

Factors influencing serum total tryptase concentrations in a general adult population

Arturo Gonzalez-Quintela^{1,*}, Luis Vizcaino²,
Francisco Gude³, Jesus Rey⁴, Luis Meijide⁴,
Carmen Fernandez-Merino⁴, Allan Linneberg⁵
and Carmen Vidal⁶

¹ Department of Internal Medicine, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain

² Department of Biochemistry, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain

³ Department of Clinical Epidemiology, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain

⁴ Primary Care Unit, A Estrada, Pontevedra, Spain

⁵ Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark

⁶ Department of Allergy, Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain

Abstract

Background: Serum tryptase measurements are increasingly being used in clinical practice. However, there is a paucity of studies focused on factors that influence tryptase concentrations. We investigated the relationships between total tryptase concentrations and age, gender, atopy, alcohol consumption, smoking, and common metabolic abnormalities in adults.

Methods: This was a cross-sectional study of a random sample from an adult population (n=420, age 18–92 years, 45% males) from a single municipality. Serum total tryptase was measured using a fluoro-enzyme immunoassay.

Results: The median tryptase concentration was 5.1 µg/L (range, <1–30.7 µg/L). There was a significant (p<0.0001) continual increase in tryptase with age; the median concentrations were 4.0 µg/L in 18–30 years old and 6.6 µg/L in those >80 years. Female gender and heavy ethanol use were negatively and independently associated with tryptase concentrations. Tryptase was higher in individuals that were non-atopic, overweight, or had metabolic syndrome compared to individuals that were atopic, normal weight, or did not have the metabolic syndrome. However, these associations were attenuated after adjusting for age.

Conclusions: Serum total tryptase concentrations in adults are not significantly modified by atopy status, but may be

modified by lifestyle factors, such as alcohol consumption and gender. Serum total tryptase concentrations are particularly dependent on age.

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Introduction

Serum (or plasma) concentrations of total tryptase are increasingly used in clinical practice. Tryptase concentrations indicate the body burden of mast cells and are useful for the diagnosis and follow-up of clonal mast cell disorders, such as systemic mastocytosis and related diseases (1, 2). Tryptase is also a potential marker for other myeloid neoplasms (3). Tryptase is increased following mast cell activation and is therefore useful in the differential diagnosis of systemic anaphylaxis (1). Among patients that are allergic to Hymenoptera venom, increased tryptase concentrations indicate the risk for severe reactions such as, for example, occurring during immunotherapy (1, 4, 5) and may be a marker for an underlying clonal mast cell disorder (6).

Several studies have investigated serum tryptase in patients with Hymenoptera venom allergy (4–6), respiratory allergy (7, 8), and healthy individuals (3, 8–10). However, no studies have been performed on representative samples from the general population. Studies performed in unselected individuals from the general population may identify common factors that might be associated with tryptase concentrations, including age, gender, lifestyle factors, and common disorders such as atopy. The relationship between gender with serum tryptase concentrations is controversial (3, 9). Also, some studies using healthy controls reported no differences in tryptase between groups with different ages (10), while others reported lower tryptase in younger (<16 years) individuals compared to older subjects (3). Preliminary studies found no differences in serum tryptase between patients with allergic rhinitis and healthy controls (7). Furthermore, recent studies did not find any differences between atopic and non-atopic children (8). However, no large study has been performed in adults. Additional common factors that might influence immunological variables include lifestyle factors, such as alcohol consumption and smoking, kidney dysfunction, and obesity/metabolic syndrome, which is considered a chronic, low-grade, inflammatory disorder (11, 12). To the best of our knowledge, no study has comprehensively evaluated the potential influence of all these factors on serum tryptase concentrations, and their confounding effects. The

*Corresponding author: Dr. A. Gonzalez-Quintela, Department of Internal Medicine, Hospital Clinico Universitario, 15706 Santiago de Compostela, Spain
Phone: +34 981 951279, Fax: +34 981 950501,
E-mail: arturo.gonzalez.quintela@usc.es
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goal of the present study was to investigate the relationships between serum total tryptase concentrations and age, gender, atopy, alcohol consumption, smoking, and common metabolic abnormalities in a general adult population.

