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Lack of oxidative stress in a selenium deficient area in Ivory Coast

Potential nutritional antioxidant role of crude palm oil

Summary *Background* Previous studies have described an important selenium deficiency in a mountain region (Glanle) in the west of Ivory Coast. *Aim of the study* To assess the antioxidant ca-

capacity of subjects from a selenium deficient area in Ivory Coast (Glanle region). *Methods* This study involved 57 subjects, 18 to 69 years old, living in the Glanle region and 56 healthy controls living in the southern coastal region (Bodou). In the Glanle region families consume basically a vegetarian and crude palm oil diet, whereas in the Bodou region, families eat a fish-based diet with principally refined palm oil. Fasting blood samples were collected to assess the following parameters: lipid status (plasma total lipids; total-, HDL- and LDL-cholesterol; triglycerides; phospholipids; fatty acid composition), plasma protein status (total protein, albumin, transthyretin, orosomucoid, CRP, transferrin), antioxidant capacity (plasma selenium, uric acid, retinol, α -tocopherol and tocotrienols levels, plasma seleno-glutathione peroxidase (GSHPx) activity) and oxidative stress markers (malondialdehyde (MDA) and advanced oxidation protein products (AOPP)). *Results* The mountain re-

gion samples (Glanle) were characterized by significantly lower plasma albumin, total-, HDL- and LDL-cholesterol, retinol and selenium levels, plasma PUFA content and GSHPx activity, but significantly higher α -tocopherol index and total tocotrienol level, than controls from the coastal area (Bodou). These results suggest a higher exposure risk to oxidative stress for the mountain region subjects. However, the absence of oxidative damage in this group provides evidence of a selenium independent protection mechanism against oxidative stress. This protection is related to lower plasma LDL cholesterol and PUFA content, and to higher α -tocopherol index, δ and total tocotrienols. *Conclusion* The long-term consumption of crude palm oil could be considered as an effective protective factor against oxidative stress.

Key words oxidative stress – selenium – palm oil – Ivory Coast

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Introduction

Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms [1]. This imbalance results in DNA damage and lipid and protein oxidation in blood and

membranes [2, 3]. Thus, oxidative stress is involved in the pathogenesis of atherosclerosis, neuro-degenerative diseases and cancer [3]. Cells have developed antioxidant defense mechanisms to prevent ROS formation and to limit their damaging effects. Nutrition plays a crucial role in antioxidant defense mechanisms by providing antioxidant vitamins (retinol, tocopherols, tocotrienols,

and ascorbic acid) and antioxidant trace elements such as selenium. Selenium, a biological trace element essential to human health, is involved in host oxidative defense by its incorporation into antioxidant enzymes as selenocysteine (SeCys), which is the redox active site of these antioxidant Se-containing enzymes, i. e. glutathion peroxidase and thioredoxine reductase. Selenium deficiency decreases the activity of these enzymes and increases oxydative stress; it can also lead to cell damage and apoptosis and is implicated in the etiology of cardiovascular diseases [4, 5]. Indeed, it is widely accepted that a low selenium serum concentration of $< 45 \mu\text{g/l}$ increases risk of cardiovascular disease and cancer [6]. Selenium deficiency in some regions of China, with extremely Se deficient soil, is the cause of endemic cardiomyopathy (Keshan disease) [7] and a type of osteoarthritis (Kahsin-Beck disease) [8]. In Ivory Coast, two authors [9, 10] have described an important selenium deficiency in the western region of the country (the Glanle region). However neither myocardiopathy nor Kahsin-Beck disease has been observed in this region. The aim of this study was to determine the nutritional status, antioxidant capacity and oxidative stress biomarkers of subjects from this region, compared with subjects from another, non-selenium deficient region (the Bodou region).

Subjects and methods

Subjects

This study involved 57 subjects from 18 to 69 years old (23 women and 34 men, mean age 38.5 ± 13.1 years, weight: 61.10 ± 6.47 kg) living in a remote mountain region in the west of Ivory Coast (the Glanle region, hereafter referred to as 'mountain region') and 56 healthy control subjects from 18 to 69 years old (24 women and 32 men, mean age 44.4 ± 16.3 years, weight: 68.76 ± 12.03 kg) living along the coast of Aby laguna in southern Ivory Coast, near Abidjan, the capital city (the Bodou region, hereafter referred to as 'coastal region'). In the mountain region families produce their own food and eat a mostly vegetarian and crude palm oil diet. In the coast region, families eat a fish-based diet and consume both crude and refined – but predominantly refined-palm oil. Subjects were chosen on a voluntary basis and the study was approved by the Medical Sciences University of Abidjan.

