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Undiagnosed Hypoglycemia Disorders in Children Detected When Hypoglycemia Occurs in the Setting of Illness: A Retrospective Study

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Abstract

Objective: Whether hypoglycemia incidentally detected during intercurrent illness in children requires an endocrine workup remains controversial. This study aimed to determine the yield of conducting a diagnostic evaluation in this setting, and to compare clinical and biochemical features between patients ultimately diagnosed with a hypoglycemic disorder and those who were not.

Design: Single-center, retrospective review of children referred between January 2013 and December 2018 for evaluation of hypoglycemia (defined as plasma glucose <3.9 mmol/L [<70 mg/dL]) in the setting of acute illness.

Results: 145 patients met eligibility criteria. A hypoglycemia disorder was identified in 12 patients (8% of the cohort, 17% of those who underwent diagnostic fast). There were no cases in which diagnosis was established in the absence of a diagnostic fast. Characteristics associated with identifying an underlying disorder included younger age (1.03 years [IQR: 0.05, 1.54] v. 2.18 years [IQR: 1.29, 3.99], p<0.001), higher bicarbonate level (22 ± 5.5 mmol/L v. 16 ± 3.6 mmol/L, p<0.001), lower frequency of elevated plasma or urine ketones (25% v. 92%, p=0.004), and lower frequency of other documented medical problems (17% v. 50%, p=0.03).

Conclusions: The yield of diagnostic evaluation among children with incidental detection of hypoglycemia in the setting of illness is not insignificant. We thus recommend that all children with hypoglycemia in the setting of illness undergo guided diagnostic evaluation. Younger age and absence of ketosis and acidosis at presentation may serve as useful predictors for establishing a diagnosis. Future studies are needed to confirm these findings.

What is already known on this topic – The prevalence of undiagnosed hypoglycemia disorders among children seen in the emergency department for any clinical reason has been reported as 10-28%. During illness, oral intake in children is often reduced. In this setting, incidentally hypoglycemia is often attributed to prolonged fasting. Determining whether children with hypoglycemia detected during illness require a dedicated endocrine evaluation has been limited by a paucity of data.

What this study adds – In this cohort, 8% of children who presented with hypoglycemia in setting of illness were found to have an underlying hypoglycemia disorder. Underlying hypoglycemia diagnoses were only established in those children who underwent a comprehensive evaluation including diagnostic fast. Younger age, higher bicarbonate level, and lower ketones at presentation were associated with establishing a hypoglycemia diagnosis.

How this study might affect research, practice or policy – All children with hypoglycemia detected in the setting of acute illness should undergo guided diagnostic evaluation.

1 Introduction

Incidental detection of hypoglycemia during childhood illness commonly occurs following prolonged starvation, in which glucose utilization exceeds glucose supply. Rarely, it may be the initial presentation of an underlying hypoglycemia disorder wherein missing the diagnosis carries a high risk of harm. The reported prevalence of undiagnosed hypoglycemia disorders among children seen in the emergency department for any reason ranges between 10 and 28% (1-3). However, these studies were not limited to children presenting with acute illness. Consequently, whether children with hypoglycemia detected during acute illness require an endocrine workup remains controversial. We sought to evaluate the yield of conducting an evaluation when hypoglycemia occurs in this setting and to describe the clinical and biochemical features of those children ultimately found to have underlying pathology.

2 Materials and Methods

A retrospective review was conducted of children referred to endocrinology for evaluation of hypoglycemia (plasma glucose <3.9 mmol/L [<70 mg/dL]) in the setting of acute illness at Children's Hospital of Philadelphia (CHOP) between January 2013 and December 2018. Billing records were utilized to obtain a list of inpatient and outpatient endocrine consults for hypoglycemia using ICD codes for "hypoglycemia, unspecified" (ICD-9 251.2 prior to October 2015, ICD-10 16.2 after October 2015). Additionally, inpatient billing records were manually searched for "hypoglycemia" as the consultation reason. Patients were included if they were <18 years of age and had both documented plasma glucose <3.9 mmol/L (<70 mg/dL) and illness symptoms (e.g., fever, vomiting, diarrhea, respiratory symptoms) at the time of presentation. Exclusion criteria included children with previously diagnosed hypoglycemia disorders, diabetes mellitus, or use of medications that can alter glucose metabolism (hypoglycemic agents, systemic steroids, chemotherapy, or beta-

blockers) within one month of presentation. A plasma glucose threshold of <3.9 mmol/L (<70 mg/dL) was utilized to define hypoglycemia in this study in keeping with established hypoglycemia definitions (4, 5), and because below this threshold, neuroendocrine responses to hypoglycemia are activated (6). Additionally, most infants and children are able to maintain plasma glucose above this threshold after 15-18 hours of fasting (7).

Demographic, clinical, and biochemical data were extracted from the electronic health record (EHR). Acute illness was categorized as: gastroenteritis, isolated vomiting, isolated diarrhea, upper respiratory infection, otitis media, fever, and other. Illness categories were not exclusive; patients were included in all categories for which there were documented symptoms. Height was categorized as: short stature (<3 percentile for age), normal stature (≥3 and <97 percentile for age), and tall stature (≥97 percentile for age). Height and weight were used to calculate weight-for-length percentiles for patients <2 years of age and body mass index (BMI) percentiles for patients ≥2 years of age. Weight was categorized as: underweight (weight-for-length/BMI <5 percentile for age), normal weight (weight-for-length/BMI ≥5 and <85 percentile for age), overweight (weight-for-length/BMI ≥85 and <95 percentile for age), and obese (weight-for-length/BMI ≥95 percentile for age). Physical examination findings of interest included dysmorphic features, hepatomegaly, and signs of suggestive of hypopituitarism (midline defects, microphallus in males).

Types of hypoglycemia evaluation performed included laboratory studies drawn at the time of presentation, non-fasting laboratory studies obtained following presentation ("baseline evaluation"), genetic testing, and diagnostic fasting studies, which were conducted as previously described (standard protocol (8)). Evaluations were conducted at the discretion of the provider. This was typically an emergency medicine provider at presentation. The decision to pursue diagnostic fasting studies was made solely by endocrinologists. Standard practice at our center is to obtain baseline

metabolic studies (acylcarnitine profile, total and free carnitine levels, and urine organic acids) prior to performing fasting studies when there is concern for a possible fatty acid oxidation disorder. To facilitate comparison between groups, urine and blood ketone levels were combined into categories wherein positive ketones were defined as either small or greater urine ketones or blood ketones ≥1 mmol/L.

The EHR was reviewed for additional episodes of hypoglycemia and for endocrine or metabolic diagnoses (hormone deficiencies, disorders of insulin secretion/signaling, glycogen storage disease, disorders of gluconeogenesis, and fatty acid oxidation disorders) made subsequent to the index event. Duration of follow-up was calculated from index event and last contact dates.

This study was determined to be exempt by the CHOP Institutional Review Board. Patients were not involved in the design or conduct of this study.

2.1 Statistical Analysis

Categorical variables were reported as proportions. Normally distributed continuous variables were summarized using mean and standard deviation. Median and interquartile range were reported for non-normally distributed continuous data. In comparing the clinical and biochemical characteristics of patients ultimately diagnosed with a hypoglycemic disorder with those who were not, and patients who underwent diagnostic fasting evaluation with those who did not, proportions were compared using Fisher's exact test, t-tests were used to compare means of normally distributed data, and Wilcoxon rank sum tests were used to compare medians of nonparametric data. All tests were two-sided with $p<0.05$ set as the threshold for statistical significance.

3 Results

A total of 1410 patients were evaluated by endocrinology for hypoglycemia at CHOP between January 2013 and December 2018. Of these, 145 patients met inclusion criteria, and their records were reviewed (Figure 1). Characteristics of the cohort at time of presentation are summarized in Table 1. Median age at presentation was 2 years and ranged from 2 days to 11 years. Abnormal findings on physical examination were uncommon. Four patients had dysmorphism, three had hepatomegaly, and one had macrocephaly. No patients had documented cleft lip or palate or microphallus.

Thirteen percent of patients presented with altered mental status and 10% presented with seizure like activity. Of the patients with a prior history of hypoglycemia, none had previously undergone a diagnostic evaluation. Thirty-four percent of patients had recurrent episodes of hypoglycemia following the index event. The median follow-up duration was 27 months (range: 0 days – 7.8 years).

3.1 Evaluations conducted

Laboratory evaluations performed at any point during follow-up varied considerably. At the time of initial presentation with hypoglycemia, urine or plasma ketones were obtained in 57% of patients, bicarbonate was obtained in 63%, transaminases were measured in 28%, and cortisol was obtained in 11%. Lactate, ammonia, insulin, c-peptide, free fatty acids, growth hormone, and metabolic studies (acylcarnitine profile, total and free carnitine levels, and urine organic acids) were each obtained in <10% of patients. Of the patients who had laboratory evaluation beyond glucose, ketones, and bicarbonate at the time of initial presentation 50% had abnormal findings. Abnormal findings included elevated transaminases (e.g., above the upper limit of normal) in 50% and elevated lactate in 18%. Cortisol was >276 nmol/L in all patients in whom it was obtained. Baseline evaluation was obtained in 59% of patients with metabolic studies performed most frequently. Baseline evaluation yielded abnormal findings in 29% of patients.

Of the 102 patients with plasma glucose <2.8 mmol/L (<50 mg/dL) on presentation, "critical sample" labs including insulin, urine or plasma ketones, lactate, ammonia, cortisol, growth factors, and acylcarnitine profile were obtained in 10%. Seventy percent of patients in whom a "critical sample" was obtained had symptomatic hypoglycemia at the time of presentation.

A diagnostic fasting test was performed, either at the time of initial presentation or during a follow-up admission, in 48% of patients. Twenty-five percent of the cohort had genetic testing performed (Supplemental Table 1). Only two children had genetic testing without also undergoing a diagnostic fast.

3.2 Identified hypoglycemia diagnoses

An underlying hypoglycemia disorder was identified in 12 patients (8%) all of whom underwent a diagnostic fast. The clinical presentation, evaluation, and course of these patients is detailed in Supplemental Table 2. The yield of performing a diagnostic fast in this study was 17%.

Hyperinsulinism was the most frequently identified etiology and was diagnosed in seven patients. Additional diagnoses included inborn errors of metabolism in three patients, growth hormone deficiency in one patient, and impaired hepatic insulin clearance due to acute hepatic insufficiency in one patient. A final diagnosis was established in two patients in whom laboratory evaluation at presentation other than glucose, ketones, and bicarbonate yielded abnormal findings. In both cases (dihydrolipoamide dehydrogenase deficiency and impaired hepatic insulin clearance), transaminases were elevated above the upper limit of normal for age.

An underlying genetic diagnosis was suggested based upon testing in four patients and included hyperinsulinism due to an autosomal dominant mutation in *ABCC8*, hyperinsulinism associated with Turner syndrome, isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency, and dihydrolipoamide dehydrogenase (DLD) deficiency.

3.3 Factors associated with identifying a specific hypoglycemia diagnosis

We compared clinical and biochemical characteristics at the time of presentation between the patients ultimately diagnosed with an underlying etiology of hypoglycemia and those who were not (Table 2). Younger age (1.03 years [IQR: 0.05, 1.54] v. 2.18 years [IQR: 1.29, 3.99], p<0.001) and higher bicarbonate level (22 ± 5.5 mmol/L v. 16 ± 3.6 mmol/L, p<0.001) were associated with identifying an underlying disorder. Patients diagnosed with a hypoglycemia disorder were less likely to have elevated plasma or urine ketones at presentation (25% v. 92%, p=0.004) and were less likely to have a documented history of other medical problems (17% v. 50%, p=0.03). No statistically significant differences were observed between groups with regard to the other clinical or biochemical features assessed.

