

Letter to the Editor

Obesity is not linked to increased whole-body mast cell burden in children

To the Editor:

Obesity is clearly associated with insulin resistance and chronic low-grade inflammation.^{1,2} Macrophages appear to be especially important in this relationship because they infiltrate adipose tissue and produce a variety of inflammatory cytokines.¹ Exploring the contribution of other immune cells to the development of obesity, Liu et al³ described a role for mast cells in the development of obesity and diabetes in mice. Using genetically modified mice and pharmacologic stabilizers of mast cells, they demonstrated that mast cells and mast cell-mediated protease expression can promote the growth of white adipose tissue. Importantly, the idea that mast cells might function in a similar manner in human obesity was suggested by the finding of increased numbers of mast cells in human white adipose tissue from obese compared with lean subjects in their study. Furthermore, mean serum tryptase levels were higher in obese (13.1 ng/mL) than lean (7.7 ng/mL) subjects, as determined by using an in-house tryptase assay. We attempted to replicate these data by comparing serum tryptase levels in obese, overweight, and lean subjects from a pediatric population.

Because the serum total tryptase level, which is comprised primarily of α and β protryptases, seems to correlate with the whole-body burden of mast cells,⁴ we measured levels of this protein in subjects 8 to 18 years old recruited through newspaper advertisement for research participation. The cohort contained a diverse group of children and adolescents with and without obesity, impaired glucose tolerance, and/or diabetes mellitus (Fig 1, A, and see Tables E1 and E2 in this article's Online Repository at www.jacionline.org). Because the body mass index (BMI) is age and sex specific in children and teens,⁵ we divided the subjects based on the BMI percentiles by age and sex into those who were of healthy weight (BMI $\geq$ 5th to <85th percentile), overweight (BMI $\geq$ 85th to <95th percentile), and obese (BMI $\geq$ 95th percentile). Comparisons were made by using the nonparametric Kruskal-Wallis test because the data were not normally distributed after standardization to percentiles. No statistical difference in the median serum tryptase level was seen among the groups (3.1, 3.5, and 2.7 ng/mL, respectively; $P = .068$; Fig 1, B). Although the BMI varies with age and sex in pediatric patients, the raw values still accurately reflect total-body fatness⁵ and thus should directly correlate with the serum tryptase levels according to the data presented by Liu et al.³ Therefore to directly compare our results with those of Liu et al, lean subjects (BMI, <26 kg/m²) were compared with overweight (BMI, $\geq$ 26 but <32 kg/m²) and obese (BMI, $\geq$ 32 kg/m²) subjects; these data were normally distributed. Again, no statistical difference among the groups was observed by means of 1-way ANOVA ($P = .449$; see Fig E1, A, in this article's Online Repository at www.jacionline.org).

Because some of the subjects in the cohort demonstrated impaired glucose tolerance or overt type 2 diabetes mellitus, the above comparisons were repeated with data from only those subjects with normal glucose tolerance. Once again, no statistical difference was seen between lean, overweight, and obese subjects, as defined either by BMI percentile ($P = .138$, Kruskal-Wallis test; Fig 1, C) or by BMI alone ($P = .386$, ANOVA; see Fig E1, B).

Finally, multiple linear regression analysis was performed on the entire cohort to determine the association of multiple different obesity-related parameters (BMI, BMI percentile, age, race, height, weight, fat mass, percentage of body fat, waist circumference, hip circumference, waist/hip ratio, visceral adipose tissue, percent visceral adipose tissue, subcutaneous adipose tissue, percent subcutaneous adipose tissue, and total adipose tissue) with serum tryptase levels. The combined model was unable to significantly predict serum tryptase levels ($R^2 = 0.043$ and $P = .997$ for all subjects and $R^2 = 0.110$ and $P = .974$ for only subjects with normal glucose tolerance). Moreover, none of the individual parameters were found to significantly correlate with serum tryptase levels.

