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Steve Turner, Ibrahim Abuzayan, Untiveros Paraskevi

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**Changes to exhaled nitric oxide in asthmatic children after drinking a caffeine-containing cola drink**

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Keywords:	asthma, caffeine, nitric oxide



Changes to exhaled nitric oxide in asthmatic children after drinking a  
caffeine-containing cola drink

I.Abuzayan, U.Paraskavi, S.W.Turner

Child Health, University of Aberdeen, Aberdeen, UK

Correspondence to Dr Turner:

Child Health

Royal Aberdeen Children's Hospital

Foresterhill

Aberdeen AB25 2ZD

Tel. +44 (0)1224 552471

Fax +44 (0)1224 551919

For Peer Review

**ABSTRACT (word count 236)**

Introduction. Exhaled nitric oxide ( $FE_{NO}$ ) may be a biomarker for airway eosinophilia and of use in the management of childhood asthma. Caffeine ingestion has been associated with changes in  $FE_{NO}$  concentration in adults. The present study tested the hypothesis that ingestion of a caffeine-containing cola drink will increase  $FE_{NO}$  in asthmatic children.

Methods. Exhaled NO was measured in children with asthma before, 30 and 60 minutes after taking a cola drink containing 2.5mg/kg caffeine. Intrasubject changes in  $FE_{NO}$  and flow independent NO parameters were determined including bronchial wall NO flux ( $J'_{awNO}$ ).

Results. Eleven children with asthma were recruited, 10 were prescribed inhaled corticosteroids and 9 were skin prick positive. The median [interquartile range, IQR]  $FE_{NO}$  at baseline was 47 parts per billion [9, 64] and this rose to 56ppb [11, 66] after 30 minutes and returned to 46ppb [9, 62] after 60 minutes, Friedman's test  $p=0.003$ .  $J'_{awNO}$  rose from a median [IQR] 2843nl/s [356, 4247] at baseline to 3304nl/s [479, 4387] after 30 minutes and returned to 2937nl/s [356, 4153] after 60 minutes, Friedman's test  $p=0.003$ . There was no significant change in other flow independent NO parameters.

Conclusions. Ingestion of a caffeine-containing cola drink was associated with a modest and transient rise in  $FE_{NO}$  which is mostly explained by increased NO production in the proximal airways. Ingestion of a caffeine-containing cola drink may result in clinically relevant acute changes in  $FE_{NO}$  for children with asthma.

Key words: asthma, caffeine, nitric oxide

## INTRODUCTION

Exhaled nitric oxide (FE<sub>NO</sub>) is a non-invasive biomarker for airway eosinophilia<sup>1</sup> and may be useful to monitor asthma control<sup>2-4</sup>. Measurements of FE<sub>NO</sub> can be variable within an asthmatic independent of symptoms<sup>5</sup> and this suggests that factors other than asthma might influence FE<sub>NO</sub> with implications for clinical application.

Factors other than asthma which are associated with altered FE<sub>NO</sub> in children include atopy<sup>6</sup>, hayfever<sup>7</sup>, age<sup>8</sup>, height<sup>6</sup>, ingestion of L-arginine<sup>9</sup> and possibly exposure to tobacco smoke<sup>10</sup>. There is at least one potential mechanism whereby caffeine might also acutely influence FE<sub>NO</sub> values in children. Caffeine ingestion has been associated with altered FE<sub>NO</sub> values in adults although the results are inconsistent; the three published studies have respectively found exposure resulting in increased FE<sub>NO</sub><sup>11</sup>, reduced FE<sub>NO</sub><sup>12</sup> or no change in FE<sub>NO</sub><sup>13</sup>. In an observational study, our group has observed an association between intake of carbonated drinks (the predominant source of caffeine in children) and increased FE<sub>NO</sub> in atopic five year olds<sup>14</sup>.

Given that many children are regularly exposed to caffeine-containing carbonated drinks<sup>15</sup> there is the potential for such exposure to confound FE<sub>NO</sub> measurements in children with asthma. We therefore designed an intervention study to test the hypothesis that exposure to a caffeine-containing carbonated drink increases FE<sub>NO</sub> values in atopic asthmatic children. Our second hypothesis was that exposure to caffeine increases proximal and not distal airway nitric oxide production.