Since a diagnostic fast was performed in all patients who ultimately had a final diagnosis established, we evaluated whether there were any characteristics at presentation associated with conducting this evaluation (Table 3). Median plasma glucose at presentation was lower in the group that underwent diagnostic fast (2.2 mmol/L [40 mg/dL], IQR: 1.8, 2.7 mmol/L [32, 49 mg/dL] v. 2.6 mmol/L [47 mg/dL], IQR: 2.3, 3.0 mmol/L [41, 55 mg/dL], p=0.002). Additionally, the proportion of patients with presenting plasma glucose <2.8 mmol/L (<50 mg/dL) was greater among those who underwent a diagnostic fast compared to those who did not (80% v 62%, p=0.03).

4 Discussion

Eight percent of children who presented with hypoglycemia in setting of illness were found to have an underlying hypoglycemia disorder. Underlying diagnoses were only established in children who underwent a diagnostic fast, which was conducted in 48% of patients. The frequency of underlying hypoglycemia disorders was thus two-fold higher (17%) among those who underwent diagnostic fast as compared to the overall cohort.

These findings are in keeping with those of White, et al., who found that among children seen in the emergency department for any reason with previously unrecognized hypoglycemia (plasma glucose <2.8 mmol/L [50 mg/dL]), 10.6% were diagnosed with a hypoglycemia disorder (3). Diagnoses were only identified in the children who underwent diagnostic evaluation (53%), such that 20% of those who had a workup were found to have a hypoglycemia disorder. These findings emphasize that without appropriate evaluation, children with underlying hypoglycemia disorders may not be identified.

In a similar cohort of all comers to the emergency room in whom plasma glucose was <2.5 mmol/L (<45 mg/dL), the frequency of previously unrecognized metabolic or endocrinologic disorders among those without infectious diseases causing prolonged fasting was 11% (9). Pershad, et al. reported that among children 1-5 years of age seen in the emergency department with an ICD code for hypoglycemia and a plasma glucose <2.2 mmol/L (<40 mg/dL) or <3.3 mmol/L (<60 mg/dL) with neuroglycopenic symptoms, 16% were diagnosed with an endocrine or metabolic disorder (1). Details on the evaluations conducted and proportion of patients that underwent evaluation were absent from these latter two studies. Notably, the frequency of finding an underlying hypoglycemia disorder in our study is akin to that reported in the broader population of children seen in the emergency department for any cause, potentially suggesting that the presence of illness symptoms may be less pertinent than other clinical factors in identifying children with underlying hypoglycemia disorders.

Weinstein, et al. found that 28% of children seen in the emergency department and incidentally detected plasma glucose <2.8 mmol/L (<50 mg/dL) had an undiagnosed endocrine or metabolic disorder (2). In this study, patients were prospectively recruited using software which permitted both unbiased subject enrollment and "critical sample" collection prior to correction of hypoglycemia. This is in contrast to the present study in which the decision to obtain a "critical sample" was at the discretion of the provider and was obtained in only 10% of those with plasma glucose <2.8 mmol/L (<50 mg/dL). The higher diagnosis rate in the Weinstein study, in which the decision to pursue evaluation was automated and not based upon provider discretion, accentuates the previously absent data to guide clinical practice in deciding which patients require further evaluation.

We found that young age and absence of acidosis and ketosis at presentation were associated with identifying an underlying hypoglycemia disorder. When hypoglycemia occurs in a child as a consequence of starvation (i.e., during illness), the child should have concomitantly elevated plasma and urine ketone concentrations and decreased serum bicarbonate concentration (5). When this does not occur, it should raise suspicion of dysregulated insulin secretion or disorders of fatty acid oxidation. Our findings may have been influenced by the inclusion of neonates in the study population, and in turn, the high proportion of children with previously undiagnosed hyperinsulinism. Neither the duration of illness nor decreased oral intake was associated with establishing a hypoglycemia diagnosis. However, the high level of missingness for these variables potentially limits interpretation of these findings. Absence of documented medical or surgical comorbidities at presentation also emerged as associated with establishing a diagnosis. Reasons for this finding are less obvious but may also stem from the inclusion of neonates.

While the patients with growth hormone deficiency and acute hepatic insufficiency had clinical features suggestive of the underlying etiology of hypoglycemia at presentation, the remainder did not. In fact, the child with hyperinsulinism in the setting of mosaic Turner syndrome, which is a

recognized association (10), did not have classic phenotypic features of Turner syndrome. In this child's case, a molecular diagnosis was incidentally uncovered during the genetic evaluation for hyperinsulinism.

A genetic diagnosis was suggested based upon testing in 25% of patients in whom a hypoglycemia disorder was identified. Overall, genetic testing yielded information supporting an underlying etiology of hypoglycemia in 11% of patients in whom it was obtained. In a prior study of children with ketotic hypoglycemia and nondiagnostic metabolic and endocrine evaluation, genetic testing revealed mutations in genes involved in glycogen synthesis and degradation in 12% (11). Interestingly, no cases of glycogen storage disease were identified in our cohort though it is notable genetic evaluation was not universally performed.

Our findings need to be interpreted in light of several limitations. As a retrospective study, data was subject to potential inconsistencies or omissions in documentation in the EHR. Although diagnoses of hypoglycemia were biochemically confirmed, it is likely that potential subjects were not identified because hypoglycemia was not listed as a diagnosis or reason for consultation. Decisions to obtain an initial plasma glucose level, consult endocrinology, and pursue diagnostic evaluation were each at the discretion of the provider. Selection bias could have resulted from differential decision-making at each of these levels. We explored potential sources of bias stemming from the latter of these by comparing those who did versus did not undergo a diagnostic fast, however this analysis fails to capture the role of unmeasured factors driving differential selection of subjects. This was a single-center study in which children were evaluated by endocrinologists with expertise in hypoglycemia disorders at a large children's hospital. Findings may not be generalizable to different populations, particularly those including children with different age distributions.

Despite these limitations, this study adds to the sparse body of literature examining the frequency of underlying pathology among children with hypoglycemia during intercurrent illness. Our findings highlight the importance of obtaining a "critical sample" or at a minimum, assays for bicarbonate and beta-hydroxybutyrate at the time of hypoglycemia as these studies are both readily available and informative in differentiating between categories of hypoglycemia disorders. This approach is in keeping with Pediatric Endocrine Society recommendations for evaluation of hypoglycemia in children (5). Without appropriate evaluation, these children may not be identified, and consequently, appropriate treatment may not be implemented.

5 Conclusions

The high frequency of hypoglycemic disorders identified in this study underscores the critical importance of investigating children with hypoglycemia during illness and argues against ascribing findings to prolonged starvation. Endocrinology should be consulted to guide the diagnostic evaluation. Young age and absence of ketosis and acidosis at presentation were identified as potential predictors. These findings need to be confirmed in future studies.

6 Funding and Competing Interests

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treating hyperinsulinism and postprandial hypoglycemia and has donated all financial proceeds from these patents to the Children's Hospital of Philadelphia. Dr. De León participates on the advisory boards of Soleno Therapeutics and the NIH: RADIANT Study and on the Scientific Advisory Board of Congenital Hyperinsulinism International. Dr. De León has received donated research supplies from Dexcom for studies not included in this manuscript. Dr. De León holds stock options at Merck. The other authors do not have any relevant disclosures to declare. The funding agencies did not have any role in study design, collection, analysis, interpretation of data, or writing of the report.

7 Author Contributions

O.A. and E.R. share first-authorship. O.A. and E.R. co-drafted the first version of the manuscript. O.A. performed electronic health record data extraction. E.R. conducted data analyses. D.D.D.L. conceptualized the work and edited the manuscript.

8 References

1. Pershad J, Monroe K, Atchison J. Childhood hypoglycemia in an urban emergency department: epidemiology and a diagnostic approach to the problem. Pediatr Emerg Care. 1998;14(4):268-71.

2. Weinstein DA, Butte AJ, Raymond K, Korson MS, Weiner DL, Wolfsdorf JI. High Incidence of Unrecognized Metabolic and Endocrinologic Disorders in Acutely Ill Children with Previously Unrecognized Hypoglycemia. Pediatr Res. 2011;49:88.

3. White K, Truong L, Aaron K, Mushtaq N, Thornton PS. The Incidence and Etiology of Previously Undiagnosed Hypoglycemic Disorders in the Emergency Department. Pediatr Emerg Care. 2020;36(7):322-6.

4. American Diabetes A. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43(Suppl 1):S66-S76.

5. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. J Pediatr. 2015;167(2):238-45.

6. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. J Clin Invest. 1987;79(3):777-81.

7. van Veen MR, van Hasselt PM, de Sain-van der Velden MG, Verhoeven N, Hofstede FC, de Koning TJ, et al. Metabolic profiles in children during fasting. Pediatrics. 2011;127(4):e1021-7.

8. Hawkes CP, Grimberg A, Dzata VE, De Leon DD. Adding Glucagon-Stimulated GH Testing to the Diagnostic Fast Increases the Detection of GH-Sufficient Children. Horm Res Paediatr. 2016;85(4):265-72.

9. Papini L, Piga S, Dionisi-Vici C, Parisi P, Ciofi Degli Atti ML, Marcias M, et al. Hypoglycemia in a Pediatric Emergency Department: Single-Center Experience on 402 Children. Pediatr Emerg Care. 2020.

10. Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, et al. Congenital Hyperinsulinism in Infants with Turner Syndrome: Possible Association with Monosomy X and KDM6A Haploinsufficiency. Horm Res Paediatr. 2018;89(6):413-22.

11. Brown LM, Corrado MM, van der Ende RM, Derks TG, Chen MA, Siegel S, et al. Evaluation of glycogen storage disease as a cause of ketotic hypoglycemia in children. J Inherit Metab Dis. 2015;38(3):489-93.

9 Supplementary Material

10 Data Availability Statement

Data that support the findings of this study are included in this article and its supplementary material file. Further enquiries can be directed to the corresponding author.

Table 1. Cohort characteristics

Patient Characteristics	N=145*
Age at presentation (years), median (IQR)	2.05 (1.21, 3.72)
Sex, % female (n)	55% (80)
Race/Ethnicity, % (n)	
White	62% (90)
Black	22% (32)
Asian	4% (6)
American Indian or Alaska Native	0.7% (1)
Other	9% (13)
Hispanic	2% (3)
Gestational age, % (n), N=138	
Preterm	16% (22)
Term	84% (116)
Birth weight (kg), mean ± SD, N=130	3.11 ± 0.72
History of perinatal stress, % (n), N=123	43% (53)
Past Medical History, % (n)	
Genetic Disorder	5.5% (8)
Neurodevelopment Disorder	16% (23)
Cardiac Disease	6.2% (9)
Pulmonary Disease	9.7% (14)
Gastroenterology Disease	20% (29)
Other	15% (22)
Weight category, % (n), N=140	
Underweight	13% (18)
Normal	71% (100)
Overweight	11% (16)
Obese	4.3% (6)
Height category, % (n), N=141	
Short stature	13% (18)
Normal stature	85% (120)
Tall stature	2.1% (3)
Prior history of hypoglycemia, % (n)	20% (29)
Presenting illness features, % (n)	
Gastroenteritis	25% (36)
Vomiting	44% (64)
Diarrhea	14% (20)
Upper respiratory tract infection	22% (32)
Otitis media	2.8% (4)
Fever	30% (44)
Other	12% (18)
Illness duration, % (n)	
1-3 days	59% (86)
≥ 4 days	26% (37)
Not recorded/unknown	15% (22)
History of decreased oral intake, % (n)	
1-3 days	46% (66)
≥ 4 days	8.3% (12)
Not recorded/unknown	46% (67)
Symptomatic hypoglycemia at presentation, % (n)	64% (93)

*unless otherwise noted

Table 2. Characteristics of patients in whom an underlying etiology for hypoglycemia was identified versus those without a diagnosis

