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Palm oil and cardiovascular disease: a randomized trial of the effects of hybrid palm oil supplementation on human plasma lipid pattern

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This study examines, for the first time, the effect of hybrid *Elaeis oleifera* x *E. guineensis* palm oil supplementation on human plasma lipids related to CVD risk factors. One hundred sixty eligible participants were randomized and assigned to one of the two treatments: 25 mL hybrid palm oil (HPO group), or 25 mL extra virgin olive oil (EVOO group) daily for 3 months. Fasting venous samples were obtained at baseline and after 1, 2 and 3 months for measurement of plasma lipids (TC, LDL-C, HDL-C and TAGs). Changes in body mass index and waist circumference were also assessed. Although there was an overall reduction in TC (7.4%, $p < 0.001$) and in LDL-C (15.6%, $p < 0.001$), no significant differences were found between the treatment groups in a repeated measures analysis of variance for TC ($p = 0.0525$), LDL-C ($p = 0.2356$), HDL-C ($p = 0.8293$) or TAGs ($p = 0.3749$). Furthermore, HPO consumption had similar effects on plasma lipids to EVOO, thus providing additional support for the concept that hybrid *Elaeis oleifera* x *E. guineensis* palm oil can be seen as the “tropical equivalent of olive oil”.

Introduction

Cardiovascular disease is the main cause of death worldwide at the turn of the XXI century. Western countries continue to exhibit high rates of cardiovascular morbidity and mortality, whilst these diseases constitute emerging and neglected epidemics in developing countries.^{1,2} With regard to the quality of dietary fat in relation to cardiovascular disease (CVD), a large number of studies have demonstrated the beneficial role of diets with relatively high monounsaturated fatty acid (MUFA) content on CVD risk factors, obesity and diabetes. These beneficial MUFA are mainly provided by the Mediterranean Diet and, specifically, by olive oil.³⁻⁵ On the other hand, diets rich in saturated fatty acids (SFA) have long been associated with increased plasma cholesterol concentrations and hence increased risk of cardiovascular disease. In 2003, the World Health Organization stated that there is convincing evidence that palmitic acid-rich oils consumption contributes to an increased risk of developing cardiovascular diseases. Additional evidence suggests that a

dietary intervention that reduces SFA intake and increases consumptions of vegetables, fruits and grains may reduce the development of diseases such as cancer and atherosclerosis.^{6,7} In this context, palm oil (PO), which is mainly extracted from the fruit of the African oil palm (*Elaeis guineensis* Jacq.), has so far been strongly attacked due to its high content of SFA, specifically palmitic acid (C16:0, ~44.3%), and its use in food products has been somewhat discouraged. However, in recent years there has been a lot of debate regarding the potential role of SFA in the pathogenesis of atherosclerosis and CVD and a more complex picture concerning the risk factors for CVD has now been developed. In fact, current evidence does not clearly support cardiovascular guidelines that encourage high consumption of polyunsaturated fatty acids and low consumption of total saturated fats.⁸ For instance, Mensink *et al.*⁹ have shown that myristic and palmitic acids had little effect on the ratio of total to HDL cholesterol, and stearic acid reduced the ratio slightly. More recently, a meta-analysis also showed that PO may produce both favorable and unfavorable changes compared with the other primary dietary SFA, MUFA and polyunsaturated (PUFA) fatty acids, and almost no changes were observed in total cholesterol/HDL cholesterol and LDL cholesterol/HDL cholesterol ratios.¹⁰ To date in Colombia more than 25000 hectares of oil palm interspecific hybrid *Elaeis oleifera* x *E. guineensis* has been planted. From an agronomic point of view, the good bud rot tolerance of the hybrids, compared to *E. guineensis*, justifies its use to replant severely decimated zones. However, the oil obtained from hybrid palm fruits may also have unexplored functional properties and health benefits, especially in

