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RESEARCH LETTER

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Tryptase reference values in a Swedish middle-aged general population and association with diabetes mellitus

To the Editor,

Mast cell (MC) tryptase is a tetrameric protease stored in the secretory granules in its active form and released upon strong activation signals which lead to degranulation. However, inactive monomeric pro-tryptases seem to be continuously secreted, and contribute to baseline serum tryptase.[1] The increasing use of serum tryptase (hereafter "tryptase") as a biomarker parallels the progress in our understanding of MC pathophysiology. Established as a biomarker of anaphylaxis and mastocytosis, tryptase is now accepted as a predictor of the risk and severity of allergic reactions.[1] Its value has also been suggested in conditions frequently affecting middle-aged subjects, such as atherosclerosis, metabolic syndrome and kidney disease.[1,2] Age-adjusted tryptase reference values are scarcely available, despite reports on the age-related increase in tryptase levels, especially after 50 years.[3,4] Here, we report on tryptase determinants in a general population cohort of middle-aged Swedes and establish reference tryptase levels in subjects free of such conditions.

The multicentric Swedish CArdioPulmonary bioImage Study (SCAPIS) consisted of randomly selected men and women aged 50–64. Participants gave their written and informed consent prior to filling in a comprehensive questionnaire (self-reported physician-diagnosed diseases) and undergoing extensive physical examination, lung function tests and blood sampling.[5] Tryptase was an add-on measurement in the Uppsala cohort, comprising 5036 subjects recruited between October 2015 and June 2018. Approval was obtained from the Regional Ethical Review Boards of Umeå (SCAPIS, 2010–228-31 M) and Uppsala (total tryptase determination for the present study, 2018–272).

Tryptase was measured by the ImmunoCAP total tryptase assay (Thermo Fisher Scientific) in 4915 subjects (121 missing samples).

Univariate analyses were performed with regard to known determinants of tryptase, mainly age, gender, body mass index (BMI), renal function[1,3] and different diseases (Table 1). In our cohort, male gender, older age, obesity (defined as BMI ≥ 30 kg/m^2), current smoking, hypertension, elevated creatinine (defined as >100 μM in men and >90 μM in women), diabetes mellitus (hereafter "diabetes") and chronic sinusitis related to increased tryptase levels. Other diseases or variables measured in the SCAPIS cohort were not related to variations in tryptase levels (Table 1).

False Discovery Rate (FDR) correction was done by the Benjamini-Hochberg method (Table 1). The findings for gender, hypertension and chronic sinusitis were not consistent after FDR adjustment. Next, a multiple linear regression model applied to variables with FDR-adjusted $p < .05$, that is, age, obesity, current smoking, creatinine and diabetes, showed that these were independently related with tryptase levels (not shown). Therefore, reference tryptase levels (median, 5th and 95th percentiles) were determined from 3057 SCAPIS participants free of tryptase-modifying conditions demonstrated in this cohort, that is, diabetes, obesity, elevated creatinine and current smoking. Using this approach, the median reference levels between 50 and 59 years were 5.41 μg/L for women and 5.64 μg/L for men, while between 60 and 65 years the corresponding levels were 5.67 μg/L for women and 5.84 μg/L for men (Table 2). These levels were higher than the 3.4 μg/L tryptase geometric mean provided by the manufacturer, underscoring the need for population-based reference values. The 95th percentile in each reference group was between 10.40 and 12.79 μg/L, also higher than most of the recent, updated values reported by the manufacturer and the literature: 8.20, 9.56 and 11.00 μg/L[3,6] (manufacturer's expected values https://dfu.phadia.com/Data/Pdf/5d23154289c2320348332cfd.pdf, https://dfu.phadia.com/Data/Pdf/5db0691d89c23208b8036f94.pdf). It is currently considered that more than 90% of people with tryptase levels greater than 11.4 μg/L carry excess α-tryptase alleles, a condition known as hereditary α-tryptasemia,[1] however, it is unknown whether age-related variations are present in people with this condition. Moreover, an estimated 1% of people with tryptase levels greater than 11.4 μg/L are affected by a myeloid neoplasm, which may be indolent and/or undiagnosed.[1]

Our findings confirm and complete a recent study on age-related tryptase variation in a Dutch cohort, which reported upper reference limits in the oldest (70 years and older) age group higher than the currently used 11.4 μg/L cut-off.[4] In addition, clinical data available for SCAPIS participants allowed us to assess tryptase determinants.

To our best knowledge, this is the first report on diabetes as an independent determinant of tryptase levels. Despite accumulating experimental data of MC involvement in the pathophysiology of diabetes, tryptase levels in patients with this condition have been

Christer Janson and Andrei Malinovschi: Shared senior authorship

seldom addressed and significant variations were not evidenced.[7] Our finding of a slight but significant increase in tryptase levels in middle-aged patients affected by diabetes, independently from cardiovascular, metabolic or renal conditions, is consistent with previous evidence of MC involvement in insulin resistance development in mice and the report of a positive association between tryptase and fasting insulin levels[2,8]. An association between pre-diabetes or diabetes with MC tryptase levels could not be demonstrated in previous studies from a Chinese team, possibly because of different trial design and laboratory methods, notably inclusion criteria focusing on diabetes epidemiology, age range including middle-aged and elderly subjects aged 55–75, and tryptase determination with an in-house ELISA.[7] In the SCAPIS age range, type 2 diabetes is more prevalent than type 1 diabetes. We speculate that the design of the

Key messages

- Serum tryptase was measured in a clinically characterized cohort of the Swedish general population aged 50–64.
- Cohort-derived reference values for this age group in the absence of tryptase-modifying conditions are provided.
- Diabetes mellitus is a novel, independent determinant for higher tryptase in the general population aged 50–64.

