Responses to Fasting and Glucose Loading in a Cohort of Well Children with Spinal Muscular Atrophy Type II

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Objective To examine the impact of fasting and glucose tolerance on selected metabolic variables in children with spinal muscular atrophy (SMA) type II in a well state, secondary to reports of glucose regulation abnormalities in SMA.

Study design In this prospective pilot study, 6 children aged 7-11 years with SMA type II participated in an oral glucose tolerance test and a supervised medical fast during 2 overnight visits at the University of Utah. At baseline, a dual-energy x-ray absorptiometry scan was performed to determine body composition. Laboratory test results were obtained at baseline and in response to the respective interventions. Data analysis was descriptive. Prefasting and postfasting data were evaluated using the Wilcoxon signed-rank test.

Results Based on the dual-energy x-ray absorptiometry scan, all 6 children were variably obese at baseline. All 6 exhibited hyperinsulinemia, and 3 of 6 met formal American Diabetes Association criteria for impaired glucose tolerance. According to homeostatic insulin resistance calculations, 5 of the 6 participants were insulin-resistant. All 6 participants tolerated a monitored fast for 20 hours without hypoglycemia (blood glucose <54 mg/dL). Free fatty acid levels increased significantly from prefasting to postfasting, whereas levels of several plasma amino acids decreased significantly during fasting.

Conclusion Children with SMA type II defined as obese using objective variables are at increased risk for impaired glucose tolerance regardless of whether or not they visually appear obese. Further studies are needed to determine the prevalence of impaired glucose tolerance and tolerance for fasting within the broader heterogeneous SMA population and to develop appropriate guidelines for intervention. *(J Pediatr 2015;167:1362-8)*.

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease resulting in progressive muscular weakness and atrophy. SMA is classified into clinical subtypes based on maximum achieved motor milestones.[1-3] Children with SMA type II typically present between 6 and 18 months of age, and achieve the ability to sit but never walk independently.[3,4] Bulbar, feeding, and respiratory insufficiency occur at some point in the majority of patients with SMA type II.[5,6] Despite the increasing life expectancy in these patients, attributed to advances and standardization of medical care,[7-9] knowledge of the altered metabolism and nutrition in SMA remains limited.

Compared with healthy peers, patients with SMA have documented decreased lean muscle mass and increased fat mass regardless of body mass index (BMI).[10,11] Increased visceral fat mass is a risk factor for insulin resistance and decreased glucose sensitivity in adults and children[12,13] and has been associated with peripheral neuropathy.[14-17] Healthy individuals rely on glycogen stores in the liver and muscle for short-term energy needs.[18,19] Little is known about how diminished lean mass affects fat lipolysis and protein catabolism during periods of fasting in children and adults with severe neuromuscular disease. Impaired fatty acid metabolism has been observed in children with SMA during fasting.[20,21] Poor tolerance for fasting (eg, hypoglycemia, coma) has been observed in adults with various neuromuscular diseases, including SMA.[22,23] SMA and survival motor neuron (SMN) gene-depleted mice have demonstrated pancreatic defects and altered glucose metabolism affecting glucose sensitivity.[24,25] Analysis of pancre-

BCAA	Branched-chain amino acid
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
DXA	Dual-energy x-ray absorptiometry
HbA1c	Hemoglobin A1c
HOMA-IR	Homeostatic model assessment for insulin resistance
NHANES	National Health and Nutrition Examination Survey
OGTT	Oral glucose tolerance test
PQAA	Plasma quantitative amino acids
SMA	Spinal muscular atrophy
SMN	Survival motor neuron

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atic tissue from infants with SMA type I has recapitulated some of these findings.[24] Given the convergence of such data, further study is warranted. The primary aim of the present study was to explore whether children with SMA type II demonstrate impaired glucose tolerance after glucose loading or intolerance of fasting in a well state.

Methods

Participants were admitted to the University of Utah's Center for Clinical and Translational Science in Salt Lake City for 2 overnight inpatient visits. These visits consisted of an oral glucose tolerance test (OGTT) visit and a fasting visit, separated by 8 weeks.

The participants were age 7-11 years, consumed at least 50% of their caloric intake by mouth, and met clinical diagnostic criteria for SMA type II. Homozygous *SMN1* deletion was documented in all participants; an *SMN2* dosage of 3 copies was confirmed at the Ohio State University Molecular Diagnostic Laboratory for all 6 participants. Exclusion criteria were acute illness, use of oral hypoglycemic agents, and previous diagnosis of impaired glucose tolerance. The University of Utah's Institutional Review Board approved the study (IRB 64793), and parental consent and assent were obtained for each participant.

