

Determinants of Serum Tryptase in a General Population: The Relationship of Serum Tryptase to Obesity and Asthma

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Key Words

Asthma • Epidemiology • Mast cells • Obesity • Tryptase

Abstract

Background: Recent studies indicate that mast cells are more abundant in the obese state. Total serum tryptase (ST) is a marker of mast cell numbers or activity. Since obesity and asthma have been consistently linked in epidemiological studies, a possible higher mast cell activity in obesity could be a factor between the two conditions. The aim of this study was to investigate determinants of ST and whether a potential association between obesity and allergic respiratory disease would be influenced by levels of ST in obese persons.

Methods: Measurements of ST (ImmunoCAP Tryptase assay), atopy (skin prick test reactivity), methacholine bronchial hyperresponsiveness (BHR), body mass index (BMI) and serum lipids were performed in a general population of 1,216 persons aged 15–69 years. **Results:** ST increased significantly with increasing BMI. The median ST level increased from 3.3 µg/l in persons with BMI <25 to 4.4 µg/l in persons with BMI >30, $p < 0.0001$. Age ($p < 0.0001$), male sex ($p = 0.0009$) and smoking ($p = 0.022$) were positively associated with ST, whereas alcohol consumption ($p = 0.005$) was inversely associated with ST. ST was not associated with atopy,

symptoms of allergic respiratory disease or BHR. A positive association between symptoms of allergic respiratory disease and obesity (OR = 1.98, 95% CI = 1.25–3.14) was not influenced by obesity-related differences in ST. **Conclusions:** Increasing BMI was significantly associated with increasing ST and the prevalence of symptoms of allergic respiratory disease. However, mast cell activity/burden (assessed by ST levels) did not influence the association between BMI and asthma/rhinitis symptoms.

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Introduction

Tryptase is a neutral protease from mast cells. From several forms and proforms, β -tryptase is the most abundant and active form of the tryptases. It is a tetramer folded by covalent binding and with the active site embedded in a central pore, which renders the active site difficult to reach and deactivate by anti-proteases in plasma [1]. Tryptase is granule stored and released with other heparin-bound compounds when mast cells are activated [2]. Therefore, total serum tryptase (ST) may be considered a measure of mast cell activity or mast cell burden [1]. ST is used in the diagnosis of mast cell disorder.

ders [3, 4], as a marker of the risk of severe allergic reactions [5, 6] and as a marker of recent allergic reactions [4]. The major physiological targets for mast cell tryptase are not known. However, tryptases have been reported to degrade many proteins leading to varying effects and may activate protease-activated receptors, PARs, for example [1, 7].

Besides their role in allergic reactions, mast cells have increasingly been associated with different inflammatory conditions as adiposity, atherogenesis [8–11] and autoimmune diseases [11]. Mast cells have been found to be more abundant in subcutaneous abdominal adipose tissue from (1) obese humans than from non-obese humans [12], and (2) from persons with the metabolic syndrome than from persons without it [13]. In mice, it was shown that active mast cells were necessary for the development of dietary-induced adiposity [12]. An increase in mast cell numbers or pronounced activation of mast cells may be reflected by higher ST levels. One study investigating ST after acute coronary syndromes did not find elevated ST levels [14], but concluded that the numbers of mast cells activated in the coronary arteries probably were too few to detect a systemic increase in ST. However, the studies on this subject have included a limited number of patients [12–14]. A Spanish general population study [15] in 420 adults found that increasing ST levels were associated with increasing BMI and the metabolic syndrome, but these associations were not statistically significant after adjustment for potential confounders. In the present study in an adult general population in Denmark, we aimed to investigate a possible association between ST and BMI. Furthermore, epidemiological studies have consistently shown a positive association between obesity and the risk of asthma [16–18]. Nevertheless, the underlying mechanisms for such a relationship are not yet known. It has been suggested that the low-grade inflammation associated with obesity could be the pathogenetic mechanism linking obesity to asthma [19]. If mast cells play a role in both obesity and asthma, ST, being a marker of mast cell activation, could theoretically represent a link between the two. Therefore, we investigated the possible association between BMI and asthma, and whether ST might act as an intermediate variable in the relationship between BMI and asthma.

Subjects and Methods

The current study was planned in 2008 and took advantage of already collected data and sera from a previously conducted study, the Copenhagen Allergy Study.