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reducing cardiovascular disease risk. In fact, in our recent study we fully characterized, for the first time, the composition and structure of triacylglycerols (TAGs) and partial glycerides of crude palm oil obtained from interspecific hybrid *E. oleifera* x *E. guineensis* grown in Colombia.¹¹ The study revealed interesting nutritional properties of oils obtained from interspecific hybrid palm compared to the oils from African palm oil. Although, only four fatty acids types (palmitic, C16:0; stearic C18:0; oleic C18:1; linolenic, C18:2) constituted more than 95% of total fatty acids of both oils, the hybrid palm oil had a higher percentage of oleic acid (54.6±1.0 vs 41.4±0.3) together with a lower palmitic (28.3±1.0 vs 40.1±0.1) and stearic (2.8±0.3 vs 5.0±0.1) acids amounts. The percentage of the essential fatty acid, linoleic acid, do not differ significantly. Furthermore, the *sn*-2 position of TAGs in hybrid palm oil (HPO) was shown to be predominantly esterified with oleic acid with only 10-15% of total palmitic acid and 6-20% of stearic acid acylated in the secondary position. These findings are very interesting, especially from a nutritional point of view since fatty acids in the *sn*-2 position of dietary triacylglycerols are preferentially absorbed through the intestinal wall. Therefore, our results demonstrate that a partial dietary replacement of saturated palmitic acid with monounsaturated oleic acid can be obtained by consuming crude HPO instead of African palm oil, without the need for any fractionation process to separate olein and stearin fractions because of HPO higher degrees of unsaturation. This also means that crude HPO could represent an extremely rich source of health-promoting minor components, since such bioactive compounds are not lost during the refining and fractionation processes required for African PO. Therefore, beside the interesting composition and structure of triacylglycerols, HPO can also provide substantial amount of antioxidants that might additionally contribute to lower the risk of developing CHD and certain cancers.¹² In fact, our latest studies^{13,14} revealed that HPO unsaponifiable matter is characterized by high content of tocotrienols (4.5 ± 1.4 mg of α -tocotrienol, 0.4 ± 0.1 β -tocotrienol, 14.8 ± 2.3 mg of γ -tocotrienol, 3.2± 0.4 mg of δ -tocotrienol per 100 g oil) with tocopherols, mainly entirely constituted of the α isomer, that accounted for 2.7 mg ± 0.7 per 100g oil. These results highlight another interesting peculiarity of HPO, since tocotrienols are now well recognized for their superior antioxidant activity compared with tocopherols.

The aim of the present paper was to investigate, for the first time, the health benefits of hybrid palm oil consumption and its potential as a functional food oil for the prevention of CVD. In a randomized trial we compared the effects of supplementation with crude hybrid palm oil (*E. oleifera* x *E. guineensis*) and extra-virgin olive oil, which is universally recognized as putative anti-atherogenic and cardioprotective oil, on human plasma lipids related to cardiovascular disease risk.

Materials and Methods

Clinical trial design

Participant Enrollment

Eligible participants were community-dwelling men and women, aged ≥ 50 years. The selection of subjects was based on the following inclusion criteria: 1) signed written informed consent; 2) stable dose of statins (for those receiving statin therapy). Exclusion criteria were: desire to not participate in the study, prior cardiovascular disease, any severe chronic illness, drug or alcohol addiction, and history of allergy to olive or palm oils. Enrollment was performed at the Polideportivo La Andrea, in the locality of USME (Bogotá - Colombia). We preselected volunteers whose clinical record, physical examination, and blood pressure were strictly normal. Eligible subjects provided written informed consent and were then randomized to one of 2 supplementation groups and scheduled for the baseline visit (see intervention section). The study was conducted in accordance with the guidelines from the Declaration of Helsinki, and all procedures involving human participants were approved by the institutional ethics committee of the Pontificia Universidad Javeriana (Acta # 11; 21-06-2011).

Intervention and measurements

Participants ($n=160$) were randomly assigned to a rich extra-virgin oil (EVOO) diet ($n=82$), or hybrid palm oil-rich (HPO) diet ($n=78$). Enrolled patients were assigned according to a computer-generated randomization scheme. Trained dieticians from the Department of Nutrition and Biochemistry, Faculty of Sciences (Pontificia Universidad Javeriana) were responsible for all aspects of the intervention. The duration of the supplementation trial was 3 months. Participants received nutritional education on either HPO or EVOO. During the intervention period, the participants were required to take 25mL/day of crude HPO or EVOO, depending on the group assignment. Dosing containers were delivered to the participants at the beginning of each intervention period. Oil supplements were incorporated into ready meals consumed throughout the day. The volunteers were advised to maintain their usual diet. Individual visits were repeated every 1 month. Each baseline and follow-up visit included: *i.* A simplified assessment of adherence to the supplementation diet; *ii.* 24-hour recall and food frequency questionnaire with foods plus vitamin supplements and alcohol consumption¹⁵; *iii.* Physical activity questionnaire; *iv.* Measurement of weight, height, and waist perimeter (only at baseline and Visit 3). *v.* Collection of fasting blood samples after an overnight fast of 12 hours and preparation of serum, plasma; *vi.* A general questionnaire collecting information on medication use; *vii.* Oil delivery.

Laboratory methods

Chemical characterization of the oils used as supplement

HPO was kindly provided by the Hacienda La Cabaña (Bogotá, Colombia) whereas EVOO was purchased from local supermarket. Total FA composition of the oils were

Table 1. Total fatty acid profile, unsaponifiable matter composition and total phenolic content of hybrid palm oil (HPO) and extra-virgin olive oil (EVOO) used as dietary oil supplements.

