The effect of caffeinated coffee on airway response to methacholine and exhaled nitric oxide

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Summary

Background: The bronchoprotective effect of caffeine on histamine challenge testing (HCT) has been studied with equivocal results. Current guidelines for bronchoprovocation testing recommend exclusion of caffeine the day of testing. The effects of caffeine on methacholine challenge testing (MCT), now more commonly performed than histamine challenge, are unknown.

Methods: Sixteen well-controlled asthmatics with a forced expiratory volume in 1 s (FEV1) > 65% predicted and methacholine provocation concentration causing a 20% fall in FEV1 (PC20) < 16 mg/ml participated in a randomized single-blind crossover study. The two treatments included 16 ounces of caffeinated and decaffeinated coffee given on two separate days. The fraction of exhaled nitric oxide (eNO) and FEV1 were measured before and 1 h after each treatment. One hour post treatment blood was drawn for serum caffeine level and the MCT was done.

Results: Fourteen subjects completed the study; there were no adverse events. No significant bronchodilation was seen between the mean FEV1 values before and after the caffeinated treatment (3.31 ± 0.75 L and 3.36 ± 0.74 L, respectively). No significant bronchoprotection was seen between the caffeinated and decaffeinated treatment’s geometric mean PC20 values (1.35 mg/ml and 1.36 mg/ml, respectively). Mean eNO values before and after caffeinated treatment were not significantly different (31.2 ± 19.6 ppb and 31.5 ± 20.4 ppb).

Conclusion: The amount of caffeine in a normal dietary serving of a 16oz cup of coffee is not enough to cause significant bronchoprotection, bronchodilation, or decrease eNO values.


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Introduction

Caffeine is found in many beverages and foods.\textsuperscript{1,2} It was once believed that the consumption of caffeine was leading to an underdiagnosis of asthma, but this has since been disproved.\textsuperscript{3–5} The bronchodilating effects of a normal dietary intake of caffeine, two cups, is not clinically significant but theophylline, which is chemically similar to caffeine, results in significant bronchodilation when administered in small doses and has been used in clinical research and as a treatment for asthma.\textsuperscript{6–7} Although a normal dietary intake of caffeine is ineffective in the treatment of asthma it is still not consumed prior to diagnostic tests to help yield accurate results.

Currently, newer techniques and tools are used in the diagnosis of asthma, such as the methacholine challenge test (MCT) and exhaled nitric oxide (eNO). Methacholine, a muscarinic receptor agonist acts on airway smooth muscle and causes bronchoconstriction in asthmatics.\textsuperscript{8–10} It is now often used instead of histamine in bronchial provocation testing to assess airway hyperresponsiveness (AHR) because of fewer systemic side effects.\textsuperscript{8–10} The ATS has guidelines in place for MCT to help with reproducibility to within one to two doubling doses between provocation challenges.\textsuperscript{9,11,12} Subjects are required to refrain from certain medications, such as bronchodilators, and foods before performing a methacholine challenge test. The ATS recommends withholding caffeinated beverages the day of the test (i.e. \( \geq 8 \) h) prior to methacholine challenge.\textsuperscript{11} The results of the MCT are expressed as the provocation concentration causing a 20% FEV\textsubscript{1} fall (PC\textsubscript{20}) which can be used to determine the degree of AHR and bronchoprotection offered by medications or other substances.

eNO is used to assess eosinophilic airway inflammation.\textsuperscript{13} Asthmatics tend to have a predominant amount of type 2 helper cells which can be triggered by various stimuli which ultimately leads to airway inflammation and an increased level of eNO.\textsuperscript{14,15} This increase in eNO is thought to be due to the high number of eosinophils and the expression of inducible nitric oxide synthase (iNOS) in the airway smooth muscle.\textsuperscript{16} Caffeine has been shown to decrease eNO\textsuperscript{17} but not in asthmatic subjects.\textsuperscript{18}

Additional investigations using current diagnostic techniques (i.e. methacholine and eNO) should be studied further and a larger sample size should be investigated. It is hypothesized that the amount of caffeine in two cups of coffee, approximately 330 mg, will not cause bronchodilation or bronchoprotection significant enough to affect methacholine PC\textsubscript{20} results or influence levels of eNO.