We did not perform histologic examination of the adipose tissue, and thus we cannot comment on the numbers of mast cells within the adipose tissue. It is quite possible that they are indeed increased, as in the Liu et al³ report. However, despite any possible increase in mast cell numbers in the adipose tissue, our data indicate that obese youth do not have a significantly increased whole-body mast cell burden that would manifest as an increased serum total tryptase level. There are several potential reasons for the conflicting results, the most obvious being the age difference of the subjects: in our study ages ranged from 8 to 18 years, whereas in the Liu et al³ report, ages ranged from 20 to 66 years. Another recent study involving subjects aged 15 to 69 years showed a positive correlation between BMI and serum tryptase level (median tryptase level, 4.7 ng/mL [BMI, <25 kg/m²], 5.2 ng/mL [BMI, 25-30 kg/m²], and 5.1 ng/mL [BMI, >30 kg/m²]),⁶ albeit with a more modest effect than in the report by Liu et al.³ In that same study age was also found to correlate with serum tryptase levels, with greater than 3 times the standardized effect of BMI. However, the link between obesity and chronic low-grade systemic inflammation has been established in children and adults, with similar immunologic mechanisms and cytokine profiles found in both populations.^{7,8} Therefore one should expect that similar mechanisms for mast cell recruitment and activation should be present in both age groups, although this does not seem to be the case.

Another potential cause for the difference in findings is unintentional selection bias. Although Liu et al³ attempted to exclude those with evidence of infection or inflammatory disease, the significantly increased serum tryptase levels seen in the obese group (up to 73 ng/mL) suggest that 1 or more of the subjects might have had an undiagnosed clonal mast cell disorder, such as systemic mastocytosis.⁹ We would also note that the tryptase assay used by Liu et al³ has been used in a limited number of studies and thus might not be well characterized or validated in a clinical setting. Indeed, the mean serum tryptase level for lean subjects in their study appears to be substantially higher than the mean level in healthy subjects, as established by other groups (3.8 ng/mL) using a commercially available assay (see www.phadia.com/en/Health-Care-Providers/Allergy/Products1/ImmunoCAP-Tryptase/). Finally, although the differences in median tryptase levels among patients in our cohort grouped by BMI percentile did not meet statistical significance, they indeed approached it, and thus one could argue that our study lacks sufficient power to pick up obesity-related differences in serum tryptase levels. However, the median tryptase level for the obese group was lower than that of