Materials and methods

Study population

This study took advantage of a survey of the general population in the municipality of A-Estrada (Spain). Detailed descriptions have been reported elsewhere (13, 14). Briefly, an age-stratified random sample ($n=720$) of the adult (>18 years) population of the municipality was obtained from the Health Care Registry, which included $>95\%$ of the population. A total of 469 individuals consented to participate in the study. The study was approved by the Institutional Review Board and conformed to the current Helsinki Declaration. A frozen serum sample (obtained under fasting conditions) for tryptase determination was available for 420 individuals. The study population was all Caucasian, with a median age of 55 years, range 18–92 years, and 45.0% were male.

Serum tryptase

Serum tryptase was measured with the ImmunoCAP Tryptase assay (Phadia, Uppsala, Sweden), which measures the total tryptase; i.e., all pro-forms and mature forms of β - and α -tryptase (1, 10). The reportable range is 1–200 $\mu\text{g/L}$. An upper reference value of 10 $\mu\text{g/L}$ is recommended by the manufacturer based on a study of 126 healthy individuals (aged 12–61 years; 48% males) (10). Some authors have adopted the 95th percentile value from that series (11.4 $\mu\text{g/L}$) as the upper reference level (5, 6), while others have adopted the upper 95% confidence limit for the mean (13.5 $\mu\text{g/L}$) from the same series (4). Other authors have suggested 15 $\mu\text{g/L}$ as the upper reference concentration (1, 3). In addition, tryptase concentrations $>20 \mu\text{g/L}$ are used as the diagnostic criterion for clonal mast cell disorders (2).

Atopy status

All participants underwent a panel of skin prick tests (SPT) to 13 aeroallergens that are common in the area including house dust mites (*Dermatophagoides pteronyssinus*), storage mites (*Lepydoglyphus destructor*, *Tyrophagus putrescentiae*), grass pollens (*Lolium perenne*), tree pollens (*Betula alba*), weed pollens (*Plantago lanceolata*, *Parietaria judaica*), molds (*Alternaria alternata*, *Aspergillus spp.*, *Penicillium notatum*, *Cladosporium herbarum*), and animal dander (dog and cat) (ALK-Abelló, Spain) (14). Individuals with at least one wheal ≥ 4 mm were considered SPT-positive or atopic (15). These allergens account for $>98\%$ of respiratory allergy cases in the area (unpublished observation). Mites are, by far, the most common sensitizers (14). Nasal and bronchial symptoms were investigated using the following yes/no questions: “have you ever had a problem with sneezing, or a runny nose, or a blocked nose when you did not have a cold or the flu?” and “have you ever had wheezing or whistling in the chest at any time in the past?” (14). Individuals with a positive history of nasal or bronchial complaints were considered symptomatic.

Serum total immunoglobulin E (IgE), an additional marker for atopy, was measured using a chemiluminiscent enzyme immunoassay (Immulite™, Siemens Healthcare Diagnostic Products, Llanberis, UK). Total IgE concentrations in this population were

previously reported elsewhere (13). Concentrations $>170 \text{ IU/mL}$ were considered abnormally increased (13).

Lifestyle factors

Alcohol consumption was evaluated as the number of standard drinking units [glasses of wine ($\sim 10 \text{ g}$), bottles of beer ($\sim 10 \text{ g}$), and spirits ($\sim 10 \text{ g}$)] regularly consumed per week. Individuals were classified as abstainers/occasional drinkers ($<10 \text{ g/week}$), light drinkers ($10\text{--}140 \text{ g/week}$) or heavy drinkers ($>140 \text{ g/week}$). Individuals that consumed at least one cigarette per day were considered smokers.

