Dysregulated Lipid Profiles of Non-Alcoholic Fatty Liver Disease (NAFLD)

High Sensitivity Lipid Quantitation using the Lipidyzer™ Platform

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is an umbrella term for a range of liver conditions affecting people who drink little to no alcohol. As the name implies, the main characteristic of nonalcoholic fatty liver disease is too much fat stored in liver cells. Nonalcoholic steatohepatitis, a potentially serious form of the disease, is marked by liver inflammation, which may progress to scarring and irreversible damage. This damage is similar to the damage caused by heavy alcohol use. At its most severe, nonalcoholic steatohepatitis can progress to cirrhosis and liver failure. However, if caught early the fatty liver and fatty liver to NASH process can be reversed.

In this pilot study, the Lipidyzer™ platform was used to evaluate two stages of NAFLD, simple steatosis and steatohepatitis to evaluate any dysregulation in complex lipid metabolism.

METHODS

In this study, plasma samples with known disease classifications were obtained from three sample groups; healthy controls (n=15), simple steatosis (n=14) and steatohepatitis (n=15). Samples were extracted using the standardized protocols and chemical standard kits of the Lipidyzer™ Platform. Quantitative profiling of over 1100 lipid species from 13 different lipid classes was performed using the Lipidyzer Platform (SCIEX), providing extensive lipid coverage. The flow injection analysis (FIA) method provided simple sample introduction and lipid molecular species were measured using MRM analysis in both positive and negative polarities. Use of SelexION® Technology provided enhanced selectivity for analyzing some of the lipid classes.

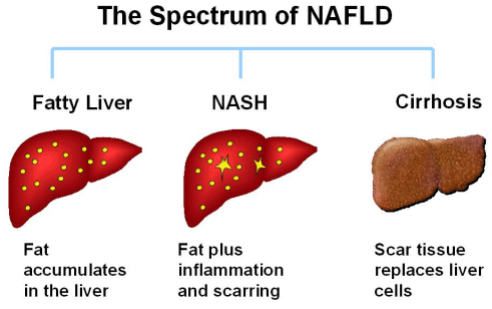

Figure 1. Non Alcoholic Fatty Liver Disease Progression. The process begins with simple steatosis (Fatty Liver) where fat begins to accumulate in the liver, followed by the onset of fat accumulation leading to inflammation and scarring (NASH). Once the scarring tissue starts to replace the liver cells, irreversible damage is done and Cirrhosis sets in.

Class	CV	IS Mix	LOT Number
CE	3.11	UF NAFLD	CHEISTLPV-100
CER	4.44	UF NAFLD	CERISTLPV-100
DAG	7.26	UF NAFLD	DAGISTLPV-100
DCER	30.73	UF NAFLD	DCERISLPV-100
FFA	3.8	UF NAFLD	FFAISTLPV-100
HCER	9.36	UF NAFLD	HCERISLPV-100
LCER	11.54	UF NAFLD	LCERISLPV-100
LPC	1.18	UF NAFLD	LPCISTLPV-100
LPE	6.91	UF NAFLD	LPEISTLPV-100
PC	3.91	UF NAFLD	PCISTLPV_100
PE	1.89	UF NAFLD	PEISTLPV_100
SM	1.77	UF NAFLD	SMISTLPV_100
TAG	5.07	UF NAFLD	TAGISTLPV_100

Table 1. Spiked QC Results Table. Spiked quality control samples were monitored throughout the study and the high accuracy obtained across replicates highlights the reproducibility of this platform for quantitative lipid profiling. The dihydroceramide class (DCER) displayed higher CVs but this is because they are present at exceedingly low levels in plasma.

RESULTS

All data obtained from the Lipidyzer Platform was automatically processed in the Lipidomics Workflow Manager (LWM). Spiked quality control samples were monitored throughout the study and the high accuracy obtained across replicates (Table 1) highlights the reproducibility of this platform for quantitative lipid profiling.

