Author’s response to reviews

Title: The Prevalence, Risk Factors, and Screening Measure for Prediabetes and Diabetes among Emirati Overweight/Obese Children and Adolescents

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Author’s response to reviews: see over
Dear Editors,

We wish to thank you very much for your kind reply and invitation to re-submit the subject manuscript. We appreciate the reviewers’ thoughtful comments and here wish to provide a point-by-point response to the concerns as detailed below. Please also note that the English grammar in the present manuscript has been reviewed as requested.

Point-by-point response:

Reviewer 1: Emmanuel MiraGLìa del GìdÌc

Major Compulsory Revisions

1. The study suffers of a limitation that I think should be clearly expressed in the text. Reading the paper and the relative flowchart I note that a potentially interesting subset of obese children with pre-diabetes has not been identified. These are obese children showing normal fasting glucose and normal HbA1C, but, eventually, abnormal OGTT (at 120 minutes more than 140 mg/dl of glucose and less than 200 mg/dl). In my experience and in a number of papers this represents a group of children with pre-diabetes not numerically negligible. In this study the Authors have missed these patients.

We totally agree with the reviewer. The study was designed so that the OGTT was used as a confirmatory test in cases of suspected diabetes and pre-diabetes based on capillary fasting blood glucose and capillary HbA1c. To
be able to find this potential group of children and adolescents with prediabetes (IGT) we would have had to screen the entire study population using OGTT, which was difficult to carry out in this case. We have revised the manuscript with a clarification in the Discussion regarding this limitation of the study;

Discussion

Line 279; “Nevertheless, the design of the study using OGTT as a confirmatory test in cases of suspected diabetes and prediabetes based on capillary fasting blood glucose and capillary HbA1c, may have missed to identify a potential group of obese children and adolescents with prediabetes showing normal fasting glucose and normal HbA1c but eventually abnormal OGTT (IGT).”

2. In the correlation I suggest to use BMIZ-score in order to test the influence of BMI on prediabetes.

Thank you for this very important input. Actually, when we tested the influence of BMI on diabetes and prediabetes using BMI Z-score there was a statistically significant difference in the BMI Z-scores between the three different groups of students: normal glycemic testing, prediabetes and type 2 diabetes (p=0.04), as shown in Table 3. The specific groups that differed were diabetic students versus those with normal glycemic testing (P=0.024).

Please note that Table 3 has been revised accordingly to these results and the manuscript has been revised as follows;

Abstract, Line 56: “Overall adiposity, family history of T2D, parents being unemployed and high levels of triglycerides were associated risk factors for abnormal glycemic testing.”

Results, Line 216: “There was a statistically significant difference in the BMI Z-scores between the three different groups of students showing normal glycemic testing, prediabetes and T2D (p=0.041), as shown in Table 3. The specific groups that differed were diabetic students versus those with normal glycemic testing (p=0.024).”

Discussion, Line 235: “We also show that glycemic status among children is significantly associated with overall adiposity (BMI Z-score), family history of T2D, and levels of triglycerides.”
3. In the correlation I suggest to use the waist-to-height ratio in order to test the influence of abdominal fat on pre-diabetes. Waist circumference, in fact, normally changes from 11 to 17 years and this change can be normalized with height.

Once again, thank you for very valuable comments. Unfortunately we could not see any statistically significant difference in waist-to-height ratio scores in between the three different groups of students (Table 3). Please note additional data on waist-to-height ratio in Table 1 and 3.

Reviewer 2: Maria Fatamone Singh

Essential Revisions

Abstract
1. “HbA1c seemed to substantially overestimate pre-diabetes (21.9%) but not diabetes.” It is possible that OGTT underestimates pre-diabetes as well as there is substantial day-to-day variability in OGTT results.

Yes definitely. None of the three methods for diagnosing diabetes and pre-diabetes are perfect. Most likely the various diagnostic methods do not overlap but instead identify different groups of participants with pre-diabetes and diabetes [34], [35]. A follow-up study would be able to clarify which of the methods to be deemed most reliable in predicting progression to diabetes among these variety of pre-diabetic children and adolescents. This is mentioned in the Discussion, Line 285. Since the present study did not follow up these children and adolescents, there is an uncertainty factor as to which of the methods which ultimately is the most sensitive to predict future diabetes onset. In light of this the conclusion has been reformulated in the Abstract and Discussion as mentioned in the last question below (Discussion/Q1).


2. “glycemic status were” should be “was”

Revised as suggested (Line 50).
3. "... parents being unemployed and with high triglyceride levels are at higher risk for developing T2D. " This is a cross-sectional study, and causality cannot be inferred from associations observed, reverse causality is also a possibility for these relationships, or linkage to a common underlying mechanistic factor.