Metabolic abnormalities

Individuals were classified according to body mass index as normal-weight ($<25 \text{ kg/m}^2$), overweight ($25\text{--}30 \text{ kg/m}^2$), or obese ($>30 \text{ kg/m}^2$). Individuals were considered to have metabolic syndrome, according to the Adult Treatment Panel III (16), when at least three of the following criteria were present: abdominal obesity (waist circumference $>102 \text{ cm}$ in males or $>88 \text{ cm}$ in females); hypertriglyceridemia [serum triglycerides $\geq 150 \text{ mg/dL}$ (1.695 mmol/L)]; low levels of high-density lipoprotein-cholesterol [$<40 \text{ mg/dL}$ (1.036 mmol/L) in males or $<50 \text{ mg/dL}$ (1.295 mmol/L) in females]; high blood pressure ($\geq 130/\geq 85 \text{ mm Hg}$ or current use of anti-hypertensive medication use); and hyperglycemia [blood glucose $\geq 110 \text{ mg/dL}$ (6.1 mmol/L) or current use of anti-diabetic therapy]. Individuals were considered to have kidney dysfunction if their glomerular filtration rate was $<60 \text{ mL/min/1.73 m}^2$ as estimated with the prediction equation from the Modification of Diet in Renal Disease Study (17).

Statistical analyses

The Mann-Whitney U-test and the Jonkheere-Terpstra trend test were used to compare tryptase concentrations. Linear regression was used for multivariate analyses. For this purpose, tryptase concentrations (dependent variable) and IgE concentrations were $\log_{10}$ -transformed in order to normalize their distributions. Cases with undetectable tryptase concentrations were assigned an arbitrary value of 1 $\mu\text{g/L}$. All covariates were forced into the equation.

Results

The distribution of serum total tryptase concentrations in the population is shown in Figure 1. From a total of 420 individuals, 29 (6.9%) presented with tryptase $>10 \mu\text{g/L}$, 18 (4.3%) presented with concentrations $>11.4 \mu\text{g/L}$, and 2 (0.5%) presented with values $>20 \mu\text{g/L}$. These two individuals, a 71-year-old female and a 43-year-old male with concentrations of 20.2 and 30.7 $\mu\text{g/L}$, respectively, were asymptomatic and had no skin disease or recent allergic reactions. Six individuals (1.4%) showed undetectable ($<1 \mu\text{g/L}$) tryptase concentrations.

Serum tryptase concentrations increased steadily with age, from a median of 4.0 $\mu\text{g/L}$ in individuals 18–30 years old to a median of 6.6 $\mu\text{g/L}$ in those >80 years old (p for trend, <0.0001 , Figure 2). The increase in serum tryptase was statistically significant ($p<0.05$) in individuals above 50 years old (Figure 2). The age of 50 years was therefore used for stratification in some analyses.

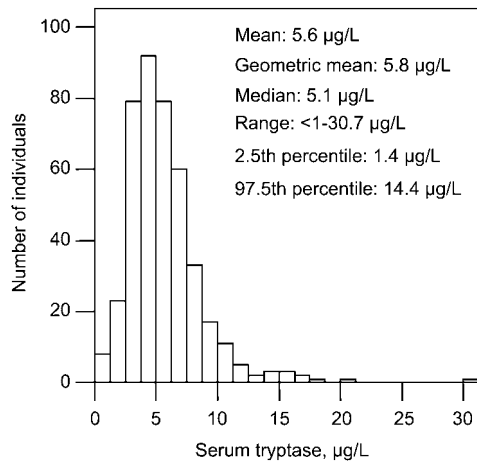


Figure 1 Histogram of serum total tryptase concentrations in the study population.

In order to calculate the geometric mean, cases with undetectable tryptase concentrations were assigned an arbitrary value of 1 µg/L.