Methods

Subjects

Sixteen non-smoking subjects with doctor-diagnosed mild to moderate asthma were recruited, both known asthmatics and volunteers. Amount of regular caffeine consumption was not considered prior to enrolment but was examined post hoc. Criteria for inclusion consisted of a diagnosis of current asthma, a tidal breathing methacholine PC\textsubscript{20} \( \leq 16 \) mg/ml, an FEV\textsubscript{1} \( \geq 65\% \) predicted, and no respiratory tract infection or allergen exposure for \( \geq 4 \) weeks; subjects were requested to refrain from caffeine-containing beverages for at least 8 h prior to all testing. All subjects signed a consent form prior to testing and the study was approved by the University of Saskatchewan Biomedical Research Ethics Board.

Methacholine challenge test

The methacholine challenges were performed using the standard 2 min tidal breathing challenge as outlined in the current ATS guidelines.\textsuperscript{11} The tidal breathing method was performed using a Bennett Twin Jet nebulizer (Puritan Bennett Corporation, Carlsbad, CA) calibrated to deliver an output of 0.13 mg/ml. The subjects wore noseclips and the aerosol was directed towards the mouth over a period of 2 min via a loose-fitting facemask. Complete baseline spirometry was initially performed in triplicate and truncated FEV\textsubscript{1} manoeuvres were performed at 30 and 90 s after the completion of each 2 min inhalation period. The next cycle started 5 min after the start of the previous cycle. Normal (0.9%) saline was inhaled first followed by doubling doses of methacholine. Concentrations from 0.03 mg/ml to 256 mg/ml were available. The change in FEV\textsubscript{1} was calculated from the lowest post-saline FEV\textsubscript{1} and lowest post-methacholine FEV\textsubscript{1}. The challenge continued until FEV\textsubscript{1} fell \( \geq \)17%. PC\textsubscript{20} was then interpolated or extrapolated from the log dose vs. response curve algebraically.

Fraction exhaled nitric oxide and blood samples

eNO was measured using a chemiluminescence gas analyzer (Niox, Aerocrine Inc., New York, NY). Subjects performed an inhalation to total lung capacity followed by an exhalation with a constant flow rate of 50 ml/s via a mouthpiece. Measurements were made in triplicate and recorded in parts per billion (ppb).

Two blood samples were collected on separate days from all consenting subjects 60 min post ingestion of caffeinated or decaffeinated treatment to capture peak serum levels of caffeine.\textsuperscript{15} The two blood samples were stored and sent to Pharmalytics (Saskatoon, SK) for the determination of serum caffeine levels.

Study design

A randomized single-blind crossover design was conducted with 16 subjects undergoing two methacholine challenges on separate days, at least 24 h apart but no more than one week apart, at the same time of day. Subjects were blinded as to which treatment they were receiving. One methacholine challenge test was performed after drinking a caffeinated (16oz) cup of coffee and the other was performed after drinking a decaffeinated cup of coffee of the same size. The caffeinated coffee had approximately 330 mg of caffeine, and the decaffeinated coffee had approximately 25 mg of caffeine. Subjects were randomly placed into caffeinated and decaffeinated treatments on their first visit; every second subject received caffeinated coffee as the first intervention. On both days of testing, eNO and FEV\textsubscript{1} were measured prior to treatment. One hour post ingestion of
either treatment, blood samples were collected and, eNO and FEV\textsubscript{1} measurements repeated. Once these two measurements were collected the MCT was done.

**Statistical analysis**

The study, with 14 subjects, had a 90% power to detect a difference in log PC\textsubscript{20} of 0.099; this represents approximately a one third doubling concentration difference which is less than the minimum clinically significant difference of a one half doubling concentration change. Differences in mean FEV\textsubscript{1} and eNO values between baseline and post treatments were compared using the Student’s paired \textit{t}-test.

Since histamine/methacholine PC\textsubscript{20} values are log-normally distributed,\textsuperscript{19} methacholine PC\textsubscript{20} values were log transformed prior to analysis. A \( p \) value < 0.05 was considered statistically significant. The correlation between broncho-protection (caffeinated minus decaf dose-shift = \( \Delta \log \text{PC}_{20}/0.3 \)) and serum caffeine level (caffeinated minus decaf) was determined using linear regression analysis.

**Results**

Two of the 16 subjects were excluded because they did not have a positive MCT. Fourteen subjects (5 males) were included in the statistical analysis. The 14 subjects completed the study without adverse event, the major adverse event of concern being severe methacholine induced bronchonstriction. Of the fourteen subjects, twelve consented to providing blood samples. Subject demographics including gender, baseline values and if they routinely ingest caffeine, a post hoc item of interest, are shown in Table 1. All subjects had a baseline % predicted FEV\textsubscript{1} \( \geq \) 65 and the majority of subjects regularly ingested caffeine on a daily basis (71%).

