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► To cite this version:

Steve Turner, Ibrahim Abuzayan, Untiveros Paraskevi. Changes to exhaled nitric oxide in asthmatic children after drinking a caffeine-containing cola drink. *Pediatric Pulmonology*, 2010, 45 (12), pp.1228. 10.1002/ppul.21313 . hal-00599821

HAL Id: hal-00599821

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Journal:	<i>Pediatric Pulmonology</i>
Manuscript ID:	PPUL-10-0138.R1
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	09-Jun-2010
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Keywords:	asthma, caffeine, nitric oxide



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

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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 (FENO) is a non-invasive biomarker for airway eosinophilia¹ and may be useful to monitor asthma control²⁻⁴. Measurements of FENO can be variable within an asthmatic independent of symptoms⁵ and this suggests that factors other than asthma might influence FENO with implications for clinical application.

Factors other than asthma which are associated with altered FENO in children include atopy⁶, hayfever⁷, age⁸, height⁶, ingestion of L-arginine⁹ and possibly exposure to tobacco smoke¹⁰. There is at least one potential mechanism whereby caffeine might also acutely influence FENO values in children. Caffeine ingestion has been associated with altered FENO values in adults although the results are inconsistent; the three published studies have respectively found exposure resulting in increased FENO¹¹, reduced FENO¹² or no change in FENO¹³. In an observational study, our group has observed an association between intake of carbonated drinks (the predominant source of caffeine in children) and increased FENO in atopic five year olds¹⁴.

Given that many children are regularly exposed to caffeine-containing carbonated drinks¹⁵ there is the potential for such exposure to confound FENO measurements in children with asthma. We therefore designed an intervention study to test the hypothesis that exposure to a caffeine-containing carbonated drink increases FENO 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 method¹⁶. 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 children¹⁷. 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 recommendations¹⁸. Baseline FE_{NO} was measured before spirometry. Exhaled NO was measured at 10, 30, 50, 100 and 200 ml/s. The nonlinear method of Silkoff¹⁶ 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 programme¹⁹.

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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Spirometry. A pneumotachygraph (Vitalograph 20/20, Vitalograph, UK) and commercially available software (Spirotrac IV version 4.22, Vitalograph, Bucks, UK) were used and international recommendations for quality control were applied.

Power calculation. Based on our previous report¹⁷ where the mean difference in repeated FE_{NO} measurements over 24 hours was 0.2ppb with a standard deviation of 2.2, a study of 10 individuals would have power at >90% to detect an intrasubject difference in FE_{NO} of 4 ppb at the 5% level of significance.

Statistics. Within subject differences were studied using Wilcoxon’s test for two variables and Friedman’s test for three variables taken from the same individual. Significance was assumed at the 5% level. A standard statistical package was used (SPSS version 17.0.0).

RESULTS

Changes in FE_{NO}

Eleven children were recruited and their details at baseline are presented in table one. Figure one shows FE_{NO} values at baseline and 30 and 60 minutes after ingestion of the cola drink. Thirty minutes after ingestion of caffeine, FE_{NO} 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_{NO} 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_{NO} 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_{NO} measurements in children¹⁴. 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_{NO} 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_{NO} values in asthmatic children treated with inhaled steroids.

The present study findings are consistent with a previous report linking elevated FE_{NO} and ingestion of carbonated drinks among atopic children¹⁴. 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 drinks¹⁴. The present intervention study is also consistent with a study where FE_{NO} was measured in adults before and after a cup of coffee¹¹. In their study of non-asthmatic adults, Warke *et al*¹¹ reported FE_{NO} 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 effect¹³ or a negative¹² effect of caffeine exposure on FE_{NO}. The reason for the different outcomes between studies is not clear but some included asthmatic¹³ and others non-asthmatic^{11 12} individuals and the dose of caffeine varied from