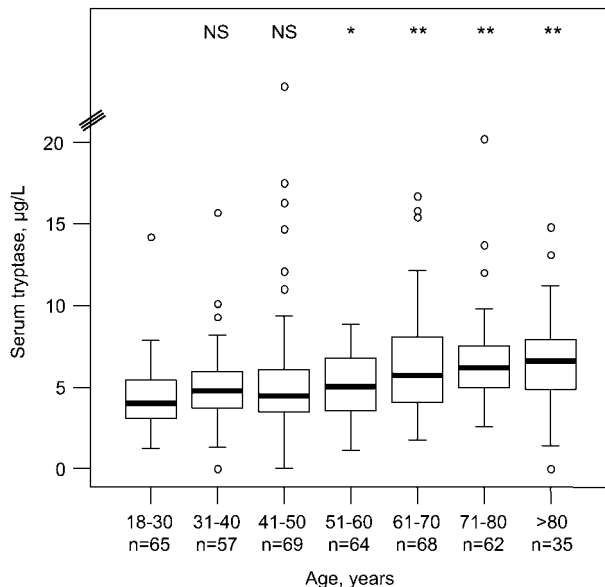


Figure 2 Serum total tryptase concentrations according to age strata.

Horizontal lines represent median values, boxes represent the interquartile range, whiskers represent the range, and dots represent extreme values (higher than the 75th percentile plus 1.5 times the interquartile range). The p-values (top of the Figure) reflect comparisons to the group of 18–30 years old. NS, not significant ($p > 0.05$); * $p < 0.01$; ** $p < 0.001$.

In univariate analyses (Table 1), serum tryptase concentrations were higher in non-atopic (SPT-negative) than in atopic (SPT-positive) individuals as a whole ($p = 0.01$). Among atopic individuals, there was no difference in serum tryptase between those that did and those that did not indicate respiratory symptoms (Table 1). The association between atopy and tryptase concentrations was attenuated after adjusting for age (Figure 3), because atopic individuals were younger than

non-atopic individuals (data not shown). Likewise, tryptase concentrations tended to be lower among individuals with high concentrations of serum IgE (Table 1), which were closely associated with atopic status (data not shown). The inverse association between IgE and tryptase concentrations was also attenuated after adjusting for confounders (Table 2).

Overweight and obese individuals had higher tryptase concentrations compared with individuals with normal weight. Similarly, individuals with metabolic syndrome had higher tryptase concentrations than those without metabolic syndrome (Table 1). However, the association of tryptase with these metabolic abnormalities was attenuated after adjusting for confounders, particularly age (Table 2). Gender and alcohol consumption were not significantly associated with tryptase concentrations in univariate analyses (Table 1). After adjusting for confounders, male gender showed a positive association and heavy drinking a negative association with tryptase concentrations (Table 2). Smoking showed no independent association with tryptase concentrations (Table 2). Kidney dysfunction, which was present in a small number of individuals, tended to be positively associated with tryptase concentrations, but the association was attenuated after adjusting for age (data not shown). The association of age with tryptase remained significant after adjusting for gender, atopy, alcohol consumption, smoking, and metabolic abnormalities (Table 2).

Discussion

This study included adults ranging from young to old individuals and showed that serum total tryptase concentrations are influenced by age. We found that individuals > 50 years old had median tryptase values $> 30\%$ higher than those in younger adults. Previous studies using smaller sample sizes did not report this phenomenon (3, 10). However, tryptase concentrations tended to be lower in healthy individuals < 16 years than in older subjects (3). Another study reported an increase in tryptase concentrations with age in patients allergic to Hymenoptera venom (5). In the present study, the effect of age was independent of potential confounders, including metabolic abnormalities and renal dysfunction. The reasons underlying this phenomenon are unknown, but could include an increased mast cell burden with ageing. This finding may be clinically important because tryptase is used as a diagnostic criterion (2) in disorders that present in distinct age groups.

Previous studies in healthy adults (3) and children that attended an allergy clinic (8) found slightly higher tryptase concentrations in males compared with females. However, that result is controversial because other studies found higher values in females than in males (9). Of note, estrogens can stimulate mast cells to release mediators, as recently reviewed (18). In our general adult population, male gender was associated with higher tryptase concentrations, but the effect was only evident after adjusting for confounders. Taken together, these results suggest that the effect of gender, if

Table 1 Serum total tryptase concentrations in relation to demographic variables, atopy, common habits, and metabolic abnormalities.

