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# A method for the direct evaluation of the fatty acid status in a drop of blood from a fingertip in humans: applicability to nutritional and epidemiological studies

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**Abstract**

Several studies have shown that the fatty acid composition of circulating lipids reflects dietary fat intake, in turn being related to health status. The fatty acid composition of plasma lipids is therefore an important parameter in studies on dietary interventions. The aim of our study was to develop a rapid and inexpensive method for the analysis of circulating fatty acids applicable to large population groups. Drops of blood collected from fingertips have been directly subjected to transmethylation for gas chromatography analysis. This new method, validated for reproducibility, has been compared with the conventional method, based on withdrawal of blood from the antecubital vein followed by lipid extraction, and identical data have been obtained with the two techniques. Observed and predicted differences between blood and plasma fatty acids are related to the contribution of circulating cell membranes in blood. Finally the application of the methods to samples from 100 healthy subjects and the assessed correlation between dietary habits and blood fatty acid profiles demonstrate the validity of the new method and its applicability to nutritional and epidemiological studies.
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The relationship between fat consumption and health has been investigated in several epidemiological and clinical studies carried out in recent decades. In particular, the intake of n-3 polyunsaturated fatty acids (PUFA)[1] with the diet has been positively associated with optimal infant development [1,2], cardiovascular protection [3], prevention of neurodegenerative diseases [4] and behavioral disorders [5], and improvement of immune defenses [6].

However, only in relatively few published human studies, data on the fatty acid composition of circulating lipids [7,8] in confirmation of dietary fat intakes and habits have been reported and, when available, data were derived from small numbers of recruited subjects. The main limiting factors in this type of study are the difficulties associated with the collection of blood from the antecubital vein in a large number of subjects, the complexity and costs of the conventional analytical methods, involving health personnel and medical facilities, and the application of time-consuming and relatively costly procedures (preparation of samples, lipid extraction, etc.).

Lands and co-workers [9] have previously described a rapid method for the analysis of the fatty acids (FA) of plasma phospholipids, based on the direct application of plasma aliquots to the silica layer of a TLC plate without previous lipid extraction, followed by a chromatographic run. The method, however, has not apparently been subsequently applied to the assessment of the fatty acid status in epidemiological and intervention studies.

The aim of our study was to develop a rapid and inexpensive method, applicable to large population groups and not requiring the intervention of specialized personnel in the collection of samples, to analyze the FA

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[1] *Abbreviations used*: PUFA, polyunsaturated fatty acids; BHT, butylated hydroxytoluene; FAME, fatty acid methyl esters; DMA, dimethylacetal; DHA, docosahexaenoic acid; AA, arachidonic acid.

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composition of circulating lipids in a drop of blood, collected from a fingertip by a noninvasive procedure and analyzed for the FA profile without lipid extraction. This analytical approach may facilitate studies devoted to the screening and follow-up of population groups of particular interest, e.g., infants, pregnant women, elderly people, or sick persons, who are not easy to study using conventional approaches and, for this reason, have not yet been adequately investigated.

## Methods

### *Subjects*

One hundred healthy subjects, aged between 22 and 72 years, 46 males and 54 females, all working at the Department of Pharmacological Sciences in Milan, and including students, technicians, researchers, administrative personnel, and professors, were recruited. They gave informed consent and were asked to answer a questionnaire on dietary intakes and lifestyle habits. Collection of all samples was carried out rapidly over a few days.

### *Methods*

Samples for the new analytical method were obtained by collecting a drop of blood from a fingertip and, for comparison, 50 μl of blood and plasma through venous blood collection. The drop of blood was obtained by punching the fingertip with an automatic lancing device equipped with a lancet, and blood was absorbed on a strip ($1 \times 4$ cm) of paper for chromatography (Schleicher Schull; Chromatography Paper, preparative, 165 gsm). This type of absorbent proved to be superior to alternative materials that were tested, e.g., cyclodextrine, silica, talc, or calcium sulfate tablets, and did not interfere with the analysis. In particular no gas chromatographic peak was observed after processing for FA analysis this absorbent alone.

