



## Case Report

# Lipid changes in the metabolome of a single case study with maple syrup urine disease (MSUD) after five days of improved diet adherence of controlled branched-chain amino acids (BCAA)



Teresa D. Douglas<sup>a,\*</sup>, L. Kristin Newby<sup>b</sup>, Julie Eckstrand<sup>b</sup>, Douglas Wixted<sup>b</sup>, Rani H. Singh<sup>a</sup>

<sup>a</sup> Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

<sup>b</sup> Duke Clinical and Translational Science Institute (CTSI), Duke University School of Medicine, Durham, NC, USA

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**Guarantor:** Rani H. Singh, PhD, RD, LD, Department of Human Genetics, Metabolic Nutrition Program Emory University, Atlanta, GA 30322.

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## ABSTRACT

**Background:** Distinguishing systemic metabolic disruptions in maple syrup urine disease (MSUD) beyond amino acid pathways is under-investigated, yet important to understanding disease pathology and treatment options.

**Methods:** An adolescent female (15 years) with MSUD without liver transplant, attended 2 study visits, 5 days apart. Medical diet adherence was determined based on her 3-day diet records and plasma branched-chain amino acid (BCAA) concentrations at both study visits. Plasma from a single age- and sex-matched control (MURDOCK Study, Duke University) and the case patient were analyzed with UPLC/MS/MS for intensity ( $m/z$ ), annotated, and normalized against a median of 1 (Metabolon, Morrisville NC). Differences between case/control and 5-day comparisons were defined as  $\geq |0.5|$ .

**Results:** 434 lipid metabolites were identified across samples; 90 (20.7%) were higher and 120 (27.6%) lower in the MSUD case at baseline compared with control. By study visit 2, plasma BCAA had declined, while 48 (53%) of elevated lipids and 14 (11.7%) of lower lipid values had moved to within  $|0.5|$  of control. Most shifts towards control by day 5 were seen in long-chain fatty acid intermediates (42%) and acylcarnitines (32%). Although androgenic (28%) and bile acid (23%) metabolites increased towards control, neither reached control level by day 5.

**Discussion:** This comparative metabolomics study in a single MSUD case and healthy control suggests intrinsic differences in MSUD lipid metabolism potentially influenced by therapeutic diet. Findings suggest influences on hormone regulation, fatty acid oxidation, and bile acid synthesis, but further studies are needed to confirm an association between MSUD and lipid dysregulation.

**Synopsis:** Within 5 days of improved dietary adherence, a single MSUD case experienced substantial changes in lipid markers potentially related to changes in plasma branched-chain amino acids.

## 1. Introduction

Maple syrup urine disease (MSUD), also known as branched-chain ketoaciduria (BCK), is an autosomal recessive inherited metabolic disorder (IMD). In MSUD, metabolism of the branched-chain amino acids (BCAA), leucine (LEU), isoleucine, (ILE) and valine (VAL), is impaired due to branched-chain  $\alpha$ -ketoacid dehydrogenase complex (BCKDH) deficiency [3]. Although typically rare (1,185,000 live births), incidence is higher (up to 1:176 live births) among old order Mennonite groups [6]. When untreated, during poor diet adherence, or times of catabolic stress, sequelae for classic MSUD may include a strong maple syrup odor to the urine, neurological and developmental delay, seizure activity, encephalopathy, and death. Newborn screening (NBS) can

detect MSUD within days of birth. Although treatment from infancy typically yields good outcomes, treatment is limited to either liver transplant or a lifelong strict low intact-protein diet with medical food (MF) that is free of BCAA [3,18].

Discovery-focused metabolomics, beyond diagnostic methodology, is seldom employed in the field of IMD, despite potential for discovering uncharacterized biochemical phenotypes and development of precision therapies [9,31]. Among the few publications, most address the disorders phenylketonuria [5,20,21,27] and mucopolysaccharidosis [13,29,30]. Ours is the first study to use metabolomics for discovery of altered metabolic pathways beyond BCAA in a case with MSUD. In collaborations on another metabolomics project, we took the opportunity to perform a pilot analysis of stored plasma available from a single

\* Corresponding author.