There were no significant differences (\( p > 0.05 \)) between pre-caffeinated and post-caffeinated coffee in FEV\textsubscript{1} or eNO (Table 2). There was no effect of decaffeinated coffee on FEV\textsubscript{1}, however there was a small (5%) reduction in eNO after decaffeinated coffee (\( p < 0.05 \), Table 2). eNO levels were also higher (\( p < 0.05 \)) both before and after ingestion of decaffeinated coffee when compared to the caffeinated day (Table 2). There was no treatment sequence effect.

In Fig. 1 the individual and mean differences in methacholine PC\textsubscript{20} between the caffeinated and decaffeinated treatments are shown. No significant difference (\( p > 0.99 \)) in log methacholine PC\textsubscript{20} values was seen. Geometric mean PC\textsubscript{20}’s were 1.35 and 1.36 mg/mL for caffeinated and decaffeinated coffee respectively.

The serum caffeine levels between the caffeinated and decaffeinated treatments were 4.33 ± 1.60 mg/mL and 1.39 ± 1.18 mg/mL respectively. Serum caffeine levels varied between each subject and caffeine was detected in

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**Table 1** Subject demographics including baseline FEV\textsubscript{1} and FeNO values.

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Gender (G)</th>
<th>Age (y)</th>
<th>Height (in)</th>
<th>Weight (lbs)</th>
<th>Baseline FEV\textsubscript{1} (Litres)</th>
<th>Predicted FEV\textsubscript{1} (%)</th>
<th>Baseline FeNO (ppb)</th>
<th>Routinely Ingest Caffeine</th>
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<tbody>
<tr>
<td>1 F</td>
<td>21</td>
<td>65</td>
<td>150</td>
<td>3.80</td>
<td>108</td>
<td>82</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>2 M</td>
<td>21</td>
<td>69</td>
<td>165</td>
<td>4.67</td>
<td>103</td>
<td>23</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>3 F</td>
<td>20</td>
<td>64</td>
<td>145</td>
<td>3.25</td>
<td>94</td>
<td>16</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>4 F</td>
<td>33</td>
<td>62</td>
<td>120</td>
<td>2.78</td>
<td>94</td>
<td>32</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>5 F</td>
<td>45</td>
<td>66</td>
<td>150</td>
<td>2.80</td>
<td>93</td>
<td>10</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>6 M</td>
<td>63</td>
<td>66</td>
<td>150</td>
<td>2.31</td>
<td>72</td>
<td>31</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>7 F</td>
<td>22</td>
<td>61</td>
<td>129</td>
<td>2.99</td>
<td>95</td>
<td>56</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>8 F</td>
<td>20</td>
<td>65</td>
<td>190</td>
<td>3.00</td>
<td>84</td>
<td>16</td>
<td>N</td>
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</tr>
<tr>
<td>9 M</td>
<td>24</td>
<td>66</td>
<td>145</td>
<td>2.77</td>
<td>67</td>
<td>47</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>10 F</td>
<td>26</td>
<td>64</td>
<td>145</td>
<td>3.28</td>
<td>99</td>
<td>16</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>11 M</td>
<td>33</td>
<td>70</td>
<td>180</td>
<td>3.69</td>
<td>85</td>
<td>27</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>12 M</td>
<td>20</td>
<td>75</td>
<td>170</td>
<td>4.96</td>
<td>95</td>
<td>41</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>13 F</td>
<td>26</td>
<td>63</td>
<td>175</td>
<td>2.70</td>
<td>84</td>
<td>21</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>14 F</td>
<td>21</td>
<td>66</td>
<td>125</td>
<td>3.52</td>
<td>98</td>
<td>19</td>
<td>Y</td>
<td></td>
</tr>
</tbody>
</table>

**Mean Values** 9F/5M 28 66 153 3.32 91 31 10Y/4N

**Table 2** Caffeinated and decaffeinated treatments (\( n = 14 \)).

<table>
<thead>
<tr>
<th></th>
<th>Caffeinated</th>
<th></th>
<th>Decaffeinated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (L)</td>
<td>3.31 ± 0.75</td>
<td>3.36 ± 0.74</td>
<td>3.31 ± 0.69</td>
<td>3.31 ± 0.76</td>
</tr>
<tr>
<td>eNO (ppb)</td>
<td>31.2 ± 19.6</td>
<td>31.5 ± 20.4</td>
<td>35.7 ± 23.5</td>
<td>33.6 ± 22.2 (^a)</td>
</tr>
<tr>
<td>Serum Caffeine (µg/mL)</td>
<td>4.33 ± 1.6</td>
<td>1.39 ± 1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log PC\textsubscript{20}</td>
<td>0.129 ± 0.45</td>
<td>0.132 ± 0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric Mean PC\textsubscript{20} (mg/mL)</td>
<td>1.35</td>
<td>1.36</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

eNO levels were higher at both times on the decaffeinated day (\( p < 0.05 \)).