Anthropometry

Weight (precision 100 g) and height (precision 1 mm) were measured for each subject using trained personnel and standard procedures, and BMI (weight/height²) was

interpreted according to the same standard cut-off points for both sexes, i. e. $< 18.5 \text{ kg/m}^2$ for undernourished, $\geq 18.5 - < 24.9 \text{ kg/m}^2$ for normals, and $\geq 25.0 \text{ kg/m}^2$ for overweight and obese subjects [11].

Blood sampling

Blood samples (10 ml) were collected after an overnight fast of 8–10 hours by venipuncture in dry (5 ml) and heparinized (5 ml) vacutainer tubes (Choay laboratories, Paris, France). Samples were stored in a refrigerated box and immediately transferred to the biochemistry laboratory of the Medical Sciences University of Abidjan. The supernatant was isolated by centrifugation at 4000 rd/min for 15 minutes and aliquoted. Aliquots were stored at -80°C and carried by plane to the biochemistry laboratory of Lapeyronie hospital, Montpellier, France. Aliquots were kept frozen at -80°C prior to analysis.

Assays

Selenium plasma concentration was determined by electrothermal atomic absorption spectrometry using a Perkin-Elmer Zeeman model 600 device (Perkin-Elmer, Paris, France). Plasma seleno-glutathione peroxidase activity was measured by the Paglia and Valentine method [12] using Randox reagents (Randox laboratories, Paris, France) adapted on a spectrophotometric autoanalyzer Cobas Mira (Roche, Paris, France).

Retinol and alpha-tocopherol were measured in plasma by high performance liquid chromatography using a UV detector according to the Driskell et al. method [13]. Plasma alpha-tocopherol index was evaluated by the ratio of plasma alpha-tocopherol to plasma total-cholesterol (TC) + triglyceride (TG) [14]. Plasma tocotrienol levels were measured by HPLC adapted from the Tan and Brzuskiwicz method [15]. A Waters Model 510 HPLC pump equipped with a Waters Spherisorb ODS II, $3 \mu\text{m}$ column ($250 \text{ mm} \times 2 \text{ mm}$ internal diameter) (Waters SA, Paris, France) and an electrochemical detector (Precision Instrument, Marseille, France) were used. The mobile phase comprised 2% (v/v) distilled water in methanol with 3 g lithium perchlorate and 1 ml acetic acid in a final volume of 1000 ml. A $50 \mu\text{l}$ aliquot of plasma was measured into a microcentrifuge tube and deproteinized by adding $100 \mu\text{l}$ ethanol and $500 \mu\text{l}$ hexane. The mixture was vortex-mixed for 5 min and then centrifuged at 4000 rd/min for 8 min at 4°C . The hexane supernatant was evaporated under a stream of nitrogen. The residue was redissolved in $100 \mu\text{l}$ methanol and $5 \mu\text{l}$ of the solution was injected for chromatography.

Plasma level of malondialdehyde (MDA) was measured by the spectrofluorimetric method of Yagi [16]. The

plasma level of advanced oxidation protein products (AOPP) was assayed by spectrophotometry at 340 nm using a method derived from Witko-Sarsat et al. [17].

Serum lipids and lipoproteins (total cholesterol, triglycerides, phospholipids, and HDL cholesterol) were analyzed by colorimetric enzymatic methods using reagents purchased from Konelab (Konelab, Paris, France). LDL cholesterol was calculated by the Friedewald equation. Fatty acids of plasma total lipids were measured by gas chromatography [18]. In brief, lipids were extracted according to the method of Folch et al. [19]. A Fisons GC 8000 (Thermo Separation Products, Les Ullis, France) gas chromatograph equipped with a WCOT fused silica capillary column (50 m × 0.32 mm internal diameter, coated with 100 % cyanopropyl siloxane 88 phase; Chromopack, Les Ullis, France) was used. Fatty acid data are expressed as weight percentage of total identified fatty acids.