The amount of blood collected, measured by the use of a microbalance, ranged from 15 to 75 μg (equivalent to the same values in μl). Full linearity in the gas chromatographic response of individual fatty acid peaks with increasing sample size was maintained within the above range. The samples were either immediately processed or stored at 4 °C in individual cellophane envelopes with airtight closure. For storage periods exceeding 2 weeks, the incorporation of 50 μg BHT, added in 10 μl ethanol, into the absorbent was found necessary to prevent the loss of polyunsaturated fatty acids. The strip of paper containing the blood drop (1 $cm^2$) was transferred to Teflon screw-capped glass vials, with 1 ml of 3 N MeOH/HCl and maintained in a dry bath at 90 °C for 1 h. Then, 2 ml of water and 2 ml of a saturated solution of KCl were sequentially added, and fatty acid methyl esters (FAME) were finally extracted using 2 ml of *n*-exane twice.

FAME were analyzed by injecting about 1/50th of each sample in a gas chromatograph (85.10; DANI Instruments S.p.A, Cologno Monzese, Italy) equipped with a 30-m capillary column (Omegawax 320 Supelco; Belafonte PA), PTV injector, FID, and a dedicated data system. Temperature programming went from 170 to 205 °C with a 5 °C/min increment and after 5-min isotherm to 220 °C at the same rate. Peaks were identified by the use of pure reference compounds. Fatty acids from 14 to 24 C were detected.

The new method was compared to the conventional one, by using whole blood and plasma samples, obtained by centrifugation of blood, drawn from the antecubital vein of six male volunteers, using ethylene diaminetetraacetic acid (EDTA) as anticoagulant; 5-ml tubes were stored at −20 °C until analyzed. Total lipids were extracted from 500 μl of plasma and whole blood with chloroform/methanol 2:1 as already described [10,11] and weighed by the use of a microanalytical balance (Cahn). FAME were prepared by transesterification of total lipid aliquots (300 μg) with 3 ml of 3 N MeOH/HCl (Supelco) at 90 °C for 1 h and subsequent extraction with 6 ml of water and 3 ml of *n*-exane three times. The gas chromatographic profiles of fatty acids in a drop of plasma using the conventional and the new procedures were substantially identical. The gas chromatographic plots of plasma and whole blood analyzed with the new procedure, obtained from the same subject, are shown in Figs. 1A and B. The same FA are present in both samples, but in whole blood lipids two minor additional peaks were detected immediately before the peaks of 16:0 and 18:0 FA, corresponding to the dimethylacetal (DMA) derivatives of plasmalogens present in red blood cell phospholipids. The chromatographic behavior of DMA was assessed after lipid extraction of a whole blood sample, acid methylation, TLC separation of FAME from DMA, and GC analysis of derivatives [12]. It was found that the DMA peaks did not overlap with any of the FA (Fig. 1B).

## Results and discussion

The first part of the study was aimed to determine the equivalence between the FA composition of plasma and blood lipids assessed by the use of the conventional analytical approach, i.e., lipid extraction followed by preparation of FAME, and the same determination with only direct methylation.

The validity of the direct method was then assessed for plasma in six subjects and, as shown in Table 1, the FA percentage levels, evaluated with the new method vs the conventional method, were substantially identical.