	No.	Median tryptase (25th–75th percentile), μg/L	p-Value ^a
Gender			
Female (reference)	231	5.0 (3.5–6.8)	0.23
Male	189	5.4 (3.9–7.0)	
Age, years			
18–50 (reference)	191	4.3 (3.2–5.9)	<0.0001
> 50	229	5.8 (4.2–7.5)	
Atopy status			
SPT-negative (reference)	309	5.3 (3.9–7.2)	0.05
SPT-positive, asymptomatic	41	4.7 (3.4–5.9)	
SPT-positive, symptomatic	70	4.6 (3.3–6.7)	
Serum total IgE, IU/mL			
< 170 (reference)	336	5.1 (3.7–4.2)	0.04
≥ 170	84	4.8 (3.3–6.1)	
Alcohol consumption			
Abstainers (reference)	195	5.2 (3.9–7.0)	0.91
Light drinkers	133	5.2 (3.5–7.4)	
Heavy drinkers	92	4.8 (3.2–6.5)	
Smoking			
Non-smokers (reference)	329	5.1 (3.8–7.1)	0.09
Smokers	91	4.7 (3.2–6.9)	
Kidney dysfunction			
Absent (reference)	409	5.0 (3.7–6.9)	0.07
Present	8	6.9 (4.9–7.9)	
Body mass index			
Normal weight (reference)	112	4.7 (3.2–6.5)	0.04
Overweight	180	5.2 (3.8–7.0)	
Obese	127	5.1 (4.0–7.2)	
Metabolic syndrome			
Absent (reference)	315	4.9 (3.5–6.7)	0.008
Present	105	5.5 (4.1–7.5)	

^aComparison with the reference category (Mann-Whitney U-test). SPT, skin prick tests; IgE, immunoglobulin E.

any, is probably small. Thus, gender should not be considered an important factor for interpretation of tryptase concentrations in routine clinical practice.

The question of the potential relationship between atopy and tryptase concentrations remains to be fully addressed. A previous study found no difference in tryptase among 62 patients with allergic rhinitis and 19 healthy controls (7). A recent study found no difference in tryptase among 153 atopic and 44 non-atopic children that attended an allergy clinic (8). The present study performed in an unselected adult population showed that atopy (SPT-positivity, with or without symptoms) was negatively associated with tryptase concentrations in univariate analyses, but the association attenuated after adjusting for age. Among these adults, age appeared to be the main determinant of tryptase concentrations. Furthermore, among individuals with atopy, serum tryptase concentrations were similar in those that reported and those who did not report respiratory symptoms. Likewise, serum total IgE, which is closely linked to atopy, was inversely associated with serum tryptase in univariate analyses. However, the association was attenuated after adjusting for confounders. Taken together, these results indicate that local mast cell activation is not reflected by tryptase concentrations in the

circulation, as previously suggested (7). This finding is clinically important for the interpretations of tryptase, a commonly requested assay in allergy clinics where the prevalence of atopy is high. Likewise, serum tryptase concentrations were similar in subjects with and without positive (but clinically irrelevant) IgE responses to Hymenoptera venoms (data not shown).

To our knowledge, no previous study investigated the relationships between tryptase concentrations and common lifestyle factors or metabolic abnormalities. Alcohol consumption has been shown to be associated with a number of immunological abnormalities (19), including high IgE concentrations and possibly allergic sensitization (13, 20). In the present study, individuals that consumed alcohol heavily tended to have lower tryptase concentrations compared with those that abstained or only occasionally consumed alcohol. This finding should be interpreted with caution because acetaldehyde (the main metabolite of ethanol) can induce mast cell degranulation in vitro (21). Metabolic syndrome includes abdominal obesity and a constellation of abnormalities related to insulin resistance (11), which in turn is related to inflammation, asthma, and allergy (12). Of note, adipose tissue from obese humans and mice contains more mast cells