Variable N=145*	Diagnosis established N=12	No diagnosis established N=133	p-value
Age at presentation (years), median (IQR)	1.03 (0.05, 1.54)	2.18 (1.29, 3.99)	<0.001
Sex, % female (n)	67% (8)	54% (72)	0.55
Race, % White (n)	75% (9)	60% (81)	0.54
Ethnicity, % Hispanic (n)	0% (0)	2.3% (3)	>0.99
Weight category, % (n), N=140			0.27
Underweight	17% (2)	13% (16)	
Normal	83% (10)	70% (90)	
Overweight/Obese	0% (0)	17% (22)	
Height category, % (n), N=141			0.74
Short stature	17% (2)	12% (16)	
Normal stature	83% (10)	85% (110)	
Tall stature	0% (0)	2.3% (3)	
Prior history of hypoglycemia, % (n)	25% (3)	20% (26)	0.71
Past medical/surgical history, % (n)	17% (2)	50% (67)	0.03
Abnormal physical examination findings, % (n)	8.3% (1)	5.3% (7)	0.51
Presenting illness features, % (n)			
Gastroenteritis	25% (3)	25% (33)	>0.99
Vomiting	25% (3)	46% (61)	0.23
Diarrhea	25% (3)	13% (17)	0.22
Upper respiratory tract infection	17% (2)	23% (30)	>0.99
Otitis media	0% (0)	3.0% (4)	>0.99
Fever	33% (4)	30% (40)	0.76
Other	17% (2)	12% (16)	0.65
Illness duration, % (n), N=123			0.45
1-3 days	56% (5)	71% (81)	
≥ 4 days	44% (4)	29% (33)	
History of decreased oral intake, % (n), N=78			0.11
1-3 days	50% (2)	86.5% (64)	
≥ 4 days	50% (2)	13.5% (10)	
Symptomatic hypoglycemia at presentation, % (n)	50% (6)	65.4% (87)	0.35
Autonomic symptoms	0% (0)	6.0% (8)	>0.99
Neuroglycopenic symptoms	50% (6)	63% (84)	0.37
Labs at initial presentation			
Plasma glucose (mmol/L [mg/dL]), median (IQR)	2.5 (1.4, 2.7) [45 (26, 49)]	2.5 (2.1, 2.9) [45 (37, 52)]	0.42
Plasma glucose <2.8 mmol/L (<50 mg/dL), % (n)	83% (10)	69% (92)	0.51
Positive plasma or urine ketones, % (n), N=82	25% (1)	92% (72)	0.004
Serum bicarbonate (mmol/L), mean ± SD, N=91	22 ± 5.5	16 ± 3.6	<0.001
Other abnormal findings on presenting or baseline evaluation,† % (n), N=111	25% (2)	44% (45)	0.46

*unless otherwise noted, †including transaminases, lactate, ammonia, cortisol, growth hormone, IGF-I, IGFBP-3, acylcarnitine profile, carnitine profile, urine organic acids

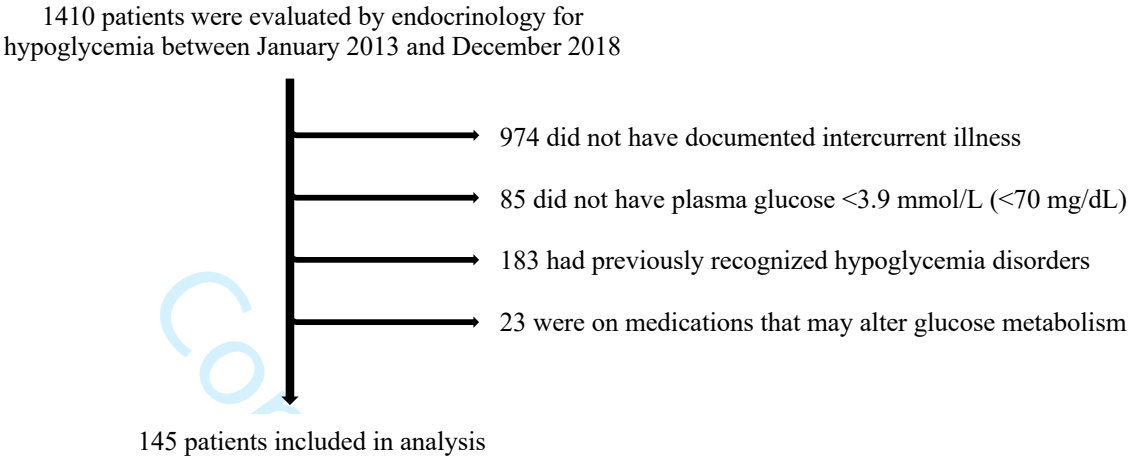
Table 3. Factors associated with performing diagnostic fast

Variable N=145*	Diagnostic fast N=69	No diagnostic fast N=76	p-value
Age at presentation (years), median (IQR)	1.94 (1.23, 3.60)	2.12 (1.20, 3.96)	0.51
Sex, % female (n)	62% (43)	49% (37)	0.13
Race, % White (n)	68% (47)	57% (43)	0.17
Ethnicity, % Hispanic (n)	0% (0)	3.9% (3)	0.25
Weight category, % (n), N=140			0.68
Underweight	15% (10)	11% (8)	
Normal	74% (50)	72% (52)	
Overweight/Obese	12% (8)	17% (12)	
Height category, % (n), N=141			0.65
Short stature	15% (10)	11% (8)	
Normal stature	83% (57)	88% (63)	
Tall stature	2.9% (2)	1.4% (1)	
Prior history of hypoglycemia, % (n)	23% (16)	17% (13)	0.41
Past medical/surgical history, % (n)	42% (29)	53% (40)	0.25
Abnormal physical examination findings, % (n)	5.8% (4)	5.3% (4)	>0.99
Presenting illness features, % (n)			
Gastroenteritis	29% (20)	21% (16)	0.34
Vomiting	36% (25)	51% (39)	0.09
Diarrhea	17% (12)	11% (8)	0.34
Upper respiratory tract infection	25% (17)	20% (15)	0.55
Otitis media	1.5% (1)	4.0% (3)	0.62
Fever	30% (21)	30% (23)	>0.99
Other	15% (10)	11% (8)	0.62
Illness duration, % (n), N=123			>0.99
1-3 days	69% (38)	71% (48)	
≥ 4 days	31% (17)	29% (20)	
History of decreased oral intake, % (n), N=78			0.77
1-3 days	86% (31)	83% (35)	
≥ 4 days	14% (5)	17% (7)	
Symptomatic hypoglycemia at presentation, % (n)	71% (49)	58% (44)	0.12
Autonomic symptoms	4.4% (3)	6.6% (5)	0.72
Neuroglycopenic symptoms	70% (48)	55% (42)	0.09
Labs at initial presentation			
Plasma glucose (mmol/L [mg/dL]), median (IQR)	2.2 (1.8, 2.7) [40 (32, 49)]	2.6 (2.3, 3.0) [47 (41, 55)]	0.002
Plasma glucose <2.8 mmol/L (<50 mg/dL), % (n)	80% (55)	62% (47)	0.03
Positive plasma or urine ketones, % (n), N=82	83% (29)	94% (44)	0.16
Serum bicarbonate (mmol/L), mean ± SD, N=91	17 ± 4.0	16 ± 4.0	0.26
Other abnormal findings on presenting or baseline evaluation,† % (n), N=111	38% (22)	47% (25)	0.34

*unless otherwise noted, †including transaminases, lactate, ammonia, cortisol, growth hormone, IGF-I, IGFBP-3, acylcarnitine profile, carnitine profile, urine organic acids

Figures and Figure Legends

Figure 1. Consort diagram



Supplemental Table 1. Details of genetic testing performed

Of the genetic testing conducted, a commercial panel testing for glycogen storage diseases (sequencing of *AGL, G6PC, GAA, GBE1, GYS2, PFKM, PHKA1, PHKA2, PHKB, PHKG2, PYGL, PYGM, SLC2A2*, and *SLC37A4*) was obtained most frequently, in 14 patients. A ketotic hypoglycemia panel (sequencing and deletion/duplication analysis of *ACAT1, AGL, G6PC, GYS2, PHKA2, PHKB, PHKG2, PYGL, SLC16A1*, and *SLC37A4*) was obtained in 10 patients, hyperinsulinism panel (including sequencing of *ABCC8, KCNJ11, GCK, GLUD1* in all cases, with the addition of sequencing and deletion/duplication analysis of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1, UCP2* depending on the panel utilized) was obtained in six patients, fatty acid oxidation defect panel (sequencing and deletion/duplication analysis of *ACADVL*) was obtained in five patients, isolated *SLC16A1* mutation analysis was performed in three patients, and one patient had a commercial metabolic hypoglycemia panel (sequencing of *ACAT1, AGL, ALDOB, FBP1, G6PC, GALT, GYS2, HMGCL, MLYCD, OXCT1, PC, PCK1, PCK2, PGM1, PHKA2, PHKB, PHKG2, PYGL, SLC16A1, SLC2A2*, and *SLC37A4*) obtained. One child had targeted testing for Russel Silver syndrome based upon clinical examination findings. Chromosomal microarray was obtained in one patient and four patients had whole exome sequencing.