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3 1 cup¹¹, to 200mls coffee^{12 13} (equivalent to 100mg caffeine) or 200mg caffeine¹².
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5 Chronic caffeine intake may possibly have confounded the acute FE_{NO} response to
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7 ingestion and atopy may be a further confounder¹⁴. Children are not small adults and it
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9 may not be appropriate to compare the present study with adult studies but the consensus
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11 of all intervention studies is that any acute change in FE_{NO} after ingestion of a caffeine-
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13 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 cells²⁰. 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 production²¹ and it is possible that the effect of cola drink on FE_{NO}
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36 and J'awNO in steroid naïve children with asthma may be more marked.
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43 We have previously reported that ingestion of L-arginine leads to an increase in FE_{NO}
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45 which was maximal after 60 minutes and which was associated with increased NO in the
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47 distal airways⁹. Here we demonstrate that a second dietary exposure also appears to
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49 influence NO metabolism in the child's airway but in a separate compartment of the
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51 airway and with different time to peak effect. Assuming that caffeine was modifying NO
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53 production in our study participants, the mechanism where exposure might influence
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3 FE_{NO} 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 activity²².
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7 Alternatively, caffeine has a weak bronchodilator action in children²³ at larger doses than
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9 used in the present study (i.e. 10mg/kg) and may elevate FE_{NO} by increasing the surface
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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 hours²³ which contrasts with the peak rise in FE_{NO} 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_{NO} 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_{NO} 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_{NO} 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_{NO} 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 $J'awNO$ ²⁴. 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 children²⁵, 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
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5 quarter of individuals²⁴. Finally, it is possible that NO may have been released into the
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7 mouth (“oral contamination”) after the cola drink and therefore falsely elevated FE_{NO}
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9 values. However, in adults FE_{NO} values are not changed after a drink of distilled water²⁶
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11 and are reduced after an antibacterial mouth wash²⁶, possibly due to a reduction in oral
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13 NO-producing bacteria. Acidity might confer antibacterial properties on a cola drink and
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15 this would reduce FE_{NO} values and thus we do not believe that oral contamination could
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17 explain the elevated FE_{NO} 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.

For Peer Review

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INTRODUCTION

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RESULTS

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Figure one shows FE_{NO} values at baseline and 30 and 60 minutes after ingestion of the cola drink. Thirty minutes after ingestion of caffeine, FE_{NO} 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_{NO} 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_{NO} 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_{NO} measurements in children¹⁴. 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_{NO} 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_{NO} 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_{NO} and ingestion of carbonated drinks among atopic children¹⁴. 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 drinks¹⁴. The present intervention study is also consistent with a study where FE_{NO} was measured in adults before and after a cup of coffee¹¹. In their study of non-asthmatic adults, Warke *et al*¹¹ reported FE_{NO} 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 effect¹³ or a negative¹² effect of caffeine exposure on FE_{NO} . The reason for the different outcomes between studies is not clear but some included asthmatic¹³ and others non-asthmatic^{11 12} individuals and the dose of caffeine varied from

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2 1 cup¹¹, to 200mls coffee^{12 13} (equivalent to 100mg caffeine) or 200mg caffeine¹².
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4 Chronic caffeine intake may possibly have confounded the acute FE_{NO} response to
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6 ingestion and atopy may be a further confounder¹⁴. 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_{NO} after ingestion of a caffeine-
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12 containing drink is transient and relatively minor.
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17 This is the first study to separately consider changes in proximal and distal airway NO
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19 production after exposure to caffeine-containing drinks. Our observation that proximal
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21 airway NO turnover (J'awNO), and not delivery of NO to the distal airway (CANO),
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23 increased after exposure is consistent with the paradigm that the excess NO in asthma
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25 arises from the proximal airways due to upregulated activity of nitric oxide synthase
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27 (NOS) in the airway epithelial cells²⁰. All but one of the study participants were treated
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29 with inhaled steroids and in adults this treatment is thought to preferentially suppress
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31 proximal airway NO production²¹ and it is possible that the effect of cola drink on FE_{NO}
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33 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_{NO}
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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 airways⁹. 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_{NO} is likely to involve adenylyl cyclases. Caffeine is a phosphodiesterase inhibitor and thus may increase cAMP which in turn induces nitric oxide synthase activity²². Alternatively, caffeine has a weak bronchodilator action in children²³ at larger doses than used in the present study (i.e. 10mg/kg) and may elevate FE_{NO} by increasing the surface area of lung from which NO can diffuse; the peak effect of caffeine on lung function is seen after two hours²³ which contrasts with the peak rise in FE_{NO} after 30 minutes reported here. This study was not designed to describe a mechanism whereby cola drink ingestion increases FE_{NO} 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_{NO} 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_{NO} measurements are reproducible over a period of hours and thus the study was powered to determine intrasubject differences in FE_{NO} 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'awNO²⁴. 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 children²⁵, the median value of CANO was relatively low and this index could not be determined in several individuals;

