are shown as means ± 95 % CI. RBC, red blood cell; WBC, white blood cell; ESR, erythrocyte sedimentation rate; TSH, thyroid-stimulating hormone.

2.3. Statistical analysis

All data were analyzed by GraphPad Prism 9.2.0 (GraphPad Software, Inc., San Diego, CA, USA). Results were expressed as means (±95 % CI) and two groups were compared by the unpaired, two-tailed Student's *t* test. Association was evaluated by calculating the prevalence risk (PR) and odds ratio (OR), and significance was defined by a *P* value of < 0.05.

3. Results

Subjects were stratified based on their FBG into NG and HG and the levels of different PUFA indices were then compared. As shown

in Fig. 1a-c, no statistically significant difference was observed in EPA, DHA, or ώ-3 index between NG and HG subjects. Significantly diminished AA was nonetheless evident, as depicted in Fig. 1d, in HG compared to NG individuals; 0.70 % (0.59–0.80 %) *vs* 0.46 % (0.38–0.53 %) who also had a significantly higher EPA/AA ratio at 0.36 (0.30–0.42) *vs* 0.69 (0.61–0.77) as seen in Fig. 1e.

The ώ-6/ώ-3 ratio was not influenced by the glycemic state (Fig. 1f) until males were considered in isolation. In Fig. 2f, significantly elevated ώ-6/ώ-3 ratio was seen in HG subjects in comparison to their NG counterparts; 0.36 (0.26–0.45) *vs* 5.68 (4.98–6.38). No other index was differentially regulated relative to FBG in males (Fig. 2a-e).

In females, both DHA at 2.91 % (2.54–3.29 %) *vs* 3.37 % (3.13–3.60 %) and ώ-3 index at 3.1 % (2.70–3.49 %) *vs* 3.63 % (3.38–3.88 %) were significantly elevated in HG as shown in Fig. 3b and c, respectively. No significant difference was detected in any other index in females (Fig. 3a, d, e, and f).

Next, we examined the prevalence rates of disturbed AA and EPA/AA in NG and HG. Table 2 shows that both reduced AA (<0.45 %) and elevated EPA/AA ratio (≥0.45) were more prevalent in HG compared to NG subjects (26.53 *vs* 28.72 and 30.61 *vs* 38.29, respectively).

To assess the risk of HG associated with low AA or high EPA/AA ratio, we measured PR and OR values as shown in Table 3. Although elevated EPA/AA ratio carried a greater risk for HG (OR = 1.41, 95 % CI: 0.67–2.94, $P = 0.3634$), no statistical significance was found and such an association may perhaps be attributed to unmeasured variables.

Importantly, we evaluated the diagnostic performance of all studied indices and their ability to discriminate HG from NG individuals using receiver operating characteristic (ROC) curve analysis. As seen in Fig. 4a-c, EPA, DHA, and ώ-3 index displayed poor classifying ability. In Fig. 4d, it is shown that AA had an area under the curve (AUC) of 0.7424 ($P < 0.0001$) with the highest sensitivity and specificity of 0.6129 and 0.7347, respectively, achieved at a cutoff of < 0.45 %. The likelihood at this cutoff was 2.310. Furthermore, as evident in Fig. 4e, the AUC of EPA/AA ratio was 0.8025 ($P < 0.0001$) and a cutoff of > 0.45 yielded sensitivity of 0.7128 and specificity of 0.6939 for HG with a likelihood ratio of 2.328. The ώ-6/ώ-3 ratio was of no diagnostic value (Fig. 4f).

4. Discussion

DM is a life-threatening, systemic, inflammatory disease prevalent in 25 % of the Saudi population which is expected to double by 2030 (Robert and Al Dawish 2020). DM significantly increases the risk of cardiovascular complications which is the cause of death in more than 50 % of Saudi diabetics (Robert and Al Dawish 2021). Institutional and individual efforts to reduce the pervasiveness of DM are, therefore, urgently needed. These must include health practitioner and patient education, enforcing consumer protection policies, and vigilant DM detection and management.

