Short-term stability of whole blood PUFA content on filter paper

Disco
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INTRODUCTION

Among the long chain-polyunsaturated fatty acids (LC-PUFA), arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are of major interest in humans. DHA is an important structural component of photoreceptor and cortical neuronal membranes and is required for optimal neural development and visual function. Reduced DHA status in newborn preterm infants has been related to impaired visual function. Plasma and red blood cell content of LC-PUFA are commonly used to evaluate certain medical conditions and to estimate nutritional status or compliance in epidemiologic and interventional studies. Finger stick assay was used in epidemiological studies in adults, children and infants and demonstrated its ability to evaluate nutrition-induced changes in DHA status as well as to show statistically significant association between the blood level of DHA and neurodevelopment scores. Data on the long-term stability of such methods is limited. In the present study we tested the stability of the long chain fatty acids in dried blood for as long as 6 months from sampling. We measured both the mol% values and the ^{13}C enrichments.

MATHERIALS AND METHODS

We collected 12 cord blood samples from the delivery suite of the "G. Salesi" Children's Hospital, Ancona, Italy. Whole EDTA blood samples, 40-50 µl aliquots were then immediately spotted onto treated filter paper squares (AHLSTROM 226,) and air dried for up to 10 minutes. From the same blood samples one 50 µl aliquot was immediately processed. BHT impregnated filter paper squares were prepared according to the method described by Ichihara *et al*. The samples were either immediately processed, or stored, in plugged test tubes, at -28°C and analyzed after: 7 days, 1 month, 3 months and 6 months. The filter paper samples were transferred to test tubes as described previously by Marangoni *et al*.

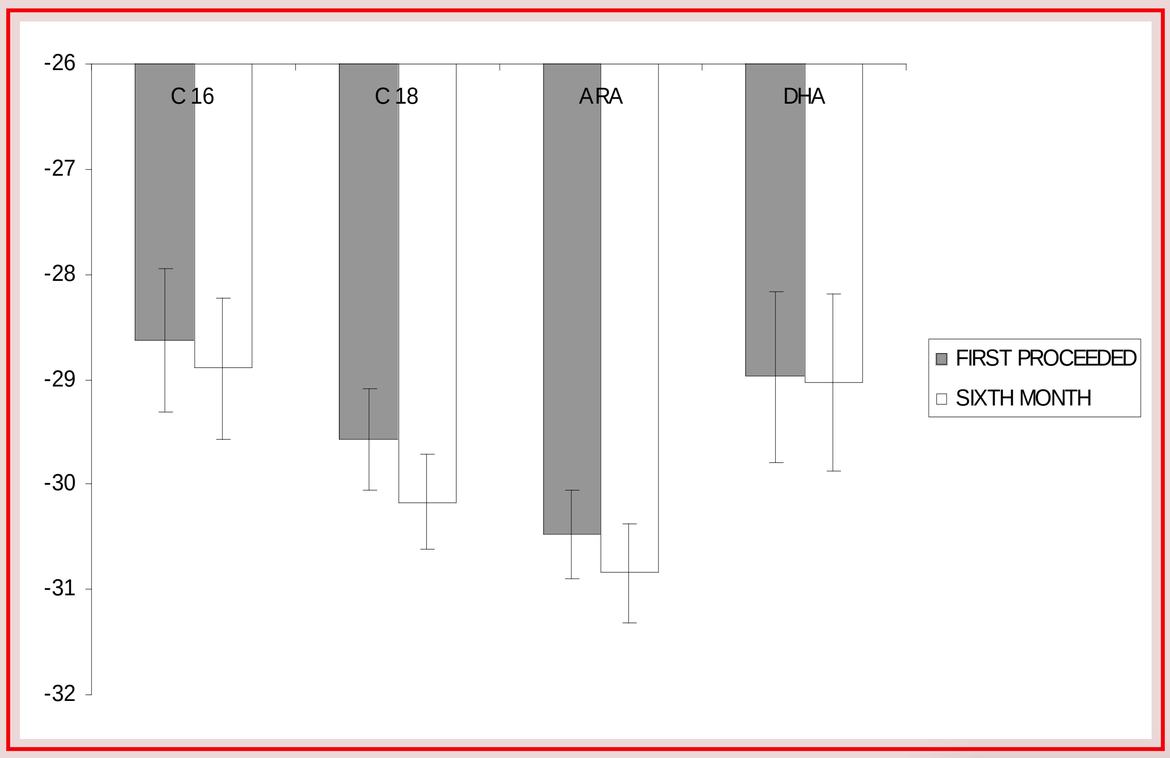
RESULTS

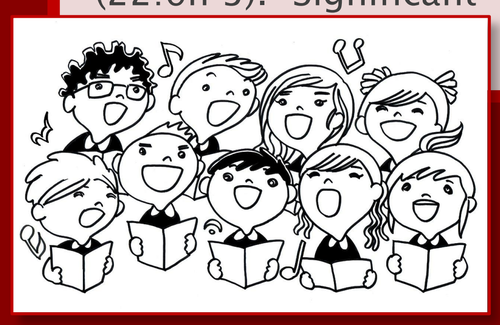
The first part of this study aimed to determine the equivalence of fatty acid composition of fresh and dried blood samples. We also wanted to evaluate the effect of short time storage on lipid composition; dried blood spots were stored at -28°C for 7 days and, 1, 3 and 6 months. There were striking differences between the lipid profile of the sample processed immediately and those of the sample processed after 6 months. There were marked decreases in the LC-PUFA ARA (20:4n-6), EPA (20:5n-3) and, DHA (22:6n-3). Significant differences in FA amount were evident even after 1 month of storage at -28°C. ARA, EPA and DHA decreased by 9%, 28% and 20% after 30 days of storage, in contrast saturated fatty acid percentage, such as C16:0, increased by 19%. The ^{13}C enrichment of blood ARA, DHA, C16:0 and C18:0 is depicted in the figure. The ^{13}C enrichment of these fatty acids did not differ significantly during the study period.

DISCUSSION (CONCLUSION)

This study provides new information on the rate of FA degradation, especially of LC-PUFA, in dried blood samples stored at -28°C. Our results indicate that a decrease in major LC-PUFA was evident even after one week of storage and became statistically significant after one month. Decreases in individual fatty acids from baseline to month 1 were 9% for ARA, 29% for EPA and 20% for DHA. Our results can lead us to conclude that higher concentration of BHT can improve the stability providing protection against PUFA degradation. We also considered that relevant changes can happen during initial blood spotting and drying phases.

The aim of the second part of this study was to monitor the effect of long time storage on the isotope ratio of individual FA at natural abundance level. We were pleased to notice that there were no differences due to degradation process occurring to blood spotting and air drying. No significant changes in ^{13}C enrichment were also seen with prolonged storage (6 months). This is very reassuring as we in the past have obtained important kinetic data on LC-PUFA metabolism from isotopic analysis at the natural abundance level.





Di chi sarà il mondo di domani?
Di chi oggi canta in coro.

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