Participants

The Copenhagen Allergy Study was a population-based study carried out between October 1997 and November 1998. The aim of the study was to assess the prevalence and risk factors of allergic diseases. The study design and general characteristics of the study population have been reported in more detail elsewhere [20, 21]. In brief, random samples of 15- to 69-year-old individuals, born in Denmark and with Danish citizenship, living in 11 municipalities in the South-Western part of Copenhagen (the Capital of Denmark), were drawn from the Civil Registration System and invited to a health examination. A total of 1,216 persons (62% of the invited) were examined. The invitation procedure was a two-stage design in order to enrich the study population with persons reporting allergic respiratory symptoms. The first stage consisted of a screening questionnaire survey of allergic respiratory symptoms. The second stage included a health examination, a self-administered questionnaire and allergy tests. Among others, the examinations included skin prick test (SPT) reactivity and serum-specific IgE positivity against common inhalant allergens, and a test of bronchial hyperreactivity (BHR), as described below. The health examination, blood sampling and SPTs were performed by nurses. Measurements of body weight and height were done with the participants in light clothing without shoes. BMI was calculated as weight/height² (kg/m²). In some of the analyses, BMI was categorized as underweight (<18.5), normal weight (18.5 to <25), overweight (25 to <30) and obese (≥30). The Copenhagen Allergy study was approved by the Ethics Committee, and written informed consent has been obtained from all participants.

Serum Tryptase

Blood was drawn on the day of the health examination during the period from October 2007 to November 2008. The participants had not been fasting overnight. Sera were stored at –20°C from the day of the blood sampling to the day of analyses. In 2010, ST was determined (in groups of 50–100 serum samples at the time) using the ST ImmunoCAP tryptase assay (Phadia, Uppsala, Sweden) in the ImmunoCap-250 analyzer (Phadia), which determines total tryptase, i.e. all pro-forms and mature forms of β - and α -tryptase [4] (Phadia AB, Immuncap®). The reportable range is 1–200 μ g/l. An upper reference level of 10 μ g/l is recommended by the manufacturer, based on a study in 126 healthy individuals (aged 12–61 years; 48% males). Some authors have adopted the 95th percentile in that series (11.4 μ g/l) as the upper reference level [22, 23], and others have adopted the upper 95% confidence limit for the mean (13.5 μ g/l) in the same series [24]. Still other authors have suggested 15 μ g/l as the upper reference concentration [4, 25].

Biomarkers

Blood samples were drawn and handled as described above. HDL cholesterol, total cholesterol and triglycerides were determined using the VITROS 950 automatic analyzer (Johnson & Johnson, Langhorne, Pa., USA). Very-low-density lipoprotein (VLDL) was calculated as triglyceride $\times$ 0.45. LDL values were calculated by subtracting HDL and the calculated VLDL from total cholesterol. Systolic and diastolic blood pressure values were each measured twice in the sitting position, and the mean of the two measurements were used, respectively.

Skin Prick Test Reactivity

The Soluprick SQ® system (ALK-Abelló, Hørsholm, Denmark) was used for SPT testing as described previously [20]. The panel of allergens comprised birch pollen (*Betula verucosa*), grass pollen (*Phleum pratense*), mugwort pollen (*Artemisia vulgaris*), animal danders (horse, cat and dog), two house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), and two moulds (*Cladosporium herbarium* and *Alternaria alternata*). A mean wheal diameter ≥ 3 mm was considered positive. SPT reactivity was defined as a positive SPT to at least 1 of 10 allergens.

Methacholine Bronchial Hyperreactivity

Methacholine BHR was measured with a modification of the method of Yan et al. [26] as described in detail elsewhere [27]. A maximal cumulative dose of 1,200 μ g methacholine was inhaled, beginning with 9.39 μ g and ending at a dose of 600 μ g, with each inhalation representing a doubling of the cumulative dose. Lung function was measured 1 min after each dose and the test was stopped if the forced expiratory volume in the 1st s (FEV₁) was 20% below the initial level (following saline inhalation). The provocative dose of methacholine required to cause a 20% fall in FEV₁ (PD₂₀) was calculated. BHR was defined as a PD₂₀ below the maximal cumulative dose of 1,200 μ g [27].