Our results indicate that ώ-6/ώ-3 ratio is elevated in hyperglycemic males while DHA and ώ-3 index are significantly elevated in hyperglycemic females. Moreover, irrespective of gender, diminished AA and elevated EPA/DHA were more

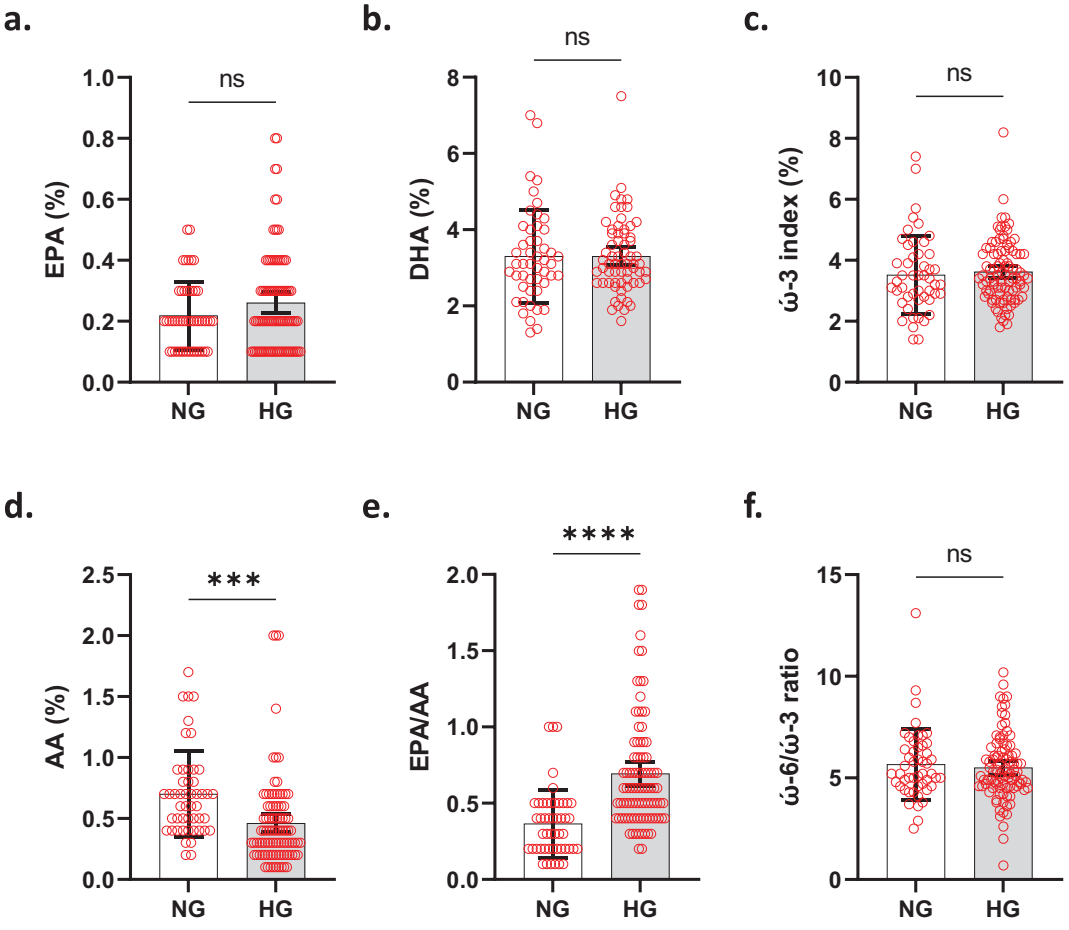

Fig. 1. PUFA indices in both genders. Mean percentages ± 95 % CI of (a) EPA, (b) DHA, (c) ω-3 index, (d) AA, (e) EPA/AA, and (f) ω-3/ω-6 ratio in NG and HG subjects. ns indicates no significance, ***($P < 0.001$), and ****($P < 0.0001$).

