Short Communication

Fatty acid profiles of blood lipids in a population group in Tibet: correlations with diet and environmental conditions

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The aim of this study was to compare blood fatty acid profiles of two population groups: Italian and Tibetan, differing with regard to ethnic, life style and environmental aspects. Additionally the collection of two staple foods provided the opportunity to analyze typical Tibetan dishes. A new, simple, rapid, and substantially non invasive method for fatty acid (FA) analysis of blood lipids was applied to healthy Italian (n= 14) and Tibetan (n= 13) subjects. Blood drops obtained from the ear lobe of Tibetans or the fingertip of Italians were adsorbed by a special strip of paper and processed for fatty acid analysis. The fatty acid profiles of the two groups are different, and environmental factors, such as dietary fats and altitudes of Milan, Italy (a low altitude site), and Lhasa, Tibet (a high altitude site) appear to contribute to these differences. More specifically, in Tibetans higher levels of monounsaturated fatty acids, including the 22 and 24 carbon molecules, were found. This appears to be derived mainly from locally consumed fats (mustard seed oil), and are associated with lower levels of total polyunsaturated fatty acids and higher levels of selected omega 3 fatty acids, when compared to the Italians. These relatively higher levels of monounsaturated fatty acids may also indicate means of adaptation to local prooxidant conditions. The observed differences in blood fatty acid profiles in Tibetans vs Italians appear to result both from dietary factors and adaptation to local environmental conditions such as the high altitude of the Tibetan location.

Key Words: Tibetans, Italians, blood fatty acids, dietary fats, altitude

INTRODUCTION

Fatty acids (FA), as glycerol esters, are major components of dietary fats, and in the body are incorporated in blood lipids, in depot fats and in structural lipids in biological membranes.

The assessment of the FA status in population groups, an index of nutritional adequacy for the prevention of certain chronic diseases (from cardiac heart disease (CHD) to neurodegenerative pathologies) is obtained through the analysis of FA in the circulatory system (plasma, erythrocytes or whole blood).

Populations living at high altitudes are exposed to reduced oxygen tension, and this is responsible for enhanced oxidative stress[1] which may activate processes that affect FA profiles. It should be considered also that populations at high altitudes,[2] such as Tibetans[3] have a high proportion of red blood cells (high hematocrit value or polycythemia) and this will in part affect the total amount of LC-PUFA in blood, since red blood cells (RBC) have higher concentrations of these compounds. However, to our knowledge, information on the FA profiles of populations living at high altitudes is very limited.

In this respect, our laboratory had the opportunity to participate into a project devoted to assess various physiological parameters in a small population group living in an area of Tibet.

The study has been carried out under rather difficult local conditions, that imposed limitations to the collection of data (number of subjects, anthropometric and physiologic parameters, as well as the estimation of dietary intakes), and of samples (blood and food items). In spite of the above limitations, the opportunity to carry out this work was facilitated by the application of a suitable strategy for the collection of blood samples and FA analysis, and has led us to present a report on this pilot study in an area that certainly deserves further attention.

In particular the subjects recruited for the study were Tibetan males living in Lhasa, a town located at the altitude of 3,650 meters at the bottom of a small basin.

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This area is crossed by the Lhasa River, running through snow-covered highly elevated peaks and gullies of the Nyainqentanglha Mountains. Results of a previous study on this population have already been published.[4]

The comparative FA profiles were assessed in drops of blood, drown from the capillary circulation (fingertip, heel or ear lobe) on a special support,[5] facilitating all the steps prior to FA analysis, even under unique conditions.[6,7,8] The procedure is rather easy, non-invasive, does not require the intervention of health operators, has simple storage and shipment requirements, and allows for the preparation of FA derivatives without lipid extraction for final analysis.

The method therefore is applicable to study populations in remote geographical areas, such as that in Lhasa. In addition to anthropometric information on the population in the study, data on the fat contents and FA composition of typical food items consumed in Tibet were obtained by analysing samples collected in the village. It is of interest that some foods are rather unique to this diet, as is the case for yak's meat and dri (female yak) derived food products, such as milk, butter, yoghurt and cheese. Yak is an animal living in Asia and is mainly raised on grass. It is part of the Tibetan culture, since it is used not only as food, but also for transport and for preparing clothes, blankets and other items. The fat contents and composition of yak's meat and dri's cheese were compared with those of corresponding food items in the Italian population. The aim of this study was thus to compare the FA status in the population group in Tibet with that of a corresponding group living in Milan.