TABLE 1 Serum tryptase levels as a function of age, gender, lifestyle and clinical conditions

Variable	Yes	No	Univariate *p*-Value	FDR-adjusted *p*-Value
Female gender	**5.77 (5.69; 5.86) (2518)**	**5.91 (5.81; 6.01) (2397)**	**.045**	.14
Age ≥ 60 years	**6.05 (5.94; 6.16) (3179)**	**5.72 (5.64; 5.81) (1736)**	**<.00001**	**.0001**
Obesity (BMI ≥ 30 kg/m^2)	**6.07 (5.92; 6.22) (1044)**	**5.78 (5.70; 5.85) (3871)**	**.0004**	**.0020**
Current smoking	**6.28 (6.02; 6.55) (407)**	**5.80 (5.73; 5.88) (4182)**	**.0002**	**.0017**
Atopy	5.76 (5.63; 5.88) (1261)	5.86 (5.79; 5.94) (3643)	.16	.31
Asthma	5.66 (5.41; 5.91) (310)	5.85 (5.78; 5.92) (4306)	.16	.31
Allergic rhinitis	5.84 (5.69; 5.99) (1034)	5.81 (5.74; 5.89) (3487)	.75	.84
Chronic sinusitis	6.40 (5.92; 6.93) (103)	5.81 (5.74; 5.88) (4470)	**.0164**	.07
Heart infarction	5.75 (5.09; 6.49) (61)	5.84 (5.77; 5.91) (4549)	.77	.84
Angina pectoris	6.50 (5.40; 7.83) (28)	5.83 (5.76; 5.90) (4582)	.16	.31
Atrial fibrillation	5.82 (5.32; 6.38) (83)	5.84 (5.77; 5.91) (4527)	.96	.96
Heart failure	6.53 (5.32; 8.01) (19)	5.83 (5.76; 5.90) (4591)	.23	.41
Valvular disease	6.33 (5.65; 7.10) (30)	5.83 (5.76; 5.90) (4580)	.27	.44
Bypass/Percutaneous coronary intervention	5.67 (4.66; 6.89) (31)	5.84 (5.77; 5.91) (4579)	.69	.82
Arterial disease	5.18 (3.35; 8.03) (9)	5.84 (5.77; 5.91) (4601)	.38	.53
Intervention of aorta aneurysm	7.25 (2.98; 17.69) (4)	5.83 (5.77; 5.90) (4606)	.28	.44
Stroke	5.97 (5.38; 6.62) (51)	5.83 (5.77; 5.90) (4559)	.69	.82
High blood pressure	5.98 (5.83; 6.13) (1018)	5.80 (5.72; 5.87) (3592)	**.035**	.13
High cholesterol	5.86 (5.66; 6.06) (568)	5.83 (5.76; 5.91) (4042)	.84	.88
Diabetes mellitus	**6.65 (6.27; 7.06) (208)**	**5.80 (5.73; 5.87) (4402)**	**<.00001**	**.0001**
High creatinine (male > 100 μM, female > 90 μM)	**6.44 (6.05; 6.86) (364)**	**5.81 (5.74; 5.88) (4433)**	**.0003**	**.0019**
Chronic obstructive pulmonary disease	6.18 (5.36; 7.13) (41)	5.83 (5.76; 5.90) (4569)	.37	.53
Respiratory disease other than asthma or COPD	6.46 (5.88; 7.10) (59)	5.83 (5.76; 5.90) (4551)	.053	.15
FEV1 < 80% predicted	5.95 (5.49; 6.45) (122)	5.83 (5.77; 5.90) (4677)	.60	.79
FEV1/FVC < 0.70	6.01 (5.75; 6.27) (364)	5.82 (5.76; 5.89) (4433)	.16	.31

Note: Serum tryptase levels (μg/L) are presented as geometric mean and 95% confidence interval. Sample size is indicated. False-discovery rate (FDR) correction was done by Benjamini-Hochberg method. *p*-Values <.05 are denoted in bold font.

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 second; FEV1/FVC ratio, Tiffeneau-Pinelli index; FVC, forced vital capacity; μM, micromoles per litre.

TABLE 2 Reference values for serum tryptase according to age and gender

	50–59 years	60–65 years
All subjects ($n = 3057$)	5.51 (3.14; 10.40) ($n = 1984$)	5.74 (3.31; 11.82) ($n = 1073$)
Male subjects ($n = 1398$)	5.64 (3.13; 10.49) ($n = 925$)	5.84 (3.25; 11.70) ($n = 473$)
Female subjects ($n = 1659$)	5.41 (3.16; 10.40) ($n = 1059$)	5.67 (3.40; 12.79) ($n = 600$)

Note: Serum tryptase levels are expressed in micrograms per litre and presented as median (5th; 95th percentiles). *n*, sample size.