Supplemental Table 2. Summary of patients with identified hypoglycemia diagnoses

History and initial presentation	Evaluation findings	Treatment and Course
Growth hormone deficiency		
Term male, history of uninvestigated neonatal hypoglycemia. Presented at 10 months with vomiting, irritability, and POC PG of 32 mg/dL. Length z score -2.24, weight-for-length 80%ile.	Ketotic hypoglycemia (PG 52 mg/dL, BOHB 2.3 mmol/L) with normal lactate and cortisol (30 mcg/dL) but low GH 0.97 ng/mL. Peak GH after stimulation (arginine/clonidine) was 9.7 ng/mL and MRI revealed a small pituitary gland with possible ectopic pituitary tissue.	Initiated GH with resolution of hypoglycemia. Remains on GH replacement at 9 years of age.
Fatty acid oxidation disorder		
23-month-old female without significant past medical history presented with seizure and POC PG 20 mg/dL in setting of gastroenteritis.	Hypoketotic hypoglycemia with hyperfattyacidemia (PG 39 mg/dL, BOHB 1.4 mmol/L, FFA 3.98 mmol/L). Acylcarnitine profile revealed mild increase of C14:1 and C14:2 and UOA showed markedly increased dicarboxylic acids. Sequencing of *ACADVL* was negative, however, fatty acid oxidation probe of fibroblasts demonstrated significantly reduced oxidation of palmitate, consistent with impaired long-chain fatty acid oxidation.	Dextrose-containing fluids every 2 hours with illness. Multiple additional episodes of hypoglycemia during illness, one requiring hospitalization.
Dihydrolipoamide dehydrogenase (DLD) deficiency		
14-month-old male without significant medical history presented with gastroenteritis, lethargy, seizures, and PG 9 mg/dL.	Hypoglycemia with lactic acidosis and abnormal urine organic acid profile (PG 40 mg/dL, BOHB 1.3 mmol/L, FFA 2.5 mmol/L, lactate 5.2 mmol/L, ammonia 18 µmol/L, UOA: increased lactate, ketone, 2OH-glutaric acid, TCA cycle intermediates, 2-keto-glutaric acid, 2OH-adipic acid and glutaric acid). WES identified compound heterozygous variants in *DLD*: Gly229Cys / Ser258Pro.	Low-protein diet. Numerous admissions for hypoglycemia and intermittent hepatic dysfunction.
3 methylcrotonyl-CoA carboxylase deficiency		
18-month-old female without significant past medical history presented with vomiting, lethargy, PG 49 mg/dL, and HCO3 16 mmol/L.	Ketotic hypoglycemia with abnormal urine organic acid profile (PG 52 mg/dL, BOHB 3.9 mmol/L, lactate 1.2 mmol/L, ammonia <9 µmol/L, acylcarnitine profile: moderate increase in C5OH-carnitine, UOA: increased 3-methylcrotonylglycine, lactic acid, 3-hydroxy-isovalerate, consistent with deficiency in 3 methylcrotonyl-CoA carboxylase. *MCCC1* sequencing identified a heterozygous novel pathogenic frameshift variant (Ser622Pro).	Limit fasting. Glucose meter and ketone meter monitoring. Multiple episodes of ketosis during illness, all managed at home.
Hyperinsulinism		
Term female born AGA, limited prenatal care. Presented at 5 days of age with jaundice and diarrhea due to rotavirus. POC PG 49 mg/dL, HCO3 23 mmol/L.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon on fast at 11 days of age (PG 57 mg/dL, BOHB 1.2 mmol/L, FFA 1.04 mmol/L, insulin <2 µIU/mL, C-peptide 0.22 ng/mL, IGFBP-1 167 ng/mL, ammonia 27 µmol/L, cortisol 25 mcg/dL, GH 10.4 ng/mL, Δ PG +45 mg/dl post-glucagon). Fasted 12 hours with PG >70 mg/dL. Genetic testing not performed. Presumed PSI-HI.	Diazoxide not initiated given fasting tolerance. Glucagon PRN, glucose meter monitoring. At 7 months of age, no PG <70 mg/dL on home monitoring.
Term female born AGA to GBS+ mother. Presented at 4 days of age with fever, irritability, POC PG 36 mg/dL, and HCO3 14 mg/dL. Infectious work-up was negative.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon on fast at 14 days of age (PG 43mg/dL, BOHB 0.8 mmol/L, FFA 0.8 mmol/L, insulin <2 µIU/mL, C-peptide 0.16 ng/mL, IGFBP-1 144 ng/mL, ammonia 39 µmol /L, cortisol 17 mcg/dL, GH 18.6 ng/mL, Δ PG +40 mg/dl post-glucagon). Fasted 8 hours with PG >70 mg/dL. Sequencing of *ABCC8*, *KCNJ11*, *GCK*, and *GLUD1* identified VUS in *GLUD1* (Ala49Thr).	Diazoxide not initiated given fasting tolerance. Limit fasting to 8 hours, glucagon PRN, glucose meter monitoring. Lost to follow-up.
Term male born SGA, history of uninvestigated neonatal hypoglycemia. Presented at 1 month of age with	Hypoketotic hypoglycemia with hypofattyacidemia (PG 50 mg/dL, BOHB 0.62 mmol/L, FFA 0.57 mmol/L, insulin <2 µIU/mL, C-peptide 0.21 ng/mL, lactate 0.8 mmol/L, ammonia 32 µmol/L, cortisol 10 mcg/dL, GH 4.08 ng/mL, glucagon stimulation not performed). Fasted 11	Glucagon PRN, glucose meter. Repeat fast at age 9 months demonstrated resolution of HI

fever, POC PG 58 mg/dL, and POC BOHB <0.3 mmol/L. + parechovirus.	hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8*, *KCNJ11* and sequencing of *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *SLC16A1*, and *UCP2* was negative.	(PG 42 mg/dL, BOHB 2.4 mmol/L, IGFBP-1 723 ng/mL).
Term male infant born with AGA. Presented at 2 days with diarrhea, irritability, and POC PG 49 mg/dL. Required max GIR 13 mg/kg/min. Found to have shigella enteritis.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon on fast at 8 days of age (PG 44 mg/dL, BOHB 0.9, FFA 0.72 mmol/L, insulin <2 µIU/mL, C-peptide 0.22 ng/mL, lactate 1.3 mmol/L, ammonia 19 µmol/L, cortisol 6 mcg/dl, GH 4.84 ng/mL, Δ PG +30 mg/dl post-glucagon). Fasted <3 hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1,* and *UCP2* and sequencing of *GCK* was negative.	Diazoxide 5 mg/kg/d, glucagon PRN, glucose meter monitoring. Transferred care to local endocrinologist at discharge.
Term female born AGA with failure to thrive and GERD. Presented at 5 months of age with fever, congestion, seizure, POC PG 42 mg/dL, HCO3 24 mmol/L, and negative urine ketones.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon (PG 42 mg/dL, BOHB <0.3 mmol/L, FFA 0.19 mmol/L, insulin <2 µIU/mL, C-peptide 0.35 ng/mL, lactate 1.3 mmol/L, ammonia 33 µmol/L, cortisol 11.6 mcg/dl, GH 8.07 ng/mL, Δ PG +45 mg/dl post-glucagon). Fasted <3 hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1,* and *UCP2* and sequencing *GCK* was negative for genes analyzed, revealed partial deletion of X chromosome, cytogenic analysis subsequently confirmed mosaicism for monosomy X and ring X confirming a diagnosis of Turner syndrome.	Enteral dextrose via G-tube. Later started lanreotide. At age 5 years, repeat fast off therapy demonstrated a safe fasting tolerance (PG >70 for 12 hours) but continued evidence of HI.
Female born at 34 weeks, SGA with heterotaxy syndrome. Presented at 18 months with fever, URI, diarrhea, POC PG 54 mg/dL, HCO3 28 mmol/L, and negative urine ketones.	Hypoketotic hypoglycemia with glycemic response to glucagon (PG 50 mg/dL, BOHB 1.7 mmol/L, FFA 2.1 mmol/L, insulin <2 µIU/mL, C-peptide 0.3 ng/mL, lactate 1.8 mmol/L, ammonia <9 µmol/L, cortisol 8.5 mcg/dL, GH 1.6 ng/mL, Δ PG >30 mg/dl post-glucagon). Fasted 3 hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1,* and *UCP2* and sequencing of *GCK* was negative.	Enteral dextrose via G-tube. Required treatment through age 3 years when demonstrated ability to fast 18 hours with PG >70 mg/dL off treatment.
Term female with history of uninvestigated neonatal hypoglycemia. Presented at 18 months with gastroenteritis and POC PG 16 mg/dL.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon (PG 45mg/dL, BOHB 0.62 mmol/L, FFA 0.5 mmol/L, insulin < 1 µIU/mL, C-peptide 0.5 ng/mL, lactate 1.2 mmol/L, ammonia 33 µmol/L, cortisol 5.1 mcg/dL, Δ PG + 68 mg/dl post-glucagon). Sequencing and del/dup of *ABCC8*, *KCNJ11* and sequencing of *GCK* and *GLUD1* identified an autosomal dominant paternally inherited mutation in *ABCC8* (pSer1387del)	Enteral dextrose via G-tube and lanreotide. Overnight enteral dextrose discontinued by 3 years of age. Remains on lanreotide.
Impaired hepatic insulin clearance		
22-month-old female with fever, URI, gastroenteritis, PG 48 mg/dl, HCO3 25 mmol/L, AST 6774 U/L, ALT 4847 U/L, and prolonged PT and PTT. Diagnosed with acute hepatic insufficiency due to rhinovirus and enterovirus. Hypoglycemia persisted despite improved liver function.	Hypoketotic hypoglycemia with elevated insulin and appropriately low C-peptide (PG 50 mg/dL, BOHB <0.3 mmol/L, FFA 2.0 mmol/L, insulin 8.6 µIU/mL, C-peptide 0.3 ng/mL, lactate 2.0 mmol/L, ammonia 32 µmol/L, cortisol 18.6 mcg/dL, GH 1.53 ng/mL, no glycemic response to glucagon, normal acylcarnitine profile and UOA). Fasting study repeated x 3 with consistent results.	Enteral dextrose via NG-tube overnight. Discontinued at 26 months of age following repeat fast demonstrating resolution of inappropriate insulin action (PG 47 mg/dL, BOHB 3.0 mmol/L, FFA 3.46 mmol/L, insulin <2 µIU/mL, and C-peptide < 0.1 ng/mL).

AGA appropriate for gestational age, ALT alanine aminotransferase, AST aspartate aminotransferase, BOHB ß-hydroxybutyrate, FFA free fatty acids, GBS Group B *Streptococcus*, GERD gastroesophageal reflux disease, GH growth hormone, GIR glucose infusion rate, G-tube gastrostomy tube, HCO3 bicarbonate, HI hyperinsulinism, IGFBP-1 insulin-like growth factor binding protein 1, MRI magnetic resonance imaging, NG nasogastric tube, PG plasma glucose, POC point-of-care, PRN *pro re nata*, PSI-HI perinatal stress induced hyperinsulinism, PT prothrombin time, PTT partial thromboplastin time, SGA small for gestational age, TCA tricarboxylic acid cycle, UOA urine organic acids, URI upper respiratory infection, WES whole exome sequencing

Response to Reviewer Comments

(1) Definition of hypoglycaemia - the authors suggest that <3.9mmol/L is hypoglycaemia, but this is not correct. Their data includes (I think) 102 patients with actual hypoglycaemia, and the analysis should be restricted to those with genuine hypoglycaemia.

Clinical hypoglycemia is defined as a plasma glucose concentration low enough to cause symptoms and/or signs of impaired brain function. However, in children, hypoglycemia may be difficult to recognize because the signs and symptoms are nonspecific. A specific plasma glucose concentration cannot easily be used to define hypoglycemia, because the thresholds for the physiologic responses to hypoglycemia occur across a range of plasma glucose concentrations and these thresholds can be altered by the presence of other metabolic fuels.

However, despite these limitations, established normative values for plasma glucose exist (3.9-5.5 mmol/L, [70-100 mg/dL]) (1, 2). These normative ranges are the same for children and adults; most infants and children are able to maintain plasma glucose above 3.9 mmol/L (70 mg/dL) after 15-18 hours of fasting (2). Many established committees and guidelines, including the American Diabetes Association, define hypoglycemia as a plasma glucose <3.9 mmol/L (<70 mg/dL) because this has been recognized as the threshold for neuroendocrine responses to falling glucose in healthy individuals. (3).

Accordingly, a plasma glucose threshold of 70 mg/dL was utilized to define hypoglycemia in this study. We aimed to cast a wide net by setting the threshold to identify our cohort at <70 mg/dL, understanding that the yield of identifying those children who had an underlying hypoglycemia disorder would be higher the lower the threshold. Rational for the plasma glucose threshold used to define hypoglycemia in this study was added to the methods section (lines 77-81).

(2) The authors need to give an estimate of how many patients have presented to their institution with acute illness and hypoglycaemia, not just those then referred for endocrine opinion. (This is giving a selection bias in the study design). This will give a better estimate of how many acutely unwell patients with hypoglycaemia have an underlying disorder.

While the authors agree that inclusion of the total number of patients seen at the institution with hypoglycemia during presentation for acute illness would provide helpful context, collection of these data was outside the scope/feasibility of the present study. The role of selection bias in this study was expanded in the discussion section (lines 254-259). Additionally, throughout the discussion, this study's findings are placed into context of existing literature, including studies in which estimates of all patients presenting to the institution with biochemical hypoglycemia are described, providing additional framework for interpretation to the reader.

(3) There is no set list of "hypoglycemic disorders", and although there is some discussion of endocrine and metabolic causes this must be delineated

Delineation of diagnoses was added to the methods section (lines 109-110).

(4) I am concerned that many patients seem to have diagnostic fasts performed on "clinician decision"

but without a clear rationale for why these are all needed. In particular, fasting a patient who may have a fatty acid oxidation defect is medically negligent and dangerous – yet the paper suggests that a diagnosis could only be reached in those who had a fasting test. It is mandatory to obtain results from an acylcarnitine profile before a fast is undertaken. The authors should clarify their institution's protocol on this.

The methods section was revised to include clarification of the institutional protocol for pursuing diagnostic fast in children in whom a disorder of fatty acid oxidation is suspected (lines 101-103)

(5) Several of the diagnoses listed in Supplemental Table 2 do not need fasting test to make the diagnosis – this can be reached on the baseline evaluations. This should be clarified.

