Evaluating the Metabolic Impact of Feeding Baboons a High Fat, High Calorie Diet Using an Accurate Mass GC/Q-TOF

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Introduction

Glucose homeostasis is achieved through a balance of several factors: the rate of consumption and intestinal absorption of dietary carbohydrate, the rate of utilization of glucose by peripheral tissues, the loss of glucose through the kidney tubule, and the rate of removal or release of glucose by the liver and kidney. To avoid hyperglycemia (uncontrolled increases in blood glucose levels following meals) and fasting hypoglycemia (decreased in blood glucose levels during periods of fasting), the body can adjust levels by a variety of cellular mechanisms (Szablewski, L., Glucose Homeostasis and Insulin Resistance, Bentham Science Publishers, 2011, pg. 46). Male baboon blood serum was analyzed with GC/Q-TOF mass spectrometry to distinguish the metabolic differences produced from drastically different diets.

Experimental

Blood was drawn from a group of 3.5 year old male baboons that were immunocompromised in utero. The protein fraction of a 30 µL aliquot of plasma was crashed and ultracentrifuged. The supernatant was collected and dried by speed vacuum, and the active functional groups were derivatized by methoximation using a saturated solution of hydroxylamine HCl in pyridine followed by silylation with N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and 1 % trimethylchlorosilane (TMCS).

This study was performed using an Agilent 7890 GC coupled to an Agilent 7200 Series Quadrupole-Time-of-Flight (Figure 1). GC and MS conditions are described in Table 1.

Figure 1. 7200 Series GC/Q-TOF system.

Experimental

GC and MS Conditions:	
Column	DP-5 MS UI, 30 meter, 0.25 mm ID, 0.25 µm film
Injection volume	1 µL
Split ratio	10:1
Split/Splitless inlet temperature	250 °C
Oven temperature program	60 °C for 1 min 10 °C/min to 325 °C, 3.5 min hold
Carrier gas	Helium at 1.2 mL/min constant flow
Transfer line temperature	290 °C
Ionization mode	EI, positive CI (20 % methane flow)
Source temperature	275°C
Quadrupole temperature	150°C
Scan range	50 to 600 m/z
Spectral acquisition rate	5 Hz, collecting both in centroid and profile modes

Table 1. GC/Q-TOF conditions used in the study.

The data were processed using the Targeted Deconvolution algorithm that is built into the MassHunter software. Targeted Deconvolution integrates and automates three processes. These processes are as follows:

- Identification and quantitation based on target and qualifier ions and ratios, combined with locked retention times.
- Deconvolution, where identification is based on comparing the "clean" full spectrum to a user library.
- Confirmation of a compound's identity based on the deconvoluted spectrum.

Results from each of these three processes are combined in an easy to read report.

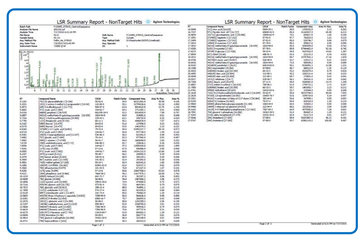

Figure 2. Targeted Deconvolution Report using the Fiehn RT Locked Database.

Targeted Deconvolution was able to distinguish 700+ unique components in each sample (Figure 2). The corresponding hits were found in the Fiehn metabolomics MS library with a Match Factor score >50 for about 40-60 components in each sample.

Experimental

RT	Compound Name	Comment
5.523	[6871] 2-hydroxypyridine [6.519]	gut microbe
8.302	[441] beta-Hydroxybutyric acid [8.264]	goes up when burning fat
9.435	[179] urea [9.599]	bad peak shape
13.469	[70289] Allothreonine 2 [13.346]	Threonine
13.270	[440015] cis-4-Hydroxyproline 3TMS [13.06]	turnover of collegen
14.393	[30320] L-glutamic acid 2 [14.398]	bad peak shape
14.487	[6140] Phenylalanine 2TMS [14.29]	showing changes in weight loss
16.597	[311] citric acid [16.615]	citrate/isocitrate
16.732	[875] Myristic Acid -027 [16.727]	Internal Standard
17.163	[1101] L- sorbose 1 [17.187]	Fructose and other ketohexoses
17.307	[448388] D-allose [17.278]	glucose and other aldohexose
17.414	[24154] galactosamine 2 [17.386]	search NIST
20.493	[1161490] trans-13-octadecenoic acid [20.608]	isomer of oleaic acid (especially if the peak is big)
23.516	[14900] alpha-Monopalmitin [23.52]	Gross contaminant on glasswaare
23.917	[446284] eicosapentaenoic acid [24.013]	light hand tail of arachidonic acid - probably coeluting
24.949	[24699] alpha-Monostearin [24.96]	affected by fat in the diet
27.429	[6033] alpha-Tocopherol 1TMS [27.50]	vitamin e
27.584	[304] Cholesterol [27.657]	Always present

Figure 3. Data curation is important to validate the quality of the data and the validity of the parameters used to extract the data.

Both identified and unidentified components were further searched against the NIST MS library for additional confirmation and identification of the components not present in the Fiehn library. In general, there is a wide distribution of the compounds identified using the Fiehn library from gut microbes, amino acids, carbohydrates, impurities like EDTA and alpha-Monopalmintin, vitamins, sterols, etc. Curating the data for known entities is important in evaluating the quality of the data and the effectiveness of the parameters used to extract the raw data.