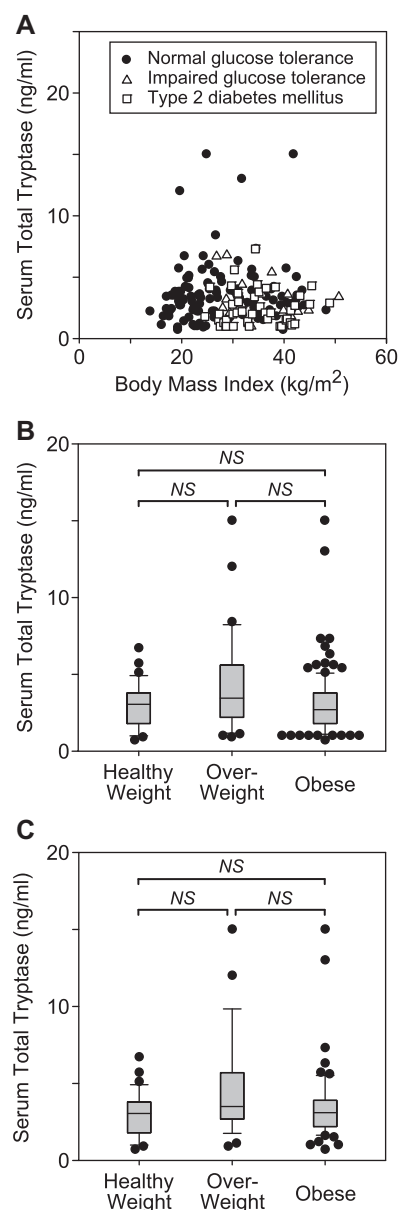


FIG 1. Serum total tryptase levels in healthy-weight, overweight, and obese pediatric patients. **A**, Serum total tryptase levels were measured in a cohort of pediatric patients with varying degrees of body fat, as approximated here by BMI. Subjects with normal glucose tolerance, impaired glucose tolerance, and overt type 2 diabetes mellitus are indicated by *solid circles*, *open triangles*, and *open squares*, respectively. **B**, Serum total tryptase levels were compared among all healthy-weight ($n = 34$), overweight ($n = 26$), and obese ($n = 117$) subjects, as defined by BMI percentile. **C**, Serum total tryptase levels were compared among only those healthy-weight ($n = 34$), overweight ($n = 21$), and obese ($n = 59$) subjects, as defined by BMI percentile, with normal glucose tolerance. Box plots (Fig 1, B and C) demonstrate the medians and 10th, 25th, 75th, and 90th percentiles, with all outliers (those data outside the 10th through 90th percentiles) plotted as *solid circles*. NS, No significant difference.

both the healthy-weight and overweight groups and not higher. Thus additional power would be unlikely to support a positive correlation between tryptase levels and obesity in our study population.

In their report Liu et al³ provided an elegant argument for the role of mast cells in murine obesity and diabetes. Because inflammatory mechanisms are known to be associated with obesity and insulin resistance in human subjects, the stated implication of their study was that mast cells might have a similar role in human and murine obesity. We agree that the potential role for mast cells in human obesity and diabetes is intriguing, but using serum tryptase levels to show an increase in total-body mast cell burden in children with increased body fat is not supported by our data. Clearly, additional studies are needed to fully establish and characterize the potential relationship between mast cells and obesity in human subjects.

Brant R. Ward, MD, PhD^a

Silva A. Arslanian, MD^b

Elisa Andreatta, MD^{b,c}

Lawrence B. Schwartz, MD, PhD^a

From ^athe Department of Internal Medicine, Division of Allergy and Immunology, Virginia Commonwealth University, Richmond, Va; ^bthe Division of Weight Management & Wellness and the Division of Pediatric Endocrinology, Metabolism, and Diabetes Mellitus, University of Pittsburgh School of Medicine, Pittsburgh, Pa; and ^cthe Department of Pediatrics, University of Verona, Verona, Italy. E-mail: lbschwar@vcu.edu.

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REFERENCES

1. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003;112:1796-808.
2. Ryden M, Arner P. Tumour necrosis factor- α in human adipose tissue—from signaling mechanisms to clinical implications. *J Intern Med* 2007;262:431-8.
3. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nat Med* 2009;15:940-5.
4. Schwartz LB. Diagnostic value of tryptase in anaphylaxis and mastocytosis. *Immunol Allergy Clin North Am* 2006;26:451-63.
5. Deurenberg P, Weststrate JA, Seidell JC. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. *Br J Nutr* 1991;65:105-14.
6. Fenger RV, Linneberg A, Vidal C, Vizcaino L, Husemoen LL, Aadahl M, et al. Determinants of serum tryptase in a general population: the relationship of serum tryptase to obesity and asthma. *Int Arch Allergy Immunol* 2011;157:151-8.
7. Roth CL, Kratz M, Ralston MM, Reinehr T. Changes in adipose-derived inflammatory cytokines and chemokines after successful lifestyle intervention in obese children. *Metabolism* 2011;60:445-52.
8. Maffei C, Silvagni D, Bonadonna R, Grezzani A, Banzato C, Tato L. Fat cell size, insulin sensitivity, and inflammation in obese children. *J Pediatr* 2007;151:647-52.
9. Valent P, Horny HP, Escibano L, Longley BJ, Li CY, Schwartz LB, et al. Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001;25:603-25.

