ABSTRACT

Background: Secretory phospholipases A2 (sPLA2) have functions relevant to asthmatic inflammation, including eicosanoid synthesis and effects on dendritic cells and T cells. The aim of this study was to measure sPLA2 activity in patients with stable and acute asthma and to assess potential associations with body mass index (BMI), and plasma cholesterol and vitamin C concentrations.

Methods: Plasma sPLA2 activity and concentrations of cholesterol and vitamin C were measured in 23 control subjects and 61 subjects with stable asthma (42 mild to moderate, 19 severe). In addition, sPLA2 activity was measured in 36 patients experiencing acute asthma and in 22 of these patients after recovery from the acute attack.

Results: sPLA2 activity was not significantly greater in severe (499.9 U; 95% confidence interval (CI) 439.4 to 560.4) compared with mild to moderate asthmatic subjects (464.8; 95% CI 425.3 to 504.3) or control subjects (445.7; 95% CI 392.1 to 499.4), although it was higher in patients with acute asthma (581.6; 95% CI 541.2 to 622.0; p<0.001). Male gender, high plasma cholesterol, increased BMI and atopy were associated with increased sPLA2 activity, while plasma vitamin C was inversely correlated with sPLA2 activity in patients with stable asthma and in control subjects. There were significant interactions between gender and plasma cholesterol and between gender and vitamin C in relation to sPLA2 activity.

Conclusions: Plasma sPLA2 may provide a biological link between asthma, inflammation, increased BMI, lipid metabolism and antioxidants. Interactions among these factors may be pertinent to the pathophysiology and increasing prevalence of both asthma and obesity.

Phospholipases A2 (PLA2) are a large group of enzymes that hydrolyse fatty acids, including arachidonic acid, from the sn-2 position of glycerophospholipids, providing the substrate for synthesis of leukotrienes, prostaglandins, platelet activating factor and lysophospholipids that are important in lung inflammation and asthma.1 The PLA2 superfamily also plays crucial regulatory roles in phospholipid metabolism, host defence and signal transduction.2 The secretory phospholipases A2 (sPLA2) are low molecular weight enzymes found in extracellular fluids, including plasma. In humans, there may be as many as six or seven of these calcium dependent enzymes that are resistant to proteolysis and denaturation.3 4 A role for these sPLA2 enzymes in inflammatory processes has been suggested by studies showing increased serum sPLA2 activity in acute pancreatitis and rheumatoid arthritis.5 6 Asthma is a chronic inflammatory disease, involving T lymphocytes, eosinophils, basophils, neutrophils and mast cells,7 and many of these cells express and release sPLA2.8 9 Furthermore, recent studies indicate that sPLA2 is likely to have numerous biological functions that are relevant to the pathogenesis of asthma, including eicosanoid synthesis,10 maturation and migration of dendritic cells,11 T cell proliferation12 as well as cytokine and chemokine production by monocytes, macrophages, neutrophils and eosinophils.13 14 15 However, patient studies directly implicating sPLA2 in the pathophysiology of asthma are limited. Increased serum and leucocyte PLA2 activity has been reported in patients with asthma and rhinitis.16 17 An increase in sPLA2 activity was also observed in nasal lavage following challenge of allergic subjects,18 and in bronchoalveolar lavage fluid following antigen challenge of patients with asthma.19 Recent epidemiological studies have identified associations between obesity and increases in the incidence and prevalence of asthma.20 21 Therefore, lipid parameters such as plasma triglyceride and cholesterol concentrations that are associated with increased body mass index (BMI)22 may also be associated with the activities of lipid modifying enzymes, such as sPLA2 in patients with asthma. Furthermore, the antioxidant vitamins C and E appear to inhibit the oxidative modification of lipoproteins that are substrates for sPLA2.23 24

We recently developed an assay for a low molecular weight sPLA2 in serum, and showed that this enzyme was associated with high density lipoproteins and was strongly correlated with total cholesterol, low density lipoprotein cholesterol and triglyceride concentrations in healthy subjects.25 The aim of the present study was to extend these findings by comparing sPLA2 activity in patients with stable, mild to moderate or severe asthma, or acute exacerbations of asthma, with that in healthy control subjects. In addition, we assessed the potential associations between sPLA2 activity and BMI, plasma total cholesterol and plasma vitamin C concentrations in patients with stable asthma and in control subjects.

