PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to a another journal from Archives of Disease in Childhood but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Paediatrics Open. The paper was subsequently accepted for publication at BMJ Paediatrics Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Undiagnosed Hypoglycemia Disorders in Children Detected When Hypoglycemia Occurs in the Setting of Illness: A Retrospective Study
AUTHORS	Rosenfeld, Elizabeth De León, Diva D. Alzahrani, Ohoud

VERSION 1 – REVIEW

REVIEWER	Reviewer name: James Davison Institution and Country: United Kingdom of Great Britain and Northern Ireland Competing interests: None
REVIEW RETURNED	4-Jul-2022

GENERAL COMMENTS	Thank you for asking me to review this manuscript. The authors' intention is to review how often a child found to have hypoglycaemia during an acute intercurrent illness may have an underlying "hypoglycaemic" disorder. They attempt to do this by a retrospective review of patients referred to endocrinology for evaluation of hypoglycaemia, then filtering for those with acute illness, and excluding those with known diagnoses or on suspicious medications. The paper then describes some of the investigations undertaken. Unfortunately the paper's structure makes it difficult to follow the process that patients went through, hampered by trying to impose a statistical analysis on a retrospective review without any formal protocol for patient evaluation. There are a number of issues that must be addressed: (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. (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.

	(3) There is no set list of "hypoglycemic disorders", and although there is some discussion of endocrine and metabolic causes this must be delineated (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. (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. (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. (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. While hypoglycaemia is an important clinical finding that does warrant careful evaluation, at present this paper in its current form is not able to address the question it sets out to answer.

VERSION 1 – AUTHOR RESPONSE

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.

VERSION 2 – REVIEW

REVIEWER	Reviewer name: James Davison Institution and Country: United Kingdom of Great Britain and Northern Ireland Competing interests: None
REVIEW RETURNED	04-Jan-2023

GENERAL COMMENTS	Thank you for asking me to review this revised manuscript. The authors have addressed my previous comments with some minor alterations to the manuscript. Definition of hypoglycaemia: While there remains open debate about what glucose concentration should be sued to define hypoglycaemia, the authors have provided a rationale of their cutoff of 3.9mmol/L. This is higher than the level I would define as significant hypoglycaemia. Only 10% of patients who had "critically low" glucose levels (<2.8mmol/L) had evidence of acutely collected samples (for insulin

	etc) as part of a "hypoglycaemia screen". It would be helpful to comment why so few had these important samples collected, and what could be done to try to improve the rate of collection of these samples which are important in identifying infants/children with hyperinsulinism etc. To address the concern that the study design included potential selection bias (since patients included only those referred to endocrinology for a consult) I had suggested giving an estimate of how many patients had presented to their institution with acute illness and hypoglycaemia, not just those then referred for endocrine opinion. This data is not available, and additional comment has been added to the discussion to address this issue. Given that the individual "hypoglycaemic disorders" are rare, having an understanding of the expected incidence/prevalence of these in the paediatric population and comparing this to the total number of patients presenting with illness and hypoglycaemia would have been helpful. The inclusion in the study of younger infants/neonates may explain the high rate of hyperinsulinism as the underlying diagnosis; this is acknowledged by the authors. The conclusion of the manuscript is correct, highlighting the importance of considering an underlying endocrine or metabolic diagnosis for infants and children presenting with hypoglycaemia. The authors may consider adding a comment to highlight the importance of obtaining time-critical diagnostic samples at the point of significant hypoglycaemia. Minor comment: Lines 104-106: The definitions here need to be clarified as the sentence is unclear. Are there two categories, one with "low" ketones and one with "high" ketones? "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."

REVIEWER	Reviewer name: Dr. Peter Flom Institution and Country: Peter Flom Consulting, 515 West End Ave, New York, United States Competing interests: None
REVIEW RETURNED	10-Jan-2023

GENERAL COMMENTS	I confine my remarks to statistical and methodological aspects of this paper. Unfortunately, I think some fairly major changes are needed. General: I didn't see a power analysis. Was one done? Lines 86 to 93: Height and weight should not be categorized. Doing so increases both type 1 and type 2 error and implies that there are big changes at the cut points but no changes within categories. Categorization after the analysis may. sometimes, be useful for presentation, but it should happen after analysis. Splines can be used to examine nonlinear relationships. See my blog post https://medium.com/@peterflom/what-happens-when-we-categorize-an-independent-variable-in-regression-77d4c5862b6c Also, BMI is not a good measure of obesity. See e,g another of my blog posts:

	https://medium.com/peter-flom-the-blog/why-bmi-is-a-bad-measure-of-obesity-and-what-is-better-f8a62fc9ca49 Lines 116-123 While these tests aren't wrong, it would be much better to do logistic regression. This lets multiple variables be considered at once. Tables 2 and 3 should have footnotes indicating what test was used to get the p values. The results for height and weight should be changed because those variables should be continuous. The same applies to the duration variables. Peter Flom

VERSION 2 – AUTHOR RESPONSE

Thank you for the insightful review of our manuscript. Revisions, detailed below, have been made in response to the reviewer's comments.