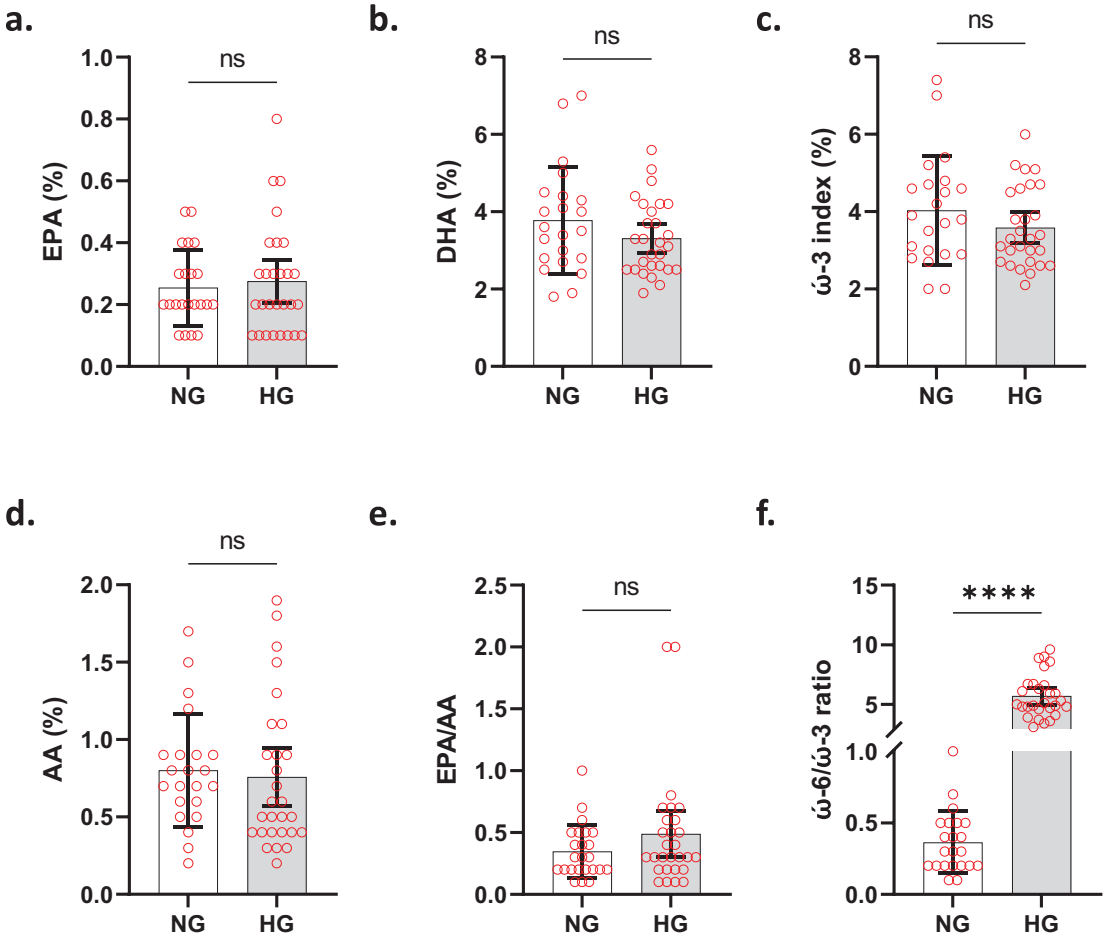

Fig. 2. PUFA indices in males. Mean percentages ± 95 % CI of (a) EPA, (b) DHA, (c) ω-3 index, (d) AA, (e) EPA/AA, and (f) ω-3/ω-6 ratio in NG and HG males. ns indicates no significance while $^{****}(P < 0.0001)$.