METHODS

Subjects

Patients with stable asthma were recruited from the databases of the Lung Institute of Western Australia and Sir Charles Gairdner Hospital, Perth.
These institutions treat patients with mild to moderate to severe asthma from the entire Perth metropolitan area. All patients had been diagnosed as asthmatic by a respiratory physician and were categorised as having severe asthma if they met at least four of the following criteria, which were modifications of the National Asthma Education and Prevention Program, Expert Panel Report II guidelines\(^5\): (1) \( \beta_2 \) agonist use \( \geq 3 \) times per day, on most days in the previous 3 months, (2) regular inhaled corticosteroid use \(( \geq 2000 \mu \text{g beclomethasone equivalent/day})\) in the previous 3 months, (3) use of oral corticosteroids within the past 12 months, (4) hospital admission for asthma in the previous 12 months, (5) \( \geq 3 \) unplanned visits to a general practitioner in the previous 12 months because of asthma exacerbations, (6) daily asthma symptoms including cough, wheeze, chest tightness and breathlessness when asthma was unstable in the previous 3 months and (7) nocturnal awakening due to asthma symptoms more than twice a week in the past 3 months. All patients were studied when their asthma was stable, and the severity categorisation was designed to reflect their chronic long term asthma severity. On this basis, 19 patients were categorised as having severe asthma, while the 42 patients who did not meet the criteria for severe asthma were categorised as having mild to moderate asthma.

In order to recruit non-asthmatic control subjects from a comparable population, letters inviting subjects to participate were mailed to addresses randomly selected from the Perth metropolitan telephone directory. The 23 non-asthmatic subjects who were recruited completed a brief questionnaire relating to their general health status. All patients and control subjects were recruited within a 6 month period. They completed a lung function test, and their atopic status was assessed by skin prick testing. A weal diameter \( \geq 3 \) mm was considered to be a positive reaction. A blood sample was taken and placed on ice for transfer to the laboratory within 2 h. The characteristics of the patient and control groups are presented in table 1.

For the study of plasma sPLA\(_2\) activity in acute asthma, patients were recruited on presentation to the emergency department at Sir Charles Gairdner Hospital with an acute exacerbation of their asthma. A blood sample was taken while awaiting treatment was commenced. These patients were followed-up 6–8 weeks later when their lung function had returned to normal or to their previous best, and at this time a second blood sample was taken. The characteristics of the patients recruited for the acute asthma study are presented in table 1. All of the research undertaken was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee and all subjects gave informed consent.

### Assay of plasma sPLA\(_2\) activity

Plasma samples were purified on heparin–sepharose affinity columns (HiTrap Heparin HP; Amersham Biosciences, Sydney, Australia) that were prewashed with 50 mM Tris-HCl, pH 7.5, before application of the plasma sample (0.5 ml). Columns were washed with 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and sPLA\(_2\) activity was eluted with 0.5 ml of 50 mM Tris-HCl, 1 M KCl. sPLA\(_2\) activity in purified plasma samples was assayed using 4-nitro-3-octanoyloxy-benzoic acid as substrate, as described previously.\(^6\) Briefly, substrate solution (180 \( \mu \text{l} \)) and purified plasma (20 \( \mu \text{l} \)) were incubated in microplate wells at room temperature for 2 h. Absorbances at 425 and 600 nm (to correct for turbidity in the sample) were then measured on a spectrophotometer (Molecular Devices, Sunnyvale, California, USA). A unit (U) of sPLA\(_2\) activity was defined as nmol of product formed by 1 ml of plasma in a 1 h incubation and was calculated as

\[
\text{nmol product formed} = \left( \text{OD}_{425\text{nm}} - \text{OD}_{600\text{nm}} \right) \times 78.62 \times 25
\]

where 78.62 is the nmol of product producing an OD\(_{425}\) of 1.0 in 0.2 ml and 25 is the correction factor for 20 \( \mu \text{l} \) of plasma to 1 ml and a 2 h incubation to 1 h.