	EVOO	HPO
Fatty acids (%)		
C16:0	12.5±0.8	27.9±1.2
C16:1	0.9±0.1	0.5±0.1
C18:0	2.5±0.4	2.9±0.3
C18:1 ^{Δ9c}	76.0±1.2	55.2±1.3
C18:2 ^{Δ9c,12c}	5.5±0.8	10.8±0.5
C18:3 ^{Δ9c,12c,15c}	0.6±0.1	0.4±0.0
C20:0	0.4±0.2	0.3±0.1
C20:1 ^{Δ11}	0.3±0.1	0.4±0.2
ΣSFA	15.4±0.8	30.8±1.6
ΣMUFA	77.2±1.2	56.1±1.0
ΣPUFA	6.1±0.4	11.1±0.7
Unsaponifiable components (mg/100g oil)		
Squalene	321± 8.6	24.7 ± 0.3
Campesterol	1.1 ± 0.4	9.8 ± 0.3
Stigmasterol	2.9± 0.1	8.2 ± 1.0
β-sitosterol	127± 11.4	39.1 ± 3.3
Δ ⁵ -avenasterol	21.3± 2.8	1.7 ± 0.2
n-Alkanols	13.2±1.4	6.2 ± 1.7
4-methylsterols	20.7±2.1	1.3 ± 0.2
4,4-dimethylsterols	135.6±9.5	7.4 ± 1.2
Tocols*	18.1±1.5	25.9 ± 4.8
Total phenolic content (ppm)		
	154±4.6	190±2.5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Legend for fatty acids - m:n Δx, m = number of carbon atoms, n = number of double bonds, x = position of double bonds. *Tocols = sum of α-Tocopherol, α-Tocotrienol, β-Tocotrienol, γ-Tocotrienol and δ-Tocotrienol contents

determined by gas chromatography with flame ionization detection (GC-FID) of fatty acid methyl esters, which were prepared by sodium methoxide-catalyzed transesterification of oil samples, as described in Christie.¹⁶ Details of instrumental conditions were reported in Mozzon *et al.*¹¹

The oil unsaponifiable matter was analysed according to the procedure described in Lucci *et al.*¹⁴

The extraction of the phenolic fraction from the oils was performed as reported by Montedoro *et al.*¹⁷ whereas the total phenolic content of the oils was determined according to the procedure reported by Loizzo *et al.*¹⁸ The results were expressed as gallic acid equivalents (mg/kg oil) based on the calibration curve ($R^2=0.996$). The estimation of total phenol content was carried out in triplicate and the results were averaged.

The total fatty acid profile, the unsaponifiable matter composition and the total phenolic content of the oils are shown in **Table 1**. It is noteworthy to underline that the amount of antioxidant compounds provided by HPO supplementation was similarly to that provided by EVOO supplementation. In fact, the dose of EVOO (25 ml/day) corresponded to a daily intake of tocopherols and phenols of 4.5 and

38.5 mg/day, respectively. Similarly, the dose of HPO (25 ml/day) corresponded to a daily intake of tocopherols and phenols of 6.5 and 47.5 mg/day, respectively. Once again, it is important to mention that in HPO tocotrienols constitute ~90% of the total tocopherols.

Blood sample collection and storage

Blood sampling was performed in the morning after a 12-hour overnight fast. Samples were drawn into EDTA tubes and centrifuged at 4°C to separate cells from plasma. Plasma samples were then stored at -80°C for later analyses.

Plasma analytical procedures

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triacylglycerols (TAGs) were measured by colorimetric methods using Spinreact kits (Spinreact). LDL-C was calculated by the Friedewald formula for subjects with triacylglycerol <400 mg/dL.

Sample Size and Power Calculation

The number of subjects was calculated to detect a difference of 15% between the two groups in plasma lipids that are clinically relevant in the improvement of CVD risk, assuming a standard deviation of 1.5, with $\alpha=5\%$ and a power of 80% (1-beta=0.8). Given these constraints, 75 evaluable subjects per group (150 in total) were required. To allow for drop-outs, a total of 160 subjects were recruited.