MATERIALS AND METHODS

Subjects

Thirteen Tibetans and fourteen Italians, all males, participated in the study. The Tibetans were physical education students in Lhasa attending courses to become "sherpas", while the Italians were students at the School of Pharmacy of the University of Milan. Volunteers gave informed consent and the study conforms to the ethical standards on human experimentation of the University of Milan; in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Blood Sample Collection and Analysis

Subjects were pricked on ear lobes and on fingertip respectively, with an automatic lancing device equipped with antiseptic lancets, and blood drops (on average 20-30 µl) were collected on a special strip of paper. The samples were preserved at 4°C until analyzed in our laboratory at the Department of Pharmacological Sciences, University of Milan.

The samples were directly transmethylated,[9] then at the end of the process, the FA methyl esters (FAME) were dissolved in 70 µL hexane and 1 µL of each sample was injected for analysis in a gas-chromatograph (GC 1000, DANI S.p.A, Monza, Italy) equipped with a 30 m capillary column (Omegawax 320 Supelco), PTV injector, FID detector and a dedicated data collection system. The column temperature was programmed to increase from 170°C to 205°C at 5°C/min and after 5 minutes to 220°C at the same rate. FAME were identified by the use of authentic standards and 14- to 24-carbon FA were detected.

Statistical differences were evaluated by using Student's t-test and ANOVA to compare data between groups.

Food Collection and Analysis

Diet in the Lhasa population is characterized by the consumption of Tibetan staple foodstuffs such as various tsampa (roasted flours), mustard oil, yak meat, dri or sheep or goat milk, yoghurt, potatoes, courgettes, green vegetables, chili, cabbages and pricks. Yak meat and dri cheese were collected during the study in an attempt to characterize their fat contents, and the samples were stored at -20°C until they were sent to the lab for analysis.

Lipids from these two staple foods were extracted[9] and FAME prepared from the extracts and analyzed by gas liquid chromatography (GLC) as previously described. Nonadecanoic acid (19:0) was added to each sample as an internal standard, for the quantitation of the compounds. Gas chromatography/electron ionization-mass spectrometry was also used for the identification of selected FA, such as the presence of odd FA and conjugated linoleic acid (CLA) in dri cheese.

RESULTS

FA composition in blood samples

The subjects were healthy young males, 13 Tibetans aged 22.6 ± 9.69 year and 14 Italians 22.2 ± 1.85 years of age (Table 1). In Table 2, FA percentage levels of blood lipids in Tibetan and Italian subjects are shown. Between the two groups significant differences were observed with regard to higher MUFA and lower PUFA levels, in Tibetans vs. Italians.

Significant differences also occurred for other FA, in particular 20:0 is higher, while 22:0 and 24:0 were lower in Tibetans vs. Italians, in association with higher levels of the corresponding monounsaturated derivatives, 22:1 and 24:1. Levels of the n–3 FA, alpha linolenic acid (ALA, 18:3 n–3), eicosapentaenoic acid (EPA, 20:5 n–3), and 22:5 n–3 and also total omega 3 were higher, while docosahexaenoic acid (DHA, 22:6 n–3) and LA (18:2 n–6) were lower, in Tibetans.

Table 1. Characteristics of subjects and of the locations

Nationality Town Number	Altitude of location	Gender	Age	Major dietary components	Smokers
Italian, Milan N = 14	121 meter	m	22.2±1.85	(pasta, rice, vegetables, legumes, cheese, meat and fish)	0/14
Tibetan, Lhasa N = 13	3650-4300 meter	m	22.6±9.69	(dri cheese, yak meat, potatoes)	2/13