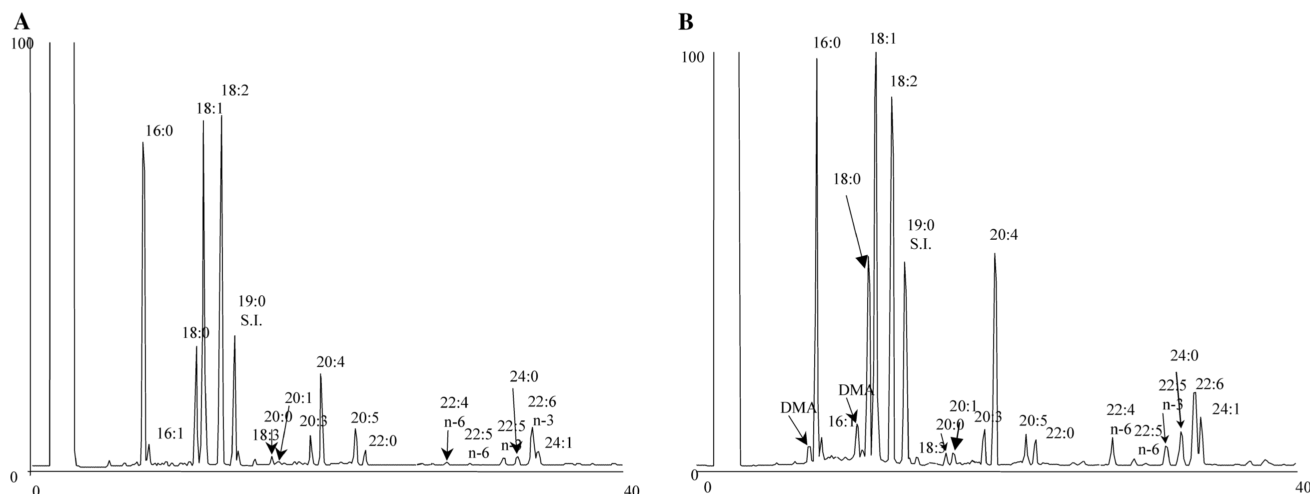


Fig. 1. Gas chromatographic profiles of FAME of plasma (A) and whole blood (B) lipids after direct transmethylation.

Table 1
FA percentage composition of plasma total lipids after extraction (A) and direct transmethylation (B) ($n = 6$), mean ± SD

| FA% | A (conventional method) | B (new method) |
|---|---|---|
| 16:0 | 21.03 ± 0.96 | 22.04 ± 1.50 |
| 18:0 | 7.05 ± 0.55 | 6.72 ± 0.33 |
| 20:0 | 0.25 ± 0.05 | 0.29 ± 0.05 |
| 22:0 | 0.44 ± 0.05 | 0.57 ± 0.12 |
| 24:0 | 0.37 ± 0.07 | 0.47 ± 0.10 |
| 16:1 | 1.10 ± 0.56 | 1.75 ± 0.41 |
| 18:1 n-9 | 29.24 ± 3.31 | 26.06 ± 5.45 |
| 18:1 n-7 | 0.89 ± 0.70 | 1.06 ± 0.68 |
| 20:1 | 0.25 ± 0.12 | 0.24 ± 0.13 |
| 24:1 | 0.64 ± 0.03 | 0.87 ± 0.13 |
| 18:2 n-6 | 25.86 ± 1.45 | 26.68 ± 1.82 |
| 20:3 n-6 | 1.59 ± 0.35 | 1.58 ± 0.23 |
| 20:4 n-6 | 5.95 ± 1.08 | 6.10 ± 1.10 |
| 22:4 n-6 | 0.15 ± 0.04 | 0.14 ± 0.05 |
| 22:5 n-6 | 0.43 ± 0.15 | 0.25 ± 0.11 |
| 18:3 n-3 | 0.41 ± 0.14 | 0.36 ± 0.16 |
| 20:5 n-3 | 1.22 ± 0.98 | 1.61 ± 0.99 |
| 22:5 n-3 | 0.47 ± 0.17 | 0.49 ± 0.16 |
| 22:6 n-3 | 2.66 ± 1.7 | 2.73 ± 1.48 |
| Saturated | 29.14 ± 0.40 | 30.09 ± 3.04 |
| Monounsaturated | 32.13 ± 3.44 | 29.97 ± 5.65 |
| Polyunsaturated | 38.73 ± 3.13 | 39.94 ± 3.69 |
| Unsaturation index | 140.76 ± 14.36 | 142.23 ± 13.44 |

The same type of comparison was then made for whole blood collected from the antecubital vein. Again, analyses with and without lipid extraction in six subjects gave comparable results (Table 2), confirming that the two methods provide substantially identical information. In addition, and more relevant for the study, the FA profiles of lipids in whole blood samples from arm and from fingertips did not reveal significant differences (not shown).

