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Amino acids and asthma: a case-control study

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Amino acids and asthma: a case-control study. A. Fogarty, E. Broadfield, S. Lewis, N. Lawson, J. Britton. ©ERS Journals Ltd 2004.

ABSTRACT: Amino acids contribute to various anti-oxidant and immunological activities relevant to asthma pathogenesis, raising the possibility that differences in amino acids may be involved in asthma aetiology. The authors hypothesised that cystine reduces the risk of asthma *via* glutathione metabolism. Methionine, glutamine, glutamic acid and glycine may have potential protective effects, whilst arginine, phenylalanine and tryptophan may have adverse effects in asthma.

Fasting plasma levels of amino acids were compared in a case-control study. A total of 89 adults, aged 18–65 yrs, with asthma controlled by inhaled corticosteroids, were recruited from a volunteer database and local primary care registers, and compared with 89 controls individually matched for age, sex and primary care centre.

Contrary to the primary hypothesis, cases had higher fasting plasma cystine levels than controls, and there was no difference between cases and controls in any of the other amino acids tested, with the exception of plasma glycine, which was associated with a strongly reduced risk of asthma (odds ratio for the highest tertile compared to lowest 0.30 (95% confidence interval (0.11–0.82)).

This study negates the hypothesis that higher fasting plasma cystine levels have a protective effect on the risk of asthma, although the inverse correlation with plasma glycine deserves further investigation.

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The hypothesis that diet can influence both the prevalence and severity of asthma has been studied over the past 15 yrs, particularly in relation to intake of anti-oxidants, magnesium and fatty acids [1–8]. To date, however, the role of amino acids in asthma and allergic disease has attracted little interest.

Amino acids are the structural units of proteins, but also have intrinsic biological properties that are only just beginning to be recognised. Of particular interest are the amino acids cystine, methionine, glycine and glutamic acid, which collectively contribute to glutathione metabolism [9], which is emerging as an important anti-oxidant that may influence susceptibility to asthma [10, 11]. In particular, glutathione depletion in mice results in a shift towards the T-helper-2 lymphocyte phenotype [10] associated with asthma [12]. Cystine is of particular interest, as it may be converted to the reduced form of cysteine by macrophages, which thus increases intracellular glutathione [13] and may therefore influence glutathione levels. Arginine, the precursor for nitric oxide, which has been shown to be elevated in asthma [14], and glutamine, which has powerful anti-bacterial properties *in vivo* [15], are also of interest. In addition, phenylalanine is potentially important since uncontrolled phenylketonuria is associated with increased plasma immunoglobulin E and atopic dermatitis [16]. Previous studies have also drawn attention to tryptophan, where the metabolic pathways may differ in asthmatic subjects as compared to control subjects, as demonstrated by elevated urinary kynurenic acid and xanthurenic acid excretion in children with asthma [17–19].

It is, therefore, possible that changes in the pattern of amino acid intake arising from an overall increase in the proportion of protein from animal sources [20] may have contributed to the rise in asthma prevalence that has occurred in most developed countries [21]. However, to the current authors' knowledge, only one previous study has explored the role of plasma amino acids in subjects with asthma and demonstrated that plasma cystine levels were lower in children with previous status asthmaticus as compared to controls [22]. Therefore, in this study, an existing database of fasting blood samples from a previous case-control study (unpublished data) was used to determine whether the risk of asthma is related to fasting plasma levels of cystine and seven other amino acids.

Subjects and methods

Subjects

Cases and controls were recruited from a previous study of erythrocyte membrane fatty acids in cases with asthma and healthy controls (unpublished data). Subjects aged 18–65 yrs, with a physician diagnosis of asthma, who were taking at least one dose of an inhaled corticosteroid daily, were identified from the City Hospital (Nottingham, UK) asthma volunteer database and from local primary care registers. Cases were excluded if they were taking a leukotriene antagonist or oral corticosteroids. One control matched for age (to within 15 yrs) and sex, without asthma, was then selected from the same primary care register as the case. Those eligible were contacted by letter with a summary of the study, inviting them to participate. In the event of a control not being able to participate, an alternative control was selected and approached in a similar manner. Cases and controls were

considered ineligible if they had other metabolic or inflammatory disorders (including hypertension or cardiovascular disease), were taking oil supplements (such as cod liver oil, cholesterol-lowering or nonsteroidal anti-inflammatory drugs), were pregnant or breast-feeding, or had a smoking history of >10-pack-yrs. Approval for the study was obtained from Nottingham City Hospital Ethics Committee.