## METHODS

Study design. At a single assessment,  $FE_{NO}$ , skin prick reactivity and spirometry were measured in children with asthma. A dose of 2.5mg/kg caffeine in a carbonated cola drink was ingested (5.5ml/kg of Diet Coke 45.6mg caffeine/100mls); for a 40kg child this was equivalent to 220mls or two thirds of a standard 330ml can. Exhaled NO was measured 30 and 60 minutes after ingestions. Proximal and distal airway contributions to exhaled NO were derived from a non-linear method<sup>16</sup>. The study was approved by the local research ethics committee; written parental consent and verbal assent from the child were obtained.

Enrolment. Children attending a hospital asthma clinic with a prior physician diagnosis of asthma were eligible. Children aged under 6 years were not eligible since  $FE_{NO}$  measurements are not obtained in a large proportion of such young children<sup>17</sup>. Children were free of respiratory symptoms on the day of testing.

Exhaled NO measurements. A chemiluminescence analyser (NIOX, Aerocrine, Sweden) was used to measure  $FE_{NO}$  in real time in accordance with international recommendations<sup>18</sup>. Baseline  $FE_{NO}$  was measured before spirometry. Exhaled NO was measured at 10, 30, 50, 100 and 200 ml/s. The nonlinear method of Silkoff<sup>16</sup> was used to derive alveolar NO (CANO, units ppb), airway wall NO flux (J'awNO, units pl/s), the mean airway wall concentration of NO (CawNO, units ppb) and total airway compartment diffusing capacity for transfer of NO from the airway wall to the gas phase (DawNO, units pl/ppb/s) using a previously validated software programme<sup>19</sup>.

Skin prick reactivity. The skin prick test was used to ascertain sensitisation to egg, house dust mite, grass and cat dander. Positive and negative controls were used and a positive

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3 reaction was defined as a weal at least 3mm in longest diameter or, in cases of  
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5 dermatographism, a weal greater than the negative control. All reagents were supplied by  
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8 Alk-Abello (Northampton, UK).  
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10 Spirometry. A pneumotachygraph (Vitalograph 20/20, Vitalograph, UK) and  
11  
12 commercially available software (Spirotrac IV version 4.22, Vitalograph, Bucks, UK)  
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14 were used and international recommendations for quality control were applied.  
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17 Power calculation. Based on our previous report<sup>17</sup> where the mean difference in repeated  
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19 FE<sub>NO</sub> measurements over 24 hours was 0.2ppb with a standard deviation of 2.2, a study  
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21 of 10 individuals would have power at >90% to detect an intrasubject difference in FE<sub>NO</sub>  
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23 of 4 ppb at the 5% level of significance.  
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26 Statistics. Within subject differences were studied using Wilcoxon's test for two  
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28 variables and Friedman's test for three variables taken from the same individual.  
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30 Significance was assumed at the 5% level. A standard statistical package was used  
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32 (SPSS version 17.0.0).  
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## RESULTS

### Changes in FE<sub>NO</sub>

Eleven children were recruited and their details at baseline are presented in table one. Figure one shows FE<sub>NO</sub> values at baseline and 30 and 60 minutes after ingestion of the cola drink. Thirty minutes after ingestion of caffeine, FE<sub>NO</sub> had risen from a median of 47ppb [inter quartile range, IQR 9, 64] to 56 ppb [11, 66] Wilcoxon's test p=0.003 equivalent to a median [IQR] % change of +15% (4, 28), table two. Sixty minutes after ingestion, the median [IQR] FE<sub>NO</sub> was 46ppb [95% CI 9, 62] Wilcoxon's test p=0.203 compared to baseline and equivalent to a median (IQR) % change of -1% (-5, 0), table two. The overall change in absolute FE<sub>NO</sub> values was significant, Freidman's test p=0.003.

### Changes in NO flow independent parameters

These data are presented in table 2. There was a significant rise in J'awNO 30 minutes after drinking the cola drink, but no other parameters were different from baseline after ingestion of cola drink.



## DISCUSSION

This intervention study was designed to address the findings of an observational study which suggested that ingestion of carbonated drinks might substantially influence FE<sub>NO</sub> measurements in children<sup>14</sup>. There were two findings which arose from the present study. First, drinking a caffeine-containing cola drink was associated with a short-lived and possibly clinically-relevant increase in FE<sub>NO</sub> values in children with asthma. Second, a change was apparent in the proximal, but not distal, delivery of NO to the airways after ingestion of the cola drink. These findings add to what is understood about NO production in the airways and how this may be affected by constituents of cola drink. Clinicians might advise that cola drinks should not be drunk within an hour of FE<sub>NO</sub> values in asthmatic children treated with inhaled steroids.