Symptoms of Allergic Respiratory Disease

Reporting symptoms of allergic respiratory disease was defined as confirmatory answers to symptoms of either allergic rhinitis, allergic asthma or both. Symptoms of allergic rhinitis were defined as a confirmative answer to at least one of the questions 'Have you within the last 12 months had an itchy or stuffy nose or have you been sneezing when (A) near grass, trees, or flowers, (B) following exposure to furry animals, e.g. horse, dog, cat, rabbit, guinea pig or hamster, or (C) following exposure to dust from cleaning or from making a bed?' Symptoms of allergic asthma were defined as a confirmative answer to at least one of the questions 'Within the last 12 months have you experienced shortness of breath or trouble breathing following exposure (A) to grass, trees or flowers, (B) to furry animals, e.g. horse, dog, cat, rabbit, guinea pig or hamster, or (C) to dust when cleaning rooms or making beds, or when in bed?'

Alcohol Intake and Smoking

The average amount and type (beer, wine or spirit) of alcoholic beverage consumed per week during the last 12 months was recorded by a number of questions. One beer, one glass of wine or one glass of spirit was counted as one standard drink (1.5 cl or 12 g of pure ethanol). The total average amount (units) consumed per week was calculated. Total alcohol intake assessed by this method has previously been found well correlated with levels of γ -glutamyl transferase [28]. Smoking status (table 1) was defined in three categories: never smokers, ex-smokers and daily smokers (≥ 1 g tobacco/day). In linear regression and logistic regression analyses (table 2, 3), smoking status was defined as current smoker or non-smoker.

Statistics

Statistics were performed using the statistical program SAS, version 9.2 (SAS Institute Inc., Cary, N.C., USA). All p values reported are two tailed and statistical significance was defined as $p < 0.05$. The Mann-Whitney U test was used to compare ST concentrations (table 1). Linear regression (table 2) and logistic re-

Table 1. ST concentrations in relation to demographic variables, atopy, lifestyle factors and BMI

	n	Median tryptase (25th–75th percentile), μ g/l	p value ^a
Sex			
Female (reference)	648	3.4 (2.3–4.9)	
Male	547	4.0 (2.6–5.7)	<0.01
Age			
15–50 years (reference)	897	3.4 (2.3–4.9)	
>50 years	298	4.4 (3.1–6.3)	<0.01
Alcohol consumption			
Abstainers (reference)	219	3.8 (2.5–5.6)	
Light drinkers 1–14 U/week	803	3.7 (2.5–5.1)	0.30
Heavy drinkers >14 U/week	159	3.7 (2.1–5.3)	0.29
Smoking			
Non-smokers (reference)	537	3.4 (2.3–4.8)	
Ex-smokers	211	4.0 (2.6–5.3)	0.03
Current smokers	435	3.8 (2.6–5.7)	<0.01
BMI			
Underweight	17	2.7 (2.2–4.3)	0.48
Normal weight (reference)	589	3.3 (2.3–4.9)	
Overweight	401	3.8 (2.5–5.6)	<0.01
Obese	188	4.4 (3.0–6.1)	<0.01
Serum HDL concentration			
Normal (reference)	1,057	3.7 (2.4–5.2)	
Too low ^b	129	3.5 (2.4–5.3)	0.86
Atopy			
SPT negative (reference)	727	3.7 (2.4–5.3)	
SPT positive	466	3.7 (2.4–5.1)	0.49
Allergic respiratory disease			
Absent (reference)	639	3.8 (2.5–5.4)	
Present	576	3.6 (2.3–5.1)	0.04
Bronchial hyperreactivity			
Absent (reference)	1,003	3.6 (2.4–5.1)	
Present	154	3.9 (2.5–5.6)	0.33

^a Comparison with the reference category (Mann-Whitney U test).

^b <1.036 mmol/l for males and <1.295 mmol/l for females.

gression (table 3) were used for multivariate analyses. p values of likelihood ratio tests were used to test for statistical significance in all multivariate analyses. Tryptase concentrations were ln transformed. Therefore, the β -coefficients of the explanatory variables can be interpreted as the percentage increase or decrease in ST for any change of 1 unit of the explanatory variable. The concentrations of the blood lipids were ln transformed in order to fulfill model assumptions of linear regression. Model assumptions of linear regression analyses were fulfilled. In order to further assess and to directly compare the importance of the explanatory variables on the ST level, the standardized effect of each of these explanatory variables on ST was calculated (table 2). The standardized effect was defined as the percentage increase or decrease in ST level that a certain difference in the explanatory vari-

Table 2. Multivariate analyses (linear regression) of factors associated with ST concentrations