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METHODS

Patient selection and characteristics

Pediatric patients were enrolled in a combined cohort as part of an ongoing National Institutes of Health–funded K24 “Insulin resistance in childhood” project at the Children’s Hospital, University of Pittsburgh Medical Center. Information regarding the patients’ age, race, height, weight, BMI, fat mass, percentage of body fat (with dual-energy x-ray absorptiometry), waist circumference, hip circumference, waist/hip ratio, visceral adipose tissue volume, percentage of visceral adipose tissue, subcutaneous adipose tissue volume, percentage of subcutaneous adipose tissue, and total adipose tissue volume (with computed tomographic scan) were collected, calculated, or both at the time of enrollment. BMI and anthropometric data were calculated by using standard formulas and techniques. Patients were categorized as lean, overweight, and obese based on both BMI percentile and BMI alone. For categorization by BMI percentile, lean subjects were defined as those with BMIs of greater than or equal to the 5th percentile but less than the 85th percentile. Overweight subjects were defined as those with BMIs of greater than or equal to the 85th percentile but less than the 95th percentile. Obese subjects were defined as those with BMIs of greater than or equal to the 95th percentile. BMI percentiles were calculated by using the reference charts published by the Centers for Disease Control and Prevention.^{E1} For categorization by BMI alone, lean subjects were defined as those with BMIs of less than 26 kg/m². Overweight subjects were defined as those with BMIs of greater than or equal to 26 kg/m² but less than 32 kg/m². Obese subjects were defined as those with BMIs of greater than or equal to 32 kg/m².

Tryptase measurement

Blood samples were collected after 10 to 12 hours of overnight fasting during research investigations for the above study. Sera from these samples

were then sent to Virginia Commonwealth University, where tryptase levels were measured with the Phadia ImmunoCAP Tryptase assay (Phadia, Uppsala, Sweden).^{E2}

Statistical analysis

Quantitative data for the groups defined by BMI alone were normally distributed, and thus comparison between these groups was performed by using standard ANOVA. Because these data were no longer normally distributed after conversion of BMI to percentiles, data for these groups were compared by using the nonparametric Kruskal-Wallis ANOVA test. Because no statistical differences were found in the serum tryptase levels among the groups, correction for repeated measures was unnecessary. Qualitative traits for each group, regardless of how the groups were defined, were compared by using the χ^2 test. Multiple regression analysis was performed by using multiple parameters (BMI, BMI percentile, age, race, height, weight, fat mass, percent body fat, waist circumference, hip circumference, waist/hip ratio, visceral adipose tissue volume, percentage of visceral adipose tissue, subcutaneous adipose tissue volume, percent subcutaneous adipose tissue, and total adipose tissue volume) as predictors of serum tryptase levels. All statistical analyses were performed by using SPSS Version 19 software (IBM Corp, Armonk, NY).

REFERENCES

- E1. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. *Adv Data* 2000;(314):1-27.
- E2. ImmunoCAP Tryptase: Quantifying total tryptase levels with ImmunoCAP Tryptase results in evaluation of risk for severe allergic reaction. Uppsala: Phadia AB; 2011. Available at: <http://www.phadia.com/en/Health-Care-Providers/Allergy/Products1/ImmunoCAP-Tryptase/>. Accessed September 7, 2011.