E-mail addresses: [tdougl@emory.edu](mailto:tdougl@emory.edu) (T.D. Douglas), [rsingh@emory.edu](mailto:rsingh@emory.edu) (R.H. Singh).

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case with MSUD. Given recent literature connecting higher BCAA in the blood with adverse health outcomes such as Alzheimer's [24], diabetes, dyslipidemia, and cardiovascular disease [1,2] in the typical population, we anticipated the metabolomics profile of a person with MSUD would reveal metabolic disruptions beyond known amino acid effects. The presented case report takes into account influence of medical diet adherence and reveals opportunities for further research into biological mechanisms of MSUD pathogenesis, potential long-term health outcomes, and therapeutic targets relevant to BCAA metabolic affects in both MSUD and the general population.

Our objectives were to determine with an opportunistic pilot analysis of the influence of a one-week in-house BCAA-controlled diet intervention on the metabolome of a patient with MSUD and to identify biochemical subgroups and specific analytes that may differ from healthy age-similar controls.

## 2. Methods

### 2.1. Study design

Metabolomics case analysis for rare diseases has precedent in the peer-reviewed literature [12,32], which guided our decision to perform a pilot investigation of stored plasma from a single case patient with MSUD. The case study patient (MSUD diagnosed via NBS), was recruited and consented at the 2017 (23rd annual) Emory Genetics Metabolic Camp. The camp takes place for 6 days every third week of June. Campers have the option to give informed consent for research participation (eIRB# 00002447), with biomarker collection taking place on the first and fifth days of camp. This time interval is sufficient for dietary management to yield impact in metabolic markers, including plasma amino acids [11,25]. During the five-day interval, the case participated in an overnight camp located at the main Emory University Campus in Atlanta, GA where adolescent and adult females with aminoacidopathies engaged in an environment designed to promote social support, healthy lifestyle, and optimal disease self-management through both fun and educational activities. Low protein meals were prepared by a culinary certified registered dietitian (RD) experienced in the nutritional needs and food prep requirements for patients with aminoacidopathies. The case was assigned to a camper subgroup led by an RD counselor. Counselors were responsible for ensuring their assigned campers were compliant with dietary expectations and MF intake as prescribed by their home dietitian.

Two samples from the individual with MSUD, collected 5 days apart at the Emory University Hospital General Clinical Research Center (EUH GCRC), were added to an analytical run with samples from another collaborative metabolomics project. The individual with MSUD had no history of liver transplant. Metabolomics outcomes for the individual were compared from camp days 1 and 5. Outcomes for both time points were compared against a single age- and sex- matched healthy control sample; provided by the Duke University Measurement to Understand the Reclassification of Disease of Cabarrus and Kannapolis (MURDOCK) Study biorepository (Kannapolis, NC). Metabolomics data from an additional 20 healthy unmatched control plasma samples (females ages 13–19 years) was provided by the MURDOCK Study from an investigative sub-cohort under 18 years of age, which we used for metabolite Z-score calculation.

### 2.2. Sample and data collection

Fasted case and control plasma samples for the metabolomics analysis were collected by venipuncture in lavender top EDTA tubes. A green top lithium heparin tube was used to collect plasma for quantified amino acids analysis in the patient with MSUD, then shipped frozen on dry ice to LabCorp. The case with MSUD had her blood drawn at the EUH GCRC in Atlanta, GA, under the Georgia Clinical and Translational Science Alliance (GCTSA). MURDOCK healthy controls had plasma

drawn from Duke University sponsored clinical sites across the regional community in Kannapolis, NC. Stored EDTA plasma aliquots (200  $\mu$ L), frozen at  $-80^{\circ}\text{C}$  were shipped on dry ice to Metabolon Inc. (Research Triangle Park, NC) for metabolome profile analysis.

Three-day diet records were collected on camp days 1 and 5 for the patient with MSUD and analyzed with MetabolicPro 1.0 by an RD. Diet records covered the 3 days prior to patient arrival at Metabolic Camp, and on days 3, 4, and 5 of Metabolic Camp. MURDOCK Study participants do not typically submit diet records as part of their study participation, although Duke does collect a qualitative questionnaire from participants that asks about general health patterns.