We totally agree with the reviewer. Please be aware that the sentence has been corrected as follows:

Abstract
Line 56; “Overall adiposity, family history of T2D, parents being unemployed and high levels of triglycerides were risk factors associated with abnormal glycemic testing.”

Methods
1. "Further, exercise habits using physical activity score based on different levels of physical activity [no activity, activity (1 time/week), regular activity (1-2 times/week), regular activity (3-5 times/week), and regular daily activity] were recorded [17].” If this questionnaire has been validated in children of this age, please cite this reference and confirm validity.

The questionnaire for physical exercise used in the present study is taken from the National Diabetes Register (NDR), [17]. NDR is one of Sweden’s national quality registers operated by the Swedish Society for Diabetology (SFD) on behalf and with the support of local authorities and the Swedish National Board of Health and Welfare. The questionnaire is validated and used for adults as well as children and adolescents (through SWEDABKIDS) to facilitate systematic quality work at the participating care units.


2. “BMI percentiles according to percentile charts for age and sex from the Centers for Disease Control and Prevention (CDC), subsequently, children’s weights were classified as underweight: BMI < 5th % ile, normal weight: BMI # 5th to <85th % ile, overweight: BMI # 85th to <95th % ile, and obese: BMI # 95th % ile”. Please comment on the validity of these CDC percentiles based on Caucasian children from USA to the cohort under evaluation.

The reference population used to construct the CDC Growth Charts for children aged 2 years to 20 years is a nationally representative sample obtained from 5 national health examination surveys conducted by NCHS from 1963 to 1994. Survey-specific sample weights were applied to the national survey sample data to assure representation of the U.S. population according to age, gender, and racial/ethnic composition at the time the surveys were conducted. CDC promotes one set of growth charts for all racial and ethnic groups. Racial- and ethnic-specific charts are not recommended because studies support the premise that differences in growth among various racial and ethnic groups are the result of environmental rather than genetic influences (Garza and de Onis, 2004; Lusky, 2000; Mei, Yip, Thowbridge, 1998; Kuczynski et al. 2002).

3. Statistical analysis. Prior to use of parametric statistics described, all continuous variables should have been inspected visually and statistically for normality of distribution, and need for transformation or use of non-parametric descriptive and analytical statistical methods determined.

Yes, the sample data was checked for normal distribution testing normality (including frequency histogram) to evaluate the validity of using parametric tests (please see below). Variables were tested for normality both visually and statistically and most of the variables lacked normal distribution as shown in the table below. Accordingly non-parametric tests were used. Additionally, Fisher’s exact test was used to compare categorical variables since one cell in the table had only n=9 observation (which is the cell with number of diabetic students). Thus, due distribution and sample size we have chosen to use non-parametric tests including Kruskal-Wallis and Fisher’s exact test (as mentioned in Statistical evaluation, line 175).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obs</th>
<th>Pr(Skewness)</th>
<th>Pr(Kurtosis)</th>
<th>adj chi2(2)</th>
<th>Prob&gt;chi2</th>
<th>comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>1.00E+03</td>
<td>0.0174</td>
<td>0.7441</td>
<td>5.74</td>
<td>0.0567</td>
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</tr>
<tr>
<td>Parameter</td>
<td>Mean</td>
<td>95% CI</td>
<td>P-value</td>
<td>Significant for lack of normality</td>
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<td></td>
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<tr>
<td>---------------------------------</td>
<td>------</td>
<td>--------</td>
<td>---------</td>
<td>-----------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>1.00E+03</td>
<td>&lt; 0.001</td>
<td>0.0001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.00E+03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>Dystolic blood pressure</td>
<td>1.00E+03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>Capillary HbA1c</td>
<td>1.00E+03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>BMIC</td>
<td>1.00E+03</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>BMIZ</td>
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<td>0.4691</td>
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<td>18.64 0.0001</td>
<td>significant for lack of normality</td>
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</tr>
<tr>
<td>Plasma Fasting blood glucose</td>
<td>348</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>57.61 0</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>Venous HbA1c</td>
<td>347</td>
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<td>&lt; 0.001</td>
<td>30.73 0</td>
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<td></td>
</tr>
<tr>
<td>2hr plasma glucose</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
</tr>
<tr>
<td>Capillary fasting glucose</td>
<td>348</td>
<td>0</td>
<td>0</td>
<td>57.61 0</td>
<td>non-significant for lack of normality</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>347</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
</tr>
<tr>
<td>LDL</td>
<td>334</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0</td>
<td>significant for lack of normality</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>347</td>
<td>0.0010</td>
<td>0.0049</td>
<td>16.02 0.0003</td>
<td>significant for lack of normality</td>
<td></td>
</tr>
</tbody>
</table>
4. **What is the definition of consanguinity for the purpose of this investigation?**

Relationship by blood is an important risk factor for type 2 diabetes to consider. Previous epidemiologic studies have shown that people with a family history of diabetes in first-degree relatives who are affected with diabetes are 2 to 6 times as likely to have the disease compared with people who have no affected relatives [37]. The United Arab Emirates has one of the highest prevalence of type 2 diabetes in the world and marriages between cousins are common, which could increase the risk of getting type 2 diabetes.