Covariates	Unadjusted analyses		Adjusted analyses		
	coefficient (slope)	p value	coefficient (slope)	p value	standardized effect, % ^a
Age, years	0.0095	<0.0001	0.010	<0.0001	15.0
Sex					
Female	(reference)		(reference)		
Male	0.1234	0.0012	0.110	0.012	10.7
BMI	0.0192	<0.0001	0.010	0.037	4.3
Serum HDL, $\mu\text{mol/l}$			-0.173	0.046	-4.2
Alcohol consumption, U/week	-0.0004	0.8812	-0.006	0.015	-5.1
Smoking					
Non-smokers	(reference)		(reference)		
Former smokers	0.0544	0.1691	-0.022	0.693	-
Current smokers			0.078	0.071	7.1
Atopy status					
SPT negative	(reference)		(reference)		
SPT positive	0.0276	0.4800	0.016	0.690	-

All covariates were used in the adjusted model: $\ln(\text{ST}) = \text{age, sex, atopy, BMI, alcohol consumption, smoking}$.

^a Percentage change in ST for 1-SD change in covariate (continuous variables) or for full change in covariate (binary variables) for the adjusted model.

Table 3. Multivariate analyses (logistic regression) of the associations of BMI with symptoms of allergic respiratory disease with methacholine BHR

BMI	Self-reported symptoms of allergic respiratory disease (n = 576//N = 1,215)		Methacholine BHR (n 159//N = 1,177)	
	model 1	model 2 including tryptase	model 1	model 2 including tryptase
Categorical				
Normal weight	1 (<i>reference</i>)	1 (<i>reference</i>)	1 (<i>reference</i>)	1 (<i>reference</i>)
Overweight	1.37 (1.01–1.86)	1.38 (1.02–1.88)	0.85 (0.56–1.29)	0.82 (0.53–1.26)
Obese	1.68 (1.13–2.50)	1.73 (1.16–2.58)	1.23 (0.749–2.014)	1.24 (0.75–2.05)
Continuous	1.05 (1.02–1.08)	1.05 (1.02–1.09)	1.03 (0.99–1.07)	1.03 (0.99–1.07)

Estimates are ORs (95% CIs). Model 1 is adjusted for age, sex, SPT reactivity and alcohol consumption. Model 2 is adjusted for all variables included in model 1 and for $\ln(\text{tryptase})$.

able would bring about. For continuous explanatory variables, the standardized effect was defined as the theoretical percentage increase or decrease in ST per standard error difference of the explanatory variable. For binary variables, the standardized effect was simply defined as the β -coefficient of the variable estimated in the model. The question of whether mast cell tryptase could represent a link between obesity and asthma was investigated in multivariate logistic regression models of the association between BMI (explanatory variable) and symptoms of allergic asthma (dependent variable) with (model 2) and without (model 1) ST in-

cluded as explanatory variable. The estimates for the effects of obesity on allergic asthma symptoms obtained in models 1 and 2 were compared. The model assumptions of the regression analyses, including linearity between logit of the dependent and independent variables, were fulfilled.

Information on all variables could not be obtained for all participants, and analyses were based on a number of participants varying between 1,150 and 1,216 as mentioned in the individual tables. Cases with undetectable tryptase concentrations were assigned an arbitrary value of 0.9 $\mu\text{g/l}$.

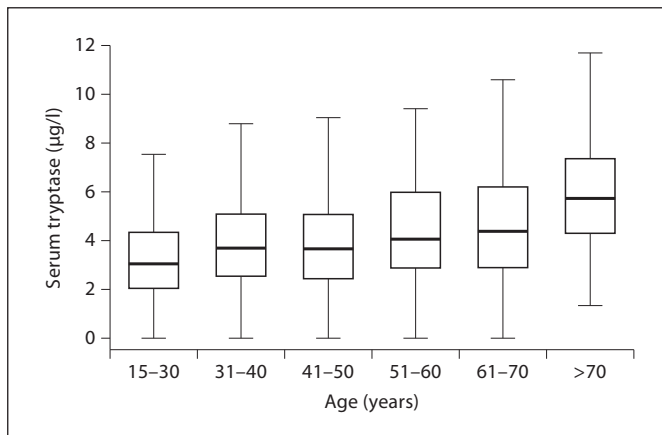


Fig. 1. ST according to age. Horizontal bold lines represent median values, boxes represent the range from the 25th to the 75th percentile. Whiskers represent the 5th and the 95th percentile. All groups were significantly different from the 15- to 30-year group ($p \leq 0.005$).