Genotype for the MSUD patient was sequenced and clinically reported in 2005 by Emory Molecular Genetics Laboratory (Atlanta, GA).

### 2.3. Metabolomics sampling and data normalization

De-identified control and patient samples were analyzed at Metabolon using UPLC-MS/MS and spiked with non-interfering standards for quality control purposes. Equipment included a Waters Acquity UPLC, Thermo Scientific High resolution/accurate MS, and Orbitrap mass analyzer at 35,000 mass resolution. Scan range covered 70–1000  $m/z$ . Peaks were annotated based on  $m/z$  and retention time index (RTI) utilizing Metabolon's combined biochemical library within their laboratory information management system (LIMS). Area under the curve (AUC) for peak quantification was used and peak data normalized against a median of 1 (termed "block correction") to control for inter-day variability and potential sample volume differences. Principle component analysis (PCA) and hierarchical clustering analysis (HCA) allowed visualization of differences in the case control matched analysis across time points.

### 2.4. Data analysis

Notable peak differences were defined as those at least  $\pm 0.5$  between days 1 and 5 for the patient with MSUD. The same criterion was used to define notable peak differences between case and control. To narrow the list of MSUD metabolites with greatest deviation from normal, consistent with prior literature [14], a Z-scale for each annotated metabolite was calculated with the data from the 20 healthy unmatched age-similar controls to determine analyte Z-score differences for the patient with MSUD on camp days 1 and 5.

## 3. Results

### 3.1. Study sample characteristics

The genotype of the patient with MSUD included two novel pathogenic allele variants (c.659C > T, c.929C > G) in the *BCKDHA E1* alpha gene. Clinical assessment based on dietary LEU tolerance and metabolic BCAA control indicates the two novel alleles yield a moderate to mild phenotype. The patient elects to take a thiamine supplement due to cofactor action of the vitamin on *BCKDH*, although phenotypic thiamine response had not been clinically determined. Table 1 provides the characteristics of the patient with MSUD, matched control, and the group of twenty age-similar female controls (age range: 14–19 years) with metabolomics profiles analyzed simultaneously. Body mass index (BMI) and BMI percentiles were determined based on age in both years and months, height (cm), and weight (kg).

PAA results and 3-day diet records for the patient with MSUD (Table 2) show overall good metabolic control. The patient's plasma LEU and dietary LEU intake complied with MSUD guidelines at both time points. Plasma VAL met guidelines although intake was below that prescribed. Plasma ILE was below guidelines and intake below prescribed (Table 2). Even with good baseline metabolic control, plasma BCAA declined markedly across the 5-day interval. Alloisoleucine (Allo-ILE), a pathognomonic marker of MSUD, likewise decreased. Table 3

**Table 1**  
Demographics and health characteristics of single case with MSUD<sup>a</sup> along with matched and unmatched MURDOCK controls.

Marker	MSUD case	Matched control	Z-score controls (n = 20)
Age (years), mean ± SD	16.6	16	17.0 ± 1.6
Sex (M/F)	F	F	F
Ethnicity	Non-Hispanic White (NHW)	NHW	19 NHW, 1 Asian
BMI (with percentile) <sup>b</sup>	21.8 (63rd)	22.3 (70th)	22.3 ± 2.3 (62nd ± 21)
Physical activity	Moderately active	Low active	Variable
Prescriptions	None		
• Hormone contraception		Yes	n = 10 (50%)
• Acne treatment		Yes	n = 3 (15%)
• Norepi inhibitor (Strattera)		No	n = 1 (0.5%)
• Antibiotic		No	n = 1 (0.5%)
Supplements		None	
• Multivitamin and iron	No		n = 1 (0.5%)
• Fish oil	Yes		n = 1 (0.5%)
• Melatonin	No		n = 1 (0.5%)
• Thiamine	Yes		None
Comorbidities	None	None	
• Iron deficiency anemia			n = 1 (0.5%)
• ADHD			n = 1 (0.5%)

<sup>a</sup> Genotype (c.659C > T, c.929C > G) in the BCKDHA (E1 alpha gene).