The discussion (lines 238-243) was edited for clarification as suggested.

(6) There is very little mention of "idiopathic ketotic hypoglycaemia" which is probably the commonest cause of hypoglycaemia presenting in an acute illness. The final explanation for the hypoglycaemia for all of the patients should be given - either "physiological hypoglycaemia" explained in a starved child with acute illness, or Ketotic Hypoglycaemia, or alternative diagnosis.

The question of distinguishing those children with appropriate fasting tolerance for age but prolonged starvation due to illness versus those with inappropriately foreshortened fasting tolerance for age gets to the heart of the rationale for this study. The authors were interested to evaluate the role duration of reduced oral intake (i.e.: "duration of fasting"), however these analyses were limited by the retrospective nature of the study. Consequently, distinguishing between children with physiological hypoglycemia due to prolonged starvation during acute illness and those with idiopathic ketotic hypoglycemia provoked by acute illness was not feasible. Future prospective studies in which these data are more uniformly collected would likely prove useful in elucidating this further. Differentiating between these groups, however, is less clinically relevant than the identification of those children with endocrine and metabolic causes of hypoglycemia that have high risk of harm if left untreated and that require specialist follow up – which this study directly addresses.

(7) The statistical analysis identifies higher bicarbonate and lower ketones as "risk factors" for being more likely to have an underlying diagnosis. The confusion over which patients were included in the various analyses (were they all hypoglycaemic?) makes this hard to interpret correctly. It would be important to give an explanation for these findings. Hypoketotic hypoglycaemia is abnormal - suggestive of hyperinsulinism or fatty acid oxidation defect. Normal bicarbonate levels may mean the blood gas was normal, or just be consistent with absence of ketosis. Further discussion is warranted.

At presentation, all patients had hypoglycemia (lines 137-138) as defined in the methods section as plasma glucose <3.9 mmol/L (<70 mg/dL). All patients for whom ketones or serum bicarbonate values were available in the medical record at the time of presentation were included in these analyses (N for each provided in Table 2). Discussion of the implication of findings of hypoketotic hypoglycemia is provided in the discussion lines 229-231.

1. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. J Clin Invest. 1987;79(3):777-81.
2. van Veen MR, van Hasselt PM, de Sain-van der Velden MG, Verhoeven N, Hofstede FC, de Koning TJ, et al. Metabolic profiles in children during fasting. Pediatrics. 2011;127(4):e1021-7.
3. American Diabetes A. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43(Suppl 1):S66-S76.

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Undiagnosed Hypoglycemia Disorders in Children Detected When Hypoglycemia Occurs in the Setting of Illness: A Retrospective Study

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Abstract

Objective: Whether hypoglycemia incidentally detected during intercurrent illness in children requires an endocrine workup remains controversial. This study aimed to determine the yield of conducting a diagnostic evaluation in this setting, and to compare clinical and biochemical features between patients ultimately diagnosed with a hypoglycemic disorder and those who were not.

Design: Single-center, retrospective review of children referred between January 2013 and December 2018 for evaluation of hypoglycemia (defined as plasma glucose <3.9 mmol/L [<70 mg/dL]) in the setting of acute illness.

Results: 145 patients met eligibility criteria. A hypoglycemia disorder was identified in 12 patients (8% of the cohort, 17% of those who underwent diagnostic fast). There were no cases in which diagnosis was established in the absence of a diagnostic fast. Characteristics associated with identifying an underlying disorder included younger age (1.03 years [IQR: 0.05, 1.54] v. 2.18 years [IQR: 1.29, 3.99], p<0.001), higher bicarbonate level (22 ± 5.5 mmol/L v. 16 ± 3.6 mmol/L, p<0.001), lower frequency of elevated plasma or urine ketones (25% v. 92%, p=0.004), and lower frequency of other documented medical problems (17% v. 50%, p=0.03).

Conclusions: The yield of diagnostic evaluation among children with incidental detection of hypoglycemia in the setting of illness is not insignificant. We thus recommend that all children with hypoglycemia in the setting of illness undergo guided diagnostic evaluation. Younger age and absence of ketosis and acidosis at presentation may serve as useful predictors for establishing a diagnosis. Future studies are needed to confirm these findings.

What is already known on this topic – The prevalence of undiagnosed hypoglycemia disorders among children seen in the emergency department for any clinical reason has been reported as 10-28%. During illness, oral intake in children is often reduced. In this setting, incidentally hypoglycemia is often attributed to prolonged fasting. Determining whether children with hypoglycemia detected during illness require a dedicated endocrine evaluation has been limited by a paucity of data.

What this study adds – In this cohort, 8% of children who presented with hypoglycemia in setting of illness were found to have an underlying hypoglycemia disorder. Underlying hypoglycemia diagnoses were only established in those children who underwent a comprehensive evaluation including diagnostic fast. Younger age, higher bicarbonate level, and lower ketones at presentation were associated with establishing a hypoglycemia diagnosis.

How this study might affect research, practice or policy – All children with hypoglycemia detected in the setting of acute illness should undergo guided diagnostic evaluation.

1 Introduction

Incidental detection of hypoglycemia during childhood illness commonly occurs following prolonged starvation, in which glucose utilization exceeds glucose supply. Rarely, it may be the initial presentation of an underlying hypoglycemia disorder wherein missing the diagnosis carries a high risk of harm. The reported prevalence of undiagnosed hypoglycemia disorders among children seen in the emergency department for any reason ranges between 10 and 28% (1-3). However, these studies were not limited to children presenting with acute illness. Consequently, whether children with hypoglycemia detected during acute illness require an endocrine workup remains controversial. We sought to evaluate the yield of conducting an evaluation when hypoglycemia occurs in this setting and to describe the clinical and biochemical features of those children ultimately found to have underlying pathology.

2 Materials and Methods

A retrospective review was conducted of children referred to endocrinology for evaluation of hypoglycemia (plasma glucose <3.9 mmol/L [<70 mg/dL]) in the setting of acute illness at Children's Hospital of Philadelphia (CHOP) between January 2013 and December 2018. Billing records were utilized to obtain a list of inpatient and outpatient endocrine consults for hypoglycemia using ICD codes for "hypoglycemia, unspecified" (ICD-9 251.2 prior to October 2015, ICD-10 16.2 after October 2015). Additionally, inpatient billing records were manually searched for "hypoglycemia" as the consultation reason. Patients were included if they were <18 years of age and had both documented plasma glucose <3.9 mmol/L (<70 mg/dL) and illness symptoms (e.g., fever, vomiting, diarrhea, respiratory symptoms) at the time of presentation. Exclusion criteria included children with previously diagnosed hypoglycemia disorders, diabetes mellitus, or use of medications that can alter glucose metabolism (hypoglycemic agents, systemic steroids, chemotherapy, or beta-

blockers) within one month of presentation. A plasma glucose threshold of <3.9 mmol/L (<70 mg/dL) was utilized to define hypoglycemia in this study in keeping with established hypoglycemia definitions (4, 5), and because below this threshold, neuroendocrine responses to hypoglycemia are activated (6). Additionally, most infants and children are able to maintain plasma glucose above this threshold after 15-18 hours of fasting (7).

Demographic, clinical, and biochemical data were extracted from the electronic health record (EHR). Acute illness was categorized as: gastroenteritis, isolated vomiting, isolated diarrhea, upper respiratory infection, otitis media, fever, and other. Illness categories were not exclusive; patients were included in all categories for which there were documented symptoms. Height and weight were used to calculate weight-for-length percentiles for patients <2 years of age and body mass index (BMI) percentiles for patients ≥2 years of age. Weight status was categorized as: underweight (weight-for-length/BMI <5 percentile for age), normal weight (weight-for-length/BMI ≥5 and <85 percentile for age), overweight (weight-for-length/BMI ≥85 and <95 percentile for age), and obese (weight-for-length/BMI ≥95 percentile for age). Physical examination findings of interest included dysmorphic features, hepatomegaly, and signs of suggestive of hypopituitarism (midline defects, microphallus in males).

Types of hypoglycemia evaluation performed included laboratory studies drawn at the time of presentation, non-fasting laboratory studies obtained following presentation ("baseline evaluation"), genetic testing, and diagnostic fasting studies, which were conducted as previously described (standard protocol (8)). Evaluations were conducted at the discretion of the provider. This was typically an emergency medicine provider at presentation. The decision to pursue diagnostic fasting studies was made solely by endocrinologists. Standard practice at our center is to obtain baseline metabolic studies (acylcarnitine profile, total and free carnitine levels, and urine organic acids) prior

to performing fasting studies when there is concern for a possible fatty acid oxidation disorder. To facilitate comparison between groups, urine and blood ketone levels were combined into categories wherein positive ketones were defined as either small or greater urine ketones or blood ketones ≥1 mmol/L; ketones were otherwise defined as negative.

The EHR was reviewed for additional episodes of hypoglycemia and for endocrine or metabolic diagnoses (hormone deficiencies, disorders of insulin secretion/signaling, glycogen storage disease, disorders of gluconeogenesis, and fatty acid oxidation disorders) made subsequent to the index event. Duration of follow-up was calculated from index event and last contact dates.

This study was determined to be exempt by the CHOP Institutional Review Board. Patients were not involved in the design or conduct of this study.

2.1 Statistical Analysis

Categorical variables were reported as proportions. Normally distributed continuous variables were summarized using mean and standard deviation. Median and interquartile range were reported for non-normally distributed continuous data. In comparing the clinical and biochemical characteristics of patients ultimately diagnosed with a hypoglycemic disorder with those who were not, and patients who underwent diagnostic fasting evaluation with those who did not, proportions were compared using Fisher's exact test, t-tests were used to compare means of normally distributed data, and Wilcoxon rank sum tests were used to compare medians of nonparametric data. All tests were two-sided with $p<0.05$ set as the threshold for statistical significance.

3 Results

A total of 1410 patients were evaluated by endocrinology for hypoglycemia at CHOP between January 2013 and December 2018. Of these, 145 patients met inclusion criteria, and their records were reviewed (Figure 1). Characteristics of the cohort at time of presentation are summarized in Table 1. Median age at presentation was 2 years and ranged from 2 days to 11 years. Abnormal findings on physical examination were uncommon. Four patients had dysmorphism, three had hepatomegaly, and one had macrocephaly. No patients had documented cleft lip or palate or microphallus.

Thirteen percent of patients presented with altered mental status and 10% presented with seizure like activity. Of the patients with a prior history of hypoglycemia, none had previously undergone a diagnostic evaluation. Thirty-four percent of patients had recurrent episodes of hypoglycemia following the index event. The median follow-up duration was 27 months (range: 0 days – 7.8 years).

3.1 Evaluations conducted

Laboratory evaluations performed at any point during follow-up varied considerably. At the time of initial presentation with hypoglycemia, urine or plasma ketones were obtained in 57% of patients, bicarbonate was obtained in 63%, transaminases were measured in 28%, and cortisol was obtained in 11%. Lactate, ammonia, insulin, c-peptide, free fatty acids, growth hormone, and metabolic studies (acylcarnitine profile, total and free carnitine levels, and urine organic acids) were each obtained in <10% of patients. Of the patients who had laboratory evaluation beyond glucose, ketones, and bicarbonate at the time of initial presentation 50% had abnormal findings. Abnormal findings included elevated transaminases (e.g., above the upper limit of normal) in 50% and elevated lactate in 18%. Cortisol was >276 nmol/L in all patients in whom it was obtained. Baseline evaluation was obtained in 59% of patients with metabolic studies performed most frequently. Baseline evaluation yielded abnormal findings in 29% of patients.

Of the 102 patients with plasma glucose <2.8 mmol/L (<50 mg/dL) on presentation, "critical sample" labs including insulin, urine or plasma ketones, lactate, ammonia, cortisol, growth factors, and acylcarnitine profile were obtained in 10%. Seventy percent of patients in whom a "critical sample" was obtained had symptomatic hypoglycemia at the time of presentation.