Serum albumin, transthyretin, orosomucoid, C-reactive protein (CRP) and transferrin were assayed by immunoturbidimetry, total protein by the biuret method and uric acid by the uricase PAP method, with an Olympus model AU2700 device using reagents and methods sold by the manufacturer (Olympus, Rungis, France).

Statistical analysis

Statistical analyses were carried out using EPI INFO 6. Data are expressed as the mean ± 1SD. The means of biochemical and anthropometric values of both samples were compared using the Mann-Whitney non-parametric test. The limit for statistical significance was set at $p < 0.05$. Logistic regression analysis between the mountain and the coastal subjects was performed selecting as independent variables the parameters that differed by $p < 0.05$ in the Mann-Whitney test. These parameters were adjusted for sex and age.

Results

Nutrition status

The mean (± SD) ages for the mountain and the coastal subjects were 38.5 ± 13.1 years vs 44.4 ± 16.3 years ($p = 0.06$) respectively. The mean (± SD) BMI of the mountain sample is significantly lower (22.8 ± 1.8 kg/m² vs 24.7 ± 3.6 kg/m² for the coastal subjects, $p < 0.001$). The distribution of BMI categories in both samples is reported in Fig. 1. No subject was underweight (BMI < 18.5 kg/m²) in either sample, while 28.5 % were overweight (BMI = [25–29.9]) and 10.7 % were obese (BMI > 30 kg/m²) in the coastal sample. The results summarized in Table 1 show that apart from triglycerides, other lipid levels were significantly lower in the mountain subjects

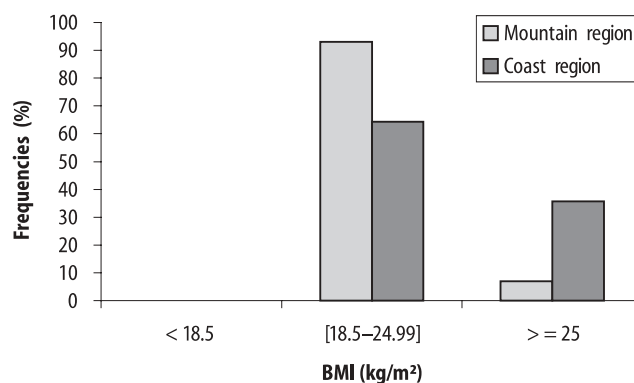


Fig. 1 Distribution of BMI in Mountain region and Coast region subjects

than in the coastal subjects. In both regions HDL concentration tends to be higher in women than in men (0.94 ± 0.28 versus 0.86 ± 0.24 and 1.29 ± 0.37 versus 1.23 ± 0.39 mmol/l in Glanle and Bodou respectively); however this difference does not reach significance. Hypcholesterolemia (< 4.1 mmol/l) is observed in both groups, but is more pronounced in the mountain sample (75 % vs 40 %, respectively).

Fatty acid analysis shows that the total lipid mono-unsaturated fatty acids (MUFA) percentage was higher in the mountain sample, and by contrast total lipid polyunsaturated fatty acids (PUFA), eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) percentages in the mountain subjects are lower than in the coastal ones.

The analysis of protein profile reported in Table 2 reveals that albumin, and total protein levels were low in the mountain subjects, compared with values of the coastal sample. No significant difference was observed for transthyretin, transferrin, orosomucoid and CRP levels between the two groups.

Antioxidant capacity

Plasma (mean ± SD) levels of vitamins are reported in Table 3. The retinol plasma level is lower in the mountain group ($p = 0.002$), but only one subject had a retinol level below the deficiency limit recognized by the World Health Organization (WHO) of $0.70 \mu\text{mol/l}$ [20]. No significant difference was observed between α -tocopherol plasma level in either group; however, the α -tocopherol/TC + TG ratio (alpha-tocopherol index) of the mountain sample is higher than that of the coastal sample (4.25 ± 0.64 vs 2.53 ± 0.56 , $p = 0.001$). Three isomers of tocotrienols (α , δ and γ isomers) have been identified in both groups of subjects (Table 3). The total tocotrienol plasma level was higher in the mountain sample compared to that of the other ($150.39 \pm 81.91 \text{ ng/ml}$ vs $94.26 \pm 55.03 \text{ ng/ml}$, $p = 0.001$). These tocotrienol

Table 1 Anthropometric characteristics, plasma lipid status and fatty acid composition of adults from the mountain and coastal regions