On the other hand, the FA composition of plasma and blood lipids showed substantial differences, as expected (Table 3) since blood lipids have higher saturated and lower monounsaturated FA levels. The similar PUFA percentages obtained in the two types of samples

Table 2
FA percentage composition of blood total lipids after extraction (A) and direct transmethylation (B) ($n = 6$), mean ± SD

| FA% | A (conventional method) | B (new method) |
|---|---|---|
| 18:0 | 11.46 ± 0.44 | 11.57 ± 0.41 |
| 18:1 n-9 | 21.93 ± 3.52 | 22.07 ± 3.77 |
| 18:2 n-6 | 18.38 ± 1.11 | 18.39 ± 0.84 |
| 20:4 n-6 | 10.46 ± 1.77 | 10.25 ± 0.44 |
| 18:3 n-3 | 0.28 ± 0.07 | 0.26 ± 0.08 |
| 20:5 n-3 | 1.25 ± 0.92 | 1.20 ± 1.07 |
| 22:5 n-3 | 1.36 ± 0.43 | 1.25 ± 0.29 |
| 22:6 n-3 | 4.59 ± 3.01 | 4.02 ± 2.10 |
| Saturated | 32.33 ± 5.38 | 32.87 ± 1.95 |
| Monounsaturated | 27.84 ± 3.24 | 27.92 ± 3.95 |
| Polyunsaturated | 39.83 ± 5.07 | 39.22 ± 2.74 |
| Unsaturation index | 160.57 ± 28.29 | 157.05 ± 13.41 |

Table 3
FA composition (%) of blood and plasma lipids after direct transmethylation

| FA% | Blood | Plasma |
|---|---|---|
| 18:0 | 11.57 ± 0.41 | 6.72 ± 0.33 |
| 18:1 n-9 | 22.07 ± 3.77 | 26.06 ± 5.45 |
| 18:2 n-6 | 18.39 ± 0.84 | 26.68 ± 1.82 |
| 20:4 n-6 | 10.25 ± 0.44 | 6.10 ± 1.10 |
| 18:3 n-3 | 0.26 ± 0.08 | 0.36 ± 0.16 |
| 20:5 n-3 | 1.20 ± 1.07 | 1.61 ± 0.99 |
| 22:5 n-3 | 1.25 ± 0.29 | 0.49 ± 0.16 |
| 22:6 n-3 | 4.02 ± 2.10 | 2.73 ± 1.48 |
| Saturated | 32.87 ± 1.95 | 30.09 ± 3.04 |
| Monounsaturated | 27.92 ± 3.95 | 29.97 ± 5.65 |
| Polyunsaturated | 39.22 ± 2.74 | 39.94 ± 3.69 |
| Unsaturation index | 157.05 ± 13.41 | 142.23 ± 13.44 |

reflected the substantially lower linoleic acid, 18:2 n-6, and higher n-6 and n-3 LC-PUFA in blood, due to the contribution of circulating cells with membranes mainly composed of phospholipids, rich in arachidonic acid (AA, 20:4 n-6) and docosahexaenoic acid (DHA, 22:6 n-3) [11]. On the other hand, as mentioned under Methods, the DMA derivatives from aldehydes in red blood cell plasmalogens do not interfere with the FA analysis.

The reproducibility of the new analytical approach, assessed through analyses of FAME prepared from the same blood sample and repeated in a sequence within the same day, or of duplicate samples collected simultaneously from the same subject and processed and

Table 4
FA percentage levels of blood samples, expressed as mean (range), standard deviation (SD), and coefficient of variation % (CV%)

| FA % | Repeated analysis of the same sample ($n = 6$) | | | Analysis of the same sample at different time periods (0, 1, and 3 weeks) | | | Analysis of different samples from the same subject ($n = 6$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean (range) | SD | CV% | Mean (range) | SD | CV% | Mean (range) | SD | CV% |
| 18:0 | 11.32 (11.08–11.55) | 0.24 | 2.09 | 11.04 (11.46–11.54) | 0.04 | 0.36 | 11.04 (10.97–11.08) | 0.06 | 0.56 |
| 18:1 | 19.38 (19.20–19.69) | 0.27 | 1.41 | 14.82 (14.56–15.00) | 0.24 | 1.59 | 19.77 (19.00–20.38) | 0.70 | 3.56 |
| 18:2 n-6 | 19.43 (19.32–19.61) | 0.15 | 0.79 | 20.75 (19.94–21.26) | 0.72 | 3.45 | 19.53(19.32–19.76) | 0.22 | 1.13 |
| 20:4 n-6 | 8.57 (8.47–8.75) | 0.16 | 1.84 | 8.75 (8.71–8.89) | 0.10 | 1.08 | 8.43 (8.42–8.47) | 0.03 | 0.36 |
| 20:5 n-3 | 0.68 (0.67–0.69) | 0.01 | 1.60 | 1.36 (1.30–1.39) | 0.05 | 3.90 | 0.68 (0.65–0.70) | 0.03 | 3.89 |
| 22:6 n-3 | 2.82 (2.73–2.88) | 0.08 | 2.97 | 4.47 (4.43–4.54) | 0.06 | 1.41 | 2.82 (2.77–2.86) | 0.04 | 1.53 |
| Sat. | 39.68 (39.06–40.00) | 0.54 | 1.36 | 39.52 (38.36–40.39) | 1.04 | 2.64 | 39.24 (38.75–39.99) | 0.66 | 1.68 |
| Mono. | 25.28 (25.14–25.45) | 0.16 | 0.62 | 21.34 (20.61–21.97) | 0.68 | 3.20 | 25.81 (25.14–26.51) | 0.68 | 2.65 |
| Poly. | 35.04 (34.75–35.49) | 0.40 | 1.13 | 39.14 (38.16–39.67) | 0.85 | 2.17 | 34.91 (34.74–35.23) | 0.25 | 0.71 |