The present study findings are consistent with a previous report linking elevated FE<sub>NO</sub> and ingestion of carbonated drinks among atopic children<sup>14</sup>. The previous observational study was not able to determine causation and was limited by only being able to consider all carbonated drinks rather than only caffeine-containing drinks<sup>14</sup>. The present intervention study is also consistent with a study where FE<sub>NO</sub> was measured in adults before and after a cup of coffee<sup>11</sup>. In their study of non-asthmatic adults, Warke *et al*<sup>11</sup> reported FE<sub>NO</sub> rising after ingestion of caffeine from 5.4ppb to 8.3ppb after 30 minutes and returned to baseline after one hour. Our results are not consistent with two studies in adults which found no effect<sup>13</sup> or a negative<sup>12</sup> effect of caffeine exposure on FE<sub>NO</sub>. The reason for the different outcomes between studies is not clear but some included asthmatic<sup>13</sup> and others non-asthmatic<sup>11 12</sup> individuals and the dose of caffeine varied from

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3 1 cup<sup>11</sup>, to 200mls coffee<sup>12 13</sup> (equivalent to 100mg caffeine) or 200mg caffeine<sup>12</sup>.  
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6 Chronic caffeine intake may possibly have confounded the acute FE<sub>NO</sub> response to  
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8 ingestion and atopy may be a further confounder<sup>14</sup>. Children are not small adults and it  
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10 may not be appropriate to compare the present study with adult studies but the consensus  
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12 of all intervention studies is that any acute change in FE<sub>NO</sub> after ingestion of a caffeine-  
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14 containing drink is transient and relatively minor.  
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20 This is the first study to separately consider changes in proximal and distal airway NO  
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22 production after exposure to caffeine-containing drinks. Our observation that proximal  
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24 airway NO turnover (J'awNO), and not delivery of NO to the distal airway (CANO),  
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26 increased after exposure is consistent with the paradigm that the excess NO in asthma  
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28 arises from the proximal airways due to upregulated activity of nitric oxide synthase  
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30 (NOS) in the airway epithelial cells<sup>20</sup>. All but one of the study participants were treated  
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32 with inhaled steroids and in adults this treatment is thought to preferentially suppress  
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34 proximal airway NO production<sup>21</sup> and it is possible that the effect of cola drink on FE<sub>NO</sub>  
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36 and J'awNO in steroid naïve children with asthma may be more marked.  
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44 We have previously reported that ingestion of L-arginine leads to an increase in FE<sub>NO</sub>  
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46 which was maximal after 60 minutes and which was associated with increased NO in the  
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48 distal airways<sup>9</sup>. Here we demonstrate that a second dietary exposure also appears to  
49  
50 influence NO metabolism in the child's airway but in a separate compartment of the  
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52 airway and with different time to peak effect. Assuming that caffeine was modifying NO  
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54 production in our study participants, the mechanism where exposure might influence  
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3 FE<sub>NO</sub> is likely to involve adenylyl cyclases. Caffeine is a phosphodiesterase inhibitor and  
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5 thus may increase cAMP which in turn induces nitric oxide synthase activity<sup>22</sup>.  
6  
7 Alternatively, caffeine has a weak bronchodilator action in children<sup>23</sup> at larger doses than  
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9 used in the present study (i.e. 10mg/kg) and may elevate FE<sub>NO</sub> by increasing the surface  
10  
11 area of lung from which NO can diffuse; the peak effect of caffeine on lung function is  
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13 seen after two hours<sup>23</sup> which contrasts with the peak rise in FE<sub>NO</sub> after 30 minutes  
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15 reported here. This study was not designed to describe a mechanism whereby cola drink  
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17 ingestion increases FE<sub>NO</sub> and since we did not measure plasma or salivary caffeine and  
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19 did not use a caffeine-free cola arm to the study we cannot be certain that the changes in  
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21 FE<sub>NO</sub> were due to caffeine exposure.  
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29 There are a number of factors which should be considered when interpreting these results.  
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31 First, we have previously demonstrated that FE<sub>NO</sub> measurements are reproducible over a  
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33 period of hours and thus the study was powered to determine intrasubject differences in  
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35 FE<sub>NO</sub> of as little as 4ppb. One limitation is that the study was not adequately powered to  
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37 study changes in flow independent parameters and it is possible that with a larger number  
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39 of participants we may have been able to detect changes in parameters in addition to  
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41 J'awNO; the limits of agreement are known to be wider for CANO compared to  
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43 J'awNO<sup>24</sup>. A second limitation is that we did not measure caffeine and therefore it is  
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45 possible that another constituent in the cola drink may have influenced the result and we  
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47 acknowledge this limitation. Third, although values of flow independent parameters are  
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49 mostly consistent with published values for asthmatic children<sup>25</sup>, the median value of  
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51 CANO was relatively low and this index could not be determined in several individuals;  
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3 it is known that flow independent parameters cannot be determined in approximately one  
4 quarter of individuals<sup>24</sup>. Finally, it is possible that NO may have been released into the  
5 mouth (“oral contamination”) after the cola drink and therefore falsely elevated FE<sub>NO</sub>  
6 values. However, in adults FE<sub>NO</sub> values are not changed after a drink of distilled water<sup>26</sup>  
7 and are reduced after an antibacterial mouth wash<sup>26</sup>, possibly due to a reduction in oral  
8 NO-producing bacteria. Acidity might confer antibacterial properties on a cola drink and  
9 this would reduce FE<sub>NO</sub> values and thus we do not believe that oral contamination could  
10 explain the elevated FE<sub>NO</sub> values seen after ingestion of the cola drink.  
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**FIGURE LEGEND**