Parameters	Mountain region	Coastal region	P ₁	P ₂
Age (Years)	38.5±13.1	44.4±16.3	NS	
Weight (kg)	61.10±6.47	68.76±12.03	< 0.001	< 0.001
BMI (kg/m ²)	22.8±1.8	24.7±3.6	< 0.001	< 0.001
Total cholesterol (mmol/l)	3.24±0.59 3.2 (1.6–5.4)	4.19±1.07 4.1 (1.9–7.8)	< 0.001	< 0.001
Triglycerides (mmol/l)	1.20±0.54 1.1 (0.4–3.1)	1.10±0.52 1.0 (0.4–3.4)	NS	
Phospholipids (mmol/l)	2.09±0.31 2.0 (1.3–2.8)	2.41±0.61 2.25 (1.4–5.0)	< 0.001	< 0.001
HDL (mmol/l)	0.89±0.27 0.90 (0.2–1.6)	1.24±0.41 1.2 (0.1–2.7)	< 0.001	< 0.001
LDL (mmol/l)	1.80±0.67 1.7 (0.1–3.8)	2.43±0.85 2.25 (0.7–4.5)	< 0.001	< 0.001
SFA (%)	34.54±2.58 34.6 (28.4–43.6)	34.8±2.41 34.25 (30.6–41.2)	NS	
MUFA (%)	31.24±4.89 31.8 (20.5–40.8)	28.89±2.98 28.9 (23.3–36.2)	0.002	< 0.001
PUFA (%)	34.17±5.68 35.0 (17.8–46.6)	36.22±4.18 36.6 (25.9–43.5)	0.04	< 0.001
EPA (%)	0.41±0.24 0.3 (0.1–1.4)	2.30±1.09 2.1 (0.7–5.8)	< 0.001	< 0.001
DHA (%)	1.35±0.57 1.3 (0.6–3.5)	4.30±1.06 4.3 (2.6–7.3)	< 0.001	< 0.001

Values are means ± SD, medians and range. HDL high density lipoprotein; LDL low density lipoprotein; SFA saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; EPA eicosapentanoic acid; DHA docosahexanoic acid

(%): Fatty acid data are expressed as weight percentage of total identified fatty acids

P₁ p-value when differences tested by the nonparametric Mann-Whitney test

P₂ p-value after adjustment by age and sex (logistic regression)

Table 2 Comparison of the plasma protein status of subjects from the mountain and coastal regions

Parameters	Mountain region	Coastal region	P ₁	P ₂
Albumin (g/l)	33.07±7.36 34.0 (15.0–48.0)	43.23±6.30 44.0 (18.0–53.0)	< 0.001	< 0.001
Transthyretin (g/l)	0.17±0.11 0.2 (0.0–0.4)	0.18±0.08 0.2 (0.0–0.08)	NS	
Orosomucoid (g/l)	0.65±0.21 0.6 (0.3–1.2)	0.57±0.26 0.6 (0.2–1.7)	NS	
CRP (g/l)	5.06±10.09 1.9 (0.3–122.8)	4.90±16.43 1.6 (0.2–69.6)	NS	
Transferrin (g/l)	2.21±0.46 2.2 (0.9–3.0)	2.31±0.57 2.3 (0.9–3.8)	NS	
Total protein (g/l)	74.42±10.96 76.0 (47.0–95.0)	80.94±9.45 81.5 (52.0–101.0)	< 0.001	< 0.001

Values are means ± SD, medians and range

P₁ p-value when differences tested by the nonparametric Mann-Whitney test

P₂ p-value after adjustment by age and sex (logistic regression)

plasma levels were nevertheless lower than α -tocopherol levels in both samples. Selenium status data are reported in Table 4. These results show that the selenium plasma level and the glutathion peroxidase activity are lower in the mountain region sample, all the subjects being selenium deficient, while none were deficient in the coastal region sample.

■ Oxidative stress biomarkers

No significant difference was observed for oxidative stress biomarkers between the two groups (Table 4).