<sup>b</sup> Percentile determined by CDC scale (<https://www.cdc.gov/healthyweight/bmi/calculator.html>). BMI for grouped controls: mean ± SD.

shows the macronutrient and energy intake (per day and per kg/day) of the case with MSUD, determined from 3-day diet records. Modest declines in intake of energy, intact protein, total fat, and total carbohydrate were noted across the 5-day camp experience.

Information from answers to the MURDOCK study qualitative health questionnaire were too limited to draw conclusions on control subject diet patterns.

### 3.2. Metabolomics outcomes

There were 891 total analytes detected and annotated, including 434 lipid species, with fatty acids up to C22. The remaining analytes included amino acids, carbohydrate molecules, nucleotides, and xenobiotics. The hierarchical clustering map (Fig. 1) of the annotated metabolomics profiles for the patient with MSUD compared with the matched control revealed marked changes in the MSUD metabolome from Day 1 to Day 5. Metabolomic differences are also present for either study day in the MSUD patient when compared to the healthy matched control values. The differences among both amino acid and lipid analytes were apparent, even though the patient with MSUD had good

control of BCAA. Notable differences in the MSUD lipid profile compared with the profile of the matched control (Fig. 1) directed additional analysis.

As a whole, 51% of lipid analytes measured differed from the matched control at baseline, and 35% differed at the day 5 measurement. Annotated lipids fell within 54 reported metabolic subpathways. Using the normalized ± 0.5 deviation value from control as the criterion for an important difference, 142 (33%) lipid analytes were lower, and 93 (21%) higher, in the patient with MSUD on Day 1 compared with the control participant. Fifty-eight percent of lipid analytes that were higher than control on Day 1 decreased by Day 5 to be comparable with control. However, the majority (85%) of analytes lower than control on Day 1 remained low on Day 5. Supplemental Table 1 lists the subpathways where differences were noted between the patient with MSUD and controls, and includes endocannabinoids, medium- and long-chain fatty acids, multiple acyl carnitines, acyl choline fatty acids, phospholipid metabolites, monoacyl and diacylglycerols, sphingolipids, steroid hormones, and bile acids. Three of the analyte subpathways had the majority of compounds near control values by Day 5, including medium-chain fatty acids, long-chain fatty acids, and several acyl

**Table 2**  
Plasma and dietary BCAA measures of adherence for days 1 and 5.

Marker	Case – Day 1	Case - Day 5	Patient Rx <sup>a</sup>	MSUD guidelines <sup>b</sup>
PAA (µmol/L)			See guidelines	
• LEU	289	216	–	75–300
• ILE	164	99	–	200–400
• VAL	324	209	–	200–400
• Allo-ILE	42	Not detected (0)	–	Not available
Reported dietary intakes				
• LEU (intact foods only) (mg/day)	1190	1167	1260	15–50 mg/kg/day
o Total per kg <sup>c</sup>	19.3	19	20.5	
• ILE (mg/day)				Total: 10–30 mg/kg/day
o Supplemental	100	100	100	
o Intact foods	535	497	828	
o Total per kg <sup>c</sup>	10.3	9.7	15.1	
• VAL (mg/day)				Total: 15–30 mg/kg/day
o Supplemental	100	100	100	
o Intact foods	716	681	979	
o Total per kg <sup>c</sup>	13.3	12.7	17.5	
• MSUD medical food (Ketonex-2) (g/day)	215	215	215	Should complete protein & energy needs

<sup>a</sup> Patient's medical diet requirements as clinically prescribed by a medical doctor or licensed RD.

<sup>b</sup> <https://southeastgenetics.org/ngp/guidelines.php/105/MSUD%20Nutrition%20Guidelines/Version%201.54>. MSUD guidelines, Table 4 (ages 14–18), recommendations 2.1 and 2.2.

<sup>c</sup> Mg/kg/day calculated from patient weight of 61.5 kg.

**Table 3**

Macronutrient and energy intake of patient with MSUD (61.5 kg weight) determined from 3-day diet records collected on Days 1 and 5 of attendance at Emory University Metabolic Camp.