Figure 1. Exhaled nitric oxide ( $FE_{NO}$ ) values before (T0) and thirty (T+30) and sixty (T+60) minutes after ingestion of cola drink in eleven children with asthma. Circles correspond to skin prick positive children and triangles skin prick negative children. Wilcoxon's test  $p=0.003$  for differences between  $FE_{NO}$  values at the three time points for a given individual.

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6 I.Abuzayan, U.Paraskavi, S.W.Turner

7  
8 Child Health, University of Aberdeen, Aberdeen, UK  
9

10  
11  
12 Correspondence to Dr Turner:

13  
14 Child Health

15  
16 Royal Aberdeen Children's Hospital

17  
18 Foresterhill

19  
20 Aberdeen AB25 2ZD

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22 Tel. +44 (0)1224 552471

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**ABSTRACT (word count 236)**

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Key words: asthma, caffeine, nitric oxide

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## INTRODUCTION

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Study design. At a single assessment, FE<sub>NO</sub>, skin prick reactivity and spirometry were measured in children with asthma. A dose of 2.5mg/kg caffeine in a carbonated cola drink was ingested (5.5ml/kg of Diet Coke 45.6mg caffeine/100mls); for a 40kg child this was equivalent to 220mls or two thirds of a standard 330ml can. Exhaled NO was measured 30 and 60 minutes after ingestions. Proximal and distal airway contributions to exhaled NO were derived from a non-linear method<sup>16</sup>. The study was approved by the local research ethics committee; written parental consent and verbal assent from the child were obtained.

Enrolment. Children attending a hospital asthma clinic with a prior physician diagnosis of asthma were eligible. Children aged under 6 years were not eligible since FE<sub>NO</sub> measurements are not obtained in a large proportion of such young children<sup>17</sup>. Children were free of respiratory symptoms on the day of testing.

Exhaled NO measurements. A chemiluminescence analyser (NIOX, Aerocrine, Sweden) was used to measure FE<sub>NO</sub> in real time in accordance with international recommendations<sup>18</sup>. Baseline FE<sub>NO</sub> was measured before spirometry. Exhaled NO was measured at 10, 30, 50, 100 and 200 ml/s. The nonlinear method of Silkoff<sup>16</sup> was used to derive alveolar NO (CANO, units ppb), airway wall NO flux (J'awNO, units pl/s), the mean airway wall concentration of NO (CawNO, units ppb) and total airway compartment diffusing capacity for transfer of NO from the airway wall to the gas phase (DawNO, units pl/ppb/s) using a previously validated software programme<sup>19</sup>.