### Cholesterol and vitamin C concentrations

These analyses were performed in the Department of Clinical Biochemistry, PathWest, Perth, Western Australia. Total plasma cholesterol was determined by a standard automated spectrophotometric method. For vitamin C analyses, plasma was deproteinised with dithiothreitol-EDTA-perchloric acid, and ascorbic acid in the supernatant was separated and quantified by reverse phase high pressure liquid chromatography using external standards (0–227 \( \mu \text{M} \)). Ascorbic acid measurements were undertaken on October 15, 2003 by guest. Protected by copyright.

### Table 1: Characteristics of patients with asthma and control subjects

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Controls</th>
<th>Mild to moderate asthma</th>
<th>Severe asthma</th>
<th>Acute asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>13/10</td>
<td>17/25</td>
<td>6/13</td>
<td>10/26</td>
</tr>
<tr>
<td>Age (y) mean (SD)</td>
<td>44.3 (9.8)</td>
<td>48.7 (13.8)</td>
<td>47.5 (12.5)</td>
<td>37.5 (16.9)</td>
</tr>
<tr>
<td>Atopic (n (%))</td>
<td>14 (60.9)</td>
<td>36 (85.7)</td>
<td>13 (68.4)</td>
<td>17 (89.5)*</td>
</tr>
<tr>
<td>Smokers (n (%))</td>
<td>5 (21.7)</td>
<td>1 (2.4)</td>
<td>3 (15.8)</td>
<td>ND</td>
</tr>
<tr>
<td>FEV(_1) (% predicted) mean (SD)</td>
<td>98.4 (14.4)</td>
<td>87.9 (16.5)</td>
<td>69.9 (15.6)</td>
<td>78.6 (25.6)*</td>
</tr>
<tr>
<td>BMI (kg/m(^2)) mean (SD)</td>
<td>23.8 (3.5)</td>
<td>25.2 (5.7)</td>
<td>28.8 (7.0)</td>
<td>ND</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Asthma medications</th>
<th>Controls</th>
<th>Mild to moderate asthma</th>
<th>Severe asthma</th>
<th>Acute asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhaled corticosteroids (n (%))</td>
<td>28 (67.7)</td>
<td>18 (94.7)</td>
<td>28 (80)</td>
<td></td>
</tr>
<tr>
<td>Daily ICS dose (( \mu \text{g} )) mean (SEM)(^6)</td>
<td>1013 (157)</td>
<td>2317 (254)</td>
<td>1566 (197)</td>
<td></td>
</tr>
<tr>
<td>Oral corticosteroids (n (%))</td>
<td>0</td>
<td>7 (36.6)</td>
<td>7 (19.4)</td>
<td></td>
</tr>
<tr>
<td>( \beta ) adrenoceptor agonists (n (%))</td>
<td>29 (69)</td>
<td>18 (94.7)</td>
<td>31 (88.6)*</td>
<td></td>
</tr>
</tbody>
</table>

*Skin prick data were available for 19 acute patients, and FEV\(_1\) when stable was measured in 20 acute patients.

\(^6\)Use of oral corticosteroids at the time of blood sampling.

\(^1\)One patient refused to use \( \beta \) \_adrenoceptor agonists and used theophylline and sodium cromoglycate instead.

\(^*\)Five acute patients were not using \( \beta \) \_adrenoceptor agonists regularly prior to the acute attack.

BMI, body mass index; FEV\(_1\), forced expiratory volume in 1 s; ICS, inhaled corticosteroid.

\( ^2 \)Adrenal agonists.

\( ^3 \)Beclomethasone equivalent dose.

\( ^4 \)Adrenal agonists.