Nutrient	Case – day 1		Case - day 5	
	Per day	Per kg/day	Per day	Per kg/day
Total protein equivalents (g) <sup>a</sup>	90	1.46	81.7	1.25
Intact food protein	25.5	0.41	17.2	0.26
Medical food protein	64.5	1.05	64.5	1.05
Total fat (g)	96.1	1.56	69	1.12
DHA (n-3)	0.24	NC	0.24	NC
EPA (n-3)	0.36	NC	0.36	NC
ALA (n-3)	1.83	NC	1.26	NC
Total MCT	1.18	NC	0.2	NC
Total LCT	94.24	NC	69	NC
Total cholesterol (mg)	44	0.71	10.6	0.17
Total carbohydrate (g)	320.5	5.21	302	4.9
Total fiber (g)	19.5	0.32	16	0.26
Total energy (kcal)	2536	41.2	2180	33.5

ALA: Alpha linolenic acid, DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, LCT: long chain triglycerides, MCT: medium chain triglycerides, NC: Not calculated.

<sup>a</sup> Protein equivalents are the gram amount of free amino acids in the MSUD medical food that equate to 1 g of intact food protein.

carnitines. Twelve lipid subpathways had no differing analytes between the patient with MSUD and controls at either time point (Supplemental Table 1).

Z-score analysis (Fig. 2) revealed that the greatest differences in lipid analytes for the patient with MSUD compared with the unmatched adolescent control cohort paralleled those observed for the matched control, albeit the number of analytes was fewer. In total, for both study visits, 31 lipid analytes in the patient with MSUD had a Z-score exceeding 2.0 SD from controls, and 11 (mostly sphingomyelins) had a Z-score below  $-2.0$ . A total of 11 analytes shifted towards control values by study Day 5 as determined by Z-score, which included carnitine adjuncts, choline, the endocannabinoid anandamide, and diacylglycerols.

## 4. Discussion

### 4.1. Interpretation of key outcomes

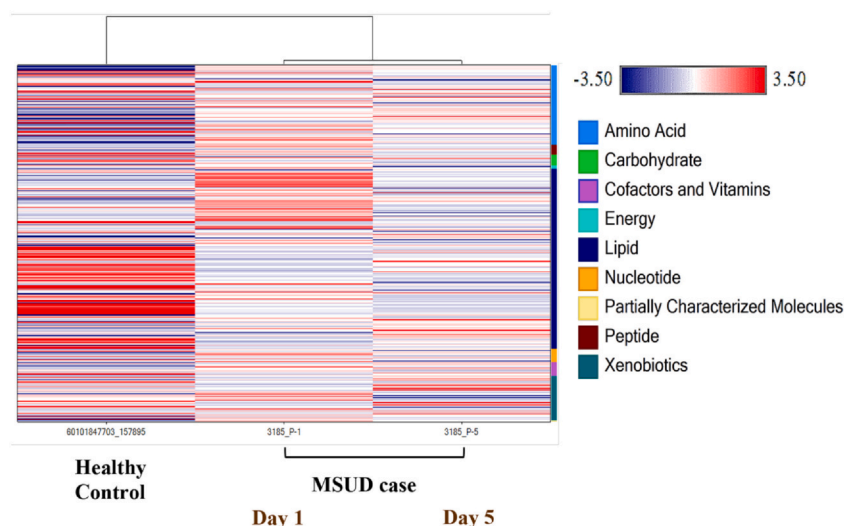
This is the first report of aberrant metabolic lipid outcomes in an MSUD case compared to a matched healthy control after non-targeted