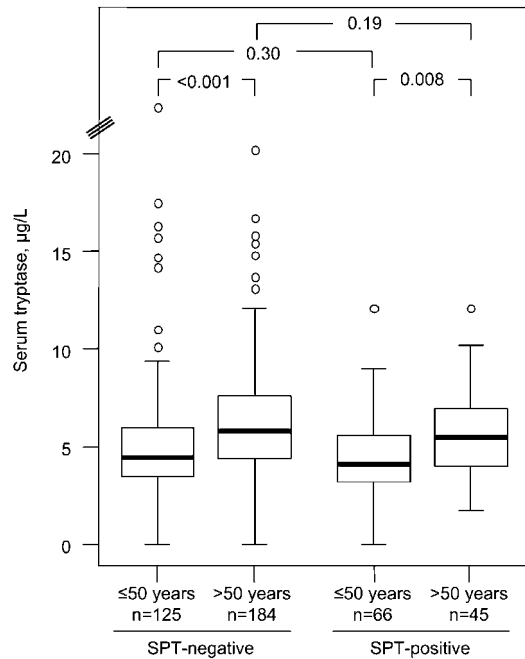


Figure 3 Serum total tryptase concentrations according to age strata and atopy (positivity of skin prick tests, SPT).

Horizontal lines represent median values, boxes represent the interquartile range, whiskers represent the range, and dots represent extreme values (higher than the 75th percentile plus 1.5 times the interquartile range).

than adipose tissue from their lean counterparts (22). In mice fed a Western diet a genetically induced deficiency of mast cells or a pharmacological stabilization of mast cells reduces body weight gain and markers of inflammation (22). This suggests that mast cells might play a role in obesity-related chronic diseases in Westernized societies. In line with this, our data showed that individuals that were obese and those with the metabolic syndrome tended to have higher tryptase concentrations than individuals without either condition. The association of metabolic syndrome with tryptase should be interpreted with caution because the statistical significance was marginal after adjusting for the confounding effect of age.

This study sample represented a general adult population that cannot be considered a reference population for the definition of normal reference ranges (23, 24). However, it should be noted that a sizeable proportion of adults with neither recent allergic reaction nor evidence of mastocytosis, the main causes for tryptase increases, presented with serum tryptase concentrations higher than the upper reference thresholds used in most studies (10 or 11.4 µg/L) (5, 6, 10). In fact, the 97.5th percentile observed in our series was closer to the 15 µg/L upper reference value that has been suggested by other authors (1, 3). In conclusion, the interpretation of serum tryptase concentrations in adults and studies designed to define reference tryptase values should take demographic factors into account, particularly age. Further studies are needed to investigate the associations between tryptase concentrations and common lifestyle and metabolic factors.

Table 2 Multivariate analysis (linear regression) of factors associated with serum total tryptase concentrations.

Covariates	Coefficient (slope)	Standard error	p-Value
Age, years	0.003	0.001	<0.0001
Gender			
Female	(reference)		
Male	0.057	0.024	0.01
Atopy status			
SPT-negative	(reference)		
SPT-positive	-0.013	0.028	0.65
Serum IgE (IU/mL, log ₁₀)	-0.025	0.022	0.25
Alcohol consumption			
Abstainers	(reference)		
Light drinkers	-0.029	0.024	0.24
Heavy drinkers	-0.090	0.031	0.003
Smoking			
Non-smokers	(reference)		
Smokers	0.023	0.029	0.43
Metabolic syndrome			
Absent	(reference)		
Present	0.043	0.025	0.08
Constant (intercept)	0.55	0.050	

Tryptase concentrations (dependent variable) and serum IgE concentrations were log₁₀-transformed in order to normalize their distribution. For that purpose, cases with undetectable tryptase concentration (n=6) were assigned an arbitrary value of 1 µg/L. All covariates entered the equation. Age was introduced in years and IgE was introduced in IU/mL; the remaining variables were introduced as “1=present or yes” and “0=absent or not”. Obesity was not introduced in the model due to its collinearity with metabolic syndrome. Kidney dysfunction was not introduced in the model due to the small number of individuals in that category. Data were available for all 420 individuals. The model explained 11.9% of the variability of serum tryptase concentrations (R², 0.119). SPT, skin prick tests; IgE, immunoglobulin E.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research granting played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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