\( ^5 \)Adrenal agonists.

\( ^6 \)Adrenal agonists.

\( ^7 \)Adrenal agonists.

\( ^8 \)Adrenal agonists.
were not corrected for recovery, which was ~90%. The inter-
analyses precision coefficient of variation was 5.4%.

Data analysis and statistics

Data are expressed as means with 95% confidence interval (CI), SD
or SEM. Differences between group mean values were assessed for
statistical significance by one way ANOVA followed by
Bonferroni’s multiple comparison test or by the unpaired t test
as appropriate. In univariate analyses, Pearson’s correlation was
used to assess the statistical significance of associations between
parameters of interest. In order to identify significant independent
predictors of sPLA2 activity after adjusting for confounding factors,
and to assess interactions, a multiple linear regression analysis was
performed. The independent variables entered into the model were
gender, age, BMI, severe asthma, atopy, use of inhaled corticoster-
oids, smoking status, and plasma cholesterol and vitamin C
concentrations. In addition, interactions between gender and
cholesterol concentration, gender and vitamin C concentration
and BMI and cholesterol concentration were assessed. All
statistical analyses were performed using SPSS forWindows
V.11.5 (SPSS Inc, Chicago, Illinois, USA) and a p value <0.05 was
considered significant.

RESULTS

The characteristics of the patients with asthma and the control
subjects are presented in table 1. Plasma sPLA2 activity in
patients with stable, mild to moderate (464.8 U; 95% CI 425.3
to 504.3) or severe asthma (499.9 U; 95% CI 439.4 to 560.4)
did not differ significantly from that in control subjects (445.7 U;
95% CI 392.1 to 499.4) (fig 1). However, among these control
subjects and patients with stable asthma, sPLA2 activity was
significantly greater in atopic (483.5 U; 95% CI 455.3 to 511.6)
than in non-atopic subjects (419.9 U; 95% CI 349.6 to 489.7;
p<0.05) and in males (501.3 U; 95% CI 455.4 to 547.3)
compared with females (442.1 U; 95% CI 409.4 to 474.8;
p<0.05). sPLA2 activity in the 15 patients with asthma who
were not using inhaled corticosteroids (516.5 U; 95% CI 452.0
to 581.1) was not significantly different compared with that in
the 46 patients who were (462.4 U; 95% CI 424.5 to 500.4), and
there was no correlation between inhaled corticosteroid dose
and sPLA2 activity.

sPLA2 activity in patients presenting with an acute exacer-
bation of asthma (581.6 U; 95% CI 541.2 to 622.0) was
significantly higher than that in control subjects or in patients
with mild to moderate asthma (p<0.001) (fig 1). However,
mean plasma sPLA2 activity measured in 22 of the 36 patients
after recovery from the acute attack (540.2 U; 95% CI 506.0 to
574.4) was not different to that measured during the acute
asthma episode (536.3 U; 95% CI 494.9 to 577.7) (fig 2). While
sPLA2 activity decreased substantially after recovery in some
patients, it increased or remained unchanged in others. There
was no correlation between inhaled corticosteroid dose and
sPLA2 activity during or after recovery from the acute attack.

Mean BMI in patients with stable, severe asthma (28.8; 95% CI
25.4 to 32.1) was significantly higher than that of control subjects

Figure 1 Plasma secretory phospholipase A2 (sPLA2) activity in control
subjects (n = 23) and in patients with mild to moderate (n = 42), severe
(n = 19) or acute asthma (n = 36). Horizontal bars indicate mean values
and 95% CI. sPLA2 activity was significantly greater in patients with an
acute attack compared with controls and compared with patients with
mild to moderate asthma. ***p<0.001.

Figure 2 Plasma secretory phospholipase A2 (sPLA2) activity in 22
patients with an acute exacerbation of asthma, and in the same patients
after they had recovered from the acute exacerbation. Mean sPLA2
activity during the acute episode (536.3 U; 95% CI 494.9 to 577.7) was
not significantly different to that after recovery (540.2 U; 95% CI 506.0 to
574.4).

