Altered Lipid Profiles of Hypertriglyceridemia

Specificity and Breadth of Lipid Quantitation using the Lipidyzer™ Platform

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A novel lipidomics platform was developed that includes simplified sample preparation, automated acquisition methods, and streamlined data processing techniques that enable facile, quantitative lipid analysis. Serum samples from individuals with known metabolic conditions were used to validate the Lipidyzer™ Platform results.

METHODS

A small plasma sample set was obtained consisting of 3 experimental groups, samples from individuals who exhibited high amounts of triglycerides in blood known as hypertriglyceridemic (n = 14), who exhibited high amount of cholesteryl esters in blood known as hypercholestberolemics (n = 14), and healthy individuals who acted as controls (n = 12). The Lipidyzer chemical standard kits were added and the lipid fraction was extracted from each sample.

The Lipidyzer™ Platform (SCIEX) was used for targeted profiling of over a thousand lipid species from 13 different lipid classes on the 40 prepared lipid samples. Using flow injection sample introduction, two MRM acquisition methods were used, one injection with SelexION® Technology ON and another with the SelexION Technology turned OFF. Positive/negative polarity switching was used in both methods.

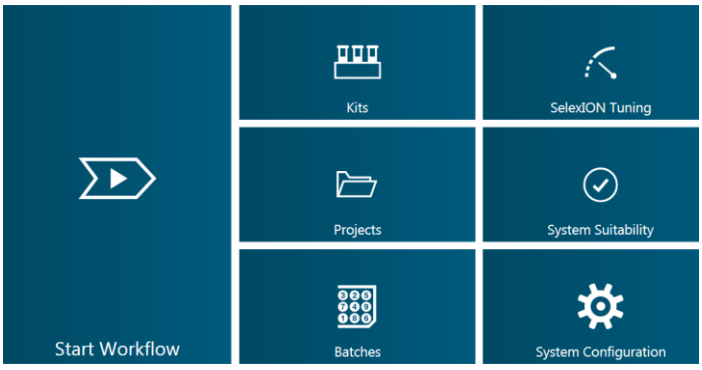
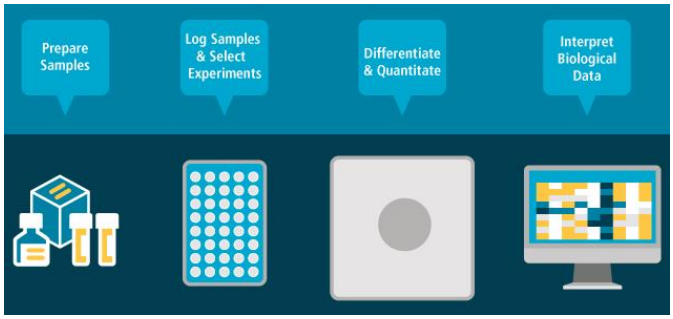


Figure 1. Lipidomics Workflow Manager. The software controlling the Lipidyzer™ Platform is the Lipidomics Workflow Manager (LWM) This software system provides LIMS capabilities for sample-tracking and workflow management, complete control of the overall system as well as the workflow. This includes automated SelexION tuning and system suitability tests than can be run as a daily or monthly check to monitor the performance of the platform. Automated data-processing for signal detection and result calculations, and reporting and visualization functionalities are all part of the software.



RESULTS

The Lipidyzer Platform involves a four-step process before samples can be acquired: (1) Kit registration for automated calculation of concentrations, (2) DMS cell tuning for class-specific compensation voltages (COV) allowing for maximum specificity, (3) system suitability testing to assess performance of the platform and the assay and (4) sample submission. All of these actions can be performed from the Lipidomics Workflow Manager (LWM) Software (Figure 1).

Once a sample set has been completed on the Lipidyzer Platform, the user has the ability to explore the results from the Project Owner summary (Figure 2). A user can view the logged samples (2b) including any metadata and if they want to add extra metadata post-acquisition this is possible. The batches acquired can be explored (2c) and here a sub-section of the spectral data can be reviewed. This includes reviewing the raw data, QC data charts as well as the processed final results (calculated to the appropriate internal standards). Finally a user can view a project report (2d), conduct any statistical analyses (2e) and publish data to access Metabolon's Surveyor Web Tools for further evaluation (2f).

Figure 3 highlights the lipid species changes which were upregulated in the hypertriglyceridemic and cholesterobolerimc