Table 2. FA percentage levels in blood lipids of Italians and Tibetans†

FA	Italians n=14 (% w/w)	Tibetans n= 13 (% w/w)
14:0	0.91±0.38	0.53±0.13**
16:0	22.7±0.68	19.8±2.05***
18:0	10.5±1.94	11.9±1.49*
20:0	0.46±0.09	0.87±0.37**
22:0	1.21±0.30	0.90±0.19**
24:0	1.88±0.77	0.98±0.45**
16:1 n–7	1.12±0.49	0.74±0.45*
18.1 n–9	19.0±2.88	19.7±5.18
18:1 n–7	1.59±0.23	2.61±0.45***
20:1 n–9	0.32±0.59	1.05±0.41**
22:1	0.50±0.74	4.18±2.34***
24:1	2.37±0.74	4.78±1.46***
20:3 n–9	0.11±0.04	0.11±0.05
18:2 n–6	22.3±5.40	17.9±3.98*
20:3. n–6	1.47±0.28	0.91±0.29***
20:4 n–6	8.91±1.70	7.91±1.95
22:4 n–6	1.06±0.61	0.98±0.37
22:5 n–6	0.25±0.12	0.26±0.13
18:3 n–3	0.34±0.12	0.69±0.27**
20:5 n–3	0.26±0.21	0.31±0.15
22:5 n–3	0.74±0.35	1.52±0.47***
22:6 n–3	2.17±0.86	1.42±0.50*
SFA	37.6±3.47	35.0±3.57*
MUFA	24.2±3.22	33.0±3.87***
PUFA	38.2±4.11	32.0±2.28**
n–6	34.0±4.23	27.92±2.65***
n–3	3.51±0.99	3.94±0.84
n–3/n–6	0.11±0.04	0.14±0.04*
n–3 HUFA	3.17±1.06	3.25±0.86
n–6 HUFA	11.7±2.31	10.1±2.53*
n–3 HUFA index	21.3±1.94	24.4±2.13*
Δ5 desaturase index	6.06±5.98	8.71±6.74

†All values are average ± SD.; *,**,*** Significantly different from Italian men: * $p < 0.05$; ** $p < 0.001$ and *** $p < 0.0001$.
FA= fatty acids; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; HUFA= highly unsaturated fatty acids

FA composition of food samples

Since the major factors responsible of the FA profiles in circulating lipids, are dietary fats, differing in the two populations, and to some extent also environmental conditions, such as the different altitudes of the locations in which the subjects live, i.e. conditions that would affect their biochemistry and physiology, these were both taken into consideration. As to the diet, in addition to the typical foods that we could analyze, other components of the local diet, that could not be directly analyzed, would appear to play significant roles.

We have, for example, compared meat and cheese consumed in Lhasa with foods commonly eaten in Milan. In Table 3, values for total lipid (TL) content and the FA percentage and contents (g/100g) of the different types of meat e.g. yak, chicken and veal are reported. In Table 4, fat and FA values for different types of cheese like dri cheese, parmigiano reggiano (parmesan) and ricotta are shown. Yak meat has a much higher fat content than meat typically consumed in the Italian diet (chicken, veal and sheep) and is rich in 18:1 n–9 (OA, oleic acid), and LA, whereas chicken contains balanced but lower proportions of both FA and in veal and sheep meat oleic acid is the major FA.

As to the cheese fat contents and FA profiles, dri and ricotta contain comparable amounts of fat, both being quite lower than parmigiano reggiano. Dri has the highest content of eicosanoic acid (20:0) and a higher content of 18:1 n–7 than the other two types of cheese. In addition traces of the long chain n–3 FA are detected in dri. The overall content of PUFA is higher in dri cheese and in this particular food we have also found an appreciable concentration of CLA, i.e. a mixture of isomers of LA, naturally produced by biosynthesis in ruminants and therefore found in milk and dairy products. The CLA (9c11t-C18:2 + 10 t12c-C 18:2) content in dri cheese was 0,26 g/100g of the product vs. practically undetectable amounts in the other two cheeses.

Table 5 shows the FA composition of different types of edible oils, which are important components in the diet of Italians and reportedly of several Asian countries including Tibet, and therefore are relevant factors in influencing

Table 3. Fat contents and FA percentage levels of different meats.

Fatty Acids	Yak Roasted		Chicken breast roasted*		Veal roasted*		Sheep w/o visible fat*	
	%	g/100g	%	g/100g	%	g/100g	%	g/100g
g LT/100g	14.4		0.9		11.5		3.1	
16:0	14.7	1.42	19.5	0.15	26.8	2.89	23.1	0.58
18:0	9.55	0.92	13.0	0.10	13.0	1.40	17.1	0.43
18:1 n–9	35.4	3.43	22.1	0.17	41.9	4.51	41.4	1.04
18:1 n–7	1.98	0.19	1.29	0.01	0.09	0.01	0.39	0.01
18:2 n–6	28.9	2.79	14.3	0.11	2.04	0.22	5.97	0.15
20:4 n–6	2.53	0.24	9.09	0.07	1.02	0.11	1.59	0.04
18:3 n–3	0.49	0.05	0.00	0.00	1.30	0.14	1.59	0.04
20:5 n–3	0.04	0.00	0.00	0.00	0.00	0.00	0.79	0.02
22:6 n–3	0.09	0.01	2.59	0.02	0.00	0.00	0.00	0.00
SFA	26.2	2.53	37.7	0.29	44.8	4.82	43.8	1.10
MUFA	40.3	3.90	29.9	0.23	50.8	5.47	44.6	1.12
PUFA	33.5	3.24	32.5	0.25	4.36	0.47	11.5	0.29

*= data from INRAN (Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione), www.inran.it
FA= fatty acids; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturateds

Table 4. Fat contents and FA percentage levels of different cheeses.