prevalent in HG and had the best diagnostic performance for HG. Data on PUFA levels in diabetic patients is extremely scarce. In one study, Poreba *et al.* found that diabetics who have atherosclerotic cardiovascular disease and $HbA_{1c} \geq 7$ % had higher LA and ώ-6/ώ-3 ratio and lower EPA, ώ-3 index, and EPA/AA ratio than those with lower HbA_{1c} (Poreba et al., 2018). In agreement with our findings, serum phospholipid LA was positively correlated with HbA_{1c} in diabetics with atherosclerotic disease (Poreba et al., 2018). Notably, elevated LA reduced the risk of DM (Forouhi et al., 2016, Wu et al., 2017), and decreased LA was related to DM and metabolic syndrome in many studies (Kurotani et al., 2012, Cho et al., 2014).

Despite the weak correlation between n-6 PUFA intake and biomarker level (Zong et al., 2019), increased consumption of LA in olive oil and full-fat dairy products, as opposed to spreads, has nonetheless been found to prevent incident DM (Riccardi 2017, Wu et al., 2017) at least in part by improving insulin sensitivity (Belury et al., 2018). In line with these findings, substituting saturated FAs and carbohydrates with PUFAs reduces glucose, HbA_{1c}, insulin, and insulin resistance (Imamura et al., 2016), which, in turn, is associated with a diminished risk for coronary heart disease (Li et al., 2015). Although the biochemical basis behind the beneficial role of LA have not been thoroughly understood, activation of glucose transporters and ion channels, regulation of gene expression (e.g., *SREBP1*), improvement of insulin receptor binding, and modulation of the gut microbiome (Devillard et al., 2009, Zong et al., 2019) are potential mechanisms.

Higher n-3 PUFAs, in particular, counteract insulin resistance and improve endothelial function in males (Leeson et al., 2002, Albert et al., 2014), and diabetics with a history of myocardial infarction had significantly lower EPA, DHA, EPA/AA, and DHA/AA compared to diabetics without a history of myocardial infarction (Takahashi et al., 2017).

Epidemiological evidence suggests that ALA and LA, but not EPA or DHA, are inversely associated with DM (Forouhi et al., 2016). No reports exist on the potential role of n-3 PUFAs in primary prevention of cardiovascular events (Siscovick et al., 2017), and studies on the potential benefit of n-3 PUFAs in preventing cardiovascular events under varying clinical contexts have produced conflicting results. Significant reduction in the risk for fatal and non-fatal cardiovascular events was noted in relation to EPA and DHA supplementation in a systematic review of eleven studies (Marik and Varon 2009). In a *meta*-analysis of eight randomized controlled trials, it was found that n-3 PUFA intake provided protection against sudden cardiac death in myocardial infarction patients, but carried a greater risk for it in those with angina (Zhao et al., 2009). Another review of twelve studies has found that n-3 PUFA intake was associated with a diminished risk of cardiac death (Leon et al., 2008). In patients with impaired glucose metabolism and coronary artery disease, six-month supplementation with EPA significantly enhanced glycemic and lipid control, and endothelial function (Sawada et al., 2016). Furthermore, oral supplementation of EPA and DHA in cod liver oil to pregnant women may reduce DM incidence in the offspring (Das 2018). Alterations of miRNA expression