Skin prick reactivity. The skin prick test was used to ascertain sensitisation to egg, house dust mite, grass and cat dander. Positive and negative controls were used and a positive

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2 reaction was defined as a weal at least 3mm in longest diameter or, in cases of  
3 dermatographism, a weal greater than the negative control. All reagents were supplied by  
4 Alk-Abello (Northampton, UK).  
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8 Spirometry. A pneumotachygraph (Vitalograph 20/20, Vitalograph, UK) and  
9 commercially available software (Spirotrac IV version 4.22, Vitalograph, Bucks, UK)  
10 were used and international recommendations for quality control were applied.  
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14 Power calculation. Based on our previous report<sup>17</sup> where the mean difference in repeated  
15 FE<sub>NO</sub> measurements over 24 hours was 0.2ppb with a standard deviation of 2.2, a study  
16 of 10 individuals would have power at >90% to detect an intrasubject difference in FE<sub>NO</sub>  
17 of 4 ppb at the 5% level of significance.  
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22 Statistics. Within subject differences were studied using Wilcoxon's test for two  
23 variables and Friedman's test for three variables taken from the same individual.  
24 Significance was assumed at the 5% level. A standard statistical package was used  
25 (SPSS version 17.0.0).  
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## RESULTS

### Changes in FE<sub>NO</sub>

Eleven children were recruited and their details at baseline are presented in table one.

Figure one shows FE<sub>NO</sub> values at baseline and 30 and 60 minutes after ingestion of the cola drink. Thirty minutes after ingestion of caffeine, FE<sub>NO</sub> had risen from a median of 47ppb [inter quartile range, IQR 9, 64] to 56 ppb [11, 66] Wilcoxon's test p=0.003 equivalent to a median [IQR] % change of +15% (4, 28), table two. Sixty minutes after ingestion, the median [IQR] FE<sub>NO</sub> was 46ppb [95% CI 9, 62] Wilcoxon's test p=0.203 compared to baseline and equivalent to a median (IQR) % change of -1% (-5, 0), table two. The overall change in absolute FE<sub>NO</sub> values was significant, Freidman's test p=0.003.

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### Changes in NO flow independent parameters

These data are presented in table 2. There was a significant rise in J'awNO 30 minutes after drinking the cola drink, but no other parameters were different from baseline after ingestion of cola drink.

## DISCUSSION

This intervention study was designed to address the findings of an observational study which suggested that ingestion of carbonated drinks might substantially influence FE<sub>NO</sub> measurements in children<sup>14</sup>. There were two findings which arose from the present study. First, drinking a caffeine-containing cola drink was associated with a short-lived and possibly clinically-relevant increase in FE<sub>NO</sub> values in children with asthma. Second, a change was apparent in the proximal, but not distal, delivery of NO to the airways after ingestion of the cola drink. These findings add to what is understood about NO production in the airways and how this may be affected by constituents of cola drink. Clinicians might advise that cola drinks should not be drunk within an hour of FE<sub>NO</sub> values in asthmatic children treated with inhaled steroids.

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The present study findings are consistent with a previous report linking elevated FE<sub>NO</sub> and ingestion of carbonated drinks among atopic children<sup>14</sup>. The previous observational study was not able to determine causation and was limited by only being able to consider all carbonated drinks rather than only caffeine-containing drinks<sup>14</sup>. The present intervention study is also consistent with a study where FE<sub>NO</sub> was measured in adults before and after a cup of coffee<sup>11</sup>. In their study of non-asthmatic adults, Warke *et al*<sup>11</sup> reported FE<sub>NO</sub> rising after ingestion of caffeine from 5.4ppb to 8.3ppb after 30 minutes and returned to baseline after one hour. Our results are not consistent with two studies in adults which found no effect<sup>13</sup> or a negative<sup>12</sup> effect of caffeine exposure on FE<sub>NO</sub>. The reason for the different outcomes between studies is not clear but some included asthmatic<sup>13</sup> and others non-asthmatic<sup>11 12</sup> individuals and the dose of caffeine varied from