FA	Dri		Parmigiano R.*		Ricotta*	
	%	g/100g	%	g/100g	%	g/100g
g TL/100g	12.02		28.10		10.9	
12:0	0.00	0.00	0.00	1.04	3.91	0.39
16:0	6.16	0.75	12.1	3.40	13.3	1.33
18:0	28.3	3.45	28.6	8.04	35.0	3.49
20:0	20.7	2.52	10.9	3.07	10.6	1.06
18:1 n–9	24.1	2.93	29.0	8.15	23.7	2.36
18:1 n–7	8.29	1.01	0.03	0.01	0.10	0.01
18:2 n–6	2.06	0.25	1.49	0.42	0.25	2.5
20:4 n–6	0.17	0.02	-	-	-	-
18:3 n–3	1.82	0.22	-	-	-	-
20:5 n–3	0.02	0.00	-	-	-	-
22:5 n–3	0.21	0.03	-	-	-	-
22:6 n–3	0.02	0.00	-	-	-	-
SFA	59.0	7.20	65.9	18.5	68.4	6.82
MUFA	35.6	4.34	31.3	8.81	27.7	2.76
PUFA	5.42	0.66	2.8	0.79	3.91	0.39

*= data from INRAN (Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione), www.inran.it
FA= fatty acids; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; TL= total lipids

Table 5. FA composition (g/100g) of different kind of oils*.

FA g/100g of oli	Mustard Oil	Canola Oil	Olive Oil	Corn Oil
14:0	1.39	0.00	0.00	0.02
16:0	3.75	4.00	11.3	10.6
17:0	0.00	0.00	0.02	0.07
18:0	1.12	1.80	1.95	1.85
20:0	0.00	0.70	0.41	0.43
22:0	0.00	0.40	0.13	0.00
24:0	0.00	0.20	0.00	0.00
16:1 n–7	0.22	0.20	1.25	0.11
17:1	0.00	0.00	0.12	0.00
18:1 undifferentiated	11.61	56.1	71.3	27.3
20:1 n–9	6.19	1.70	0.31	0.13
22:1 undifferentiated	41.2	0.60	0.00	0.00
18:2 undifferentiated	15.3	20.3	9.76	53.5
18:3 undifferentiated	5.90	9.30	0.76	1.16

*= data from USDA (United States Department of Agriculture)(last update 2005) www.usda.gov/wps/portal/usdahome.
FA= fatty acids; SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids

the FA profiles of the subjects. A major seed oil consumed in Tibet, and in most Asian Countries, is mustard seed oil, with a FA composition particularly rich in 22:1 (erucic acid) similar to the FA composition of typical rapeseed oil. This oil contains also appreciable levels of OA and LA. Canola oil, i.e. the erucic acid deprived rapeseed oil, is instead very rich in 18:1, and in addition to LA contains also ALA. Olive oil is typically rich in OA.

DISCUSSION

The differences in whole blood FA profiles showed in Table 2 can be attributed to dietary habits but the different local environmental conditions in Lhasa, such as the high altitude, may also play a role.

Several epidemiologic and controlled studies [10] have shown the influence of dietary fats on blood FA levels in circulating lipids (plasma, red blood cells or whole blood), but data on the FA status in populations living at high altitudes are, to our knowledge, non existing. In our study we observed that the levels of selected FA are significantly different in the Tibetan vs. the Italian subjects. In particular, the level of 22:1 n–9 is higher in Tibetans than in Italians and this could have resulted from the influence of the local diet, as well as a possible general metabolic trend towards the accumulation of MUFA vs. PUFA in populations living at high altitudes. Unfortunately no dietary questionnaire could be applied to the subjects in Lhasa, but the data on FA contents and profiles in the foods that we analyzed, and information on the major type of vegetable fat consumed locally, indicate that certain characteristics of dietary FA may influence the FA concentrations in whole blood (WB).

The relatively higher levels of 22:1 in WB FA in Tibetans, for instance, suggests that mustard seed oil was part of their diet (as in several Asian countries). In fact this oil is rich in erucic acid (22:1) and the level of 22:1 and 24:1, derived from 22:1, in Tibetans are correspondingly elevated.

The difference in WB LA levels between the two groups may be attributed to different intakes, whereas the comparable levels of AA and the similar ratios between 20: 4 n–6 and 18:2 n–6 and between 22:5 n–3 and 18:3 n–3, for both Italians and Tibetans, suggest that the conversions in the two series n–6 and n–3 are relatively unaffected.

