

Metabolomics Abstracts' Talks Sessions

Session 1

SIMULTANEOUS MEASUREMENT OF THREE TOCOPHEROLS, ALL-TRANS-RETINOL, AND EIGHT CAROTENOIDS IN HUMAN SERUM BY ISOCRATIC REVERSED-PHASE LIQUID CHROMATOGRAPHY.

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We describe a simplified reversed-phase HPLC method for the simultaneous determination of several fat-soluble antioxidants and vitamins in human serum. The method uses a single C18 reverse phase column (4.6 x 250mm, 3µm) coupled with a photodiode array and fluorescence detection to separate and quantify 4 vitamins (all-*trans*-retinol, α-, δ-, and γ- tocopherols), and 8 carotenoids (lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, all-*trans*-lycopene, 5-*cis*-lycopene, α-carotene, and β-carotene), along with 3 internal standards (retinyl acetate, α-tocopherol acetate, and echinenone) within 35 min. The chromatographic separation is performed by isocratic elution with a mixture of acetonitrile and methanol (65:35,v/v) containing 0.065% of triethylamine at a flow rate of 1.5 mL/min. The linear ranges of the calibration curves were 0.02-6.0 µg/mL for all-*trans*-retinol, β-cryptoxanthin and α-carotene; 0.01-3.0 µg/mL for δ-tocopherol, lutein and lycopene; 0.08-24.0 µg/mL for γ-tocopherol; 0.3-90.0 µg/mL for α-tocopherol; 0.005-1.5 µg/mL for zeaxanthin and canthaxanthin; and 0.04-12.0 µg/mL for β-carotene. The recoveries of the assay through different concentrations were from 95% to 104% for all analytes. The coefficients of variation were generally < 5% for intra-batch assay and < 10% for inter-batch assay. This improved method provides a simple approach to separate and quantify three tocopherols, all-*trans*-retinol and eight carotenoids with high precision and accuracy, and is suitable for large epidemiological studies.

Session 2

APPLICATION OF MASS SPECTROMETRY TO IDENTIFY PROTEIN TARGETS AND BIOMARKERS OF UNBALANCED DIETARY N-3 POLYUNSATURATED FATTY ACID DEFICIENCY IN RATS.

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Biomarkers indicative of deficiency or excess of n-6 and n-3 polyunsaturated fatty acids (PUFA) are lacking. To address this, rats were fed through gestation and lactation with isocaloric diets differing only the PUFA composition, one adequate and one deficient in n-3 PUFA. Earlier work revealed that the low n-3 PUFA diet alters neurogenesis in the embryonic rat brain. Shotgun LC-MS analysis of plasma in combination with principal component analysis reveals significant differences in specific lyso-phosphatidylcholines. In concurrent experiments initial protein expression studies of embryonic rat liver employing 2D gel electrophoresis show significant differences in at least 25 proteins between the two groups. The identity of these proteins is currently being investigated by mass spectrometry. This study is a first step towards the application of mass spectrometry to understanding of how dietary essential PUFA regulate biochemical pathways during early development stages, and plasma biomarkers of metabolic changes in organs inaccessible in human studies.

Session 3

UNEXPECTED DIETARY-ASSOCIATED CHANGES IN THE METABOLOME.

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Little information is available regarding non-food components of the diet that cross the gut epithelium in mammals. It is known that a number of polyphenolic compounds such as flavonoids cross often using known transporters such as the glucose transporter-1. We were interested in the effects of broccoli sprout consumption since we had previously shown that consumption of broccoli sprouts rich in sulforaphane glucosinolate results in decreased oxidative stress in spontaneously hypertensive stroke-prone rats (SHRsp). The decreased oxidative stress

was associated with improved blood pressure and less tissue inflammation. We reasoned that the decreased oxidative stress should also be associated with improvements in other physiological parameters. Plasma and liver extracts were examined using Fourier Transform Mass Spectrometry. We found, not surprisingly, that there were significant metabolomic differences between male and female on both control and broccoli sprout-containing diets (n=6/gender/diet) as well as between diets. Even though there are no fats in the sprouts, one of the most striking changes seen was an increase of free polyunsaturated fatty acids and triacylglycerols in plasma suggestive of increased lipolysis – this was more pronounced in males than in females. A curious finding was the presence of compounds in the liver whose molecular masses indicated that they were gut bacterial fermentation products of cellulose (e.g., celloheptaose), xylan (e.g., D-Xylopyranosyl-D-xylose), or starch (maltopentaose). In addition, a number of plant oligosaccharides such as stachyose and raffinose were also present in liver. These latter compounds were not detected in plasma suggesting efficient liver uptake mechanisms. If the assigned identities to the molecular masses are correct our study brings up a number of questions such as how do these compounds cross the gut epithelium, what mechanisms are used by the liver for their sequestration and is the liver capable of metabolizing these compounds. We are carrying out additional mass spec analyses to unequivocally identify these compounds.