A diagnostic fasting test was performed, either at the time of initial presentation or during a follow-up admission, in 48% of patients. Twenty-five percent of the cohort had genetic testing performed (Supplemental Table 1). Only two children had genetic testing without also undergoing a diagnostic fast.

3.2 Identified hypoglycemia diagnoses

An underlying hypoglycemia disorder was identified in 12 patients (8%) all of whom underwent a diagnostic fast. The clinical presentation, evaluation, and course of these patients is detailed in Supplemental Table 2. The yield of performing a diagnostic fast in this study was 17%.

Hyperinsulinism was the most frequently identified etiology and was diagnosed in seven patients. Additional diagnoses included inborn errors of metabolism in three patients, growth hormone deficiency in one patient, and impaired hepatic insulin clearance due to acute hepatic insufficiency in one patient. A final diagnosis was established in two patients in whom laboratory evaluation at presentation other than glucose, ketones, and bicarbonate yielded abnormal findings. In both cases (dihydrolipoamide dehydrogenase deficiency and impaired hepatic insulin clearance), transaminases were elevated above the upper limit of normal for age.

An underlying genetic diagnosis was suggested based upon testing in four patients and included hyperinsulinism due to an autosomal dominant mutation in *ABCC8*, hyperinsulinism associated with Turner syndrome, isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency, and dihydrolipoamide dehydrogenase (DLD) deficiency.

3.3 Factors associated with identifying a specific hypoglycemia diagnosis

We compared clinical and biochemical characteristics at the time of presentation between the patients ultimately diagnosed with an underlying etiology of hypoglycemia and those who were not (Table 2). Younger age (1.03 years [IQR: 0.05, 1.54] v. 2.18 years [IQR: 1.29, 3.99], p<0.001) and higher bicarbonate level (22 ± 5.5 mmol/L v. 16 ± 3.6 mmol/L, p<0.001) were associated with identifying an underlying disorder. Weight-for-age percentile was lower in patients diagnosed with a hypoglycemia disorder (13.1 [IQR: 1.7, 23.8] v. 31.0 [14.0, 59.5], p=0.02) but weight status (i.e., weight adjusted for length/height) did not statistically significantly differ between those who were diagnosed with a hypoglycemia disorder and those who were not. Patients diagnosed with a hypoglycemia disorder were less likely to have elevated plasma or urine ketones at presentation (25% v. 92%, p=0.004) and were less likely to have a documented history of other medical problems (17% v. 50%, p=0.03). No statistically significant differences were observed between groups with regard to the other clinical or biochemical features assessed.

Since a diagnostic fast was performed in all patients who ultimately had a final diagnosis established, we evaluated whether there were any characteristics at presentation associated with conducting this evaluation (Table 3). Median plasma glucose at presentation was lower in the group that underwent diagnostic fast (2.2 mmol/L [40 mg/dL], IQR: 1.8, 2.7 mmol/L [32, 49 mg/dL] v. 2.6 mmol/L [47 mg/dL], IQR: 2.3, 3.0 mmol/L [41, 55 mg/dL], p=0.002). Additionally, the proportion of patients

with presenting plasma glucose <2.8 mmol/L (<50 mg/dL) was greater among those who underwent a diagnostic fast compared to those who did not (80% v 62%, p=0.03).

4 Discussion

Eight percent of children who presented with hypoglycemia in setting of illness were found to have an underlying hypoglycemia disorder. Underlying diagnoses were only established in children who underwent a diagnostic fast, which was conducted in 48% of patients. The frequency of underlying hypoglycemia disorders was thus two-fold higher (17%) among those who underwent diagnostic fast as compared to the overall cohort.

These findings are in keeping with those of White, et al., who found that among children seen in the emergency department for any reason with previously unrecognized hypoglycemia (plasma glucose <2.8 mmol/L [50 mg/dL]), 10.6% were diagnosed with a hypoglycemia disorder (3). Diagnoses were only identified in the children who underwent diagnostic evaluation (53%), such that 20% of those who had a workup were found to have a hypoglycemia disorder. These findings emphasize that without appropriate evaluation, children with underlying hypoglycemia disorders may not be identified.

In a similar cohort of all comers to the emergency room in whom plasma glucose was <2.5 mmol/L (<45 mg/dL), the frequency of previously unrecognized metabolic or endocrinologic disorders among those without infectious diseases causing prolonged fasting was 11% (9). Pershad, et al. reported that among children 1-5 years of age seen in the emergency department with an ICD code for hypoglycemia and a plasma glucose <2.2 mmol/L (<40 mg/dL) or <3.3 mmol/L (<60 mg/dL) with neuroglycopenic symptoms, 16% were diagnosed with an endocrine or metabolic disorder (1). Details on the evaluations conducted and proportion of patients that underwent evaluation were absent from these latter two studies. Notably, the frequency of finding an underlying hypoglycemia

disorder in our study is akin to that reported in the broader population of children seen in the emergency department for any cause, potentially suggesting that the presence of illness symptoms may be less pertinent than other clinical factors in identifying children with underlying hypoglycemia disorders.

Weinstein, et al. found that 28% of children seen in the emergency department and incidentally detected plasma glucose <2.8 mmol/L (<50 mg/dL) had an undiagnosed endocrine or metabolic disorder (2). In this study, patients were prospectively recruited using software which permitted both unbiased subject enrollment and "critical sample" collection prior to correction of hypoglycemia. This is in contrast to the present study in which the decision to obtain a "critical sample" was at the discretion of the provider, typically an emergency medicine provider, and was obtained in only 10% of those with plasma glucose <2.8 mmol/L (<50 mg/dL). Reasons for the low rate of "critical sample" collection are unclear. The majority of children for whom a "critical sample" was obtained had symptomatic hypoglycemia, and it is possible that prompt treatment of hypoglycemia was prioritized over obtaining laboratory assessment in children able to tolerate oral carbohydrate whereas "critical sample" laboratories were more likely to be obtained in children in whom administration of intravenous dextrose was considered. The higher diagnosis rate in the Weinstein study, in which the decision to pursue evaluation was automated and not based upon provider discretion, accentuates the previously absent data to guide clinical practice in deciding which patients require further evaluation.

We found that young age and absence of acidosis and ketosis at presentation were associated with identifying an underlying hypoglycemia disorder. When hypoglycemia occurs in a child as a consequence of starvation (i.e., during illness), the child should have concomitantly elevated plasma and urine ketone concentrations and decreased serum bicarbonate concentration (5). When this does not occur, it should raise suspicion of dysregulated insulin secretion or disorders of fatty acid

oxidation. Our findings may have been influenced by the inclusion of neonates in the study population, and in turn, the high proportion of children with previously undiagnosed hyperinsulinism. Neither the duration of illness nor decreased oral intake was associated with establishing a hypoglycemia diagnosis. However, the high level of missingness for these variables potentially limits interpretation of these findings. Children in whom a diagnosis was established had lower weight-for-age, but weight status (weight adjusted for length/height) did not statistically significantly differ between groups. Absence of documented medical or surgical comorbidities at presentation also emerged as associated with establishing a diagnosis. Reasons for this finding are less obvious but may also stem from the inclusion of neonates.

While the patients with growth hormone deficiency and acute hepatic insufficiency had clinical features suggestive of the underlying etiology of hypoglycemia at presentation, the remainder did not. In fact, the child with hyperinsulinism in the setting of mosaic Turner syndrome, which is a recognized association (10), did not have classic phenotypic features of Turner syndrome. In this child's case, a molecular diagnosis was incidentally uncovered during the genetic evaluation for hyperinsulinism.

A genetic diagnosis was suggested based upon testing in 25% of patients in whom a hypoglycemia disorder was identified. Overall, genetic testing yielded information supporting an underlying etiology of hypoglycemia in 11% of patients in whom it was obtained. In a prior study of children with ketotic hypoglycemia and nondiagnostic metabolic and endocrine evaluation, genetic testing revealed mutations in genes involved in glycogen synthesis and degradation in 12% (11). Interestingly, no cases of glycogen storage disease were identified in our cohort though it is notable genetic evaluation was not universally performed.

Our findings need to be interpreted in light of several limitations. As a retrospective study, data was subject to potential inconsistencies or omissions in documentation in the EHR. Although diagnoses of hypoglycemia were biochemically confirmed, it is likely that potential subjects were not identified because hypoglycemia was not listed as a diagnosis or reason for consultation. Decisions to obtain an initial plasma glucose level, consult endocrinology, and pursue diagnostic evaluation were each at the discretion of the provider. Selection bias could have resulted from differential decision-making at each of these levels. We explored potential sources of bias stemming from the latter of these by comparing those who did versus did not undergo a diagnostic fast, however this analysis fails to capture the role of unmeasured factors driving differential selection of subjects. This was a single-center study in which children were evaluated by endocrinologists with expertise in hypoglycemia disorders at a large children's hospital. Findings may not be generalizable to different populations, particularly those including children with different age distributions.

Despite these limitations, this study adds to the sparse body of literature examining the frequency of underlying pathology among children with hypoglycemia during intercurrent illness. Our findings highlight the importance of obtaining a "critical sample" or at a minimum, assays for bicarbonate and beta-hydroxybutyrate at the time of hypoglycemia as these studies are both readily available and informative in differentiating between categories of hypoglycemia disorders. This approach is in keeping with Pediatric Endocrine Society recommendations for evaluation of hypoglycemia in children (5). Without appropriate evaluation, these children may not be identified, and consequently, appropriate treatment may not be implemented.

5 Conclusions

The high frequency of hypoglycemic disorders identified in this study underscores the critical importance of investigating children with hypoglycemia during illness and argues against ascribing

findings to prolonged starvation. Endocrinology should be consulted to guide the diagnostic evaluation. Young age and absence of ketosis and acidosis at presentation were identified as potential predictors. These findings need to be confirmed in future studies.

6 Funding and Competing Interests

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7 Author Contributions

O.A. and E.R. share first-authorship. O.A. and E.R. co-drafted the first version of the manuscript. O.A. performed electronic health record data extraction. E.R. conducted data analyses. D.D.D.L. conceptualized the work and edited the manuscript.

8 References

1. Pershad J, Monroe K, Atchison J. Childhood hypoglycemia in an urban emergency department: epidemiology and a diagnostic approach to the problem. Pediatr Emerg Care. 1998;14(4):268-71.

2. Weinstein DA, Butte AJ, Raymond K, Korson MS, Weiner DL, Wolfsdorf JI. High Incidence of Unrecognized Metabolic and Endocrinologic Disorders in Acutely Ill Children with Previously Unrecognized Hypoglycemia. Pediatr Res. 2011;49:88.

3. White K, Truong L, Aaron K, Mushtaq N, Thornton PS. The Incidence and Etiology of Previously Undiagnosed Hypoglycemic Disorders in the Emergency Department. Pediatr Emerg Care. 2020;36(7):322-6.

4. American Diabetes A. 6. Glycemic Targets: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43(Suppl 1):S66-S76.

5. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations from the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. J Pediatr. 2015;167(2):238-45.

6. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic thresholds for activation of glucose counterregulatory systems are higher than the threshold for symptoms. J Clin Invest. 1987;79(3):777-81.

7. van Veen MR, van Hasselt PM, de Sain-van der Velden MG, Verhoeven N, Hofstede FC, de Koning TJ, et al. Metabolic profiles in children during fasting. Pediatrics. 2011;127(4):e1021-7.

8. Hawkes CP, Grimberg A, Dzata VE, De Leon DD. Adding Glucagon-Stimulated GH Testing to the Diagnostic Fast Increases the Detection of GH-Sufficient Children. Horm Res Paediatr. 2016;85(4):265-72.