Formatting Amendments (where applicable):

1. Supplementary file / Appendix

Please be informed that this should be in PDF Format
Supplemental files uploaded in PDF format.

Editor in Chief Comments to Author
Please respond in full to the reviewers, esp the stats reviewer who will see the revised paper

Reviewer: 1
James Davison

Only 10% of patients who had "critically low" glucose levels (<2.8mmol/L) had evidence of acutely collected samples (for insulin etc) as part of a "hypoglycaemia screen". It would be helpful to comment why so few had these important samples collected, and what could be done to try to improve the rate of collection of these samples which are important in identifying infants/children with hyperinsulinism etc.
Reasons for the low rate of "critical sample" collection in the present study are unclear. The decision to obtain a "critical sample" was at the discretion of the provider, typically an emergency medicine provider. 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 intravenous dextrose administration was considered. Discussion of the low rate of critical sample attainment has been added to lines 227-232.

The conclusion of the manuscript is correct, highlighting the importance of considering an underlying endocrine or metabolic diagnosis for infants and children presenting with hypoglycaemia. The authors may consider adding a comment to highlight the importance of obtaining time-critical diagnostic samples at the point of significant hypoglycaemia.
As recommended, comment regarding the importance of obtaining time-critical diagnostic samples at the time of hypoglycemia is on lines 276-279.

Minor comment:
Lines 104-106: The definitions here need to be clarified as the sentence is unclear. Are there two categories, one with "low" ketones and one with "high" ketones?
"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 definition of “positive ketones” was clarified as suggested, revised line 106.

Reviewer: 2
Dr. Peter Flom, Peter Flom Consulting

I confine my remarks to statistical and methodological aspects of this paper. Unfortunately, I think some fairly major changes are needed.

General: I didn't see a power analysis. Was one done?
A power analysis was not conducted a priori. All individuals meeting eligibility criteria were included in the sample. Sample size was thus determined by the total number of such individuals evaluated at our institution over the study period.

Lines 86 to 93:

Height and weight should not be categorized. Doing so increases both type 1 and type 2 error and implies that there are big changes at the cut points but no changes within categories. Categorization after the analysis may. sometimes, be useful for presentation, but it should happen after analysis. Splines can be used to examine nonlinear relationships.
As suggested, height and weight are now presented as continuous variables – revised Tables 1, 2, and 3. Weight categorization (i.e., weight adjusted for length/height) is standardly calculated as weight-for-length for children <2 years of age and as BMI for children ≥2 years of age. To permit comparison between groups across the age range included in this study, a categorical variable corresponding to underweight, normal weight, overweight, and obese was used. The authors acknowledge that BMI is a flawed measure of obesity. Weight and height are measured in clinical practice, whereas waist and hip circumference are not routinely measured in children. Consequently, waist to hip ratio was not available in the medical record in this retrospective study.

See my blog post
https://nam10.safelinks.protection.outlook.com/?url=https%3A%2F%2Fmedium.com%2F%40peterflom%2Fwhat-happens-when-we-categorize-an-independent-variable-in-regression-77d4c5862b6c&data=05%7C01%7Crosenfelde%40chop.edu%7C7c29d0e3108748dadfa308daf4063cf8%7Ca611241607b041a59bb1d146b575c975%7C0%7C0%7C638090604912126306%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=y4KygfkSWL7NqSuv7nsjnXUeyGxIYq4P6Hq5ZIYh%2FN0%3D&reserved=0

Also, BMI is not a good measure of obesity. See e,g another of my blog posts:
https://nam10.safelinks.protection.outlook.com/?url=https%3A%2F%2Fmedium.com%2Fpeter-flom-the-blog%2Fwhy-bmi-is-a-bad-measure-of-obesity-and-what-is-better-f8a62fc9ca49&data=05%7C01%7Crosenfelde%40chop.edu%7C7c29d0e3108748dadfa308daf4063cf8%7Ca611241607b041a59bb1d146b575c975%7C0%7C0%7C638090604912126306%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=YoNKE1SziLfYkVy3loaHoCfPKz0gu4iLzx6N6w3wDO0%3D&reserved=0

Lines 116-123 While these tests aren't wrong, it would be much better to do logistic regression. This lets multiple variables be considered at once.
The authors acknowledge the strengths of logistic regression. Given the low number of events (n=12 patients with an identified hypoglycemia disorder), the number of predictor variables that can be reliably included in the model without overfitting is limited, and univariate analysis was presented.

Tables 2 and 3 should have footnotes indicating what test was used to get the p values. The results for height and weight should be changed because those variables should be continuous.
Revised as recommended.

The same applies to the duration variables.
Duration of illness and duration of decreased oral intake are now presented as continuous variables – revised Tables 1, 2, and 3, as recommended.

VERSION 3 – REVIEW

REVIEWER	Reviewer name: Dr. Peter Flom Institution and Country: Peter Flom Consulting, 515 West End Ave, New York, United States Competing interests: None
REVIEW RETURNED	26-Jan-2023

GENERAL COMMENTS	The authors have addressed my concerns and I now recommend publication.

VERSION 3 – AUTHOR RESPONSE