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2 1 cup<sup>11</sup>, to 200mls coffee<sup>12 13</sup> (equivalent to 100mg caffeine) or 200mg caffeine<sup>12</sup>.  
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4 Chronic caffeine intake may possibly have confounded the acute FE<sub>NO</sub> response to  
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6 ingestion and atopy may be a further confounder<sup>14</sup>. Children are not small adults and it  
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8 may not be appropriate to compare the present study with adult studies but the consensus  
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10 of all intervention studies is that any acute change in FE<sub>NO</sub> after ingestion of a caffeine-  
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12 containing drink is transient and relatively minor.  
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16 This is the first study to separately consider changes in proximal and distal airway NO  
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18 production after exposure to caffeine-containing drinks. Our observation that proximal  
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20 airway NO turnover (J'awNO), and not delivery of NO to the distal airway (CANO),  
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22 increased after exposure is consistent with the paradigm that the excess NO in asthma  
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24 arises from the proximal airways due to upregulated activity of nitric oxide synthase  
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26 (NOS) in the airway epithelial cells<sup>20</sup>. All but one of the study participants were treated  
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28 with inhaled steroids and in adults this treatment is thought to preferentially suppress  
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30 proximal airway NO production<sup>21</sup> and it is possible that the effect of cola drink on FE<sub>NO</sub>  
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32 and J'awNO in steroid naïve children with asthma may be more marked.  
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37 We have previously reported that ingestion of L-arginine leads to an increase in FE<sub>NO</sub>  
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39 which was maximal after 60 minutes and which was associated with increased NO in the  
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41 distal airways<sup>9</sup>. Here we demonstrate that a second dietary exposure also appears to  
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43 influence NO metabolism in the child's airway but in a separate compartment of the  
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45 airway and with different time to peak effect. Assuming that caffeine was modifying NO  
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47 production in our study participants, the mechanism where exposure might influence  
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FE<sub>NO</sub> is likely to involve adenylyl cyclases. Caffeine is a phosphodiesterase inhibitor and thus may increase cAMP which in turn induces nitric oxide synthase activity<sup>22</sup>. Alternatively, caffeine has a weak bronchodilator action in children<sup>23</sup> at larger doses than used in the present study (i.e. 10mg/kg) and may elevate FE<sub>NO</sub> by increasing the surface area of lung from which NO can diffuse; the peak effect of caffeine on lung function is seen after two hours<sup>23</sup> which contrasts with the peak rise in FE<sub>NO</sub> after 30 minutes reported here. This study was not designed to describe a mechanism whereby cola drink ingestion increases FE<sub>NO</sub> and since we did not measure plasma or salivary caffeine and did not use a caffeine-free cola arm to the study we cannot be certain that the changes in FE<sub>NO</sub> were due to caffeine exposure.

There are a number of factors which should be considered when interpreting these results. First, we have previously demonstrated that FE<sub>NO</sub> measurements are reproducible over a period of hours and thus the study was powered to determine intrasubject differences in FE<sub>NO</sub> of as little as 4ppb. One limitation is that the study was not adequately powered to study changes in flow independent parameters and it is possible that with a larger number of participants we may have been able to detect changes in parameters in addition to J'awNO; the limits of agreement are known to be wider for CANO compared to J'awNO<sup>24</sup>. A second limitation is that we did not measure caffeine and therefore it is possible that another constituent in the cola drink may have influenced the result and we acknowledge this limitation. Third, although values of flow independent parameters are mostly consistent with published values for asthmatic children<sup>25</sup>, the median value of CANO was relatively low and this index could not be determined in several individuals;



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2 it is known that flow independent parameters cannot be determined in approximately one  
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4 quarter of individuals<sup>24</sup>. Finally, it is possible that NO may have been released into the  
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6 mouth (“oral contamination”) after the cola drink and therefore falsely elevated FE<sub>NO</sub>  
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8 values. However, in adults FE<sub>NO</sub> values are not changed after a drink of distilled water<sup>26</sup>  
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10 and are reduced after an antibacterial mouth wash<sup>26</sup>, possibly due to a reduction in oral  
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12 NO-producing bacteria. Acidity might confer antibacterial properties on a cola drink and  
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14 this would reduce FE<sub>NO</sub> values and thus we do not believe that oral contamination could  
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16 explain the elevated FE<sub>NO</sub> values seen after ingestion of the cola drink.  
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## FIGURE LEGEND

Figure 1. Exhaled nitric oxide (FE<sub>NO</sub>) values before (T0) and thirty (T+30) and sixty (T+60) minutes after ingestion of cola drink in eleven children with asthma. Circles correspond to skin prick positive children and triangles skin prick negative children.

Wilcoxon's test p=0.003 for differences between FE<sub>NO</sub> values at the three time points for a given individual.