Components such as EDTA and N-(2-hydroxyethyl) iminodiacetic acid, a thermal breakdown product of EDTA, are contaminants. This anticoagulant is massively present in plasmas prepped with purple tops in the Vacutainer BD (formerly Becton-Dickinson) line of phlebotomy products. These can be identified and removed from consideration.

The sugars elute around 17 minutes using the Fiehn protocol. This section of the chromatographic run is complicated with a fair amount of coelution. Initially fructose and other isomeric ketohexoses elute before glucose and other isomeric aldohexoses. The Fiehn methodology would have to be adjusted to improve chromatographic resolution. The calibration file allows for adjusting RT locked methods as long as a RI calibration file is used to correct for the differences.

Mass Profiler Professional (MPP), a multivariate statistical package, was used for evaluation of the data. MPP includes Pathway Architect (PA), a module used to place the mass spectral data into biological context. PA integrates data from databases such as: KEGG, WikiPathways, BioCyc, and PathVisio custom pathways as well as GMPL and BioPAX formats. It also has the ability to resolve the nomenclature inconsistencies between the same compound in various databases by using the integrated BridgeDB.

Results and Discussion

Using multivariate statistics, we found that only a small subset of the 500+ found entities to be changing significantly (fold change >2) between the baseline (BL) and the 7 week condition sets (7WK). This interesting result is explained by considering how tightly energy metabolism is regulated. This suggests that the bulk of observed changes were in relative concentrations of known metabolites. This observation is born out when looking at the Hierarchical Clustering (Figure 4).

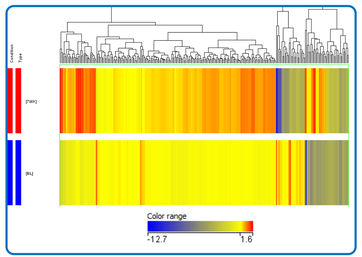

Figure 4. Hierarchical clustering on both conditions and entities show that the bulk of the changes are minor shifts in relative concentration(Red - Yellow and Red - Green) but only a handful of components change dramatically Red-Blue.

There are various ways to focus on the small subset of entities that are changing significantly. Creation of PCA Plots, Significance Testing (simple paired T-test), Volcano Plots, Venn Diagrams, or use of Sample Class Prediction to highlight these specific components can be performed. In this case, Venn Diagrams (Figure 5) were used to evaluate the Fold Change >2 correlations. Note that in Figure 5 there are 83 components that are up-regulated, 28 components that are down-regulated, and 159 of the components fall below the Fold Change > 2 threshold fold increase across the sample groups.

Although the bulk of the components did not have a Fold Change > 2 when averaged across the specimens, it is important not to discount them because they turn out to be interesting, consistent, and compelling on a paired analysis. To evaluate these minor changes, a semi-quantitative approach (Figure 6) was employed to evaluate the changes that were consistent between paired specimens. Table 2 highlights a clear increase in free glycerol and a concurrent decrease in the amino acid concentrations. This result is consistent across all the specimens in this small study set.

Results and Discussion

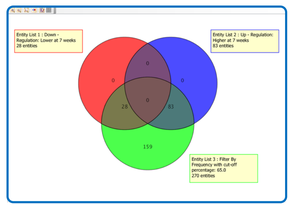

Figure 5. A Venn Diagram showing the different fold change relationships. Up-regulated is colored blue, down-regulated is colored red, and < 2 fold change is colored green.

Name	28609 BL Area	28609 7wk Area	% Change
[753] glycerol [9.941] Results	807498	3455680	328
[24749] D-glucose 1 [17.426] Results	6061647	6009452	-1
[107689] L-(+) lactic acid [6.851] Results	12362714	11315297	-8
[791] DL-isoleucine 2 [10.225] Results	733025	126619	-83
[6287] L-valine 2 [9.151] Results	2338508	1265943	-46
[5962] L-lysine 2 [17.643] Results	583160	73538	-87
[6057] L-tyrosine 2 [17.856] Results	743289	93778	-87

Table 2. Dramatic changes found in free glycerol and amino acid concentrations.

It was discovered that serum amino acid concentrations dropped between 25 and 90 % after 7 weeks on a high fat, high sugar diet (Figure 6). According to Pitkanen et al (Amino Acids, 2003 Jun; 24(4):413-21), lower serum amino acid concentrations correlate with aging. An additional observation was a three- to five-fold increase in free glycerol which is considered an important factor in lipid metabolism and cardiovascular disease risk (Table 2).

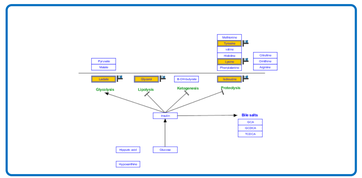

Figure 8. Pathway Architect is used to place the results into biological context. It is clear from the results generated in MPP that that glucose homeostasis is an interesting pathway to evaluate with this experiment. It shows that glucose homeostasis by lipidolysis is strongly up-regulated while other pathways were not statistically impacted or only slightly down-regulated.

Conclusions

There were two main conclusions drawn from the statistical analysis of baboon blood serum:

- We identified the main mechanism of glucose homeostasis in baboons fed a high fat, high caloric diet to be lipidolysis. The main indicator of this was a three- to five-fold increase in free glycerol.
- We also found that serum amino acid concentrations dropped between 25 and 90 % after 7 weeks on a high fat, high sugar diet. In general, lower serum amino acids are thought to correlate with aging.

Although this was a pilot study, the semi-quantitative results were consistent across the study specimens.

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