Plasma Metabolic Profiling Suggests Enrichment of Glycolytic Pathways Over Fatty Acid Oxidation in Becker Muscular Dystrophy

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1. Abstract

Becker muscular dystrophy (BMD) is a serious, progressive muscle myopathy caused by mutations in the dystrophin gene resulting in a truncated protein, which leads to enhanced contraction-induced injury, muscle degeneration and muscle replacement with fat and fibrotic tissue. We sought to understand whether alterations in metabolic signatures were observed in BMD. Plasma samples from 16 healthy volunteers were compared to 7 age-matched BMD patient plasma samples using an analysis platform created by Metabolon Inc (Durham, NC). Each sample was analyzed on both LC/MS/MS and Polar LC platforms. Results were compared to a library of standards for metabolite identification and for metabolite quantitation by peak area integration. Principal Component Analysis (PCA) of all samples showed healthy and BMD individuals clustered with clear separation between groups suggesting a metabolic shift in BMD patients. The metabolic shifts identified in BMD plasma were further supported by statistical summary count tables which revealed 30% of biochemicals within the library were significantly changed in BMD subjects relative to healthy volunteer controls. Within the identified biochemical changes, 88 (21.5%) were upregulated while 322 (78.5%) were downregulated in the BMD cohort compared to healthy controls. Notably, multiple free fatty acids (FFAs) were decreased with many reaching statistical significance in BMD subjects compared to controls. Conversely, glucose and several glycolytic intermediates were either significantly increased or were trending higher in BMD patients. These metabolic shifts in BMD compared to controls, including, changes in FFA, fatty acid oxidation, and carbohydrate metabolism potentially reflect key energetic changes in skeletal muscle and may represent novel disease signature biomarkers for BMD that merit further investigation.

2. Study Design

Plasma samples were collected from healthy control (CTRL) Becker muscular dystrophy (BMD) subjects (Table 1). Age range of study participants was 20-47 and all subjects were male.

	N	% Male	Age (Years) ± SD
Healthy	16	100	35.7 ± 7.8
BMD	7	100	33.8 ± 5.7

Table 1. Demographics of study participants. Age for each group is represented as average ± standard deviation (SD).

3. Methodology

Metabolic Evaluation:

Untargeted metabolomic profiling was performed at Metabolon, Inc (Morrisville, NC, USA) using a combination of LC-MS methods as described (1). All methods utilized a Waters ACQUITY UPLC and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. Briefly, the sample extract was dried then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. Based on Metabolon, Inc protocols and previously published methods, the first aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. The second aliquot was also analyzed using acidic positive ion conditions; however, it was chromatographically optimized for more hydrophobic compounds. The third aliquot was analyzed using basic negative ion optimized conditions and a separate dedicated C18 column. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1 × 150 mm, 1.7 µm) using a gradient consisting of water and acetonitrile with 10 mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion. The scan range varied slighted between methods but covered 70–1000 m/z. Raw data files are archived and extracted as described below. Metabolites were identified by comparison to a referenced library of chemical standards, and area-under-the-curve analysis was performed for peak quantification and normalized to day median value. To ensure high quality of the dataset, control and curation processes were subsequently used to ensure true chemical assignment and remove artefacts and background noise. Metabolites were scaled by run-day medians and log-transformed before statistical analysis.

4. Results

Process Evaluation:

The total number of metabolites detected in this study are outlined in Table 2. This total corresponds to many biochemicals that matched a named structure in Metabolon's reference library. The remaining represent distinct chemical entities., but they do not currently match a named biochemical in Metabolon's library. These unknown molecules represent a single molecule of discrete molecular formula and structure, but currently, remain unnamed.

Metabolite Classification	EDTA Plasma
Total	1388
Named/Positively Identified	1114
Unnamed	274

Table 2. Total number of biochemicals identified in the currently study. Named biochemicals are those that were matched in Metabolon's reference library while unnamed biochemicals do not currently match biochemicals in the reference library.

4. Results (Continued)

Principal Component Analysis.

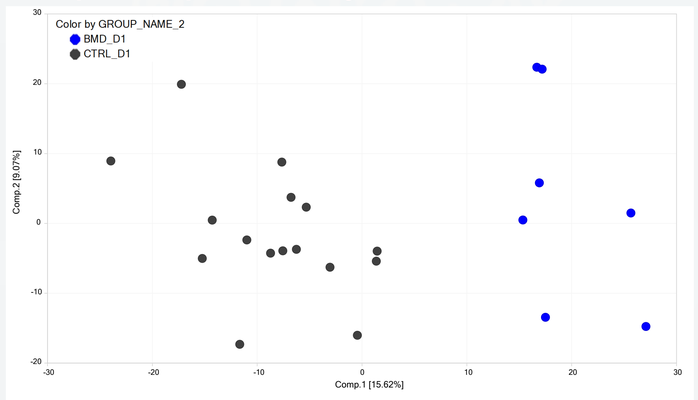

Figure 1. Principal Component Analysis. Principal component analysis shows clear metabolic differences between healthy and BMD individuals. Each subject is represented.