Figure 3 Plasma secretory phospholipase A2 (sPLA2) activity in control
subjects and in patients with mild to moderate and severe asthma with a
body mass index (BMI) <25 (n = 45), BMI 25–30 (n = 29) and BMI
>30 (n = 10). Horizontal bars indicate mean values and 95% CI. sPLA2
activity was greater in subjects with BMI >30 compared with those
with BMI 25–30. *p<0.05.
sPLA₂ activity was significantly greater in subjects who were clinically obese (BMI >30: 552.4 U; 95% CI 455.6 to 649.1) compared with those who were overweight (BMI 25–30: 442.1; 95% CI 399.2 to 485.0; \( p = 0.05 \)) (fig 3). Plasma total cholesterol concentration was significantly higher in subjects with BMI >25 (5.78 mM; 95% CI 5.42 to 6.13) compared with those with BMI \( \leq 25 \) (5.24 mM; 95% CI 4.98 to 5.50; \( p = 0.02 \)). Plasma cholesterol also tended to be higher in patients with severe asthma (5.96 mM; 95% CI 5.46 to 6.46) compared with controls (5.26 mM; 95% CI 4.89 to 5.65) or patients with mild to moderate asthma (5.41 mM; 95% CI 5.09 to 5.73), although the differences were not statistically significant.

Plasma vitamin C concentration was significantly lower in patients with severe asthma (33.1 \( \mu \text{M}; \) 95% CI 23.4 to 42.8) compared with those with mild to moderate asthma (49.6 \( \mu \text{M}; \) 95% CI 43.5 to 55.6; \( p = 0.01 \)) and control subjects (49.9 \( \mu \text{M}; \) 95% CI 41.4 to 58.5; \( p = 0.02 \)) (fig 4). Vitamin C concentration was also lower in males (38.4 \( \mu \text{M}; \) 95% CI 31.1 to 45.7) compared with females (51.4 \( \mu \text{M}; \) 95% CI 46.0 to 56.7; \( p < 0.005 \)) (fig 4).

Univariate analysis of the data showed weak but statistically significant positive correlations between sPLA₂ activity and BMI (\( r = 0.23; p < 0.05 \)) and also between sPLA₂ activity and cholesterol (\( r = 0.26; p < 0.02 \)) (fig 5A) in control subjects and in those with stable asthma. The correlation between sPLA₂ activity and cholesterol was, however, stronger in patients with severe asthma (\( r = 0.62; p = 0.005 \)). In contrast with these positive associations, sPLA₂ activity was inversely correlated with plasma vitamin C concentration (\( r = -0.33; p = 0.002 \)) (fig 5B).

A multiple linear regression analysis to identify factors independently associated with sPLA₂ activity in the control subjects and in patients with stable asthma showed that male gender and higher plasma cholesterol were independently associated with greater sPLA₂ activity (table 2). In addition, there were indications that atopy and increased BMI, although not reaching statistical significance in this model, were associated with increased sPLA₂ activity. Although there was no independent association between sPLA₂ activity and plasma vitamin C, there were significant interactions between gender and plasma vitamin C and also between gender and plasma cholesterol in relation to sPLA₂ activity.

**DISCUSSION**

Cytosolic PLA₂ plays a major role in the production of arachidonic acid derived lipid mediators, such as prostaglandins and leucotrienes, that contribute to airway inflammation in asthma. However, recent evidence suggests that secretory PLA₂ isozymes may also have an important role in eicosanoid synthesis and amplification of the inflammatory process, both directly and by functional coupling with cytosolic PLA₂ and cyclooxygenase-2. In the present study, the activity of potentially proinflammatory plasma sPLA₂ enzymes with heparin binding domains was measured in patients with stable asthma and patients presenting with acute asthma, in whom upregulation of inflammatory pathways might be expected. We previously measured “total” sPLA₂ activity in unpurified serum samples whereas in the present study samples were purified on heparin affinity columns and the sPLA₂ activity measured was therefore much less. However, it was more likely to represent the activities of groups IIA, IID and V sPLA₂ that interact with...
The finding of a significant increase in plasma sPLA2 activity in patients with severe asthma, but that the present study was inadequately powered to detect this increase, is supported by previous reports that serum PLA2 activity was significantly increased in patients with acute asthma.16 However, in that study, it is likely that total sPLA2 activity was measured, rather than the activity of heparin binding isoforms, and it is possible that other isoforms of sPLA2 excluded by heparin affinity purification may be differentially expressed in patients with severe asthma. Furthermore, the patients in that study had not received corticosteroids, which may not suppress inflammatory processes such as sPLA2 activity in patients with asthma.