851 lipid molecular species were detected and quantified from this plasma dataset. Using the supplied metadata for sample classification, fold change and p-values were computed for the lipid molecular species and the results were visualized as a heat map (Table 2). Top 10 up-regulated and down-regulated lipid molecular species are shown, comparing the samples according

CLASS	CHEMICAL_NAME	HMDB	KEGG	LIPID_MAPS	SIMPLE_CONTROL (PVAL)	SIMPLE_CONTROL (FOLD)	HEPATI_CONTROL (PVAL)	HEPATI_CONTROL (FOLD)
DAG	DAG(20:0/20:0)	HMDB07368		LMGL02010117	0.4635	-1.2157	0.0197	-7.8795
CE	CE(20:0)	HMDB06740		LMST01020010	0.5813	-1.3317	0.0273	-6.8060
CE	CE(20:1)	HMDB05193		LMST01020011	0.5911	-1.0985	0.0143	-5.9178
CE	CE(24:1)	HMDB06728		LMST01020020	0.6845	-1.2740	0.0199	-4.8855
TAG	TAG52:1-FA20:0	HMDB02212	C06425	LMFA01010020	0.4091	-1.0222	0.0493	-3.8536
CE	CE(22:2)	HMDB06737		LMST01020017	0.3406	1.0153	0.0440	-3.7752
TAG	TAG54:1-FA20:0	HMDB02212	C06425	LMFA01010020	0.3913	1.1040	0.0387	-3.4095
TAG	TAG56:2-FA20:0	HMDB02212	C06425	LMFA01010020	0.5095	1.3085	0.0133	-3.3280
TAG	TAG54:2-FA20:0	HMDB02212	C06425	LMFA01010020	0.3739	1.2018	0.0389	-3.2076
CE	CE(22:0)	HMDB06727		LMST01020016	0.5601	-1.3746	0.0042	-2.7190
DAG	DAG(18:1/20:4)	HMDB07228		LMGL02010121	0.4316	1.1492	0.0473	1.9157
TAG	TAG54:4-FA20:4	HMDB01043	C00219	LMFA01030001	0.1219	1.4603	0.0313	1.9782
TAG	TAG54:6-FA20:4	HMDB01043	C00219	LMFA01030001	0.2617	1.2880	0.0299	2.0144
TAG	TAG50:1-FA16:1	HMDB03229	C08362	LMFA01030056	0.1014	1.6623	0.0463	2.0155
TAG	TAG54:6-FA16:1	HMDB03229	C08362	LMFA01030056	0.4253	1.2494	0.0251	2.0268
TAG	TAG54:7-FA16:1	HMDB03229	C08362	LMFA01030056	0.5134	1.2381	0.0173	2.0422
TAG	TAG48:1-FA16:1	HMDB03229	C08362	LMFA01030056	0.1870	1.9032	0.0486	2.13
TAG	TAG54:5-FA20:4	HMDB01043	C00219	LMFA01030001	0.1830	1.35	0.0371	2.2240
PE	PE(16:0/22:5)	HMDB08945			0.0338	2.1798	0.0012	2.2463
TAG	TAG52:5-FA20:4	HMDB01043	C00219	LMFA01030001	0.2482	1.4537	0.0468	3.3239

Table 2. Heat Map Generated using the Lipidomics Workflow Manager Highlighting the Differentially Regulated Lipid Molecular Species. Plasma samples were classed according to their phenotype, previously determined by accepted clinical techniques. Both disease samples were compared to healthy controls and the major lipid differences were determined. Much more significant lipid changes were found when comparing the Steatohepatitis samples (Hepati) to the controls. The largest upregulated lipids in disease are shown in red and the larges down-regulated lipids are shown in blue.

to their phenotype; simple (simple steatosis) and hepati (steatohepatitis) vs control. All species in the last column in the table were upregulated between the control and steatohepatitis. However it can be seen that these species do not appear statistically significant when comparing the control to the simple steatosis group. This signals that there is a significant shift in lipid molecular species, when the disease moves from simple steatosis to steatohepatitis.

CONCLUSIONS

The Lipidyzer™ Platform was used to profile the changes in complex lipid metabolism that occur across the stages of NAFLD, using plasma samples from 3 known disease groups. The platform quantitated 851 lipid molecular species across the sample set analyzed with good reproducibility. There were a number of lipid species that had statistically significant changes from control as the disease progressed to steatohepatitis.

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