5. **Was there any instruction given for CHO consumption of a minimum amount in the 3 days prior to the OGTT? Were there any instructions given regarding exercise on the day prior to morning of the OGTT or the fasting glucose tests? How was 10 hr fasting confirmed?**

This is a very relevant question. OGTT has poor reproducibility, why standardized conditions are important. Excessive physical activity the days before the test may affect the outcome as excessive food intake the night before. Even an abnormally low carbohydrate intake the days before the OGTT may paradoxically affect the result giving elevated blood glucose readings. Smoking stimulates gastric emptying which may contribute to low postprandial values.
Therefore, the participants were instructed to live as normal as possible in respect to diet and physical activity the days before the OGTT. The test was postponed to another day in the event of ongoing infection. They were also instructed not to exercise and to abstain from food, fluids (except water) and tobacco from 10 pm the night before the test. The 10 hr fasting was confirmed by asking both the participants and their parents.

6. How similar was the HbA1c measured on capillary blood vs. venous sampling?

Although the two HbA1c values were not taken at the same occasion reported values are shown as below.

<table>
<thead>
<tr>
<th></th>
<th>Ven HbA1c</th>
<th>Ven HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>Cap HbA1c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>201</td>
<td>77</td>
</tr>
<tr>
<td>Normal</td>
<td>27</td>
<td>40</td>
</tr>
</tbody>
</table>

Sensitivity 201/228 = 0.88. Specificity 40/117 = 0.34

7. Why was it decided up front that OGTT was the gold standard, and therefore used to compare to risk factors rather than comparing both HbA1c and OGTT definitions of pre-diabetes/diabetes to see which definition was most closely related to known risk factors (convergent validity).

When this study was originally planned and carried out, OGTT was still considered as the gold standard for diagnosing diabetes (1). HbA1c as a screening method for diabetes and prediabetes was being introduced. Additionally, there was (and still are) an ongoing debate whether HbA1c could be considered as reliable for the diagnosis of prediabetes and diabetes in children. In light of this, we chose to consider OGTT as the gold standard for diagnosing diabetes and prediabetes in children and adolescents when comparing with the HbA1c method.

Mary E. Cox and David Edelman. Tests for Screening and Diagnosis of Type 2 Diabetes. Clinical Diabetes October 2, 2009 vol. 27 no. 4 132-138. ADA
Results

1. Figure 1 is missing from my copy of the paper, so I cannot tell if this information is presented there. “From the school health records, we identified 1436 Emirati students aged 11-17 years who were either overweight or obese according to our inclusion/exclusion criteria”. What was the overall cohort from which these 1436 students were identified? How accurate was the height and weight in the school records compared to that measured in this study? If not accurate, is it possible that additional overweight/obese children were not identified for this reason?

From the school health records of 16 schools (the overall cohort n=7088 students), we identified 1436 Emirati students aged 11-17 years who were either overweight or obese according to our inclusion/exclusion criteria. The School health Program in the UAE have a national physical examination screening program including measuring height and weight and charting BMI on growth charts for all students. The procedure is standardized and conducted by trained school nurses. Instructions, like students being bare-foot and in minimal clothing, are given before weight is measured with electronic scales that are calibrated periodically by biomedical engineering Department. List of due date for calibration of electronic scales are maintained in the school health Department.

2. Were students/parents asked why they did not want to participate? Were the 28% who did not participate different in terms of age, gender, or BMI compared to those who consented? This is important for determination of the external validity of the sample recruited and should be presented in results and discussion.

Letters of invitation were sent to consent parents on using their children’s data. Those who did not respond were sent further second and third reminders. If still not responding, the wish of parents was respected and other details of the student were not captured (blood pressure, glycemic testing etc.). We were not able to identify anything distinctive for those children and adolescents concerning age, gender, and BMI and we don’t have further data in order to analyze the characteristics of non-respondents in comparison with respondents. Although we cannot be sure about the students’ reason for not participating in the study, we assume the main reason to be unwillingness to be exposed to finger-pricking.

