Author's response to reviews

Title: Use of Dried Blood for Measurement of Trans Fatty Acids

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Version: 3 **Date:** 21 May 2009

Author's response to reviews:

Dear Editor,

Please find herewith reply to the Reviewer's Valued Comments point wise.

Reviewer: Staub Christian

Some validation data are missing: intraday precision (analysis in triplicate is not sufficient) and inter day precision for example.

Answer: For separation of fatty acids from one sample injected into a 100 m column, 120 min is required. On a day maximum of 4 samples can be run of which one run is that of a standard. Therefore for intraday comparison we have only 3 runs of a sample. For Inter day precision, same sample was run on six different days and the coefficient of variation calculated. For saturated fatty acid the CV was 1.52%, for monounsaturated it was 1.55%, for polyunsaturated 0.66% and for total trans 13.25%. [This data has now been incorporated into text]

The correlations between dried blood and whole blood are not, from my opinion, so good. What about % content of monounsaturated, where the slope is around 0.52 ? I am not sure that a method where the bias is about 48% is a good method. The authors should explain this result in details. For the other compounds, the slope is between 0.8487 and 1.1403, which is acceptable.

Answer: On closer look of the data it was observed that there were two outliers for monounsaturated trans fatty acids which when removed resulted in a change of the slope to 0.65 from 0.52, The 'Pearson correlation' also improved from 0.70 to 0.80. Since the slope is 0.8 to 1.0 for other fatty acids we believe that the dried blood fatty acids correlate well with whole blood fatty acids. The lower slope in case of trans mono unsaturated fatty acids could be possibly an artifact arising out of errors in identification of peaks accurately especially when the levels are

very low. No scientific reasoning could be given for a lower correlation only in case of monounsaturated fatty acids when good correlation is seen with other fatty acids [the modified Fig 2a RTF file with outliers removed is attached]

From the results presented, it is very difficult to say that trans fatty acids are stable in dried blood up to 15 days when stored at 4 oC . The authors should present analysis of the same sample for this period of time.

Answer: The purpose of the study was not to demonstrate long term stability of dried blood for fatty acid estimation. Stability of trans fatty acid in blood stored at – 70 0C is well documented. Dried blood was stored at 40C for 15 days to check the feasibility of usage of dried blood for transportation in epidemiological studies. The whole blood sample and dried blood samples were analyzed as pairs at the same time.

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Reviewer: Prem Pais

The authors state repeatedly that the gold standard for estimation of fatty acids is sampling of adipose tissue. The present paper compares dried blood spot to venous blood. I was unable to find any comment on how accurate venous blood is as compared to adipose tissue sampling

Fasting whole blood can be a reliable sample of choice for short term dietary intake for epidemiological studies. The values compare well with adipose tissue as cited in the reference given below.

Reference: A Baylin, MK Kim, A Donovan-Palmer, X Siles, L Dougherty, P Tocco and H Compos. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. American Journal of Epidemiology 2005;162 :373-381.

While the abstract states that blood was stored at -70 degrees the methods section speakes of -80 degrees.

Necessary correction made in the manuscript. (Corrected: – 70 0C) [Marked in red]

It is stated in the discussion (last paragraph) that "traditional risk factors are not able to explain the high rate of premature CAD..". This is not accurate. The INTERHEART study and a substudy of south Asians reported that 90% of risk of MI can be explained by traditional risk factors

Although the studies mentioned demonstrate the importance of traditional risk factors the role of diet in modifying lipid levels (which is one of the traditional risk factors) cannot be ignored.

The abbreviation list is incomplete e.g. ICC

Necessary correction made in the manuscript. (Added: ICC-Intraclass Correlation Coefficient) [Marked in red]

Thanks and regards,

Dr. R. Lakshmy