The Impact of Processing Methods on Free Fatty Acids Measurements in Type 2 Diabetes

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BACKGROUND

Type 2 diabetes (T2D) is associated with an increase in plasma free fatty acid (FFA) levels and elevated risk of cardiovascular disease. Links to inflammation and dyslipidemia lead to cellular impairment.

Treatment strategies aimed at improving patients' lipid profile rely on detection and follow up measurements.

AIM

We aim to investigate the effect of blood collection and storing methods on measuring the FFA alterations in patients with T2D.

METHODS

We conducted a review of observational studies and secondary data to explore reasons for variability of FFA results obtained with different methods. We searched PubMed for original articles that reported variations in FFA concentrations based on different techniques.

CONCLUSIONS

FFA detection is highly dependent on the appropriate collection and processing techniques. Orlistat/ THL addition to collection tubes provides lipolytic inhibition with easier technical manipulation than Paraoxon.

Immediate measurements or freezing samples at -70°C for storage improved accuracy.

RESULTS

Our review revealed that plasma FFA levels can be affected by collection and processing methods. Strong associations were reported between high saturated FFA levels and impaired insulin sensitivity and with HbA1c in patients with poor diabetes control. Paraoxon or Tetrahydrolipstatin(THL) additives prevented lipolysis.

Microfluorimetric measurements were accurate and less costly than spectrofotometrics(Miles)

Miles J, Glasscock R, Aikens J, Gerich J, Haymond M.: A microfluorometric method for the determination of free fatty acids in plasma. J Lipid Res. 1983 Jan;24(1):96-9. PMID: 6833886.

Samples frozen with Orlistat or THL showed significantly lower FFA, 28.4%, P < 0.008(Krebs) compared to samples without THL. Significant FFA differences(p<0.05 , p<0.01) during heparin and TG infusions (Krebs) - Figure A.

Immediate testing or freezing plasma and Paraoxon addition improved accuracy of results(Zambon) - Figure B.

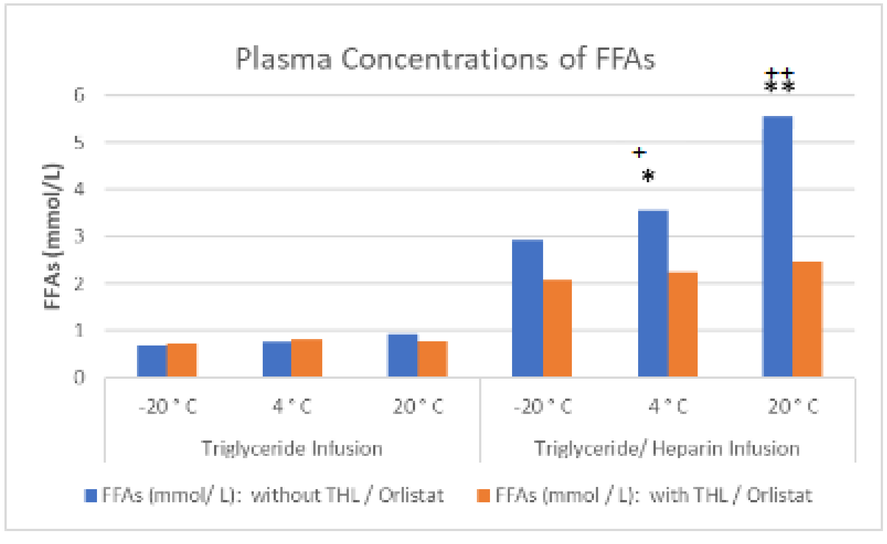

Figure A

Plasma concentrations of FFA in samples collected with and without THL during infusion of triglycerides, and during infusion of triglycerides plus heparin. FFA results from aliquotes processed at -20°C, 4°C and +20°Celsius. Compared with immediately frozen aliquot(-20C): * P<0.05; ** P<0.01. Compared with samples collected without THL: + P <0.01; ++ P<0.001.

Michael Krebs, Peter Nowotny, Daneil Weghuber, Martin Bischof: Prevention of in Vitro Lipolysis by Tetrahydrolipsraring, Clinical Chemistry (2000): 950-954. https://pubmed.ncbi.nlm.nih.gov/10894838/

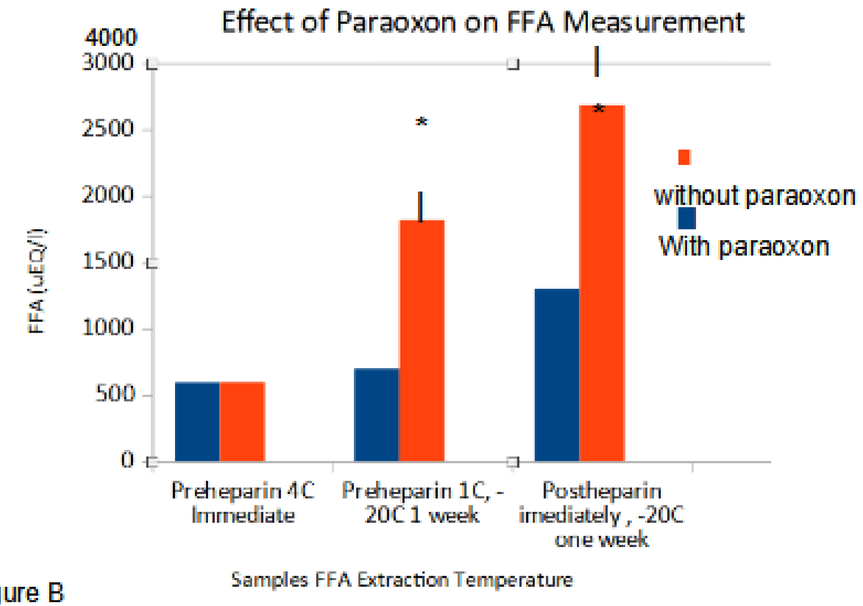

Figure B

Paraoxon pretreatment effect on plasma FFA measurements: FFA levels in pre and post heparin samples assayed under various conditions of temperature and pre-extraction timing: Preheparin 4°C, postheparin 1°C/or kept at +20°C for one week, Postheparin processed immediately /or kept at -20°C for one week. Significant FFA difference in samples collected with paraoxon versus without paraoxon based on t-test, * P <0.005, n=12

Alberto Zambon, Steven I. Hashimoto, and John D. Brunzell: Analysis of techniques to obtain plasma for measurement of levels of free fatty acids. *Journal of Lipid Research* (1993): 1021-1028. https://pubmed.ncbi.nlm.nih.gov/8354949/.