Table 3 Plasma antioxidant parameters in adults from the mountain and coastal regions

Parameters	Mountain region	Coastal region	P ₁	P ₂
Retinol (μmol/l)	1.53±0.45 1.5 (0.8–2.8)	1.8±0.57 1.75 (0.5–4.1)	0.002	< 0.01
α-tocopherol (μmol/l)	18.68±3.81 18.6 (12.3–27.6)	19.10±5.33 18.3 (11.6–24.4)	NS	
Tocopherol Index	4.25±0.64	2.53±0.56	< 0.001	< 0.001
δ-tocotrienol (ng/ml)	137.05±72.14 123.4 (24.9–332.3)	81.98±49.37 71.2 (15.0–270.1)	< 0.001	< 0.001
γ-tocotrienol (ng/ml)	11.57±17.57 5.9 (0.0–83.6)	10.42±10.00 6.35 (0.0–56.2)	NS	
α-tocotrienol (ng/ml)	1.76±2.22 0.9 (0.0–11.7)	1.84±2.03 1.1 (0.0–10.4)	NS	
Total tocotrienol (ng/ml)	150.39±81.91 138.9 (26.1–382.0)	94.26±55.03 83.9 (25.5–295.0)	< 0.001	< 0.001
Selenium (μmol/l)	0.37±0.21 0.3 (0.1–1.1)	1.80±0.31 1.8 (1.0–2.2.6)	< 0.001	< 0.001
GSH Px activity (UI/l)	352.31±176.12 247.0 (96.0–697.0)	812.17±310.21 748.0 (517.0–1527.0)	< 0.001	< 0.001
Uric acid (μmol/l)	233.87±63.68 236.0 (96.0–376.0)	251.03±82.75 247.0 (121.0–439.0)	NS	

Values are means ± SD, medians and range

GSH-Px glutathion peroxidase

P₁ p-value when differences tested by the nonparametric Mann-Whitney test

P₂ p-value after adjustment by age and sex (logistic regression)

Table 4 Oxidative stress biomarkers in adults from the mountain and coastal regions

Parameters	Mountain region	Coastal region	P ₁
MDA (μmol/l)	1.00±0.87 1.0 (0.3–1.5)	0.99±0.25 1.0 (0.5–1.5)	NS
AOPP (μmol/l)	34.18±17.44 28.7 (12.5–109.7)	39.49±21.27 35.55 (7.8–122.8)	NS

Values are means ± 1SD, medians and range

P₁ p-value when differences tested by the nonparametric Mann-Whitney test

Discussion

■ Nutrition status

BMI is a good indicator of nutritional status that permits the identification of underweight, overweight and obesity in a population. According to the WHO classification reported in Fig. 1, overt undernutrition is absent in both samples, although BMI, as well as serum albumin, retinol, and total protein levels are low in the mountain subjects. The adults from the coastal region are more inclined to overweight and obesity, which may be a consequence of dietary change in this rural population resulting from its proximity to the capital Abidjan. By contrast, the subjects sampled in the mountain region were located far from an urban area, in an isolated mountain and forest area. Their low serum albumin level was not associated with inflammation or hypo-

tranthyreteinemia. Since an inflammatory component could be ruled out, this hypoalbuminemia might reflect a low protein intake in this population. Our results indicated the existence of hypocholesterolemia in both the mountain and the coastal adults. Indeed, the levels of total cholesterol were lower in both groups than were the levels generally observed in healthy black African subjects [21, 22]. This hypocholesterolemia was more pronounced however in the mountain region. The mechanisms responsible for hypocholesterolemia are not yet well established. Despite controversy [23], inflammation and malnutrition have been suggested as the predominant causes of hypocholesterolemia [24, 25]. If an inflammatory origin appears as unlikely in our study, lower protein and calorie intakes could be a possible explanation for hypocholesterolemia as well as hypoalbuminemia in the mountain subjects. Moreover, an hypocholesterolemic effect of palm oil has been observed previously [26] and could account for the observed low cholesterol. The hypocholesterolemic property of palm oil is due to the relative neutrality towards cholesterolemia of the most important saturated fatty acids (palmitic acid (44 %) and stearic acid (5 %)) as shown by several authors [27, 28]. Another factor is the presence of high levels of tocotrienols, the unsaturated analogues of tocopherols in palm oil [29–31]; it has been shown that tocotrienols exert a significant hypocholesterolemic effect in human and animal models by decreasing HMG CoA reductase activity, as confirmed by Theriault et al. [32]. The fatty acid composition of one's usual diet is

generally thought to be reflected in the pattern of serum fatty acids [33, 34]. In this study, we observed a significant difference in the serum fatty acid composition between both samples, especially EPA and DHA correspond with the fish and vegetarian diets observed respectively in the coastal and the mountain regions. Indeed, the same difference in fatty acid composition between fish-based and vegetarian diets has been found in Tanzania [35].