metabolomics analysis. The results suggest that BCAA metabolic control may affect the lipid metabolome in someone with mild to moderate MSUD. Remarkably, only a few days of supervised diet adherence and declining BCAA in an already metabolically compliant patient correlated with substantial changes in the lipid metabolome, with some analytes approaching control values, in particular medium- to long-chain fatty acids and acyl carnitines. Most other analytes that were different between case and controls on Day 1 (Fig. 2, Supplemental Table 1), did not change for the patient with MSUD, regardless of diet. As BCAA intake and plasma BCAA dropped, medium- and long-chain fatty acids, along with acyl carnitines, in the study case drew closer to control. This may indicate a role for dietary adherence and metabolic control in the flux of these lipid analytes. Where lipid analytes were markedly different between case and control, with no change occurring after 5 days of intervention (such as with bile acids and acylglycerols), the differences may be specifically intrinsic to MSUD pathophysiology, independent from dietary and metabolic control. This highlights the need for further research to distinguish the individual roles of the intrinsic physiology of MSUD and medical diet adherence on lipid regulation.

### 4.2. Hypothesis of peroxisomal and mitochondrial interaction with BCAA

Analytes affected in this case report of an MSUD metabolome suggest peroxisomal dysfunction may be occurring during the metabolism and oxidation of lipid compounds, with possible downstream effects on mitochondrial metabolism. Decades of research support the crucial role of peroxisomes in catabolism and anabolism of lipid species. This includes alpha-oxidation of branched-chain fatty acids, beta-oxidation of long-chain and very long-chain fatty acids, bile acid synthesis, and ether lipid synthesis (i.e., plasmalogens and sphingomyelins) (Lodhi and Semenkovich 2014; Cipolla and Lodhi 2017). Since lipids serve many physiological purposes (i.e., white matter formation, cell membranes, vitamin transport, digestion, hormone regulation, and cardiovascular disease risk), chronically abnormal lipid metabolism that results from peroxisomal dysfunction in MSUD could have undetermined long-term health implications and may contribute to some of the early pathophysiology.

Human studies on the interaction between BCAA and peroxisome function are poorly represented in the literature and absent on the topics of peroxisomal and lipid status in MSUD. However, listed in Supplemental Table 2 are some studies, including animal studies, that demonstrate interactivity between BCAA metabolism, peroxisomal function, and lipid status. The close proximity and cross-metabolic



**Fig. 1.** Euclidian hierarchical metabolome clustering of MSUD case at both time points compared to single healthy matched control.