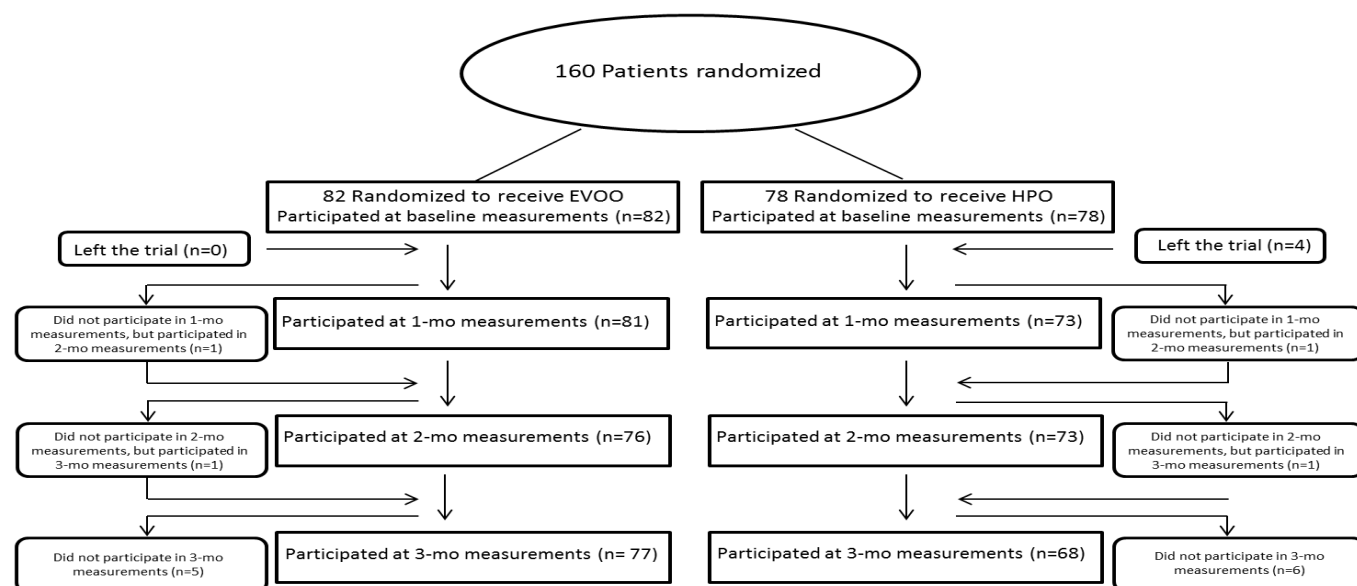
Statistical Analyses

Comparisons between the two groups were evaluated using Student's t-test for paired samples. A p value of less than 0.05 (2-sided) was adopted to determine significant differences. For the differences between treatments, taking into account the visits, repeated measures ANOVA was performed, with the baseline values as covariates. Interactions were considered between treatment and visits, and sex, age and BMI were also explored for effects. A p value <0.05 was considered significant. The statistical analyses were carried out with the STATA 13.0 software (StataCorp LP).

Results

The subjects' baseline characteristics are presented in **Table 2**. At baseline, there was no significant difference ($p<0.05$) between the group randomized to HPO or EVOO for any of the plasma measurements (TC, HDL-C, LDL-C, TAGs), body mass index (BMI) and waist perimeter. The mean ages of the HPO subjects and EVOO subjects were 64.3±8.5 and 62.8±6.1 years, respectively. The mean BMI in the study population was 28.3±3.8 with a waist circumference of 87.9±9.1. Analysis of the participants' lipid profile at the start of the trial (baseline) indicated that subjects had normal to high LDL-C, TC and TAGs

Figure 1. Study flow diagram



values. The mean TC values of the HPO subjects and EVOO subjects were 206.5 ± 39.1 and 203.8 ± 36.9 mg/dL, respectively, whereas average population levels of 120.4 ± 38.2 mg/dL for LDL-C and of 44.9 ± 12.4 mg/dL for HDL-C were found. Overall, more than 35% of the population had hypercholesterolemia (LDL-C >130 mg/dL) and more than 43% showed high levels of triglycerides (TAGs >200 mg/dL). Data from 24-hour recall and food frequency questionnaires showed no significant difference in the baseline and follow-up dietary intake of total energy, protein, total fat, carbohydrate, and alcohol between the two study groups (supplementary material 1). 11/160 (6.8%) patients did not complete the trial (Figure 1). Four subjects (2.5%) dropped out during the first month (HPO group) as they were unwilling to continue to participate in the study. In each group, one subject did not attend the visit planned for the first or second assessment (1-mo and 2-mo measurements). However, these participants were included in the analyses as they continued to participate in the

assessments at 2-mo and 3-mo. The dropout rates observed after three months of oil supplementation (3-mo measurements) were 6.1% ($n=5$) and 7.7% ($n=6$) for EVOO and HPO groups, respectively. We found no evidence of any adverse effects related to the oils.

As we can see from Table 3, there were final differences for all fractions, although significant only in the univariate analysis (considering only the treatment effect and not interactions) for TC and for LDL-C levels (Baseline vs Visit 3).

In general, there was an initial rise, for both treatments, in TC, LDL-C and HDL-C levels, followed by reductions for both groups (Figure 2). These results are confirmed by the changes observed throughout the experiment in the overall population (Table 4). A possible explanation for this finding is that in the case of insulin and leptin resistance, monounsaturated oils supplementation increases liver fat content, that may cause an increased release of lipids from the liver to prevent fatty liver development.¹⁹ As a result, a temporary increase in serum

Table 2. Baseline demographic and clinical characteristics of study subjects.