Please note the additional text in Discussion, Line 321: “A potential limitation of studies in pediatric subjects and adolescents could be difficulties in achieving a good response rate with the risk of sampling bias. In our study, the response rate was 72% at the first invitation and 79% in the second phase (Figure 1). Although we cannot be sure about the student’s reason for not
participating in the study and there might be a possibility of selection bias, we assume the main reason to be unwillingness to be exposed to finger pricking."

3. Is the high rate of consanguinity unusual for this cultural setting?

Please see the answer to question 4 as described above (Methods/Q4).

4. "However, given the small sample size, we could not compare the two methods for sensitivity and specificity". There were sufficient cases of pre-diabetes to compare the two methodologies; in fact this is the stated purpose of the investigation. This should be done.

We have added data on sensitivity and specificity in the results section of the manuscript on HbA1c in relation to the OGTT (gold standard) for the diagnosis of pre-diabetes in children and adolescents. Because of the low number of diabetes cases in this study (small sample size), it becomes more uncertain to make calculations on HbA1c in relation to OGTT for the diagnosis of diabetes in children and adolescents.

Results
Line 205; "In comparison with the OGTT (gold standard) the HbA1c method showed a sensitivity of 0.52 and a specificity of 0.34 for the diagnosis of pre-diabetes in children and adolescents. However, given the small sample size, it was not possible to compare the two methods for sensitivity and specificity for the diagnosis of diabetes."

5. "relative with diabetic" should be "relative with diabetes" in text and table.

Revised as suggested.

6. Table 4 shows that parental employment was related to 79% higher prevalence of diabetes but introduction states that increased affluence in the region has been linked to the diabetes epidemic. This discrepancy needs to be discussed in the discussion section. However, it is not really clear whether the author mean that "emp pby m ent" or "unemp pby m ent" is the risk factor from the way that it is written.

This is a very good observation. Please be aware that the following have been added to the discussion:
Line 300; “Furthermore, having both parents not working was related to 79% higher prevalence of diabetes compared to having one or both parents employed. Although unemployment is often associated with economic inactivity, it may rather be an expression of economic independence and a sedentary lifestyle in a region with increased affluence linked to the diabetes epidemic.”

7. 21% of students with abnormal capillary results on 2 occasions did not show up for venous blood sampling in the hospital. How did these students differ from the full sample? This loss needs to be commented on in terms of generalizability, as now there is a loss of 28% of overweight/obese who did not consent, in addition to 21% of those 72% who consented and were subsequently found to be hyperglycemic.

Please see the answer to question 2 as described above (Results/Q2).

Discussion
1. The conclusion that HbA1c “overdiagnosed” pre-diabetes appears to be not completely justified. A newer study in Saudi Arabia indicated that based on a review of HbA1C, FPG and OGTT:

“Within our population, we would have missed the diagnosis of pre-diabetes in 469 (25.8%) patients if we had relied only on 2-h O GT T rather than A1c.”

The above mentioned study is a retrospective study on adults in a Saudi population (mean age 54.3 ± 13.6 years). Our study includes only children and adolescents (age 11–17 years). Several studies have shown discrepancy between the results of HbA1c on children when compared with OGTT. The question is whether HbA1c as a diagnostic method is as reliable in children as it is in adults and how to interpret the outcome of the results. As mentioned in the Discussion, the American Diabetes Association (ADA) have published revised and modified diagnostic guidelines recommending that HbA1c tests should also be used for diagnosing diabetes (HbA1c ≥ 6.5% or ≥ 48 mmol/mol) and prediabetes (HbA1c = 5.7%–6.4% or 38–47 mmol/mol) in both adults and children. However, these recommendations have been questioned as they were considered being based strictly on data from adult studies and lack any input from pediatric research. In fact, the HbA1c method has been claimed to represent a poor diagnostic tool in children and adolescents due to a relatively lower test performance compared with adults [29], [30]. Several studies published on the topic indicate that using adult cut-off points for HbA1c values to predict prediabetes or diabetes may judge the prevalence of these conditions in the pediatric and adolescent population [29], [30], [31], [32], [33]. Consequently, a different HbA1c cut-off point has been proposed for children. However, since
the present study did not follow up these children and adolescents, there is an uncertainty factor as to which of the methods which ultimately is the most sensitive to predict future diabetes onset. In light of this the conclusion has been reformulated in the Abstract and Discussion as follows;

Abstract

Line 46: "HbA1c showed a considerable discrepancy regarding the prevalence of prediabetes (21.9%), but not diabetes"

Line 55: "The numbers for prediabetes were considerably higher when using HbA1c as compared to OGTT."

Discussion

Line 233: "We report that the prevalence of prediabetes and T2D among overweight and obese children and adolescents is high based on OGTT as well as HbA1c, on which the HbA1c method show significantly higher rates for prediabetes but not diabetes."


Sincerely

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