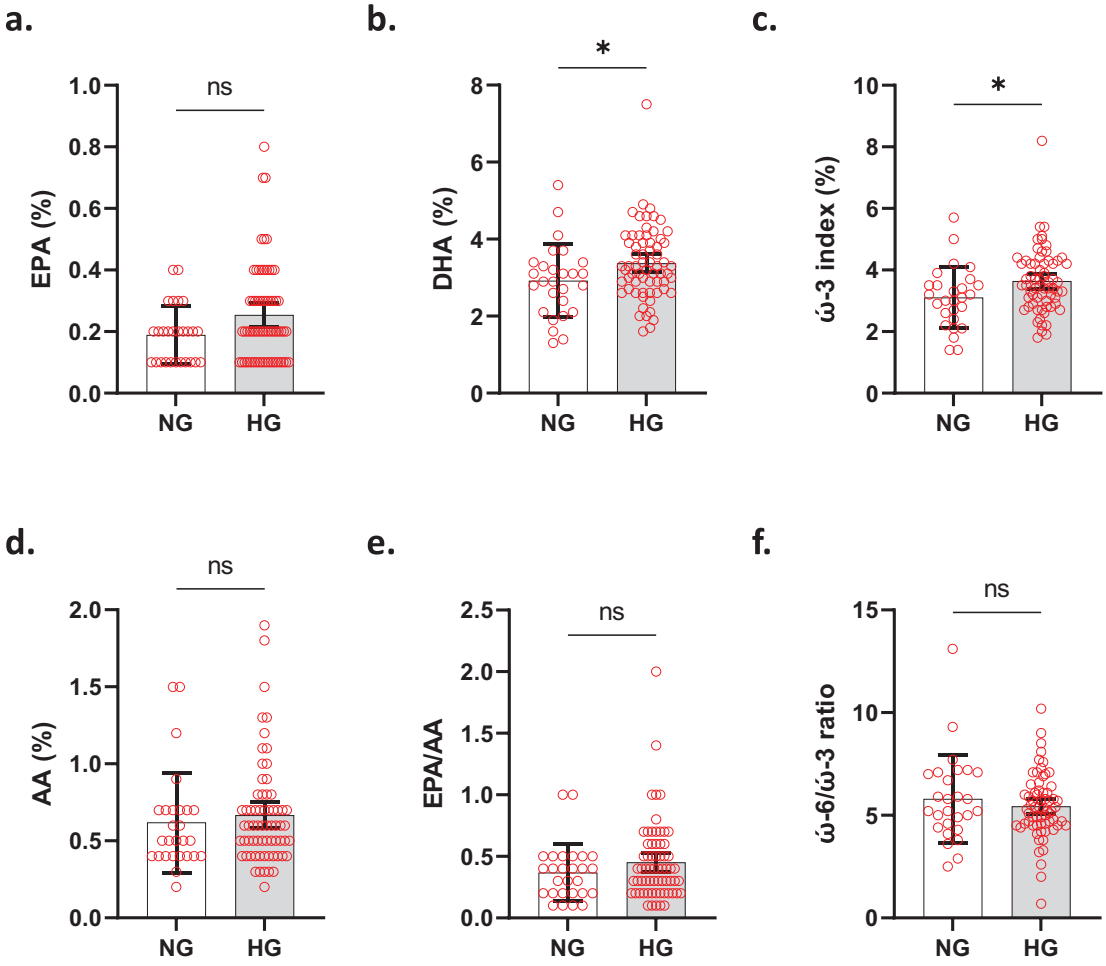

Fig. 3. PUFA indices in females. Mean percentages ± 95 % CI of (a) EPA, (b) DHA, (c) ω-3 index, (d) AA, (e) EPA/AA, and (f) ω-3/ω-6 ratio in NG and HG females. ns indicates no significance while *($P < 0.05$).

Table 2
Prevalence of reduced AA and elevated EPA/AA in NG and HG.

Parameter	All subjects	NG	HG
Reduced AA	27.97	26.53	28.72
Elevated EPA/AA	35.66	30.61	38.29

Prevalence is expressed as a percentage of all subjects in each group. AA, arachidonic acid; EPA, eicosapentaenoic acid. NG, normoglycemia; HG, hyperglycemia.

have been noted following n-3 PUFA administration to rats (Zheng et al., 2015).