Variable	Baseline population	EVOO (n = 82)	HPO (n = 78)	EVOO vs HPO p value
Age in years – mean (S.D.)	63.5 (7.2)	62.8 (6.1)	64.3 (8.5)	0.3591
Gender (% Male)	13 (8.1%)	6 (7.4%)	7 (8.9%)	0.0710
Body mass index – mean (S.D.)	28.3 (3.8)	28.1 (3.7)	28.5 (3.8)	0.4148
Waist perimeter – mean (S.D.)	87.9 (9.1)	87.2 (8.8)	88.6 (9.4)	0.3405
Hypercholesterolemia (%)	56 (35.2%)	26 (32.1%)	30 (38.4%)	0.3738
Hypertriglyceridemia (%)	70 (43.8%)	43 (53.0%)	27 (34.6%)	0.5802
Cholesterol Total – mean (S.D.)	205.1 (37.9)	203.8 (36.9)	206.5 (39.1)	0.6429
Cholesterol, HDL – mean (S.D.)	44.9 (12.4)	43.5 (10.4)	46.3 (14.2)	0.1689
Cholesterol, LDL – mean (S.D.)	120.4 (38.2)	116.9 (38.1)	124.0 (38.1)	0.2464
Triglycerides – mean (S.D.)	218.4 (128)	242.0 (155.6)	194.9 (85.7)	0.0698

Sample sizes in parenthesis, S.D.= standard deviation.

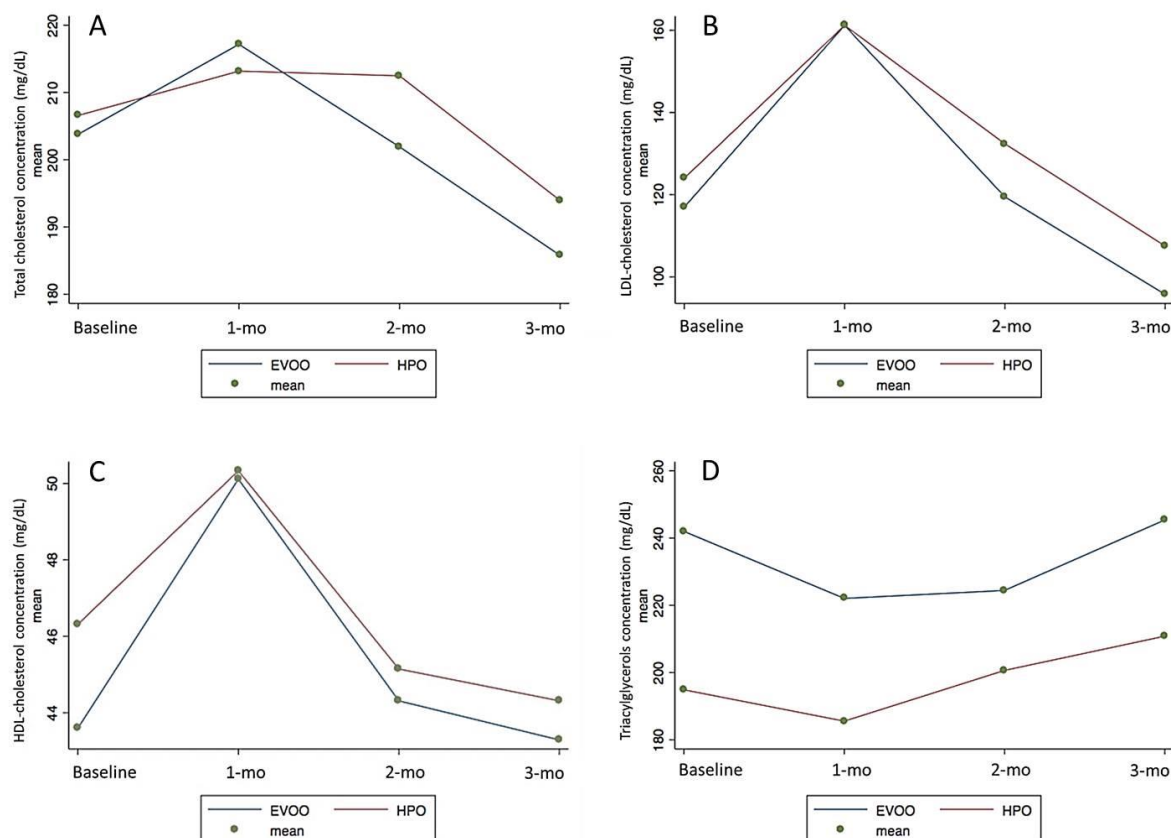
EVOO= extra-virgin olive oil

HPO= hybrid palm oil

lipids could be observed.