OGTT Visit

The first visit involved body composition analysis using dual-energy x-ray absorptiometry (DXA) and a formal OGTT. The previous evening, participants consumed a standardized meal and snack (14% protein, 54% carbohydrate, and 32% fat).

Following a 10-hour overnight fast, baseline blood samples were collected for hemoglobin A1c (HbA1c), insulin-like growth factor 1, blood glucose, insulin, glucagon, plasma quantitative amino acids (PQAA), and cortisol. Samples were analyzed by ARUP Laboratories (Salt Lake City, Utah) following standardized clinical protocols. For safety purposes, baseline and final glucose tests were also analyzed at bedside (2300 Stat Plus biochemistry analyzer; YSI, Yellow Springs, Ohio). Participants consumed an oral glucose load of 1.75 g glucose/kg body weight (maximum dose 75 g) after blood sample collection for baseline laboratory tests. Subsequent blood samples were collected at 30, 60, 90, 120, and 180 minutes and analyzed for glucose, insulin, glucagon, PQAA, and cortisol. The first and last urine voids were collected and assessed for urinary ketones using Ketostix reagent strips (Bayer HealthCare, Mishawaka, Indiana). OGTT test results were evaluated based on American Diabetes Association guidelines and reference values for nonobese children.[26,27]

DXA and Anthropometric Measures

Norland DXA (XR-36 software version 3.3.1, Fort Atkinson, Wisconsin) for small subjects was used to assess whole-body composition (percent body fat). Body fat percentiles developed for children aged 8-11 years using 1999-2004 National Health and Nutrition Examination Survey (NHANES) data from whole-body DXA scans (Hologic; Bedford, Massachusetts) were used to assign overweight (>85th percentile) and obese (>95th percentile) classifications based on sex.[28]

Additional anthropometric measures included segmental length, arm span, weight, abdominal circumference, chest circumference, ulnar length, mid-arm circumference, and triceps skinfold thickness. Length and circumference measures were obtained to the nearest millimeter using a nonstretchable tape measure. Triceps skinfold thickness was measured to the nearest millimeter on the right side using a Lange skinfold caliper (Beta Technology, Santa Cruz, California). All measurements were obtained by trained study staff using standard assessment methods.[29]

BMI-for-age percentiles were determined using Centers for Disease Control and Prevention (CDC) growth charts. In accordance with CDC criteria, a BMI for age >85th percentile was considered overweight, and a BMI >95th percentile was considered obese.

Three-Day Dietary Record

A 3-day dietary record for 2 weekdays and 1 weekend day was obtained from each participant before the OGTT visit. Diet records were analyzed using Food Processor version 10.5.2 nutrition analysis software (ESHA Research, Salem, Oregon).

Insulin Resistance and Hyperinsulinemia Standards

The homeostatic model assessment for insulin resistance (HOMA-IR) was used to evaluate insulin resistance. Insulin resistance was calculated as follows: fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.[30] Insulin resistance levels were compared with HOMA-IR cutoff values in obese children and adolescents (2.67 for prepubertal children, 3.82 for pubertal females, and 5.22 for pubertal males).[31] Insulin levels at 0, 30, 60, 90, and 120 minutes during the OGTT were summed[32]; hyperinsulinemia was defined as an insulin sum >300 μU/mL.[31]

Fasting Visit

During the second visit, participants underwent a medically supervised 20-hour fast after receiving the same standardized evening meal with a snack. Initial fasting blood samples were analyzed for insulin, glucose, epinephrine, norepinephrine, cortisol, glucagon, free fatty acids, and PQAA. Blood samples were collected every 2 hours for glucose, insulin, and cortisol. Additional samples were collected every 4 hours for glucagon, free fatty acids, and PQAA. Epinephrine and norepinephrine samples were collected at 4, 12, 16, and at 20 hours after the start of the fast. Glucose levels were analyzed at the bedside using a YSI monitor. All other laboratory values were analyzed by ARUP Laboratories. Each void was tested for urinary ketones using Ketostix. For initial and final voids, urine samples were collected and sent to ARUP Laboratories for complete urinalysis.