Data collection

Data were collected on past medical history, medication, smoking, height, weight and social status. Social class of each participant was defined as the higher of the participant′s or partner′s occupation, categorised according to the Registrar General′s criteria [23]. Allergen skin sensitivity to *Dermatophagoides pteronyssinus*, grass pollen and cat fur with histamine and saline controls (Diagenics, Newark, UK) was measured by skin-prick testing, defining sensitisation as an average saline-adjusted skin wheal diameter to any allergen of $\geqslant$3 mm. Dietary intake of vitamin C and magnesium was estimated using a semi-quantitative food frequency questionnaire (Diet Q; Tinuviel Software, Warrington, Warrington, UK).

Plasma samples and data entry

All participants were asked to refrain from eating, or drinking anything except water, from 22:00 h the night before a morning appointment at which a fasting venous blood sample was obtained and transferred to the laboratory for analysis. Within 2 h of receipt, these were centrifuged and the plasma was stored at -70°C until analysis. Plasma amino acid levels were determined by ion exchange chromatography (Biochrom 20 Automated Amino Acid Analyser; Biochrom Ltd, Cambridge, UK).

Quantification of the individual amino acids was automated and, in the event of difficulties in interpretation of the readout due to moving baseline on the readout, the values were recorded individually by a clinical chemist blinded to the case-control status of the sample. Data were entered manually and outlying values were checked. The period of interview was categorised into four seasons (January–March, April–June, July–September, October–December).

Data analysis

The primary hypothesis tested in this study (and defined *a priori* in the protocol) was that the risk of asthma is reduced in those with higher fasting plasma cystine. The secondary hypotheses involved the analysis of the seven other amino acids with potential effects in asthma: methionine, glutamine, glutamic acid and glycine (plasma levels hypothesised to be lower in asthma); and arginine, phenylalanine and tryptophan (levels expected to be higher in asthma). The effect of tertiles of amino acid levels on risk of asthma was assessed using conditional logistic regression on matched pairs. In the case of cystine, 41% of values were zero, so the bottom "tertile" comprised all zero values, and the next tertile was correspondingly small, comprising 26% of observations. Statistical analysis of the linear trend in risk of asthma, through increasing tertiles of each amino acid, was assessed using the likelihood ratio test and the conventional level of significance ($p<0.05$). The current sample size provided 80% power to detect a trend, with an odds ratio (OR) for asthma of 3 in the highest tertile compared to the lowest. Each potential confounding factor was added to the primary model of exposure of plasma cysteine exposure (in tertiles) on the risk of asthma and retained in the model if the OR changed by $\geqslant$10%. The potential confounding factors identified for the final model for the effect of the primary exposure (plasma cystine) were also used in the final models for the effect of the secondary exposures (the other seven amino acids) on the risk of asthma, to permit a simplified and standardised approach to model generation. This was considered preferable to the testing of multiple models with each individual amino acid.

Results

Out of the 426 potential cases and 510 controls approached, 195 (46%) and 156 (31%), respectively, agreed to participate, and 96 cases and 100 controls met the entry criteria. Matched controls were not recruited for seven cases, therefore, 89 matched pairs were included in the final analysis. A total of 88 were matched for age within 10 yrs and one within 15 yrs.