In HPO group, the initial level of TAGs (194.9 ± 85.7 mg/dL) remained relatively unchanged with a final 3-mo concentration of 210.8 ± 136.4 mg/dL. The same trend has been observed in EVOO group with TAGs values of 242.0 ± 155.6 mg/dL and 245.6 ± 122.9 mg/dL at baseline and month 3, respectively. In subjects who received HPO, the HDL-C concentration increased by 6.1% at month 1, followed by a decrease gradually leading to the final value (3-mo) of 44.3 ± 17.9 mg/dL (**Table 3; Figure 2C**). A similar pattern was observed in the EVOO group with plasma HDL-C levels of 43.5 ± 10.4 and 43.2 ± 16.6 , respectively, at the beginning and the end of the

study. After 1 month of HPO supplementation, plasma LDL-C concentration was higher than at baseline (124.0 ± 38.1 mg/dL compared with 161.2 ± 34.7 mg/dL). At month 2, however, LDL-C decreased to 132.3 ± 36.1 mg/dL, and this trend continued over the next month reaching a final 3-mo value of 107.2 ± 36.4 (**Figure 2B**). Therefore, during the study period, LDL-C concentration in patients receiving HPO decreased 13.3%. Furthermore, it should be stressed that reduction in LDL-C of HPO subjects was similar to that observed in the patients who received EVOO. In fact, the EVOO group showed a decrease in LDL-C from 116.9 ± 38.1 mg/dL at baseline to 95.7 ± 33.8 mg/dL at the end of the study. In HPO

Figure 2. Changes in plasma lipid levels in both groups throughout the experiment. (A) TC levels; (B) LDL-C levels; (C) HDL-C levels; (D) TAGs levels

patients, plasma TC concentrations decreased from baseline (206.5±39.1 mg/dL) to month 3 (193.4±31.5 mg/dL), a reduction of 6.3%. Again, as with LDL-C, a small increase of TC level was observed at both month 1 and month 2: the TC level

Table 3. Changes in plasma lipids during the 3-months of EVOO or HPO oil supplementation

Variable	Baseline [†]		Visit 1		Visit 2		Visit 3		Baseline vs Visit 3 p value [#]		p value*
	EVOO (n=82)	HPO (n=78)	EVOO (n=81)	HPO (n=73)	EVOO (n=76)	HPO (n=73)	EVOO (n=77)	HPO (n=68)	EVOO	HPO	
Body mass index	28.1 (3.7)	28.5 (3.8)	NA	NA	NA	NA	27.7 (4.1)	28.3 (3.3)	--	--	0.2185
Cholesterol Total	203.8 (36.9)	206.5 (39.1)	217.2 (35.4)	213.2 (33.1)	201.9 (35.3)	212.5 (34.2)	185.8 (28.4)	193.9 (31.5)	●	○	0.0525
Cholesterol, HDL	43.5 (10.4)	46.3 (14.2)	50.11 (14.1)	50.3 (16.6)	44.3 (17.5)	45.1 (14.4)	43.2 (16.6)	44.3 (17.9)	--	--	0.8293
Cholesterol, LDL [‡]	116.9 (38.1)	124.0 (38.1)	161.1 (38.2)	161.2 (34.7)	119.4 (36.6)	132.3 (36.1)	95.7 (33.8)	107.2 (36.4)	●	○	0.2356
Triglycerides	242.0 (155.6)	194.9 (85.7)	222.1 (111.4)	185.5 (83.3)	224.4 (110.5)	200.6 (87.8)	245.4 (122.9)	210.8 (136.5)	--	--	0.3749
Total:HDL cholesterol	4.97 (1.89)	4.71 (1.20)	4.61 (1.31)	4.61 (1.56)	5.09 (1.75)	5.22 (1.99)	5.34 (2.98)	5.36 (2.48)	--	--	0.4031
LDL:HDL cholesterol	2.91 (1.63)	2.84 (1.06)	3.48 (1.37)	3.58 (1.59)	3.58 (1.59)	3.11 (1.53)	2.93 (2.22)	3.18 (2.87)	--	--	0.5344

Sample sizes in parenthesis; Data are expressed as mean (standard deviation).

EVOO= extra-virgin olive oil

HPO= hybrid palm oil

NA=not assessed

[†] Number of participants who were receiving stable doses of statin across the study: EVOO (n=37), HPO (n=39).

[‡] Number of participants for whom LDL-C levels were measured by enzymatic colorimetric methods because of TAGs levels higher than 400 mg/dL: Baseline [EVOO (n=14), HPO (n=7)]; Visit 1 [EVOO (n=15), HPO (n=7)]; Visit 2 [EVOO (n=14), HPO (n=6)]; Visit 3 [EVOO (n=13), HPO (n=7)].

[#] p value for univariate analysis [(considering only the treatment effect and not interactions; Significance (p < 0.05) = ○; Significance (p < 0.01) = ●]

*p value for multivariate analysis [(considering interactions among visits and treatment; Significance (p < 0.05)]