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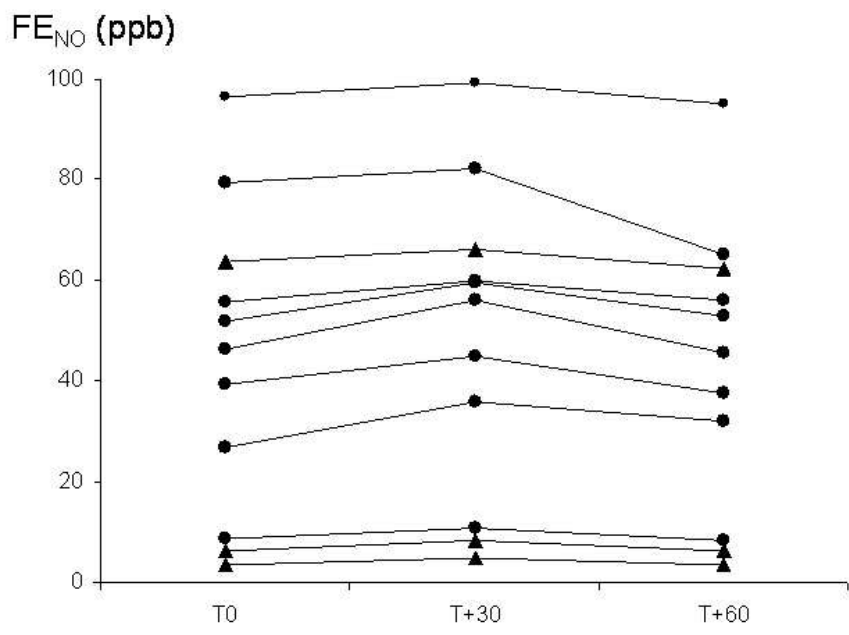
Male gender	8/11 (72%)
Mean age (SD), years	11.1 (2.1)
Inhaled corticosteroid treatment	10/11 (90%)
Skin prick positivity	9/11 (82%)
Mean % predicted FEV <sub>1</sub> (SD)	81 (9)
Median FE <sub>NO50</sub> (interquartile range), ppb	47 (9, 64)
Median J'awNO (interquartile range), pl/s	2843 (356, 4247)
Median CANO (interquartile range), ppb	0.34 (0, 2.15)
Median DawNO (interquartile range), pl/ppb/s	21.0 (1.6, 29.7)
Median CawNO (interquartile range), ppb	184 (112, 214)

Table 1. Details of study participants.

	<i>Baseline</i>	<i>+30 minutes</i>	<i>+60 minutes</i>	<i>Trend test*</i>
Median percentage change in FE <sub>NO</sub> (IQR)	0%	+15% (4, 28)	-1% (-5, 0)	p=0.003
<i>Median FE<sub>NO</sub> (IQR), ppb</i>	47 (9, 64)	56 (11, 66)	46 (9, 62)	<i>p=0.003</i>
Mean percentage change in J'awNO (IQR)	0%	+11% (3, 35)	-2% (-7, 3)	p=0.003
<i>Median J'awNO (IQR), pl/s</i>	2843 (356, 4247)	3304 (479, 4387)	2937 (356, 4153)	<i>p=0.003</i>
Mean percentage change in CANO (IQR)	0%	+23 (-80, +100)	-62 (-100, 5)	p=0.128
<i>Median CANO (IQR), ppb</i>	0.34 (0, 2.15)	0.15 (0, 1.63)	0 (0, 1.57)	<i>p=0.115</i>
Mean percentage change in DawNO (IQR)	0%	+7% (-12, 199)	-6 (-12, 30)	p=0.131
<i>Median DawNO (IQR), pl/ppb/s</i>	21.0 (1.6, 29.7)	24.1 (13.5, 31.2)	25.9 (6.6, 27.9)	<i>p=0.441</i>
Mean percentage change in CawNO (IQR)	0%	0% (-54, 12)	+1% (-20, 12)	p=0.859
<i>Median CawNO (IQR), ppb</i>	184 (112, 214)	127 (70, 195)	165 (90, 214)	<i>p=0.695</i>

Table 2. Median percentage changes and absolute values of FE<sub>NO</sub>, proximal airway NO flux (J'awNO), alveolar NO (CANO), diffusion capacity for transfer of NO from airway wall to lumen (DawNO) and concentration of NO in the airway wall (CawNO) before and 30 and 60 minutes after ingestion of caffeine-containing carbonated drink. IQR= interquartile range. \*Freidman's test for comparison of absolute values (e.g. FE<sub>NO</sub> at baseline and after 30 and 60 minutes) and Wilcoxon signed ranks test for comparison of percentage changes (e.g. % change in FE<sub>NO</sub> 30 and 60 minutes after ingestion).