The characteristics of study participants are shown in table 1. The time period of interview was identified as a potential confounding factor and all subsequent analyses were adjusted for this. The risk of asthma in different tertiles of fasting plasma amino acid levels is presented in table 2. Contrary to the primary hypothesis, the risk of asthma was positively associated with the level of cystine, this was significant after adjustment for season (p=0.02). There was also an inverse association with glycine (p=0.02), the OR for the highest tertile compared to the lowest being 0.30 (95% confidence interval (CI) 0.11–0.82). No statistically significant difference was seen for glutamic acid, methionine, tryptophan, phenylalanine, arginine or glutamine. Adjustment for body mass index, smoking history in pack-years, social class,

Table 1. – Study participant characteristics

	Nonasthmatic controls	Cases with asthma
Participants	89	89
Males	23 (26)	23 (26)
Age	43.0±9.9	42.8±9.7
Social class %		
1	13 (14.6)	10 (11.2)
2	35 (39.3)	35 (39.3)
3	36 (40.4)	41 (46.1)
4	4 (4.5)	2 (2.2)
5	1 (1.1)	1 (1.1)
Current smokers	1 (1.1)	0 (0)
Total pack-years	1.4±3.1	1.3±2.9
Using inhaled steroids		
Beclomethasone	0	64
Budesonide	0	18
Fluticasone	0	7
Season seen		
Winter	7 (7.9)	30 (33.7)
Spring	50 (56.2)	18 (20.2)
Summer	19 (21.3)	22 (24.7)
Autumn	13 (14.6)	19 (21.3)
Atopy	21 (23.6)	72 (80.9)
BMI $kg \cdot m^{-2}$	25.4±4.7	25.5±5.2
Daily energy intake MJ	7.9±2.2	8.1±2.6
Daily protein intake g	76.7±17.1	80.1±21.2
Daily dietary vitamin C mg	101.6±48.6	103.0±52.6
Daily dietary magnesium mg	332.9±102.4	322.6±113.3

Data are presented as n (%) or mean±SD. BMI: body mass index; MJ: megajoule.

Table 2. – Effect of plasma amino acids (in tertiles) on odds of having a diagnosis of asthma

Amino acid	Tertile	Range of plasma levels $\mu mol \cdot L^{-1}$	Odds of asthma for unadjusted model (95% CI)	Odds of asthma for adjusted model[¶] (95% CI) p-value for trend
Cystine	1[#]	0	1	1
	2	0.63–2.43	1.25 (0.60–2.63)	2.55 (0.64–6.82)
	3	2.48–38.04	1.30 (0.63–2.70)	3.49 (0.86–11.22)
			p=0.46	p=0.02
Methionine	1	10.85–18.77	1	1
	2	18.80–21.96	1.12 (0.51–2.43)	1.29 (0.49–3.42)
	3	21.98–35.52	0.71 (0.32–1.54)	0.69 (0.26–1.84)
			p=0.38	p=0.45
Glutamine	1	181.04–376.56	1	1
	2	377.10–501.79	0.78 (0.40–1.55)	0.51 (0.22–1.20)
	3	502.29–724–20	0.50 (0.24–1.04)	0.44 (0.17–1.13)
			p=0.07	p=0.07
Glutamic acid	1	0–67.49	1	1
	2	67.49–120.78	0.99 (0.49–2.03)	0.73 (0.29–1.80)
	3	125.29–724.20	2.22 (0.95–5.18)	1.05 (0.36–3.08)
			p=0.09	p=0.99
Glycine	1	7.42–202.62	1	1
	2	203.85–249.48	0.76 (0.35–1.62)	0.66 (0.27–1.65)
	3	251.13–562.89	0.41 (0.19–0.90)	0.30 (0.11–0.82)
			p=0.02	p=0.02
Arginine	1	11.72–36.29	1	1
	2	36.49–50.23	1.02 (0.51–2.07)	0.77 (0.32–1.85)
	3	50.33–98.27	0.96 (0.44–2.09)	0.68 (0.25–1.81)
			p=0.92	p=0.45
Phenylalanine	1	34.95–49.89	1	1
	2	50.29–56.07	0.85 (0.43–1.66)	0.65 (0.28–1.49)
	3	56.21–94.67	0.76 (0.35–1.66)	0.95 (0.22–1.57)
			p=0.49	p=0.29
Tryptophan	1	6.51–34.76	1	1
	2	34.87–44.84	1.77 (0.88–3.56)	0.99 (0.41–2.38)
	3	44.99–86.87	1.39 (0.68–2.86)	0.56 (0.21–1.48)
			p=0.32	p=0.24

Analysis was performed using conditional logistic regression. [#]: bottom category comprised all zeros (41% of values); [¶]: model adjusted for season only.

energy intake, daily dietary vitamin C and magnesium intake had a negligible effect on these outcomes.