Table 4. Overall changes from baseline by visit, for TC, LDL-C, HDL-C and TAGs

	TC		LDL-C		HDL-C		TAGs	
	Change (mg/dL)	95% Conf. Int.	Change (mg/dL)	95% Conf. Int.	Change (mg/dL)	95% Conf. Int.	Change (mg/dL)	95% Conf. Int.
Visit 1	9.99	3.96 to 16.01	40.67	33.94 to 47.39	5.24	2.21 to 8.28	-14.31	-29.18 to 0.55
Visit 2	1.94	-4.14 to 8.03	5.55	-1.23 to 12.35	-0.11	-3.17 to 2.95	-7.87	-22.91 to 7.17
Visit 3	-15.08	-21.94 to -8.94	-18.68	-25.53 to -11.83	-1.07	-4.16 to 2.01	9.19	-5.98 to 24.37

was 206.5±39.1 mg/dL at baseline, increased to 213.2±33.1 mg/dL at month 1 and to 212.5±34.2 mg/dL at month 2, then decreased to 193.9±31.5 mg/dL by month 3 (**Figure 2A**). The plasma levels of TC in the EVOO group also showed a reduction over the study period. However, in contrast to the LDL-C results, the reduction in TC was more pronounced in subjects who received EVOO. In fact, plasma levels of TC increased at month 1 (203.8±36.9 mg/dL) followed by a decline at the second and third assessment times (201.9±35.3 and 185.8±28.4), an overall reduction of approximately 9%. With regard to the TC/HDL-C and LDL-C/HDL-C ratios, used as predictors of ischemic heart disease risk,²⁰ only a small but not significant change has been observed. TC/HDL-C ratio increased from 4.71 to 5.36 over the study period (from 4.97 to 5.34 in EVOO group) and a small effect has also been detected for LDL-C/HDL-C ratio (2.84 baseline vs 3.18 month 3) (**Table 3**). In the same way, triglycerides to HDL-cholesterol ratio (TG/HDL-c), which has been proven to be strongly correlated with the plasma level of small, dense LDL atherogenic particles (phenotype B), showed little increase from 4.20 to 4.75 and from 5.56 to 5.68 for HPO and EVOO group, respectively.

No significant differences were observed for BMI between baseline and month 3 in both groups (**Table 3**). There were significant changes in all fractions when time was considered (across visits) for TC ($p<0.001$), LDL-C ($p<0.001$), HDL-C ($p<0.0005$) and TAGs ($p=0.0191$), independently of the treatment received (**Table 5**).

On the other hand, no statistically significant differences were found between the two groups for TAGs ($p=0.043$), TC ($p=0.2898$), LDL-C ($p=0.0993$) or HDL-C ($p=0.5466$). Finally, the repeated measures ANOVA shows that no differences were detected between the two treatment groups for TC ($p=0.0525$), LDL-C ($p=0.2356$), HDL-C ($p=0.8293$) or TAGs ($p=0.3749$), once the interaction between treatment and time (visits) was taken into account (**Table 3** and **5**).

Discussion

In the present study, we describe for the first time the functional effect of crude hybrid palm oil (*E. oleifera* x *E. guineensis*) supplementation on human plasma lipids related to cardiovascular disease risk factors. The results obtained were compared to those achieved from individuals who received an equivalent amount of extra-virgin olive oil. A daily 25-mL dose of hybrid palm oil, which is comparable to the daily consumption of EVOO recommended by the U.S. Food and Drug Administration²¹ was shown in our study to have similar effects on plasma lipids to EVOO, which is universally recognized as an (putative) anti-atherogenic and cardioprotective oil.²²⁻²⁴ Several studies have in fact shown an inverse relation between consumption of oleic acid-rich olive oil and incidence of coronary heart disease. For instance, Nagyova *et al.*²⁵ published a study focused on the effects of EVOO supplementation on plasma lipids, total antioxidant capacity and indices of serum lipid oxidizability, among others. A total of 26 patients (mean age 69 years) with combined hyperlipidemia consumed approximately 20g/day of extra virgin olive oil for 6 weeks. Plasma TC and LDL-C decreased significantly after 6 weeks of dietary intervention. These results, together with those from several other studies, are mainly attributed, even not limited, to the ability of oleic acid to increase the resistance of human LDL oxidation which is known to play an important role in the development of atherosclerotic lesions.²⁶⁻²⁸ In our study, the finding that HPO consumption had a favorable effect on plasma lipids and that this effect was not statistically different from that of EVOO, can also be partially attributed to the particular fatty acid composition and triglyceride structure of HPO.¹¹ In fact, we have recently reported that crude hybrid palm oil possesses a high percentage of oleic acid (54.6±1.0) together with low saturated fatty acid content (33.5±0.5). Another promising

Table 5. Significance of differences by treatment and by visit

	TC	LDL-C	HDL-C	TAGs
Treatment	0.2898	0.0993	0.5466	0.043
Visit	<0.001	<0.0001	<0.0005	0.0191
Interaction (between treatment and visit)	0.0525	0.2356	0.8293	0.3749

ANOVA analysis of repeated measures: significance ($p < 0.05$)

outcome was that the *sn*-2 position of TAGs was predominantly esterified with oleic acid (64,7-66,0% mol) with only 10-15% of total palmitic acid and 6-20% of stearic acid acylated in the secondary position. These results indicate that HPO glyceridic composition and structure is quite similar to that of EVOO, even if the latter is characterized by an even higher content of oleic acid as well as by a higher proportion of oleic acid in the position *sn*-2 of TAGs.²⁹ Nevertheless, our analysis highlighted that HPO should be considered a monounsaturated oleic acid-rich tropical oil rather than a saturated fat, especially from a nutritional point of view. The present study provides further evidence to support this claim. In fact, in our investigation, a daily 25mL dose of HPO, had a partially favorable impact on cardiovascular risk-factor profiles: after three months of HPO consumption, the TC levels decreased by 6.3%, whereas LDL-C concentration decreased by more than 13%.