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Reviewer: 1

Comments to the Author

*Some issues to consider that might improve the paper:*

*1] Methods, line 8 " 2.5 ml/kg" may be "2.5 ml/kg". It would be helpful if a statement could be added to say what this equates to in volume of Diet Coke taken by the children - so that readers could relate this to eg drinking a 330ml can of Diet Coke*

We have made these changes on page 8

*2] I think Fig 1 would be better with the actual value of FENO on the 'y'-axis with a connector line linking individual patients values at baseline, 30 and 60 mins. This is important so that the change from initial baseline value can be seen ie are the bigger rises at 30mins mainly in those with high initial values. This also allows the reader to see the changes that have occurred in children whose baseline value is at or near the cutoffs for high/low eg between 20-30ppb.*

The figure and accompanying legend has been amended as suggested by the reviewer

*3] I think the authors may be understating or even dismissing the magnitude of the rise in FENO observed at 30 mins - this finding would make me want to recommend that FENO is measured at least 60 mins after a drink of Diet Coke !*

We have amended the conclusions in the abstract and discussion in light of the conclusions made by both reviewers

Reviewer: 2

Comments to the Author

*To investigate the effect of caffeine-containing drinks, the author should have used the methods of Bruce et al. (Thorax 2002) comparing 3 groups with either caffeine containing drink (coffee) + placebo capsule, decaffeinated drink + caffeine capsule and decaffeinated drink + placebo capsule. Using this approach, it would be easier to tell, if caffeine really changes FeNO also in children. Thus, we cannot be sure, what constituent in the cola really led to changes in FeNO. And this is, in my opinion, the more interesting question.*

Our research question was Does exposure to caffeine-containing carbonated drink increase FE<sub>NO</sub> values? We believe our study was designed appropriately and addressed this question. The reviewer raises a second question of "Does exposure to caffeine increase FE<sub>NO</sub> values" and we agree this is an interesting question but as the reviewer notes, our study was not designed to address this question. In our original manuscript we addressed this potential limitation and stated that "This study was not designed to describe a mechanism whereby cola drink ingestion increases FE<sub>NO</sub> and since we did not measure plasma or salivary caffeine and did not use a caffeine-free cola arm to the study we cannot be certain that the changes in FE<sub>NO</sub> were due to caffeine exposure." We believe our pragmatic study has addressed a clinically relevant issue but we acknowledge that the underlying mechanism is still unexplained.



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3 *It is furthermore not clear, if oral contamination can explain at least a part of the FeNO*  
4 *changes, although this is unlikely as standardized procedures for FeNO-testing were*  
5 *used.*

6  
7 We agree with the reviewer that oral contamination of FENO is unlikely to have  
8 explained part of the FENO change since we did use the standard methodology. Mouth  
9 wash has been with a reduction in FENO rather than an elevation such as we observed.  
10 We have amended the text to address this issue (last sentence of discussion)  
11

12  
13 *A further suggestion would be to enroll steroid naive asthmatics, as the influence of ICS*  
14 *on FeNO is likely much bigger than that of caffeine.*

15 We agree that a study of the role of caffeine exposure on FENO in steroid naive  
16 asthmatic children is of interest. However the majority of children with asthma are  
17 treated with inhaled steroids and the present results are therefore relevant to many  
18 children  
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20  
21 *The authors state that the changes observed were not clinically meaningful, although*  
22 *they showed changes of as much as 35% (Figure 1) and therefore they can be meaningful*  
23 *as a confounder for testing.*

24 We have amended the conclusions in the abstract and discussion in light of the  
25 conclusions made by both reviewers  
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27  
28 *In summary, although the study raises an interesting question (influence of caffeine on*  
29 *FeNO measures), it does not use the proper methodology to answer it.*

30 As previously discussed, the present study was not designed to relate caffeine exposure to  
31 FENO per se, rather exposure to cola drink to FENO  
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