Table 5
Percentage levels of all FA in blood total lipids from 100 volunteers and of major FA of the same subjects subdivided into groups according to fish and meat consumption

| FA% | Volunteers ($n = 100$) | Low fish consumers ($n = 40$) (<1 serving/week) | High fish consumers ($n = 47$) (1–2 servings/week) | Low meat consumers ($n = 33$) (1 serving/week) | High meat consumers ($n = 61$) (2–3 servings/week) |
|---|---|---|---|---|---|
| 16:0 | $25.31 \pm 4,23$ | | | | |
| 18:0 | $13.92 \pm 4.75$ | $15.59 \pm 4.73$ | $13.57 \pm 5.57$ | $13.24 \pm 5.39$ | $13.57 \pm 5.51$ |
| 20:0 | $0.57 \pm 0.19$ | | | | |
| 22:0 | $1.29 \pm 0.50$ | | | | |
| 24:0 | $1.35 \pm 0.58$ | | | | |
| 16:1 | $2.74 \pm 1.25$ | | | | |
| 18:1 n-9 | $21.03 \pm 3.53$ | $21.48 \pm 3.94$ | $20.61 \pm 3.77$ | $21.10 \pm 3.51$ | $20.86 \pm 3.79$ |
| 18:1 n-7 | $2.19 \pm 0.73$ | | | | |
| 20:1 | $0.29 \pm 0.11$ | | | | |
| 24:1 | $1.60 \pm 0.72$ | | | | |
| 18:2 n-6 | $16.74 \pm 5.86$ | $14.64 \pm 5.54$ | $17.72 \pm 6.95$ | $17.92 \pm 9.24$ | $15.84 \pm 5.54$ |
| 20:3 n-6 | $1.15 \pm 0.40$ | | | | |
| 20:4 n-6 | $6.65 \pm 2.47$ | $6.06 \pm 2.37$ | $6.91 \pm 2.48$ | $6.35 \pm 2.37$ | $8.10 \pm 2.48^{**}$ |
| 22:4 n-6 | $1.03 \pm 0.49$ | | | | |
| 22:5 n-6 | $0.31 \pm 0.23$ | | | | |
| 18:3 n-3 | $0.61 \pm 0.43$ | | | | |
| 20:5 n-3 | $0.59 \pm 0.47$ | $0.49 \pm 0.36$ | $0.72 \pm 0.52$ | $0.63 \pm 0.47$ | $0.64 \pm 0.48$ |
| 22:5 n-3 | $0.87 \pm 0.57$ | | | | |
| 22:6 n-3 | $1.75 \pm 0.95$ | $1.22 \pm 0.74$ | $2.00 \pm 1.09^{*}$ | $1.71 \pm 1.03$ | $1.67 \pm 1.05$ |
| Saturated | $42.44 \pm 6.56$ | $44.75 \pm 7.18$ | $41.57 \pm 6.94$ | $41.37 \pm 7.57$ | $43.56 \pm 7.20$ |
| Monounsaturated | $27.86 \pm 4.10$ | $28.89 \pm 4.28$ | $27.09 \pm 4.20$ | $27.93 \pm 4.01$ | $27.75 \pm 4.23$ |
| Polyunsaturated | $29.70 \pm 8.13$ | $26.35 \pm 8.39$ | $31.34 \pm 8.42$ | $30.69 \pm 10.52$ | $28.69 \pm 8.29$ |
| Unsaturation index | $116.71 \pm 22.13$ | | | | |

$^{*}$ $p \leqslant 0.001$.
$^{**}$ $p \leqslant 0.05$.