But the observed "positive" effect of HPO consumption may also be associated with the intake of antioxidants contained in crude HPO. As stated before, consistent evidence now suggests that vitamin E, carotenoids and other antioxidant nutrients such as phenolic compounds also offer protection against CVD by decreasing oxidative damage. Thus, nutritional properties of edible oil depend not only on its glyceridic oil composition and structure but also on its potential as a source of health-promoting minor components. In fact, oxidation of LDL-C leads to a change in the lipoprotein conformation by which LDL cholesterol can better enter into the monocyte-macrophage system of the arterial wall and promote the atherosclerotic process. As mentioned previously, no previous studies have been undertaken to determine the health effects of HPO consumption. Studies reported in the literature are almost exclusively focused on refined palm oil fractions and based on the use of animal models. To our knowledge, our study constitutes the first human intervention trial with crude hybrid palm oil. It is certainly true that the effects of refined African palm olein on human plasma lipids and lipoproteins have been relatively well investigated in the past.³⁰⁻³⁴ However, palm olein, which is the liquid fraction obtained by fractionation of palm oil after crystallization at controlled temperatures, completely loses nutritionally important components such as carotenes, tocopherols and polyphenols. For instance, tocotrienol-enriched fraction from palm oil has been shown to decrease TC and LDL-C plasma levels.³⁵ The favorable effect of crude HPO consumption on plasma lipids can therefore be also attributed to its high content of polyphenols (190 ppm) and tocopherols (>25 mg/100g) (Table 1), as well as by its high carotenoid concentration (>1038 mg/100g)¹³ which may play a crucial role by improving plasma antioxidant defenses and lipids profiles. In fact, because of the greater degree of unsaturation of HPO compared to African palm oil, HPO can be directly consumed without the need for any fractionation or refining process to separate olein and stearin fractions.

Our positive findings of crude HPO on plasma lipids are also consistent with previous animal model studies focused on a different kind of mildly refined palm oil called "red palm olein"

(RPO) which suggested favorable cardiovascular effects of the oil. Contrarily to palm olein, more than 80% of the vitamin E and 75% of carotenoids present in crude palm oil is retained in red palm olein. For instance, Boon *et al.*³⁶ recently reported changes in serum lipid profiles of red palm olein treated hypertensive rats, with a significant reduction in LDL-cholesterol level and TC/HDL ratio (atherogenic index) compared to untreated ones. Szucs *et al.*³⁷ recently investigated the effects of dietary RPO supplementation in a cholesterol-enriched diet-induced hyperlipidemic rat model showing attenuation of the increased susceptibility of the hearts in cholesterol fed rats to ischaemia/reperfusion injury. RPO-supplementation also altered pre-ischaemic levels of matrix metalloproteinase-2 (MMP2), thus indicating that myocardial MMP2 may be implicated as a possible role player in RPO mediated protection against ischaemia/reperfusion injury in the hearts of cholesterol supplemented rats. Previously, Esterhuysen *et al.*³⁸ also suggested that dietary RPO supplementation can improve reperfusion aortic output through mechanisms that may include activation of the NO-cGMP and inhibition of the cAMP pathway.

Conclusions

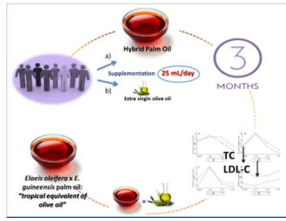
Our study provides evidence that the consumption of 25 mL/day of crude hybrid *Elaeis oleifera* x *E. guineensis* palm oil for a period of 3 months has similar effects on human plasma lipids to EVOO, which is considered the "gold standard" for heart disease prevention amongst all edible oils. The positive effects of HPO can be attributed to its high percentage of oleic acid (esterified mainly at the *sn*-2 position of TAGs) as well as to its elevated content of health-promoting bioactive minor components, especially tocotrienols, that probably lead to further benefits on LDL-cholesterol levels and oxidative damage in addition to those resulting from its remarkable glyceridic composition and structure. Finally, the results obtained in this study provide additional support for the concept that hybrid *Elaeis oleifera* x *E. guineensis* palm oil can be seen as the "tropical equivalent of olive oil".

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Hybrid palm oil supplementation (25 mL/day – 3 months) positively influences cardiovascular risk-factor profiles by significantly decreasing TC and LDL-C levels