9. Papini L, Piga S, Dionisi-Vici C, Parisi P, Ciofi Degli Atti ML, Marcias M, et al. Hypoglycemia in a Pediatric Emergency Department: Single-Center Experience on 402 Children. Pediatr Emerg Care. 2020.

10. Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, et al. Congenital Hyperinsulinism in Infants with Turner Syndrome: Possible Association with Monosomy X and KDM6A Haploinsufficiency. Horm Res Paediatr. 2018;89(6):413-22.

11. Brown LM, Corrado MM, van der Ende RM, Derks TG, Chen MA, Siegel S, et al. Evaluation of glycogen storage disease as a cause of ketotic hypoglycemia in children. J Inherit Metab Dis. 2015;38(3):489-93.

9 Supplementary Material

10 Data Availability Statement

Data that support the findings of this study are included in this article and its supplementary material file. Further enquiries can be directed to the corresponding author.

Table 1. Cohort characteristics

Patient Characteristics	N=145*
Age at presentation (years), median (IQR)	2.05 (1.21, 3.72)
Sex, % female (n)	55% (80)
Race/Ethnicity, % (n)	
White	62% (90)
Black	22% (32)
Asian	4% (6)
American Indian or Alaska Native	0.7% (1)
Other	9% (13)
Hispanic	2% (3)
Gestational age, % (n), N=138	
Preterm	16% (22)
Term	84% (116)
Birth weight (kg), mean ± SD, N=130	3.11 ± 0.72
History of perinatal stress, % (n), N=123	43% (53)
Past Medical History, % (n)	
Genetic Disorder	5.5% (8)
Neurodevelopment Disorder	16% (23)
Cardiac Disease	6.2% (9)
Pulmonary Disease	9.7% (14)
Gastroenterology Disease	20% (29)
Other	15% (22)
Weight-for-age percentile, median (IQR), N=140	29.5 (11.5, 58.1)
Height-for-age percentile, median (IQR), N=141	25.0 (8.3, 57.0)
Weight status category, % (n), N=140	
Underweight	13% (18)
Normal	71% (100)
Overweight	11% (16)
Obese	4.3% (6)
Prior history of hypoglycemia, % (n)	20% (29)
Presenting illness features, % (n)	
Gastroenteritis	25% (36)
Vomiting	44% (64)
Diarrhea	14% (20)
Upper respiratory tract infection	22% (32)
Otitis media	2.8% (4)
Fever	30% (44)
Other	12% (18)
Illness duration (days), median (IQR), N=123	2 (1, 4)
History of decreased oral intake (days), median (IQR), N=78	2 (1, 3)
Symptomatic hypoglycemia at presentation, % (n)	64% (93)

*unless otherwise noted

Table 2. Characteristics of patients in whom an underlying etiology for hypoglycemia was identified versus those without a diagnosis

Variable N=145*	Diagnosis established N=12	No diagnosis established N=133	p-value
Age at presentation (years), median (IQR)	1.03 (0.05, 1.54)	2.18 (1.29, 3.99)	<0.001†
Sex, % female (n)	67% (8)	54% (72)	0.55‡
Race, % White (n)	75% (9)	60% (81)	0.54‡
Ethnicity, % Hispanic (n)	0% (0)	2.3% (3)	>0.99‡
Weight-for-age percentile, median (IQR), N=140	13.1 (1.7, 23.8)	31.0 (14.0, 59.5)	0.02†
Height-for-age percentile, median (IQR), N=141	15.5 (5.6, 23.3)	29.0 (9.0, 59.0)	0.10†
Weight status category, % (n), N=140			0.27‡
Underweight	17% (2)	13% (16)	
Normal	83% (10)	70% (90)	
Overweight/Obese	0% (0)	17% (22)	
Prior history of hypoglycemia, % (n)	25% (3)	20% (26)	0.71‡
Past medical/surgical history, % (n)	17% (2)	50% (67)	0.03‡
Abnormal physical examination findings, % (n)	8.3% (1)	5.3% (7)	0.51‡
Presenting illness features, % (n)			
Gastroenteritis	25% (3)	25% (33)	>0.99‡
Vomiting	25% (3)	46% (61)	0.23‡
Diarrhea	25% (3)	13% (17)	0.22‡
Upper respiratory tract infection	17% (2)	23% (30)	>0.99‡
Otitis media	0% (0)	3.0% (4)	>0.99‡
Fever	33% (4)	30% (40)	0.76‡
Other	17% (2)	12% (16)	0.65‡
Illness duration (days), median (IQR), N=123	2 (1, 6)	2 (1, 4)	0.82†
History of decreased oral intake (days), median (IQR), N=78	3.5 (1, 6)	2 (1, 3)	0.47†
Symptomatic hypoglycemia at presentation, % (n)	50% (6)	65.4% (87)	0.35‡
Autonomic symptoms	0% (0)	6.0% (8)	>0.99‡
Neuroglycopenic symptoms	50% (6)	63% (84)	0.37‡
Labs at initial presentation			
Plasma glucose (mmol/L [mg/dL]), median (IQR)	2.5 (1.4, 2.7) [45 (26, 49)]	2.5 (2.1, 2.9) [45 (37, 52)]	0.42†
Plasma glucose <2.8 mmol/L (<50 mg/dL), % (n)	83% (10)	69% (92)	0.51‡
Positive plasma or urine ketones, % (n), N=82	25% (1)	92% (72)	0.004‡
Serum bicarbonate (mmol/L), mean ± SD, N=91	22 ± 5.5	16 ± 3.6	<0.001§
Other abnormal findings on presenting or baseline evaluation,‖ % (n), N=111	25% (2)	44% (45)	0.46‡

*unless otherwise noted, †Wilcoxon rank sum test, ‡Fisher's exact test, §t-test, ‖including transaminases, lactate, ammonia, cortisol, growth hormone, IGF-I, IGFBP-3, acylcarnitine profile, carnitine profile, urine organic acids

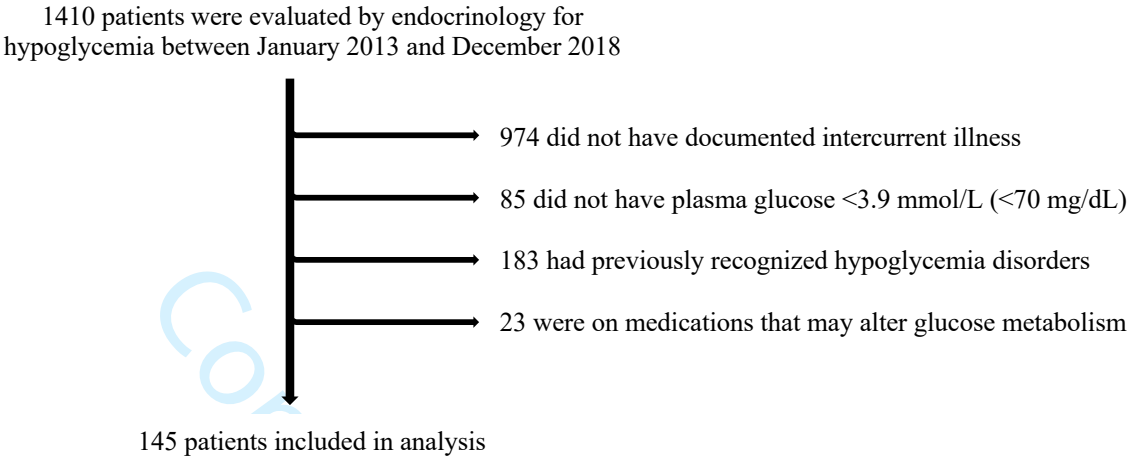
Table 3. Factors associated with performing diagnostic fast

Variable N=145*	Diagnostic fast N=69	No diagnostic fast N=76	p-value
Age at presentation (years), median (IQR)	1.94 (1.23, 3.60)	2.12 (1.20, 3.96)	0.51†
Sex, % female (n)	62% (43)	49% (37)	0.13‡
Race, % White (n)	68% (47)	57% (43)	0.17‡
Ethnicity, % Hispanic (n)	0% (0)	3.9% (3)	0.25‡
Weight-for-age percentile, median (IQR), N=140	29.2 (10.5, 59.5)	30.5 (14.0, 54.5)	0.61†
Height-for-age percentile, median (IQR), N=141	22.0 (7.2, 55.0)	29.3 (9.0, 60.0)	0.55†
Weight status category, % (n), N=140			0.68‡
Underweight	15% (10)	11% (8)	
Normal	74% (50)	72% (52)	
Overweight/Obese	12% (8)	17% (12)	
Prior history of hypoglycemia, % (n)	23% (16)	17% (13)	0.41‡
Past medical/surgical history, % (n)	42% (29)	53% (40)	0.25‡
Abnormal physical examination findings, % (n)	5.8% (4)	5.3% (4)	>0.99‡
Presenting illness features, % (n)			
Gastroenteritis	29% (20)	21% (16)	0.34‡
Vomiting	36% (25)	51% (39)	0.09‡
Diarrhea	17% (12)	11% (8)	0.34‡
Upper respiratory tract infection	25% (17)	20% (15)	0.55‡
Otitis media	1.5% (1)	4.0% (3)	0.62‡
Fever	30% (21)	30% (23)	>0.99‡
Other	15% (10)	11% (8)	0.62‡
Illness duration (days), median (IQR), N=123	2 (1, 5)	2 (1, 4)	0.58†
History of decreased oral intake (days), median (IQR), N=78	2 (1, 3)	2 (1, 3)	0.73†
Symptomatic hypoglycemia at presentation, % (n)	71% (49)	58% (44)	0.12‡
Autonomic symptoms	4.4% (3)	6.6% (5)	0.72‡
Neuroglycopenic symptoms	70% (48)	55% (42)	0.09‡
Labs at initial presentation			
Plasma glucose (mmol/L [mg/dL]), median (IQR)	2.2 (1.8, 2.7) [40 (32, 49)]	2.6 (2.3, 3.0) [47 (41, 55)]	0.002†
Plasma glucose <2.8 mmol/L (<50 mg/dL), % (n)	80% (55)	62% (47)	0.03‡
Positive plasma or urine ketones, % (n), N=82	83% (29)	94% (44)	0.16‡
Serum bicarbonate (mmol/L), mean ± SD, N=91	17 ± 4.0	16 ± 4.0	0.26§
Other abnormal findings on presenting or baseline evaluation,‖ % (n), N=111	38% (22)	47% (25)	0.34‡

*unless otherwise noted, †Wilcoxon rank sum test, ‡Fisher's exact test, §t-test, ‖including transaminases, lactate, ammonia, cortisol, growth hormone, IGF-I, IGFBP-3, acylcarnitine profile, carnitine profile, urine organic acids

Figures and Figure Legends

Figure 1. Consort diagram



Supplemental Table 1. Details of genetic testing performed

Of the genetic testing conducted, a commercial panel testing for glycogen storage diseases (sequencing of *AGL, G6PC, GAA, GBE1, GYS2, PFKM, PHKA1, PHKA2, PHKB, PHKG2, PYGL, PYGM, SLC2A2*, and *SLC37A4*) was obtained most frequently, in 14 patients. A ketotic hypoglycemia panel (sequencing and deletion/duplication analysis of *ACAT1, AGL, G6PC, GYS2, PHKA2, PHKB, PHKG2, PYGL, SLC16A1*, and *SLC37A4*) was obtained in 10 patients, hyperinsulinism panel (including sequencing of *ABCC8, KCNJ11, GCK, GLUD1* in all cases, with the addition of sequencing and deletion/duplication analysis of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1, UCP2* depending on the panel utilized) was obtained in six patients, fatty acid oxidation defect panel (sequencing and deletion/duplication analysis of *ACADVL*) was obtained in five patients, isolated *SLC16A1* mutation analysis was performed in three patients, and one patient had a commercial metabolic hypoglycemia panel (sequencing of *ACAT1, AGL, ALDOB, FBP1, G6PC, GALT, GYS2, HMGCL, MLYCD, OXCT1, PC, PCK1, PCK2, PGM1, PHKA2, PHKB, PHKG2, PYGL, SLC16A1, SLC2A2*, and *SLC37A4*) obtained. One child had targeted testing for Russel Silver syndrome based upon clinical examination findings. Chromosomal microarray was obtained in one patient and four patients had whole exome sequencing.