■ Antioxidant capacity

Oxidative stress arises when antioxidant systems fail. In this study selenium status, glutathione peroxidase (GSHPx) activity and alpha-tocopherol, retinol, tocotrienols, uric acid levels in blood have been evaluated in order to estimate the antioxidant capacity of both groups of subjects. Our results show a selenium deficiency and a lower GSHPx activity in the mountain region sample. These results confirm those of J. Arnaud et al. [10], and strongly support the hypothesis that the lower GSHPx activity in these subjects is the consequence of a long-term selenium deficiency. Selenium deficiency might reflect the low selenium content of the soil in this region as well as a low selenium content of drinking water as indicated by Kouame [9]. On the other hand both mountain and coastal region subjects have low levels of plasma alpha-tocopherol when compared with usual laboratory norms ($29.5 \pm 5 \mu\text{mol/l}$). Such relative deficiencies in alpha-tocopherol were reported in other African populations [36, 37]. However in the mountain region, the alpha-tocopherol index appears higher than in the coastal region subjects. This difference results from the lower cholesterol level observed in the mountain group. Total tocotrienol levels were also higher in this group, in likely relation to their exclusive consumption of crude palm oil. By contrast, coastal region subjects consume both crude and refined palm oil, but predominantly refined oil. Refined palm oil does not contain significant amounts of tocotrienols, whereas these antioxidants remain in crude palm oil [26]. The palm oil consumed is poor in tocopherols and rich in tocotrienols; however in our two groups of subjects the reverse was observed, i.e. a higher tocopherols:tocotrienols ratio. This could be related to the low affinity of tocotrienols with the alpha tocopherol protein transfer [38, 39]. However, Hayes et al. [40] have detected tocotrienols in Hamster tissues after palm oil intake. They suggested that plasma tocotrienol levels were low because these tocopherol analogues rapidly penetrate into

cells. Noguchi et al. [41] have shown that after exposure of HUVECs to $0.5 \mu\text{M}$ or $5 \mu\text{M}$ vitamin E analogs for 24 hours the intracellular concentrations of tocotrienols were higher than tocopherol concentrations. These findings might also explain the low plasma concentration of tocotrienols in our study population. Considering the Se-dependent parameters (plasma selenium and GSHPx activity), mountain subjects seemed to be more exposed to oxidative stress. However, the other antioxidant parameters (tocopherol index, retinol, tocotrienols, uric acid) do not support this point of view. To explore this question, oxidative stress biomarkers were compared in both groups.

■ Oxidative stress biomarkers

To evaluate the oxidative stress effects, MDA and AOPP were assayed. MDA is known as a lipoperoxidation marker [16] and AOPP as a protein oxidation marker [17]. The results of MDA and AOPP analysis, as summarized in Table 4, clearly show that there was no more oxidative stress damage in the mountain region subjects than in the coastal subjects. By contrast a survey of residents in endemic and non-endemic areas of Keshan disease, in China, showed that blood selenium content and GSHPx activity were lower while the plasma lipid peroxide concentration was higher in endemic areas compared to non-endemic areas [42]. The absence of oxidative stress in our mountain subjects could be explained by the antioxidant actions of the palm oil tocotrienols [43, 44] and by the lower PUFA percentage observed in this group. Our results might also indicate that the level of alpha-tocopherol found in this mountain region is enough to protect against oxidative stress.

In summary, the mountain region subjects were characterized by significantly lower plasma levels of selenium, GSHPx activity, HDL cholesterol and albumin than the coastal region subjects, suggesting a higher exposure risk to oxidative stress. However, the absence of oxidative stress damage in our mountain adults when compared to coastal subjects provides evidence of an effective protection resulting, on the one hand, from significantly reduced risk factors, i.e. lower plasma LDL and PUFAs and higher MUFAs, and, on the other hand increased protection by higher alpha-tocopherol index, delta and total tocotrienols. Thus the long-term consumption of crude palm oil could be regarded as a protective factor against oxidative stress. If confirmed, this finding could be considered in regions where populations are submitted to high oxidative stress.

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