it is known that flow independent parameters cannot be determined in approximately one quarter of individuals²⁴. Finally, it is possible that NO may have been released into the mouth (“oral contamination”) after the cola drink and therefore falsely elevated FE_{NO} values. However, in adults FE_{NO} values are not changed after a drink of distilled water²⁶ and are reduced after an antibacterial mouth wash²⁶, possibly due to a reduction in oral NO-producing bacteria. Acidity might confer antibacterial properties on a cola drink and this would reduce FE_{NO} values and thus we do not believe that oral contamination could explain the elevated FE_{NO} 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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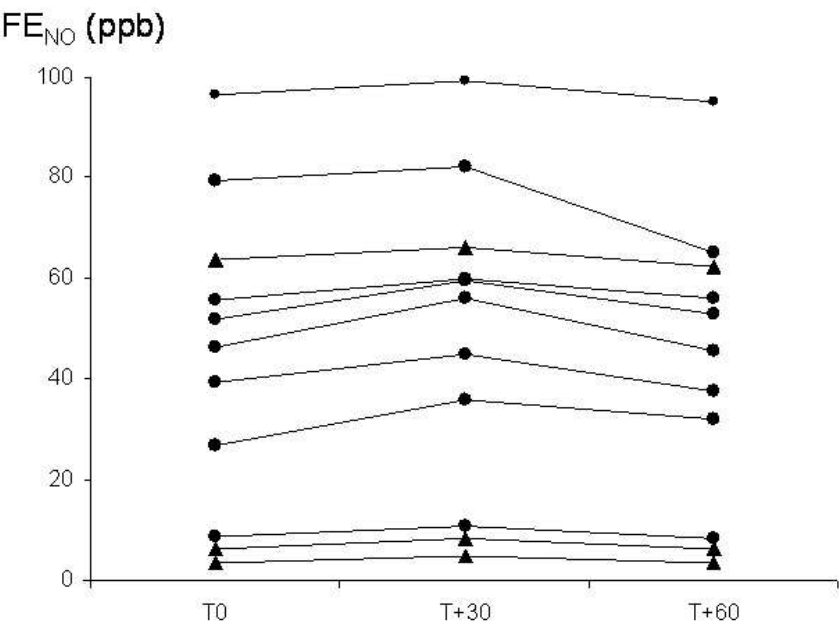
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Male gender	8/11 (72%)
Mean age (SD), years	11.1 (2.1)
Inhaled corticosteroid treatment	10/11 (90%)
Skin prick positivity	9/11 (82%)
Mean % predicted FEV ₁ (SD)	81 (9)
Median FE _{NO50} (interquartile range), ppb	47 (9, 64)
Median J'awNO (interquartile 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 _{NO} (IQR)	0%	+15% (4, 28)	-1% (-5, 0)	p=0.003
<i>Median FE_{NO} (IQR), ppb</i>	47 (9, 64)	56 (11, 66)	46 (9, 62)	p=0.003
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)	p=0.003
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)	p=0.115
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)	p=0.441
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)	p=0.695

Table 2. Median percentage changes and absolute values of FE_{NO}, 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_{NO} at baseline and after 30 and 60 minutes) and Wilcoxon signed ranks test for comparison of percentage changes (e.g. % change in FE_{NO} 30 and 60 minutes after ingestion).



254x190mm (72 x 72 DPI)

Reviewer: 1

Comments to the Author

Some issues to consider that might improve the paper:

1] Methods, line 8 "2.5 mk/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_{NO} 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_{NO} 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_{NO} 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_{NO} 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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It is furthermore not clear, if oral contamination can explain at least a part of the FeNO changes, although this is unlikely as standardized procedures for FeNO-testing were used.

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

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

We agree that a study of the role of caffeine exposure on FENO in steroid naive asthmatic children is of interest. However the majority of children with asthma are treated with inhaled steroids and the present results are therefore relevant to many children

The authors state that the changes observed were not clinically meaningful, although they showed changes of as much as 35% (Figure 1) and therefore they can be meaningful as a confounder for testing.

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

In summary, although the study raises an interesting question (influence of caffeine on FeNO measures), it does not use the proper methodology to answer it.

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