The previously observed correlation between sPLA2 activity and total cholesterol25 was confirmed in the present study, but the correlation was much stronger in patients with severe asthma. In addition, mean BMI was significantly higher in patients with severe asthma, while plasma cholesterol was increased in overweight subjects and sPLA2 activity was greater in obese subjects and positively associated with increased BMI. These results are strongly suggestive of interactions between increased BMI, dysregulation of lipid metabolism and more severe asthma, lending support to the large number of recent epidemiological studies that have reported associations between the prevalence and incidence of asthma, and increased BMI in both adults and children (reviewed by Ford25).

There are a number of plausible mechanisms that may explain the association between increased BMI and asthma.26 In obese subjects, adipose tissue may contribute significantly to inflammation through production of IL6, tumour necrosis factor α (TNFα), transforming growth factor β1 and eotaxin, as well as the adipokine, leptin, which has been shown to regulate T cell proliferation and activation.26 37 The results from the present study suggest that upregulation of sPLA2 may be a proinflammatory consequence of increases in BMI and adipose tissue metabolism, similar to the increases in other inflammatory associated molecules, such as TNFα, IL6, leptin and C reactive protein, that have been observed in obese as well as asthmatic subjects.38 40 However, it is interesting that in this study,
sPLA2 activity was higher in atopic subjects whereas increased levels of C reactive protein were associated with non-allergic asthma. Upregulation of sPLA2 activity may have important functional consequences for inflammatory processes in both obesity and asthma. Specifically, lipoprotein associated sPLA2 may increase the catabolism of phospholipids to biologically active lipids, including prostaglandins, leukotrienes, platelet activating factor and lysophospholipids, which are likely to play a role in chronic inflammation in asthmatic and obese subjects. In addition, sPLA2, like leptin, has been implicated in T cell proliferation and cytokine and chemokine production.

sPLA2 activity was inversely correlated with plasma vitamin C concentration, although the latter was not an independent predictor of lower sPLA2 activity. Importantly, however, there was a marked gender interaction, with sPLA2 activity being higher and vitamin C concentration lower in males compared with females. Lower plasma vitamin C concentrations may contribute to increased oxidative modification of lipoproteins and a resultant increase in the activity of enzymes such as sPLA2 that catalyse the hydrolysis of lipoproteins. In contrast, the multiple regression analysis performed in the present study indicated that the positive association between sPLA2 activity and plasma cholesterol was stronger in females than in males. This is in keeping with observations from a number of studies suggesting a stronger association between asthma and obesity and vitamin C in severe asthma, are likely to be confounding factors in the associations among asthma, increased BMI, sPLA2 activity and cholesterol and vitamin C concentrations.

In conclusion, this study has demonstrated a significant upregulation of plasma sPLA2 activity in patients with acute asthma. In addition, the study has identified potentially important associations between sPLA2 activity, increased plasma cholesterol, increased BMI and atopy, as well as gender interactions in the positive association of cholesterol and the inverse association of vitamin C with sPLA2 activity. sPLA2 may therefore be one of the biological links between asthma, inflammation, increased BMI, lipid metabolism and antioxidants. Further studies, possibly with larger numbers of subjects, are required to unravel these complex interactions and the mechanisms by which they may contribute to the pathophysiology and increasing prevalence of both asthma and obesity.

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REFERENCES