\(^a\)There was a slight (5%) reduction in eNO after decaffeinated coffee (\( p < 0.05 \)).
the decaffeinated group, presumably due to the small amount of caffeine in the decaffeinated coffee or to residual levels from chronic consumption.

**Discussion**

The results support the hypothesis that the ingestion of two cups of caffeinated coffee does not change the outcomes of FEV₁, eNO or methacholine PC₂₀ values when compared to decaffeinated coffee. We found no significant difference in the mean FEV₁ values (i.e. no bronchodilation); no significant difference in methacholine PC₂₀ (i.e. no bronchoprotection) and no effect on eNO levels following caffeine intake. Therefore individuals who regularly consume two cups of coffee do not need to withhold ingestion prior to pulmonary function testing.

This study followed the ATS guidelines in avoiding caffeine (except for the small amount in the decaffeinated coffee) for ≥8 h. Measurements were made at 1 h which approximates peak caffeine levels which are reported to occur 30–120 min following consumption. The half life of caffeine is quite variable but in the range of 3–6 h. Thus the (minimum) 24 h washout in non-coffee drinkers would be in the range of the 6 half lives which is considered an adequate washout time. Since most subjects were tested in the mornings and had avoided coffee for more than 12 h, the washout in our coffee drinking subjects was likely in the range of 3 half lives. The small level of serum caffeine in the placebo period is, therefore, most likely due to the residual amount of caffeine in the decaffeinated preparation. We do not believe this is a major weakness, as the caffeine levels were much greater after active treatment.

Taylor et al. concluded that caffeinated beverages did not alter eNO levels in asthmatics. These findings were inconsistent with previous research that showed caffeine decreased eNO levels. Our findings showed that eNO values did not differ significantly after caffeine treatment. We did not anticipate caffeine to have an anti-inflammatory effect, and would not expect an anti-inflammatory treatment to have an effect on eNO after a single dose. The small 5% statistically significant decrease in eNO levels following the decaffeinated treatment is likely a chance occurrence of little clinical relevance given that the magnitude of the decrease would not alter the interpretation of the test.

It has been suggested that caffeine offers bronchoprotection to the histamine challenge test (HCT). By comparison, we did not detect an inhibitory effect of caffeine on MCT (Fig. 1). The difference between study outcomes is important given that MCT has largely replaced HCT in both clinical and research applications. If current guidelines and recommendations for MCT are based solely on literature investigating the effect of caffeine on HCT additional research and an update may indeed be
necessary. Other possible factors that could account for the differences between our results and those of previous investigations include the dose of caffeine, sample size and the timing of the methacholine challenge post caffeine consumption. We looked at a dose that is considered "normal dietary intake" in a larger sample size and performed MCT at 60 min post caffeine ingestion whereas others have used higher doses in fewer subjects and performed bronchoprovocation testing at 2.5 and 4 h after caffeine ingestion.

A recent meta-analysis of 7 studies concluded that caffeine has a small (approximately 5%) bronchodilator effect.21 Our study was not designed to address bronchodilation, and with baseline FEV1 at 91% predicted, this would be a poor population in which to demonstrate bronchodilation. These subjects are, however, typical of those referred for diagnostic methacholine challenge testing, ie subjects with symptoms and normal spirometry.

Although this study has offered novel insight on how MCT is affected by the consumption of caffeine and how research subjects or patients need to prepare for MCT, future dose response investigations and, if warranted, response kinetics (i.e. onset and threshold) may be beneficial.

In conclusion, the amount of caffeine in a normal dietary serving of coffee (2 cups or 16 ounces) does not alter eNO values or cause significant bronchoprotection or bronchodilation. These results suggest the possibility that it may not be necessary to avoid this amount of caffeine prior to bronchoprovocation testing.

Conflict of interest statement

The authors (MTY, BED, and DWC) declare that they have no conflict of interest, financial or otherwise, related to this study.

References