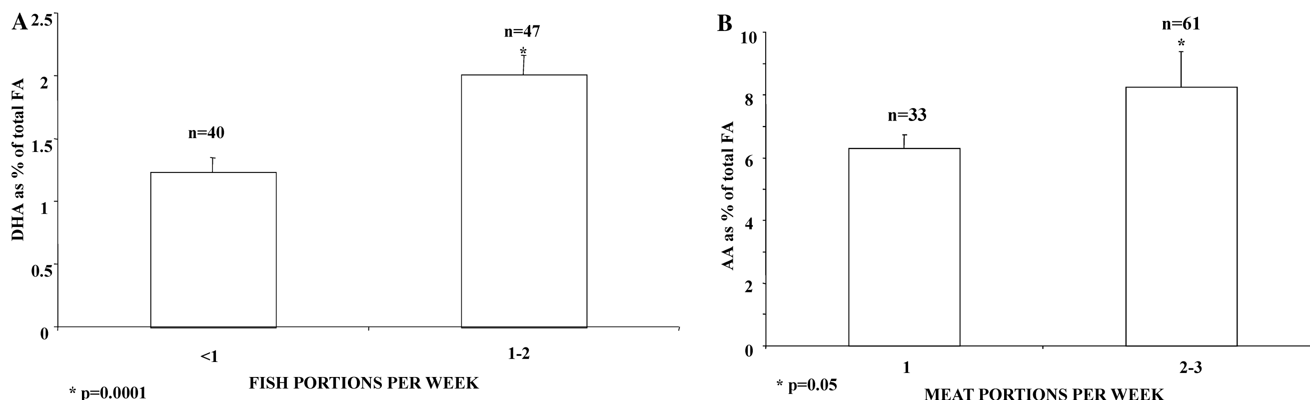


Fig. 2. (A) DHA levels in blood lipids of self declared low and high fish consumers. (B) AA levels in blood lipids of self declared low and high meat consumers.

analyzed at three time points (immediately, and after 1- and 3-week storage periods at 4 °C in the presence of BHT), or of triplicate samples collected from the same subject at three time periods during the day (including before and 2 h after a meal) is very high with the coefficient of variation (expressed as SD/mean %) on the average around 2%. These assessments are shown in Table 4.

The aim of the second part of the study was to validate our method through application to the analysis of a large number of samples and to verify its feasibility to assess possible relationships between whole blood FA composition and dietary habits. The average composition of total FA in the whole blood from all subjects and the levels of the major FA in subgroups are presented in Table 5. As a first simplified approach to this type of evaluation, subgroups were defined on the basis of the data provided by the dietary questionnaires into low and high consumers of fish or meat. DHA, mainly contained in fish, was the FA most influenced by fish consumption, as shown by the significantly higher levels in subjects who ate fish once or twice per week vs those who had less than one portion of fish per week. AA levels were significantly higher in blood of heavy (two or more portions per week) vs low (one portion per week) meat consumers. The significantly higher AA levels in the heavy meat consumers may be attributed to higher intakes of AA supplied by meat lipids. These findings are summarized in Figs. 2A and B. Although the self-declared fish and meat consumption cannot be considered an exact quantitative assessment, the analytical data showed distinct differences in the levels of specified fatty acids.

These results lead us to conclude that the newly described method is valid, noninvasive, and both time and cost saving and that it can be applied to the FA analysis of large numbers of samples. In particular its application does not necessitate the involvement of health operators in the collection of the blood sample and it markedly simplifies the analytical procedure. Finally, population groups such as pregnant women, infants, and aged persons, which would not be easily accessible to conventional procedures for blood collection, could be subjected to investigation.

Moreover, the whole blood FA composition reflects the dietary fat intake, as demonstrated for fish and meat. The method therefore provides useful information on the dietary habits of a population and on the FA profile of an individual, justifying the validity of its application to preventive and epidemiological studies on a vast scale focused on the role of dietary fats.

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