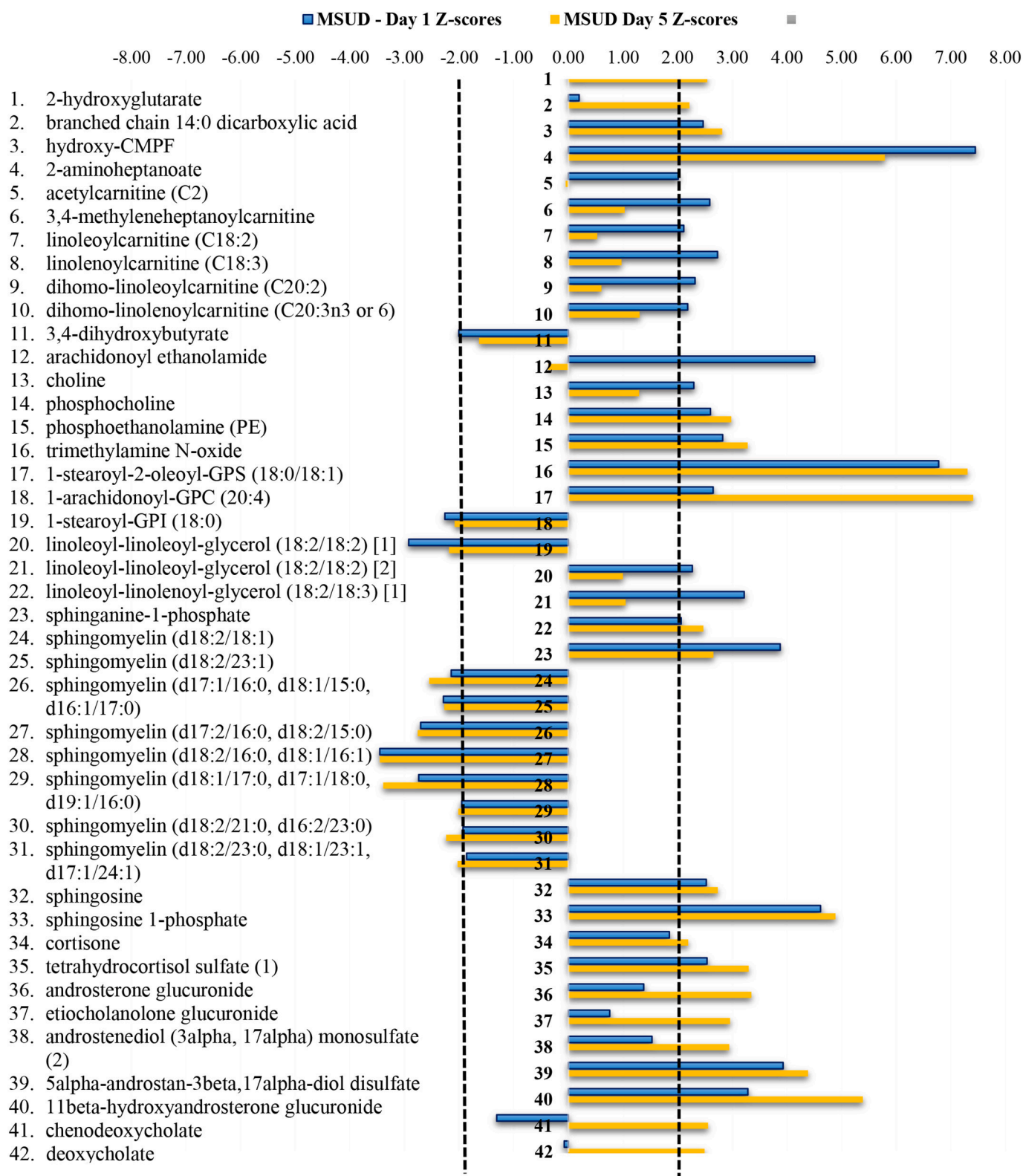


Fig. 2. Lipid analyte Z-scores of MSUD case at Day 1 and Day 5 study visits based on healthy control data from 20 adolescent females.

interactions of peroxisomes with smooth endoplasmic reticulum (ER), mitochondria, and lipid droplets stresses the importance of peroxisomal function in whole cell lipid metabolism [10,23].

Thus, an interconnection between aberrant BCAA metabolism, peroxisomal function, and lipid status in humans could explain the observations in our MSUD case study (Supplemental Fig. 1). For

example, our case individual with MSUD exhibited elevated bile acids, dicarboxylic acids, and downstream sphingosines in comparison with our control subjects (Fig. 2), all lipid species which are dependent upon peroxisomal activity. Interestingly though, branched-chain fatty acid metabolites detected in the metabolomics analysis were not different between the case and control subject or across time points

(Supplemental Table 1).

The possible influence of elevated BCAA on mitochondrial function could stem from more than one mechanism. A 2016 study revealed the role of excess keto-isocaproic acid, a LEU catabolite, on impaired mitochondrial function with rescue by metformin [26]. In addition, since several peroxisomal alpha- and beta-oxidation products require transport into mitochondria, it is reasonable that an imbalance in the metabolism of these intermediate substrates within the peroxisome, and in the transport into the mitochondria, could lead to downstream disturbances in mitochondrial beta-oxidation and energy metabolism. Further, BCAA can cross into the mitochondria through the hydrophobic membrane [8], and higher BCAA levels have been associated with mitochondrial respiratory chain disease and hyperlipidemia [7]. Thus, an overabundance of BCAA crossing the mitochondrial membrane along with reduced synthesis of succinyl-CoA and acetyl-CoA for the tricarboxylic acid cycle could result in a breakdown of mitochondrial oxidative function [28].

The authors also would like to highlight the prevalence of ether-phospholipid analytes that fell below 2 SD (Fig. 2) compared with control Z-scores. The role of the ER in utilizing peroxisomal substrates for synthesis of ether-phospholipids is well established [23]. Thus, in our MSUD case study, elevated BCAAs could be influencing ER activity, either indirectly through compromised peroxisomal activity or through an undefined action of the BCAA on the ER itself.