Results

The distribution of ST among the 1,195 participants is shown in figure 1. ST varied from less than an undetectable level of 1 to 104 $\mu\text{g/l}$. The mean was 4.47 $\mu\text{g/l}$, the median was 3.67 $\mu\text{g/l}$ and the 5th and 95th percentiles were 1.02 and 9.56 $\mu\text{g/l}$, respectively.

ST increased significantly with increasing age (table 1, 2; fig. 1). The estimate for the association between age and ST was essentially similar in unadjusted and adjusted analyses (table 2). Furthermore, males had around 13% higher ST levels than women (table 2). BMI and ST were positively and significantly associated (table 1; fig. 2). Although the increase in ST in relation to BMI appeared to be stronger in men than in women, there was no statistically significant interaction ($p = 0.27$). With every increase of 1 kg/m^2 in BMI, the ST increased with approximately 1.2%. The standardized effect of BMI on ST was approximately 4% (table 2), since for a standard error difference of BMI of 4.6, ST would increase by approximately 4.3%. The ST level was significantly higher among smokers than among non-smokers (table 1). This was confirmed in the multivariate analyses, which estimated that smokers had an ST level around 9% higher than non-smokers (table 2). Alcohol was inversely associated with ST. The standardized effect of alcohol on ST was approximately of the same magnitude as the positive standardized effect of BMI on ST (table 2).

Among the different markers of dyslipidemia, serum (S)-HDL was the only one that was significantly associ-

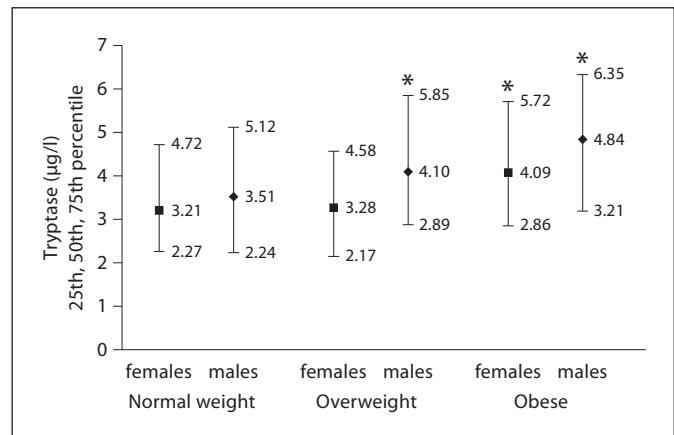


Fig. 2. ST according to BMI and gender. * $p \leq 0.008$ vs. normal-weight group; trend tests: females $p = 0.0014$ and males $p = 0.0005$.

ated with ST. S-LDL, serum total cholesterol and serum triglycerides did not appear to be associated with the level of ST (data not shown). S-HDL and ST were inversely associated. For every 1-unit increase in $\ln(\text{S-HDL})$, ST decreased with 17% [β -coefficient of $\ln(\text{S-HDL})$: -0.171]. The standardized effect of $\ln(\text{S-HDL})$ on ST was -4.2% . Thus, in this study population, the association between ST and S-HDL was approximately of equal magnitude to (and independent of) the association between ST and BMI. SPT reactivity, BHR and asthma/rhinitis symptoms were not significantly associated with ST levels, neither in univariate nor in multivariate analyses (table 1, 2). All estimates of associations between variables mentioned in table 2 and ST were similar with and without SPT reactivity included in the analyses.

There was a statistically significant association between BMI and self-reported symptoms of allergic asthma and rhinitis (table 3). Thus, the risk of reporting symptoms of asthma or rhinitis increased with increasing categories of BMI. There was a tendency towards an association between self-reported asthma within the last 12 months and BMI. However, ST did not seem to explain any of these associations. In univariate analyses (table 1), the level of tryptase was slightly lower in persons with symptoms of rhinitis compared to those without. Nevertheless, in multivariate analyses (table 3), ST levels were not significantly associated with symptoms of allergic rhinitis ($p = 0.15$), symptoms of allergic asthma ($p = 0.10$) or with self-reported asthma within the last 12 months ($p = 0.43$). Furthermore, adding ST levels to the logistic

model left the estimates of the associations between BMI and symptoms of allergic asthma/rhinitis or 'asthma within the last 12 months' essentially unchanged. Neither BMI nor ST was associated with methacholine BHR.

Discussion

We found that age, male sex, BMI and smoking were positively and alcohol drinking inversely associated with ST levels. Furthermore, S-HDL was inversely associated with ST levels, and the association was independent of BMI. In contrast, asthma and rhinitis phenotypes, SPT reactivity to inhalant allergens and BHR were not significantly associated with ST levels.