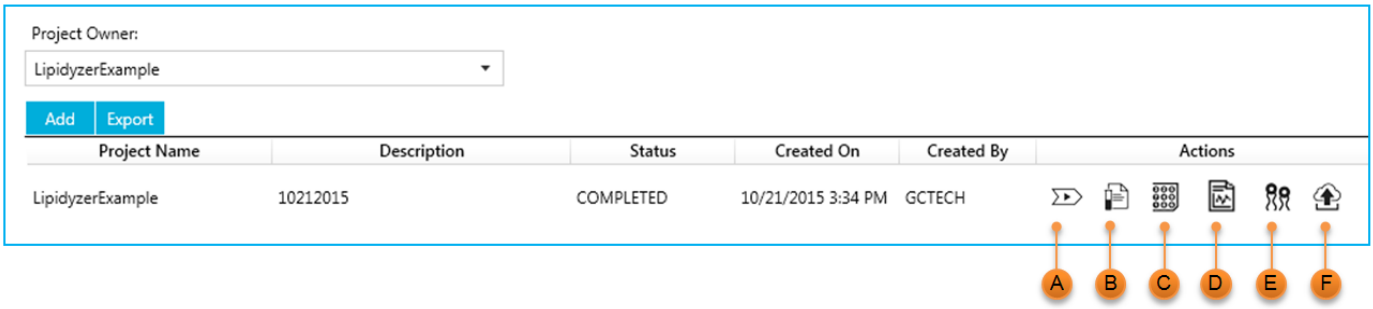


Figure 2. Project Status in the Lipidomics Workflow Manager. The above project has been marked as completed and all six icons are available for user interaction. Here a workflow was started, samples were logged, batches were created and data acquired, a report was generated, statistical analysis was performed. Finally the data can be sent to Metabolon's Surveyor Web Tools for further evaluation (Available as an add-on).

CLASS	SUB_CLASS	CHEMICAL_NAME	HMDB	KEGG	LIPID_MAPS	HIGH_TAG_NORMAL(FOLD)
TAG	ester	TAG48:0-FA16:0	HMDB00220	C00249	LMFA01010001	15.5289
TAG	ester	TAG46:0-FA14:0	HMDB00806	C06424	LMFA01010014	15.0773
TAG	ester	TAG46:0-FA16:0	HMDB00220	C00249	LMFA01010001	15.0112
TAG	ester	TAG46:1-FA16:1	HMDB03229	C08362	LMFA01030056	13.9059
TAG	ester	TAG48:1-FA16:1	HMDB03229	C08362	LMFA01030056	13.8266
TAG	ester	TAG49:0-FA16:0	HMDB00220	C00249	LMFA01010001	13.1025
TAG	ester	TAG50:0-FA18:0	HMDB00827	C01530	LMFA01010018	12.7304
TAG	ester	TAG49:0-FA17:0	HMDB02259	C17714	LMFA01010017	12.7061
TAG	ester	TAG47:0-FA16:0	HMDB00220	C00249	LMFA01010001	12.4392

CHEMICAL_NAME	HMDB	KEGG	LIPID_MAPS	HIGH_CE_NORMAL(FOLD)
CE(18:4)	-			5.4032
CE(14:1)	HMDB10367		LMST01020021	4.4896
CE(16:1)	HMDB00658		LMST01020006	3.8689
CE(14:0)	HMDB06725		LMST01020004	3.1034
CE(18:3)	HMDB10370		LMST01020009	2.9469
CE(20:5)	HMDB06731		LMST01020015	2.8251
CE(12:0)	HMDB02262		LMST01020001	2.3344
CE(20:3)	HMDB06736		LMST01020013	2.2789

Figure 3. Lipidyzer Platform Demonstrates Expected Changes in Triacylglycerols (TAGs) and Cholesteryl Esters (CEs). Here the groups were classed with their phenotype, high TAG for hypertriglyceridemia, high CE for the hypercholestberolemics and normal for the controls using the traditional colorimetric test. Thirty molecular species of TAGs had a fold change higher than ten in hypertriglyceridemic samples vs healthy. 22 CEs species also significantly showed higher levels in the hypercholestberolemics relative to healthy (2-5x higher).

samples. However when TAGs are measured in the clinic they are done using a colorimetric test which measures free glycerol which could be coming from anywhere in the body. What this colormetric TAG test couldn't highlight was the novel results found in the down regulation of the hexosylceramides (HCER) and lactosylceramides (LCER) highlighting altered glycosphingolipid metabolism.

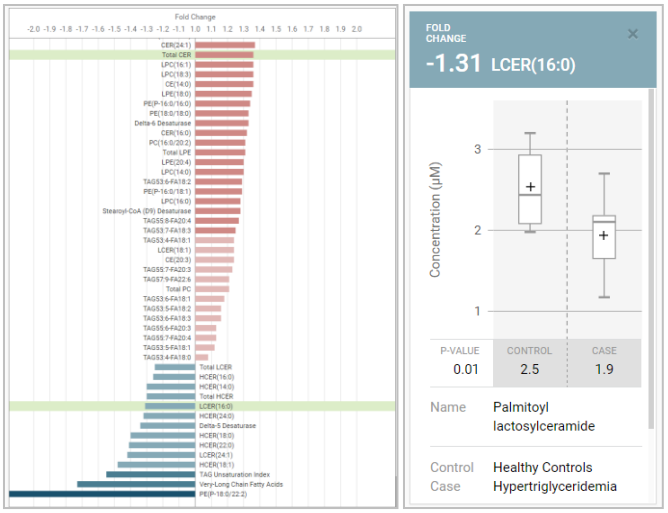


Figure 4. Novel Glycosphingolipid Metabolism. Down-regulated hexosylceramides (HCER) and lactosylceramides (LCER) were found in both of the disease groups, the highlighting altered glycosphingolipid metabolism that could not be found using the traditional colorimetric test.

CONCLUSIONS

This serum data set demonstrates that the Lipidyzer™ Platform provides similar findings to the accepted colorimetric test on samples with known metabolic. Both triglycerides and cholesteryl esters correlated with the known characterization of the samples. The specificity and breadth of the Lipidyzer Platform assay also provided novel findings which was not possible with current tests.

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