

## Lipidomics Revealed Dairy Fat Intake Biomarkers and Nitro Metabolites of Conjugated Linoleic Acid (cLA) in a cLA Depletion-Repletion Clinical Trial.

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Recent studies have shown a strong link between subclinical inflammation and metabolic diseases such as obesity. Investigating biomarkers of inflammation in asymptomatic individuals helps understanding the role of diet in general and dairy fat in particular in preventing these diseases. Despite being rich in saturated fatty acids, dairy fat may prevent metabolic disorders, such as odd-chain and branched-chain fatty acids, conjugated linoleic acid, milk polar lipids, among other lipids in dairy. Conjugated linoleic acid (cLA), particularly in its nitrated form (nitro-cLA), has anti-inflammatory and anti-adipogenic properties. In this study we aimed at investigating lipid biomarkers of dairy fat intake in plasma using untargeted and targeted lipidomics based on liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Twenty-nine healthy adults participated in a cLA depletion-repletion clinical trial; cLA repletion was achieved via intake of a cLA-bioenriched butter. After baseline data and blood collection, subjects restricted dairy fat intake for 8 weeks (depletion) followed by intake of cLA-bioenriched butter for 8 weeks (repletion). Blood samples were collected at three points: at baseline and at the end of both the depletion and repletion phases. Lipids were extracted from 50  $\mu$ L of plasma by the Folch method (chloroform:methanol, 2:1 v/v), and an internal standard mix with deuterated lipids (SPLASH® Lipidomix solution) were added from the extraction start; after centrifugation (21,000 rpm, 5 min), an aliquot of the lower phase was collected and then dried in a vacuum-centrifugal concentrator. Finally, the extracts were suspended in 100  $\mu$ L of a mixture of isopropanol:acetonitrile:water (2:1:1 v/v/v) and analyzed by LC-Orbitrap-HRMS. Multivariate analysis revealed five metabolites were significantly different ( $p < 0.05$ ) between the depletion and repletion phases. These metabolites were putatively annotated as sphingomyelin (SM) (1), triacylglycerols (TG) (3), and phosphatidylcholine (PC) (1). SM 32:1 and PC 35:1 biomarkers, notably down-regulated in the depletion phase, were associated with dairy intake. Additionally, TG 50:4 and TG 52:4 were significantly increased in the repletion phase, suggesting that cLA supplementation might influence the presence of trans-palmitoleic acid in plasma. Trans-palmitoleic have been shown to be positively correlated to the consumption of whole dairy products. Intriguingly, TG 48:2 was found to significantly decrease in the repletion phase; this lipid species in plasma was suggested as a possible biomarker for hepatic steatosis. Our findings highlight the importance of bioactive lipids in the diet. The species of TG and PC containing nitro-cLA annotated in the present study could be associated with anti-inflammatory effects resulting from cLA intake. In conclusion, these findings contribute to a better understanding of the effects of dairy intake on circulating lipid biomarkers, highlighting the need for further investigations to validate these findings and elucidate the underlying mechanisms of these effects.

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