In contrast, *meta*-analysis of fourteen randomized trials failed to establish sufficient evidence for a protective role of n-3 PUFA supplements against cardiovascular events (Kwak et al., 2012). Likewise, supplementation with high-dose n-3 PUFAs had no appreciable influence on the inflammatory status and coagulation markers in diabetics (Poreba et al., 2017). Furthermore, a low-dose, daily regimen, consisting of 900 mg ethyl esters of n-3 PUFAs, did not influence the rate of cardiovascular events in diabetics and prediabetics (Investigators et al., 2012).

Inconsistent findings in the literature regarding PUFAs and DM may be attributed to a number of factors. These include background diet, inflammation status, source, dosage, composition, and length of PUFA regimen, medication intake, genetic predisposition, ethnicity, medical history, and number of subjects (Simopoulos 2016). Primarily due to individual variation in FA metabolism (von Schacky 2015), dietary intake may not be the best estimate of PUFA concentrations in the body, and blood levels provide more accurate determination (Superko et al., 2013, Coelho et al., 2017).

We also found that AA is lower and EPA/AA and ώ-6/ώ-3 ratio are higher in HG compared to NG subjects. AA, primarily obtained in the diet from animal-based food, is a major component of cell membrane phospholipids, and serves as a precursor to both inflammatory and anti-inflammatory mediators such as eicosanoids and lipoxins (Das 2022). In experimental studies, it has been

Table 3
Risk assessment of reduced AA and elevated EPA/AA for HG.

Parameter	PR	95 % CI	*P*	OR	95 % CI	*P*
Reduced AA	1.03	0.80–1.34	0.7782	1.12	0.51–2.42	0.7816
Elevated EPA/AA	1.12	0.88–1.42	0.3486	1.41	0.67–2.94	0.3634

AA, arachidonic acid; EPA, eicosapentaenoic acid.