Discussion

In this study, the hypothesis that amino acids may modulate the risk of asthma, based on their known biological properties, has been tested. This study did not support the hypothesis that higher fasting plasma levels of cystine are associated with a lower risk of asthma and found no evidence of an effect for the six other amino acids tested (glutamic acid, methionine, tryptophan, phenylalanine, arginine and glutamine). There was, however, a strong inverse relationship between fasting plasma glycine levels and asthma risk.

A matched case-control design was used to minimise the risk of confounding due to age, sex and primary care centre, but the possibility of bias or overmatching can not be eliminated. Selection bias is a particular problem in case-control studies and this cannot be excluded in this study, as only 17% of controls invited to participate in the study did so, due, at least in part, to the requirement for fasting samples and the general inconvenience of the study to the individual. Those who did enter the study tended to be from higher socio-economic groups and this may lead to the possibility of overmatching, as diet is also associated with higher socio-economic status [24]. Case selection was based on clinical criteria, including those with mild-to-moderate asthma, although no physiological measurements of asthma were obtained. No data on antioxidant supplements or other dietary antioxidants, such as carotenoids, were available, and these cannot be excluded as potential confounding factors, as they may modulate asthma activity [25, 26].

The primary study hypothesis was negated by the finding of no association of asthma with fasting plasma cystine. Conversely, cystine levels were significantly higher in cases than controls. It remains possible that this is a false-negative finding, since the power of the study was limited by the number of fasting samples available. Multiple hypotheses were tested by studying eight amino acids, and this raises the possibility of type I statistical error and it is possible that the positive association with cystine and/or the protective effect seen with glycine on risk of asthma fall into this category.

However, it is also plausible that glycine protects against asthma, since glycine is required for the formation of haem, creatine, collagen, nucleic acids and bile salts, as well as being a component of glutathione [27, 28], a molecule that regulates intracellular oxidation status and may be important in the pathophysiology of asthma. Glycine is linked to γ-glutamylcystine by the enzyme glutathione synthetase to create the molecule glutathione [29], in the rate-limiting step of glutathione synthesis [30–32]. *In vitro* studies in rat liver demonstrate that glycine prevents a reduction in glutathione in response to toxic stimuli [33], while glycine appears to have immunomodulatory properties, reducing production of superoxide and tumour necrosis factor from alveolar macrophages [34]. This effect may have implications for therapeutic developments in a variety of diseases, including asthma. In

a rat model of haemorrhagic shock, a glycine-supplemented diet reduced oxidative stress and augmented anti-oxidant enzyme activities, while it also downregulated the expression of nuclear factor-κB and inducible nitric oxide synthetase [35], both of which are increased in the inflammation associated with asthma [9]. In humans with malnutrition, supplemental glycine leads to increased levels of the blood anti-oxidant glutathione [27]. The only comparable study in asthma compared nonfasting amino acids in 27 children with asthma with seven controls and demonstrated no difference in plasma glycine between the two groups [22], while no difference in glycine, measured in bronchoalveolar lavage samples, was observed between 10 atopic adults with asthma and six nonasthmatic controls [36].

In conclusion, the data presented here provide preliminary evidence against a major role for cystine in the pathogenesis of asthma, but they do indicate that the effect of glycine and indeed of the other amino acids identified merit further study in independent datasets. If replicated elsewhere, further studies are required to explore if any therapeutic benefits in asthma can be derived by manipulation of plasma amino acids, possibly by using dietary supplements.

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