In the present study, body constitution reflected by BMI was significantly associated with ST, and this was independent of possible confounding factors. This confirms the results of the Spanish population study [15] that found a tendency towards higher ST levels in obese persons and persons with the metabolic syndrome. The Spanish study included one third of the number of participants compared to the present study. Consequently, the statistical tests used in the Spanish study were less powerful, possibly explaining the significant associations found in the present but not in the Spanish study. All in all, essentially similar results were noted in two different European populations, and, thus, the results seem to be independent of environmental and cultural differences between Northern and Southern Europe. Our results are also in line with studies reporting that mast cells are more abundant in the abdominal subcutaneous adipose tissue in persons with the metabolic syndrome [13] and in obese persons [12]. In the latter study, it was also shown that in mice active mast cells were necessary for a high weight gain [12]. In comparison with mast cell-competent mice, mice genetically lacking mast cells or mice with pharmacologically stabilized mast cells did not gain weight when fed a Western diet. Thus, even though mice genetically lacking mast cells may have many other health problems, this mouse study suggests a plausible biological mechanism for the observed associations between ST and BMI.

Our results suggested a significant association between S-HDL and ST similar to the association between BMI and ST. Interestingly, total cholesterol and S-LDL were not associated with ST. This is consistent with some results in mice [8] in which mast cell activation did not influence total cholesterol and S-LDL, but conflicting with other studies in mice [9]. The inverse association between ST and S-HDL could be due to the finding that

mast cell tryptase can degrade the apolipoproteins on HDL [5] and thereby decrease the function of HDL [10, 29]. The difference in HDL levels among different individuals has also been suggested to depend on the genotype of a mast cell chymase gene [30]. Unfortunately, we did not have data on glucose or waist circumference so we could not determine the metabolic syndrome [31] for many of the participants. However, a summary of the results suggests that mast cells could play an important role in obesity, e.g. as a pro-inflammatory mediator, thereby extending the pathophysiological role of mast cells.

There seems to be an individual baseline tryptase level which increases with age and which is higher in men than in women. Other studies have also noticed a steady increase in ST level with age [6, 15, 32]. Our data from a general population indicate that age is such an important factor that different ST reference levels for younger and older persons may be warranted. Furthermore, ST has been shown to be a predictor of severity of reactions in hymenoptera venom allergy [5, 6, 32, 33]; therefore, a more accurate reference level may help the clinician deciding when to start immunotherapy. It is not clear whether elderly individuals have more mast cells, if the mast cells are more active or whether they simply degrade tryptase slower than younger individuals.

In our study, ST levels were higher in men than in women, in agreement with the study by Komarow et al. [34] in atopic children and the Spanish study [15]. Alcohol consumption was negatively associated whereas smoking was positively associated with ST levels. Even though it could have been expected that atopy (SPT reactivity) would be associated with mast cell activity and, thus, higher ST levels, this notion was not confirmed by our data. Results from other studies on this issue have also been conflicting [34, 35], but one of the studies [35] that reported an association between SPT reactivity and ST did not take age, a strong potential confounder, into account.

There have been several epidemiological studies reporting a positive relationship between BMI and asthma [16–18]. In this study, we partly confirmed these findings because increasing BMI seemed to be associated with a higher prevalence of symptoms of allergic respiratory disease. Assuming that the association between BMI and asthma (in this study: allergic respiratory disease) is caused by obesity-induced inflammation, we hypothesized that mast cells could be part of the link between the two conditions. Thus, obesity might increase the number of mast cells, which in turn would make the individual more prone to a mast cell-dependent [36, 37] asthmatic

response on allergen exposure. However, we did not find any indication that ST influences the link between obesity and allergic respiratory disease. There are several possible explanations for these findings. First, an increased number of mast cells in the white adipose tissue may not reflect an increased number of mast cells in the lungs, or active mast cells in one site of the body, e.g. in the white adipose tissue, do not reflect mast cell activation in other sites of the body. Second, even if mast cell numbers or activity in white adipose tissue is related to a tendency to a mast cell-driven asthmatic response, this would not necessarily be reflected by ST levels.

In conclusion, serum levels of mast cell tryptase increase with increasing BMI, indicating that either mast cell numbers or mast cell activity vary with body constitution. Further, it raises the hypothesis that mast cells

may be involved in the pathological mechanisms in obesity-related diseases. However, these obesity-related differences in ST levels did not appear to influence the association between BMI and allergic respiratory disease. Finally, we confirmed that ST is positively associated with increasing age, male sex and smoking, but inversely associated with alcohol intake.