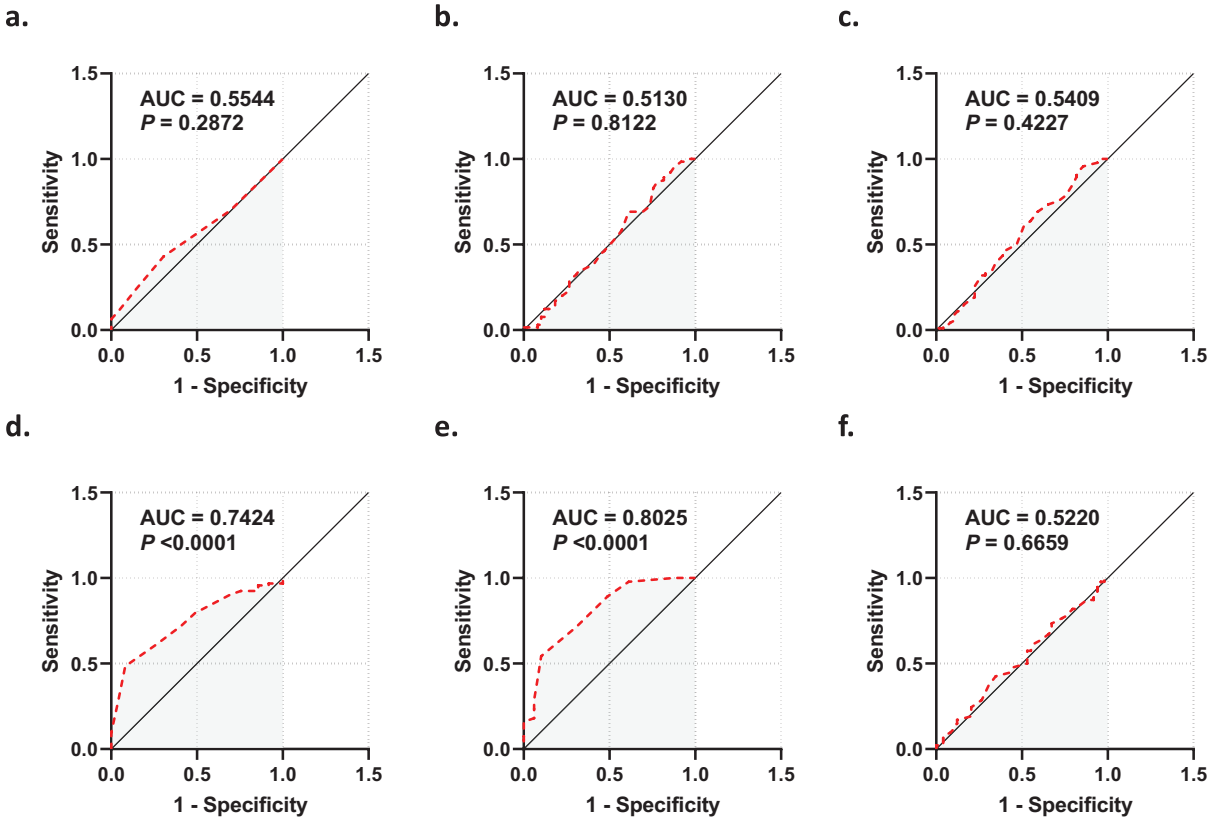

Fig. 4. Diagnostic accuracy of PUFA indices. ROC curves of (a) EPA, (b) DHA, (c) ω-3 index, (d) AA, (e) EPA/AA, and (f) ω-3/ω-6 ratio for NG and HG subjects.

reported that AA prevents streptozotocin-induced DM (Das 2017) possibly by alleviating dysregulated redox homeostasis and restoring NF-ƙB, tumor-necrosis factor-α, cyclooxygenase, nitric oxide, and Nrf2 to physiological levels (Das 2018). Notably, AA is converted to epoxyeicosatrienoic acids (EETs) through the cytochrome *P*-450 (CYP) pathway. EETs have been implicated in enhanced insulin sensitivity by activating nitric oxide synthase (Xu et al., 2011). In support of this protective role of EETs against DM, preserving the pool of available EETs, either by inhibiting or silencing epoxide hydrolase, prevents islet cell death, increases insulin secretion and sensitivity, and decreases circulating glucose levels (Luo et al., 2010, Zong et al., 2012).

It is important not to overlook the role of AA in promoting oxidative stress; an essential mechanism underlying DM. AA influences the activity of proton pumps which are central to reactive oxygen species production by NADPH oxidase (Sonnweber et al., 2018). This is detrimental as oxidative stress decreases insulin secretion and glucose uptake, and precipitates insulin resistance (Tallima and El Ridi 2018). In man, AA had an inverse association with the risk of DM (Wu et al., 2017) and coronary heart disease (Wang et al., 2003), and low blood levels of AA were negatively associated with diabetic nephropathy (Okamura et al., 2021). Although n-6 PUFAs improve insulin resistance and lower serum LDL-C, a large body of evidence indicates that n-6 PUFA metabolites participate in inflammatory damage and oxidative injury (Salas-Salvado et al., 2011, Poreba et al., 2018).

Unlike AA, EPA serves as a precursor to anti-inflammatory mediators, and compete with AA for cyclooxygenase and lipoxygenase (Nelson and Raskin 2019). Diminished EPA/AA ratio increases prostaglandin, interleukin, and thromboxane synthesis and release, and, for this reason, the EPA/AA ratio is used as a marker of chronic inflammation. Lower EPA/AA has also been demonstrated to predispose to higher cardiac risk (Itakura et al., 2011), coronary artery lesion (Hayakawa et al., 2012), acute coronary syndrome (Serikawa et al., 2014, Sakamoto et al., 2016), and chronic heart failure (Watanabe et al., 2016). In elderly diabetics, reduced EPA/AA was associated with coronary heart disease, impaired renal function, and angiopathy (Ito et al., 2014). Likewise, Elevated EPA/AA ratio in diabetics was associated with left ventricular wall thickness (Nelson and Raskin 2019). EPA/AA was superior than EPA or AA alone in predicting weight loss (Nakanishi et al., 2019), and this may be secondary to the anti-inflammatory role of EPA through adiponectins or to the release of glucagon-like peptide-1; both of which known to exert anti-obesogenic effects. Very recently, Soldavini *et al.* demonstrated that pregnant women with gestational diabetes who received ώ-3 FA supplements for 12 weeks had significantly higher EPA/AA ratio and lower platelet-activating factor levels, which increases the requirement for pharmacological intervention (Soldavini et al., 2022).