Supplemental Table 2. Summary of patients with identified hypoglycemia diagnoses

History and initial presentation	Evaluation findings	Treatment and course
Growth hormone deficiency		
Term male, history of uninvestigated neonatal hypoglycemia. Presented at 10 months with vomiting, irritability, and POC PG of 32 mg/dL. Length z score -2.24, weight-for-length 80%ile.	Ketotic hypoglycemia (PG 52 mg/dL, BOHB 2.3 mmol/L) with normal lactate and cortisol (20 mcg/dL) but low GH 0.97 ng/mL. Peak GH after stimulation (arginine/clonidine) was 9.7 ng/mL and MRI revealed a small pituitary gland with possible ectopic pituitary tissue.	Initiated GH with resolution of hypoglycemia. Remains on GH replacement at 9 years of age.
Fatty acid oxidation disorder		
23-month-old female without significant past medical history presented with seizure and POC PG 20 mg/dL in setting of gastroenteritis.	Hypoketotic hypoglycemia with hyperfattyacidemia (PG 39 mg/dL, BOHB 1.4 mmol/L, FFA 3.98 mmol/L). Acylcarnitine profile revealed mild increase of C14:1 and C14:2 and UOA showed markedly increased dicarboxylic acids. Sequencing of *ACADVL* was negative, however, fatty acid oxidation probe of fibroblasts demonstrated significantly reduced oxidation of palmitate, consistent with impaired long-chain fatty acid oxidation.	Dextrose-containing fluids every 2 hours with illness. Multiple additional episodes of hypoglycemia during illness, one requiring hospitalization.
Dihydrolipoamide dehydrogenase (DLD) deficiency		
14-month-old male without significant medical history presented with gastroenteritis, lethargy, seizures, and PG 9 mg/dL.	Hypoglycemia with lactic acidosis and abnormal urine organic acid profile (PG 40 mg/dL, BOHB 1.3 mmol/L, FFA 2.5 mmol/L, lactate 5.2 mmol/L, ammonia 18 µmol/L, UOA: increased lactate, ketone, 2OH-glutaric acid, TCA cycle intermediates, 2-keto-glutaric acid, 2OH-adipic acid and glutaric acid). WES identified compound heterozygous variants in *DLD*: Gly229Cys / Ser258Pro.	Low-protein diet. Numerous admissions for hypoglycemia and intermittent hepatic dysfunction.
3 methylcrotonyl-CoA carboxylase deficiency		
18-month-old female without significant past medical history presented with vomiting, lethargy, PG 49 mg/dL, and HCO3 16 mmol/L.	Ketotic hypoglycemia with abnormal urine organic acid profile (PG 52 mg/dL, BOHB 3.9 mmol/L, lactate 1.2 mmol/L, ammonia <9 µmol/L, acylcarnitine profile: moderate increase in C5OH-carnitine, UOA: increased 3-methylcrotonylglycine, lactic acid, 3-hydroxy-isovalerate, consistent with deficiency in 3 methylcrotonyl-CoA carboxylase. *MCCC1* sequencing identified a heterozygous novel pathogenic frameshift variant (Ser622Pro).	Limit fasting. Glucose meter and ketone meter monitoring. Multiple episodes of ketosis during illness, all managed at home.
Hyperinsulinism		
Term female born AGA, limited prenatal care. Presented at 5 days of age with jaundice and diarrhea due to rotavirus. POC PG 49 mg/dL, HCO3 23 mmol/L.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon on fast at 11 days of age (PG 57 mg/dL, BOHB 1.2 mmol/L, FFA 1.04 mmol/L, insulin <2 µIU/mL, C-peptide 0.22 ng/mL, IGFBP-1 167 ng/mL, ammonia 27 µmol/L, cortisol 25 mcg/dL, GH 10.1 ng/mL, Δ PG +45 mg/dl post-glucagon). Fasted 12 hours with PG >70 mg/dL. Genetic testing not performed. Presumed PSI-HI.	Diazoxide not initiated given fasting tolerance. Glucagon PRN, glucose meter monitoring. At 7 months of age, no PG <70 mg/dL on home monitoring.
Term female born AGA to GBS+ mother. Presented at 4 days of age with fever, irritability, POC PG 36 mg/dL, and HCO3 14 mg/dL. Infectious work-up was negative.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon on fast at 14 days of age (PG 43mg/dL, BOHB 0.8 mmol/L, FFA 0.8 mmol/L, insulin <2 µIU/mL, C-peptide 0.16 ng/mL, IGFBP-1 144 ng/mL, ammonia 39 µmol /L, cortisol 17 mcg/dL, GH 18.3 ng/mL, Δ PG +40 mg/dl post-glucagon). Fasted 8 hours with PG >70 mg/dL. Sequencing of *ABCC8, KCNJ11, GCK*, and *GLUD1* identified VUS in *GLUD1* (Ala49Thr).	Diazoxide not initiated given fasting tolerance. Limit fasting to 8 hours, glucagon PRN, glucose meter monitoring. Lost to follow-up.
Term male born SGA, history of uninvestigated neonatal hypoglycemia. Presented at 1 month of age with	Hypoketotic hypoglycemia with hypofattyacidemia (PG 50 mg/dL, BOHB 0.62 mmol/L, FFA 0.57 mmol/L, insulin <2 µIU/mL, C-peptide 0.21 ng/mL, lactate 0.8 mmol/L, ammonia 32 µmol/L, cortisol 10 mcg/dL, GH 4.08 ng/mL, glucagon stimulation not performed). Fasted 11	Glucagon PRN, glucose meter. Repeat fast at age 9 months demonstrated resolution of HI

fever, POC PG 58 mg/dL, and POC BOHB <0.3 mmol/L. + parechovirus.	hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11* and sequencing of *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *SLC16A1*, and *UCP2* was negative.	(PG 42 mg/dL, BOHB 2.4 mmol/L, IGFBP-1 723 ng/mL).
Term male infant born with AGA. Presented at 2 days with diarrhea, irritability, and POC PG 49 mg/dL. Required max GIR 13 mg/kg/min. Found to have shigella enteritis.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon on fast at 8 days of age (PG 44 mg/dL, BOHB 0.9, FFA 0.72 mmol/L, insulin <2 µIU/mL, C-peptide 0.22 ng/mL, lactate 1.3 mmol/L, ammonia 19 µmol/L, cortisol 6 mcg/dl, GH 4.84 ng/mL, Δ PG +30 mg/dl post-glucagon). Fasted <3 hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1*, and *UCP2* and sequencing of *GCK* was negative.	Diazoxide 5 mg/kg/d, glucagon PRN, glucose meter monitoring. Transferred care to local endocrinologist at discharge.
Term female born AGA with failure to thrive and GERD. Presented at 5 months of age with fever, congestion, seizure, POC PG 42 mg/dL, HCO3 24 mmol/L, and negative urine ketones.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon (PG 42 mg/dL, BOHB <0.3 mmol/L, FFA 0.19 mmol/L, insulin <2 µIU/mL, C-peptide 0.35 ng/mL, lactate 1.3 mmol/L, ammonia 33 µmol/L, cortisol 11.6 mcg/dl, GH 8.07 ng/mL, Δ PG +45 mg/dl post-glucagon). Fasted <3 hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1*, and *UCP2* and sequencing *GCK* was negative for genes analyzed, revealed partial deletion of X chromosome, cytogenic analysis subsequently confirmed mosaicism for monosomy X and ring X confirming a diagnosis of Turner syndrome.	Enteral dextrose via G-tube. Later started lanreotide. At age 5 years, repeat fast off therapy demonstrated a safe fasting tolerance (PG >70 for 12 hours) but continued evidence of HI.
Female born at 34 weeks, SGA with heterotaxy syndrome. Presented at 18 months with fever, URI, diarrhea, POC PG 54 mg/dL, HCO3 28 mmol/L, and negative urine ketones.	Hypoketotic hypoglycemia with glycemic response to glucagon (PG 50 mg/dL, BOHB 1.7 mmol/L, FFA 2.1 mmol/L, insulin <2 µIU/mL, C-peptide 0.3 ng/mL, lactate 1.8 mmol/L, ammonia <9 µmol/L, cortisol 8.5 mcg/dL, GH 1.6 ng/mL, Δ PG >30 mg/dl post-glucagon). Fasted 3 hours with PG >70 mg/dL. Sequencing and del/dup of *ABCC8, KCNJ11, GLUD1, HADH, HNF1A, HNF4A, INSR, SLC16A1*, and *UCP2* and sequencing of *GCK* was negative.	Enteral dextrose via G-tube. Required treatment through age 3 years when demonstrated ability to fast 18 hours with PG >70 mg/dL off treatment.
Term female with history of uninvestigated neonatal hypoglycemia. Presented at 18 months with gastroenteritis and POC PG 16 mg/dL.	Hypoketotic hypoglycemia with hypofattyacidemia and glycemic response to glucagon (PG 45mg/dL, BOHB 0.62 mmol/L, FFA 0.5 mmol/L, insulin < 1 µIU/mL, C-peptide 0.5 ng/mL, lactate 1.2 mmol/L, ammonia 33 µmol/L, cortisol 5.1 mcg/dL, Δ PG + 68 mg/dl post-glucagon). Sequencing and del/dup of *ABCC8, KCNJ11* and sequencing of *GCK* and *GLUD1* identified an autosomal dominant paternally inherited mutation in *ABCC8* (pSer1387del)	Enteral dextrose via G-tube and lanreotide. Overnight enteral dextrose discontinued by 3 years of age. Remains on lanreotide.
Impaired hepatic insulin clearance		
22-month-old female with fever, URI, gastroenteritis, PG 48 mg/dl, HCO3 25 mmol/L, AST 6774 U/L, ALT 4847 U/L, and prolonged PT and PTT. Diagnosed with acute hepatic insufficiency due to rhinovirus and enterovirus. Hypoglycemia persisted despite improved liver function.	Hypoketotic hypoglycemia with elevated insulin and appropriately low C-peptide (PG 50 mg/dL, BOHB <0.3 mmol/L, FFA 2.0 mmol/L, insulin 8.6 µIU/mL, C-peptide 0.3 ng/mL, lactate 2.0 mmol/L, ammonia 32 µmol/L, cortisol 18.6 mcg/dL, GH 1.53 ng/mL, no glycemic response to glucagon, normal acylcarnitine profile and UOA). Fasting study repeated x 3 with consistent results.	Enteral dextrose via NG-tube overnight. Discontinued at 26 months of age following repeat fast demonstrating resolution of inappropriate insulin action (PG 47 mg/dL, BOHB 3.0 mmol/L, FFA 3.46 mmol/L, insulin <2 µIU/mL, and C-peptide < 0.1 ng/mL).

AGA appropriate for gestational age, ALT alanine aminotransferase, AST aspartate aminotransferase, BOHB ß-hydroxybutyrate, FFA free fatty acids, GBS Group B *Streptococcus*, GERD gastroesophageal reflux disease, GH growth hormone, GIR glucose infusion rate, G-tube gastrostomy tube, HCO3 bicarbonate, HI hyperinsulinism, IGFBP-1 insulin-like growth factor binding protein 1, MRI magnetic resonance imaging, NG nasogastric tube, PG plasma glucose, POC point-of-care, PRN *pro re nata*, PSI-HI perinatal stress induced hyperinsulinism, PT prothrombin time, PTT partial thromboplastin time, SGA small for gestational age, TCA tricarboxylic acid cycle, UOA urine organic acids, URI upper respiratory infection, WES whole exome sequencing