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References

- Payne V, Kam PC: Mast cell tryptase: a review of its physiology and clinical significance. *Anaesthesia* 2004;59:695–703.
- Schwartz LB, Min HK, Ren S, Xia HZ, Hu J, Zhao W, Moxley G, Fukuoka Y: Tryptase precursors are preferentially and spontaneously released, whereas mature tryptase is retained by HMC-1 cells, Mono-Mac-6 cells, and human skin-derived mast cells. *J Immunol* 2003;170:5667–5673.
- Metcalfe DD: Mast cells and mastocytosis. *Blood* 2008;112:946–956.
- Schwartz LB: Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am* 2006;26:451–463.
- Guenova E, Volz T, Eichner M, Hoetzenecker W, Caroli U, Griesinger G, Burow G, Mitev V, Biedermann T: Basal serum tryptase as risk assessment for severe Hymenoptera sting reactions in elderly. *Allergy* 2010;65:919–923.
- Rueff F, Przybilla B, Bilo MB, Muller U, Scheipl F, Aberer W, Birnbaum J, Bodzenta-Lukaszyk A, Bonifazi F, Bucher C, Campi P, Darsow U, Egger C, Haeblerli G, Hawranek T, Korner M, Kucharewicz I, Kuchenhoff H, Lang R, Quercia O, Reider N, Severino M, Sticherling M, Sturm GJ, Wuthrich B: Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase – a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol* 2009;124:1047–1054.
- Meyer MC, Creer MH, McHowat J: Potential role for mast cell tryptase in recruitment of inflammatory cells to endothelium. *Am J Physiol Cell Physiol* 2005;289:C1485–C1491.
- Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, MacFarlane LA, Malen-St Clair J, Shi GP: Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat Med* 2007;13:719–724.
- Heikkilä HM, Trosien J, Metso J, Jauhiainen M, Pentikainen MO, Kovanen PT, Lindstedt KA: Mast cells promote atherosclerosis by inducing both an atherogenic lipid profile and vascular inflammation. *J Cell Biochem* 2010;109:615–623.
- Judstrom I, Jukkola H, Metso J, Jauhiainen M, Kovanen PT, Lee-Rueckert M: Mast cell-dependent proteolytic modification of HDL particles during anaphylactic shock in the mouse reduces their ability to induce cholesterol efflux from macrophage foam cells *ex vivo*. *Atherosclerosis* 2010;208:148–154.
- Kalesnikoff J, Galli SJ: New developments in mast cell biology. *Nat Immunol* 2008;9:1215–1223.
- Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, Sukhova GK, Wolters PJ, Du J, Gorgun CZ, Doria A, Libby P, Blumberg RS, Kahn BB, Hotamisligil GS, Shi GP: Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med* 2009;15:940–945.
- Chaldakov GN, Fiore M, Stankulov IS, Hristova M, Antonelli A, Manni L, Ghenev PI, Angelucci F, Aloe L: NGF, BDNF, leptin, and mast cells in human coronary atherosclerosis and metabolic syndrome. *Arch Physiol Biochem* 2001;109:357–360.
- Kervinen H, Kaartinen M, Makynen H, Palosuo T, Manttari M, Kovanen PT: Serum tryptase levels in acute coronary syndromes. *Int J Cardiol* 2005;104:138–143.
- Gonzalez-Quintela A, Vizcaino L, Gude F, Rey J, Meijide L, Fernandez-Merino C, Linneberg A, Vidal C: Factors influencing serum total tryptase concentrations in a general adult population. *Clin Chem Lab Med* 2010;48:701–706.
- Shore SA: Obesity, airway hyperresponsiveness, and inflammation. *J Appl Physiol* 2010;108:735–743.
- Shore SA: Obesity and asthma: possible mechanisms. *J Allergy Clin Immunol* 2008;121:1087–1093.
- Beutner DA: Obesity and asthma. *Clin Chest Med* 2009;30:479–488, viii.
- Hersoug LG, Linneberg A: The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? *Allergy* 2007;62:1205–1213.
- Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T: Smoking and the development of allergic sensitization to aeroallergens in adults: a prospective population-based study. *The Copenhagen Allergy Study. Allergy* 2001;56:328–332.
- Linneberg A, Nielsen NH, Madsen F, Frolund L, Dirksen A, Jorgensen T: Increasing prevalence of specific IgE to aeroallergens in an adult population: two cross-sectional surveys 8 years apart: the Copenhagen Allergy Study. *J Allergy Clin Immunol* 2000;106:247–252.