SCAPIS study, addressing a homogeneous population within a narrow age range matching type 2 diabetes onset, favoured its identification as a new determinant.

Another new finding is the association of higher tryptase levels and chronic sinusitis. MC involvement was described in endotype T2 of chronic sinusitis.[9] However, our finding was weaker and not consistent after adjustment for multiple testing (*p* value .07). Asthma, which is frequently associated with a T2 response, and atopy alike did not affect tryptase levels in our cohort, in line with reports from other cohort studies.[3]

As expected from previous literature reports, tryptase levels were higher in smokers, in obese participants and in people with elevated creatinine levels.[1,3] Our results on the association of diabetes and higher tryptase levels were however consistent after adjusting for current smoking, elevated creatinine and obesity.

The strengths of our study comprise the assessment of a large sample of general population with in-depth clinical characterization, focusing on a middle-age interval (50–64 years), performed within a short time frame (32 months between 2015 and 2018), and relying on the same, most recent tryptase assay version. The main weakness is the lack of screening for primary MC-related conditions, above all hereditary α-tryptasemia, which can be diagnosed using a digital droplet PCR method, as published in 2016,[1] when the SCAPIS study had already been designed and the sampling was ongoing (www.scapis.org). Finally, the Scandinavian make-up of the SCAPIS cohort can be viewed as a strength since it allows comparison with data from other national cohorts.

Taken together, we report here the determinants and reference values for tryptase in a middle-aged North European population. We showed that diabetes is a novel, independent determinant for higher tryptase levels, in keeping with MC involvement in systemic and local inflammatory processes. This finding is an incentive for further studies addressing MC involvement in diabetes pathophysiology and the possible use of tryptase as a diabetes biomarker. We demonstrated that the 95th percentile tryptase levels measured in people aged 60–64 free of tryptase-modifying metabolic and lifestyle conditions (obesity, smoking, high creatinine, diabetes) are greater than the 11.4 μg/L default upper reference level and provided reference values for men and women aged 50–59 and 60–64 respectively. These reference values should prove useful in the context of the expanding use of tryptase as a cardiovascular and metabolic biomarker, of tryptase genotyping for hereditary α-tryptasemia, and of better knowledge of tryptase increase in indolent myeloid neoplasms. Finally, the proposed reference values underscore the importance of performing paired tryptase sampling in systemic hypersensitivity reactions including anaphylaxis in middle-aged populations, given the prevalence of high baseline tryptase levels.

AUTHOR CONTRIBUTIONS

JV, AS, NR, MM, GP, JH, RM, CJ and AM participated in the study design, interpretation of the data, drafting the manuscript and approved the final version of the manuscript. JV, CJ and AM prepared the first draft of the manuscript. AM performed the statistical analysis. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CONFLICT OF INTEREST

AS, NR, RM and MM are employed by Thermo Fisher Scientific. JV reports speaker and consultancy fees in the past 5 years from Astra Zeneca, Meda Pharma (Mylan), Novartis, Sanofi, Thermo Fisher Scientific, outside the submitted work. The other authors declare no competing interests in relation to this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly

available due to privacy or ethical restrictions. More information can be found at http://scapis.org/

Joana Vitte[1,2]
Anders Sjölander[3]
Niclas Rydell[3]
Magnus Molin[3]
Gunnar Pejler[4]
Jenny Hallgren[4]
Robert Movérare[3,5]
Christer Janson[5]
Andrei Malinovschi[6]

[1]*Aix-Marseille Univ, IRD, MEPHI, IHU Méditerranée Infection, Marseille, France*
[2]*University of Montpellier, IDESP, INSERM UMR UA11, Montpellier, France*
[3]*Thermo Fisher Scientific, Uppsala, Sweden*
[4]*Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden*
[5]*Department of Medical Sciences Respiratory, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden*
[6]*Department of Medical Sciences Clinical Physiology, Uppsala University, Uppsala, Sweden*

Correspondence
Joana Vitte, IDESP – Institut Desbrest d'Épidémiologie et de Santé Publique Campus Santé, IURC, 641 avenue du Doyen Gaston Giraud, Montpellier 34090, France.
Email: joana.vitte@inserm.fr

ORCID

Joana Vitte https://orcid.org/0000-0002-3344-9408
Anders Sjölander https://orcid.org/0000-0002-6155-8505
Niclas Rydell https://orcid.org/0000-0001-5661-1343
Magnus Molin https://orcid.org/0000-0002-5859-4767
Gunnar Pejler https://orcid.org/0000-0002-6779-391X
Jenny Hallgren https://orcid.org/0000-0002-3685-5364
Robert Movérare https://orcid.org/0000-0001-6611-5036
Christer Janson https://orcid.org/0000-0001-5093-6980
Andrei Malinovschi https://orcid.org/0000-0002-4098-7765

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