Fatty Acid Metabolism

Sub Pathway	Biochemical Name	BMD CTRL (D1)
Short Chain Fatty Acid	butyrate/isobutyrate (4:0)	1.03
	valerate (5:0)	0.81
Medium Chain Fatty Acid	caproate (6:0)	1.07
	cis-4-decenoate (10:1n6)*	0.67
	(2 or 3)-decenoate (10:1n7 or n8)	0.38
	laurate (12:0)	0.44
	5-dodecenoate (12:1n7)	0.63
Long Chain Saturated Fatty Acid	myristate (14:0)	0.42
	pentadecanoate (15:0)	0.51
	palmitate (16:0)	0.60
	margarate (17:0)	0.45
	stearate (18:0)	0.62
	nonadecanoate (19:0)	0.63
Long Chain Monounsaturated Fatty Acid	myristoleate (14:1n5)	0.37
	palmitoleate (16:1n7)	0.44
	10-heptadecenoate (17:1n7)	0.40
	oleate/vaccenate (18:1)	0.58
	10-nonadecenoate (19:1n9)	0.57
	eicosenoate (20:1)	0.70
Long Chain Polyunsaturated Fatty Acid (n3 and n6)	tetradecadienoate (14:2)*	0.52
	stearidonate (18:4n3)	0.39
	eicosapentaenoate (EPA; 20:5n3)	0.44
	docosapentaenoate (n3 DPA; 22:5n3)	0.43
	docosahexaenoate (DHA; 22:6n3)	0.48
	hexadecadienoate (16:2n6)	0.45
	linoleate (18:2n6)	0.55
	linolenate [alpha or gamma; (18:3n3 or 6)]	0.50
	dihomo-linoleate (20:2n6)	0.64
	dihomo-linolenate (20:3n3 or n6)	0.59
	arachidonate (20:4n6)	0.58
	adrenate (22:4n6)	0.51
	docosapentaenoate (n6 DPA; 22:5n6)	0.49
	docosadienoate (22:2n6)	0.81
	mead acid (20:3n9)	0.53

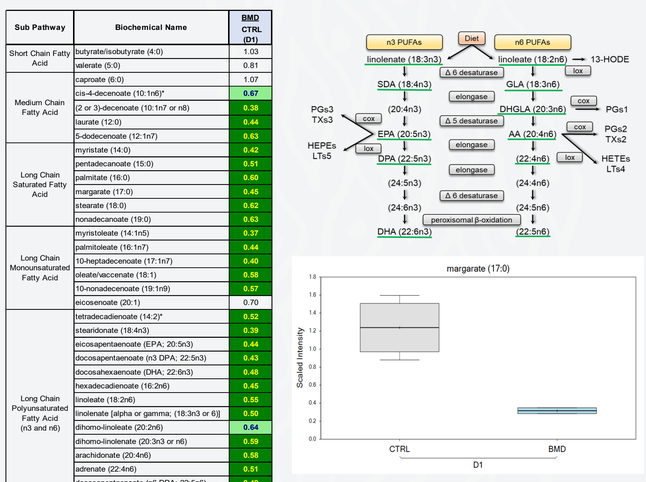

Figure 2. Fatty Acid Metabolism. Biochemical heat map, pathway map, and representative box plots for free fatty acid. BMD biomarkers that are significantly downregulated compared to control are dark green (p≤0.05) and light green represents biochemicals which are trending downward (0.05<p<0.10).

Glucose Metabolism

Sub Pathway	Biochemical Name	BMD CTRL (D1)
Glycolysis and Pyruvate Metabolism	glucose	1.10
	3-phosphoglycerate	1.44
	pyruvate	1.70
	lactate	1.51
Disaccharides and Oligosaccharides	lactose	2.46
	sucrose	1.28
Fructose, Mannose and Galactose Metabolism	fructose	1.47
	mannitol/sorbitol	1.05
	mannose	0.96
	galactonate	5.39
Pentose Metabolism	arabinose	1.19

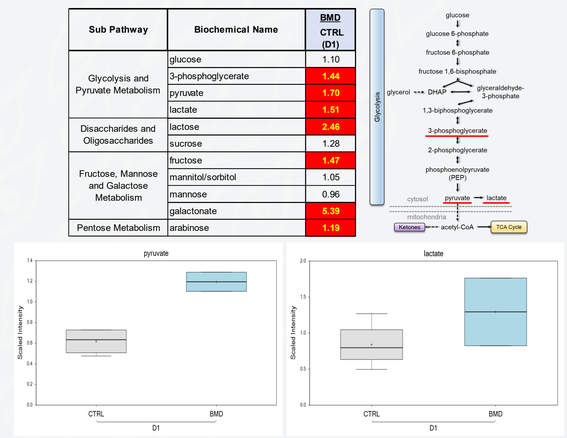

Figure 3. Glycolysis and Sugar Metabolism. Biochemical heat map, pathway map, and representative box plots for metabolites involved in glycolysis and sugar metabolism. Red represents BMD biochemicals significantly different from control (p≤0.05).

4. Conclusions

A number of metabolic differences were observed between BMD subjects compared to healthy subjects. Out of the 1114 identified biochemicals, 88 were upregulated while 322 were downregulated in the BMD cohort compared to healthy controls. Many of those significant changes occurred in the fatty acid and glycolytic metabolic pathways. This unique metabolic profile in BMD patients may represent key energetic changes in skeletal muscle and merit further investigation as potential biomarkers for disease progression.

At Edgewise, patients are at the core of everything we do.

References: 1) Evans AM, et al. High resolution mass spectrometry improves data quantity and quality as compared to unit mass resolution mass spectrometry in high-throughput profiling metabolomics. Metabolomics. 2014;4:1000132

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