Another relevant observation concerns the levels of n–3 FA in the Tibetans: in fact while total n–3 and n–3 HUFA are substantially similar in both groups, selected differences are found with regard to individual FA. ALA and 22:5 n–3 (DPA) are higher while DHA is lower in Tibetans when compared to Italians.

The higher levels of ALA may be related to local dietary habits including the consumption of yak meat and cheese, containing appreciable amounts of n–3 FA. In turn, the ingestion of higher levels of PUFA, of the n–3 series, associated with lower levels of saturated fats from animals fed on grass with high contents of n–3 FA (a reflection of adaptation of plants to local climatic conditions), may be responsible for the appreciable levels of these compounds, both in meat and cheese from local animals.[11] Another feature of local cheese (dri) is the presence of CLA that have been shown in animal studies to exert potential anticarcinogenic activity on skin, mammary, colon cancer[12] and appears also to favourably influence the atherosclerotic process.[13] Concentrations of about 0.2–0.3 g CLA (9c11t + 10t12c 18:2) per100 g cheese were found in Dri cheese vs. only traces in the Italian cheeses.

As to the low DHA/DPA ratio, somewhat indicative of the conversion of DPA to DHA through a complex pathway involving a final peroxisomal beta oxidation step[14], suggests that the final steps for DHA synthesis from precursors, is less efficient in Tibetans. In this population, a presumed lower intake of preformed DHA, a LC-PUFA that cannot be efficiently synthesized from ALA and EPA[15] and is, locally unavailable, as it is mainly provided by seafoods, may also contribute to the lower DHA levels in WB FA, in spite of the polycytemia.[2]

However it can be hypothesized that the enhanced oxidative stress occurring at high altitudes, consequent to the overproduction of free radicals, reactive oxygen species (ROS) and lipid peroxides is partly counterbalanced by increased superoxide dismutase (SOD) activity and antioxidant enzymes[4]. This may be associated with the adaptive metabolic processes. These would favour the accumulation of long chain MUFA vs. LC-PUFA under these prooxidant conditions, being thus responsible for the lower levels of DHA in whole blood lipids in Tibetans.

In conclusion, the whole blood FA profiles obtained in subjects living in Lhasa (Tibet), a rather unique environment, in comparison with Italians suggest that various factors may be responsible for the different blood FA profiles. The elevation of MUFA, especially the long chain ones, could reflect the high intakes of these compounds through locally available oils, and this could apply also to the relatively higher blood levels of ALA, apparently more available in local foods, including cheese and meat. Similarly, the lower levels of blood LA may suggest lower intakes in the Tibetan population. The elevation of the ALA derivative DPA associated with the lower values of DHA, may indicate that the conversion of ALA to DPA did not decrease, while the subsequent step leading to DHA and the direct intake of this FA with the diet were reduced.

AUTHOR DISCLOSURES

Patrizia Risé, Franca Marangoni, Antonella Martiello, Claudio Colombo, Cristina Manzoni, Claudio Marconi, Flaminio Cattabeni and Claudio Galli, no conflicts of interest.

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一個西藏族群血脂的脂肪酸組成：飲食與環境狀況的關聯

從種族、生活型態和環境的觀點，本研究的目的為比較義大利人和西藏人兩族群之血中脂肪酸組成。此外，也收集了兩種主食，提供分析西藏典型食物的機會。使用一種新式、簡單、迅速且不具侵入性的方法，分析健康的義大利人（14 人）和西藏人（13 人）的血脂中脂肪酸組成。西藏人從耳垂血滴或義大利人的指尖血滴，以一種特殊的試紙吸收後進行脂肪酸分析。兩組人的脂肪酸的組成是不同的，環境因素，諸如膳食脂肪和義大利米蘭的海拔（低海拔地區）和西藏拉薩（高海拔地區）似乎都與這些差異有關。更具體來說，西藏人有較高的單不飽和脂肪酸濃度，包括 22 和 24 碳分子。與義大利人比較，西藏人下總多元不飽和脂肪酸較低，omega 3 脂肪酸較高，應該主要源於當地食用的脂肪(芥子油)。這些相對高的單不飽和脂肪酸也可能適應當地稀氧狀況的方法。西藏人和義大利人血脂肪酸組成的差異，應該是飲食因子和當地環境狀況適應兩者的結果，例如西藏地區的高海拔地勢。

關鍵字：西藏人、義大利人、血脂肪酸、膳食脂肪、海拔。