#### 4.3. Clinical implications

If our case study findings are later confirmed by analysis in a larger MSUD cohort, it would suggest that the pathology and clinical course of patients with MSUD may result, to some extent, from sub-clinical peroxisomal dysfunction with secondary effects on mitochondria and possibly the ER. Provided this connection exists, it could reveal opportunities for therapies targeting regulation of peroxisome function and for additional dietary interventions to reduce adverse events in MSUD. For example, research into the therapeutic actions of pharmaceutical peroxisome proliferator-activated receptor (PPAR) agonists demonstrate the interplay between BCAA, peroxisome activity, and lipid metabolism [4,16,17]. Perhaps glitazones, fibrates, and other PPAR agonists could be useful for therapeutic lowering of BCAA in patients with MSUD while correcting fatty acid metabolic dysregulation, as has already been shown in patients with non-alcoholic steatohepatitis (NASH) [17].

More knowledge about, and potential solutions for, long-term risk of comorbid chronic diseases may also stem from future research into BCAA and organelle interaction. Laboratory study methods could reveal the mechanisms of how BCAA interact with peroxisomes and mitochondria to affect MSUD phenotype. Future analysis of peroxisomal function biomarkers in human cases or mouse models with MSUD would shed light on the potential influence of BCAA on peroxisomal lipid metabolism. The information may even reveal how these mechanisms impact the risk and pathophysiology of other medical diseases including peroxisomal disorders, cachexia, insulin resistance, heart disease, Alzheimer's disease, and geriatric health) [15,19,22].

#### 4.4. Study limitations

Due to the single MSUD case in this pilot analysis, statistical associations could not be determined and data were not analyzed for potential confounders (e.g., medication and supplement use, sleep habits, or socioeconomic status). Also, given the single MSUD sample evaluated, it is possible some analyte differences may be due to individual variation not specific to MSUD. For example, data on menstrual cycle was not available and oral contraceptive prescription status differed between subject and control. Additionally, lipid species > C22 (very long-chain fatty acids) were not included in the metabolomics report due to limits of detection and would require a more targeted analytical

method. Although dietary information for the control subjects were unavailable, diet comparison across time for the study case provided insight into what metabolites may respond synergistically with dietary shifts and PAA status in MSUD. Comparison with a larger cohort of patients with MSUD is important for validating the findings in this case report, while adjusting for differences in medical diet adherence and phenotype. Despite these shortcomings, the information gained from the case study findings are useful to better understand the complex etiology of MSUD beyond LEU toxicity, but still relevant to BCAA metabolism. This case report may also stimulate research that can reveal other biomolecular mechanisms capable of influencing short-term and long-term health outcomes in MSUD, which in turn may lead to targeted therapeutics.

#### 4.5. Conclusion

Our single case study revealed differences in nearly half the metabolome lipid analytes measured in a female patient with MSUD compared with a healthy matched control, as well as with a group of unmatched age-similar female controls. The majority of the differences were in lipids known to be associated with peroxisome metabolism. However, to understand the full value of our observations, the outcomes of this pilot single case analysis should be confirmed in a larger research cohort that controls for genotype, dietary intake, and metabolic compliance.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ymgmr.2020.100651>.

#### Author contributions

*Study concept and design:* TD Douglas, RH Singh.

*Interpretation of data:* TD Douglas, LK Newby, J Eckstrand, D Wixted.

*Original and revised manuscript drafts:* TD Douglas.

*Critical review of the manuscript:* RH Singh, LK Newby, J Eckstrand, D Wixted.

*Statistical analysis:* TD Douglas.

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#### Details of ethics approval

Ethical approval for the study was obtained from the Emory University Institutional Review Board for the Emory Metabolic Camp protocol (IRB # 2447). Ethical approval was also received from the Duke University Institutional Review Board.

#### Patient consent statement

Both the case study and matched control samples represented in the manuscript were obtained with patient and legal guardian written informed consent and/or assent as age appropriate.

#### Documentation of approval for care and use of laboratory animals

N/A.

## Declaration of Competing Interest

Authors have no relevant financial relationships or conflicts of interest to disclose.

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