- 22 Kucharewicz I, Bodzenta-Lukaszyk A, Szymanski W, Mroczko B, Szmitskowski M: Basal serum tryptase level correlates with severity of hymenoptera sting and age. *J Invest Allergol Clin Immunol* 2007;17:65–69.
- 23 Bonadonna P, Perbellini O, Passalacqua G, Caruso B, Colarossi S, Dal FD, Castellani L, Bonetto C, Frattini F, Dama A, Martinelli G, Chilosi M, Senna G, Pizzolo G, Zanotti R: Clonal mast cell disorders in patients with systemic reactions to Hymenoptera stings and increased serum tryptase levels. *J Allergy Clin Immunol* 2009;123:680–686.
- 24 Haeberli G, Bronnimann M, Hunziker T, Muller U: Elevated basal serum tryptase and hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin Exp Allergy* 2003;33:1216–1220.
- 25 Sperr WR, El-Samahi A, Kundi M, Girschikofsky M, Winkler S, Lutz D, Endler G, Rumpold H, Agis H, Sillaber C, Jager U, Valent P: Elevated tryptase levels selectively cluster in myeloid neoplasms: a novel diagnostic approach and screen marker in clinical haematology. *Eur J Clin Invest* 2009;39:914–923.
- 26 Yan K, Salome C, Woolcock AJ: Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760–765.
- 27 Elberling J, Linneberg A, Dirksen A, Johansen JD, Frolund L, Madsen F, Nielsen NH, Mosbech H: Mucosal symptoms elicited by fragrance products in a population-based sample in relation to atopy and bronchial hyper-reactivity. *Clin Exp Allergy* 2005;35:75–81.
- 28 Linneberg A, Hertzum I, Husemoen LL, Johansen N, Jorgensen T: Association between alcohol consumption and aeroallergen sensitization in Danish adults. *Clin Exp Allergy* 2006;36:714–721.
- 29 Lee M, Sommerhoff CP, von EA, Zettl F, Fritz H, Kovanen PT: Mast cell tryptase degrades HDL and blocks its function as an acceptor of cellular cholesterol. *Arterioscler Thromb Vasc Biol* 2002;22:2086–2091.
- 30 Fukuda M, Ohkubo T, Katsuya T, Hozawa A, Asai T, Matsubara M, Kitaoka H, Tsuji I, Araki T, Satoh H, Higaki J, Hisamichi S, Imai Y, Ogiwara T: Association of a mast cell chymase gene variant with HDL cholesterol, but not with blood pressure in the Ohasama study. *Hypertens Res* 2002;25:179–184.
- 31 Sanchez-Chaparro MA, Calvo-Bonacho E, Gonzalez-Quintela A, Fernandez-Labandera C, Cabrera M, Sainz JC, Fernandez-Meseguer A, Banegas JR, Ruilope LM, Valdivielso P, Roman-Garcia J: Occupation-related differences in the prevalence of metabolic syndrome. *Diabetes Care* 2008;31:1884–1885.
- 32 Dugas-Breit S, Przybilla B, Dugas M, Arnold A, Pfundstein G, Kuchenhoff H, Rueff F: Serum concentration of baseline mast cell tryptase: evidence for a decline during long-term immunotherapy for Hymenoptera venom allergy. *Clin Exp Allergy* 2010;40:643–649.
- 33 Ludolph-Hauser D, Rueff F, Fries C, Schopf P, Przybilla B: Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to Hymenoptera stings. *Lancet* 2001;357:361–362.
- 34 Komarow HD, Hu Z, Brittain E, Uzzaman A, Gaskins D, Metcalfe DD: Serum tryptase levels in atopic and nonatopic children. *J Allergy Clin Immunol* 2009;124:845–848.
- 35 Min HK, Moxley G, Neale MC, Schwartz LB: Effect of sex and haplotype on plasma tryptase levels in healthy adults. *J Allergy Clin Immunol* 2004;114:48–51.
- 36 Reuter S, Heinz A, Sieren M, Wiewrodt R, Gelfand EW, Stassen M, Buhl R, Taube C: Mast cell-derived tumour necrosis factor is essential for allergic airway disease. *Eur Respir J* 2008;31:773–782.
- 37 Reuter S, Taube C: Mast cells and the development of allergic airway disease. *J Occup Med Toxicol* 2008;3(suppl 1):S2.