Resistin is an adipokine that aggravates inflammation and insulin resistance. Chiefly secreted from monocytes, resistin contributes to DM, atherosclerotic lesions, and cardiovascular disease. We have recently shown that the monocyte-lymphocyte ratio is disrupted in HG subjects (Alfhili et al., 2022b) which may be related to dysregulated resistin levels. Of note, resistin also have an inverse relation with EPA/AA ratio (Higashioka et al., 2020), further highlighting the importance of a balanced PUFA intake to combat inflammation.

Although the diagnostic utility of ώ-6/ώ-3 ratio is ill-defined (Harris 2006), a higher ratio is associated with an increased risk of coronary events. A ratio of 1:1 is ideal, but studies have found it to be alarmingly up to 15–20:1 in the Western diet (Simopoulos 2008, Husted and Bouzinova 2016). Since LA and ALA converge at Δ^6-desaturase (Simopoulos 2016); a common metabolic nexus of *de novo* PUFA synthesis, increased EPA/AA and ώ-6/ ώ-3 ratio may thus be attributed to compromised ALA metabolism.

The role of the intestinal microbiome in diabetes and related conditions has recently gained considerable interest (Li et al., 2020), as gut microbes influence key metabolic intermediates involved in glucose homeostasis including lipopolysaccharides (Yoo and Kim 2016) and short-chain FAs (Gholizadeh et al., 2019). Moreover, intestinal microflora are pivotal mediators of the consequences of dietary intake, and are able to alter PUFA metabolism and utilization. Interestingly, PUFAs have also been shown in numerous studies to modulate the gut microbiota and secreted hormones (Yu et al., 2014, Yan et al., 2016). In a recent report by Miyamoto *et al*., it was reported that 10-hydroxy-*cis*-12-octadecenoic acid synthesized by gut microbes from ώ-6 FAs antagonize obesity in mice (Miyamoto et al., 2019). Also, distinct microbial strata have been associated with γ-linolenic acid in subjects who developed DM (Miao et al., 2020). Burgeoning evidence thus necessitates the integration of microbiome studies with the risk of DM and related complications, most notably dyslipidemia and anemia (Alfhili et al., 2022c). Further characterization of novel compounds with anti-α-glucosidase activity is equally important (Hussain et al., 2022, Khan et al., 2022, Mumtaz et al., 2022).

Strengths of the current study include the negligible analytical variability in PUFA determination and the assessment of risk measures and diagnostic performance of each PUFA index. Limitations include the relatively small sample size, lack of anthropometric measurements, dietary and medication intake, and relevant history. Also, no cause-and-effect relationship could be established between HG and alterations in PUFA indices.

5. Conclusions

In conclusion, this report provides preliminary evidence of the perturbed AA, EPA/AA, and ώ-6/ώ-3 ratios in HG, which underscores their potential as biomarkers of glycemic control. Our study also highlights gender-specific disturbances in distinct PUFA species in Saudi subjects which warrants further interrogation. Future studies should elucidate the molecular basis upon which PUFAs modulate insulin sensitivity and energy turnover, and investigate novel inflammatory markers, such as gasdermins, and new modalities of cell death, including ferroptosis and cuproptosis, in relation to PUFA intake, indices, and glucose homeostasis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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