Session 4

METABOLITE BIOMARKERS OF SCLERODERMA ELUCIDATED USING 1H NMR METABOLOMICS.

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Objective: A metabolic profiling approach used to investigate systemic sclerosis (SSc) compared with normal controls, as well as rheumatoid arthritis with the goal of identifying specific SSc metabolite biomarkers and metabolic pathways. **Methods:** Sera from 25 SSc patients were analyzed and compared to samples from eleven normal adults and ten rheumatoid arthritis (RA) patients. The sera were analyzed using 1H NMR spectroscopy, and concentrations of metabolites determined using a targeted profiling approach. **Results:** A metabolite bioprofile of SSc was identified which included metabolites from a diverse set of pathways including amino acids, short chain fatty acids, purines, and energy-related metabolites. Each metabolite was found to be individually statistically significant relative to control levels

using ANOVA testing ($p < 0.05$). MANOVA testing of the bioprofile indicates that the overall profile was highly discriminatory ($p = 2.8 \times 10^{-7}$). This result was corroborated by multivariate analysis using orthogonal partial least squares discriminant analysis (OPLS-DA). A model was generated which explained 93.8% of the data, with excellent predictive value ($Q^2 = 0.938$). **Conclusions:** The scleroderma metabolite “bioprofile” identified in this study suggests alterations in several significant areas of metabolism. • Elevation of the short chain fatty acids indicates elevated omega-oxidation and impaired mitochondrial beta-oxidation, and are unique to SSc. • Decreased levels of oxidative stress related metabolites are indicative of damage by reactive oxygen species. There are some similarities to RA in the identified oxidative stress metabolites. • Bioprofiling may have diagnostic and/or prognostic value in the clinical setting. Moreover identifiable patterns may assist towards individualized medicine, including monitoring disease activity and response to treatment. Our studies are ongoing and current data will be discussed.

Session 5

AFFINITY CAPTURE OF THE FC RECEPTOR COMPLEX FROM THE SURFACE OF LIVE CELLS.

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Nano or micro particles from environmental pollution may play a key role in atherosclerosis leading to heart attack and stroke. Macrophages may engulf foreign particles derived from diet or inhaled from the environment to form giant foam cells that comprise the core of atherosclerotic plaques. The innate phagocytic receptors such as the Fc receptor may play a key role in particle engulfment. An assembled and activated Fc receptor complex was captured from the surface of live cells using the receptor’s own ligand affixed to micro beads. The ligand of the Fc class of receptors (IgG) was affixed to a 2 micron bead and permitted to bind the surface of macrophage cells on ice. The cells were briefly warmed, disrupted and the receptor complex captured on the beads. The proteins bound to the beads were discovered by liquid chromatography and tandem mass spectrometry and compared to control beads incubated with crude extracts or growth media. The assembled and activated Fc receptor complex was captured from the surface of live cells using the receptor’s own cognate ligand affixed to micro beads. The ligand of the Fc class of receptors (IgG) was affixed to a 2 micron bead and permitted to bind the surface of macrophage cells on ice. The cells were briefly warmed, disrupted and the receptor complex captured on the beads. The proteins bound to the beads were discovered by liquid chromatography and tandem mass spectrometry and

METABOLOMICS ABSTRACTS' TALKS

compared to control beads incubated with crude extracts or growth media. Proteins and isoforms of the receptor complex including specific members of the Fc, src, syk, plc, pi3k, ship, pkc, tec, rho, rhoGEF, ship, rap, pak, gab, snap, ELMO, DOCK180, crk and gpi linked proteins were observed by mass spectrometry and were confirmed at the site of receptor activation by microbeads by confocal microscopy using immunofluorescence or live cell imaging of fluorescent fusion proteins. In addition some isoforms of pak, Band4.1, ARP, cofilin, intermediate filaments, myosins, MLCK, tropomyosins or other proteins that function to generate motive forces were specifically associated with the receptor complex. The effect of drugs on the receptor function can be measured precisely at the activated receptors ligated where the bead contacts the cell. Expression of mutations, deletions or silencing RNA was used to show a functional role for RhoG, P115 RhoGEF, and CrkL in micro particle engulfment by macrophages.