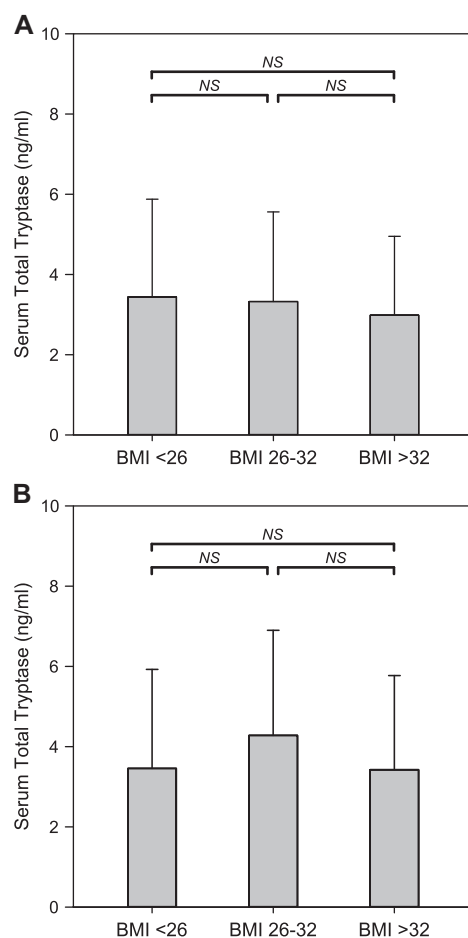


FIG E1. Serum total tryptase levels in pediatric patients stratified by BMI alone. **A** and **B**, Serum total tryptase levels were compared between subjects with a BMI of less than 26 kg/m², those with a BMI of greater than or equal to 26 kg/m² but less than 32 kg/m², and those with a BMI of greater than or equal to 32 kg/m². Comparisons were made among all subjects in the cohort (n = 58, 48, and 78 for lean, overweight, and obese subjects, respectively; Fig E1, *A*), as well as among only those subjects with normal glucose tolerance (n = 56, 20, and 39 for lean, overweight, and obese subjects, respectively; Fig E1, *B*). Bars indicate means $\pm$ SDs. NS, No significant difference.

TABLE E1. Clinical and biological parameters of patients by BMI percentile

Parameter	Lean* (median [range])	Overweight† (median [range])	Obese‡ (median [range])	P value§
No. of subjects	34	26	117	
Age (y)	13.2 (9.3-17.8)	13.0 (8.7-17.6)	14.6 (9.3-18.3)	.015
Sex (female/male)	18/16	20/6	47/70	.002
Race (white/others)	17/17	13/13	55/62	.931
Glucose tolerance (normal/impaired/type 2 diabetes)	34/0/0	21/1/4	59/16/42	<.001
BMI (kg/m ²)	19.8 (16.1-23.6)	24.4 (19.7-28.6)	34.4 (23.6-48.9)	<.001
BMI percentile	57.0 (11.0-84.3)	91.0 (85.0-94.8)	99.0 (95.0-99.9)	<.001
Height (cm)	159.1 (133-182)	158.4 (139-174)	166.4 (135-193)	<.001
Weight (kg)	49.8 (29-64)	62.2 (38-77)	97.4 (47-151)	<.001
Fat mass (kg)	10.3 (2.7-21.0)	20.5 (6.1-29.9)	39.1 (12.2-62.8)	<.001
Body fat (%)	22.1 (6.1-35.6)	35.2 (9.9-40.5)	42.7 (16.3-53.9)	<.001
Waist circumference (cm)	70.5 (60.0-83.0)	79.0 (65.0-96.0)	104.4 (76.0-142.5)	<.001
Hip circumference (cm)	85.0 (70.5-99.0)	93.5 (69.5-106.0)	113.0 (86.0-146.0)	<.001
Waist/hip ratio	0.8 (0.75-0.94)	0.9 (0.76-0.99)	0.9 (0.74-1.10)	<.001
Visceral adipose tissue (cm ²)	17.4 (5.6-49.1)	27.7 (13.8-87.0)	70.2 (14.7-242.4)	<.001
Visceral adipose tissue (%)	21.0 (6.9-62.5)	12.3 (6.3-29.5)	12.9 (4.6-50.0)	<.001
Subcutaneous adipose tissue (cm ²)	70.8 (7.0-242.1)	222.8 (38.5-413.2)	473.2 (84.2-875.8)	<.001
Subcutaneous adipose tissue (%)	79.0 (37.5-93.1)	87.7 (70.5-93.7)	87.1 (50.0-94.8)	<.001
Total adipose tissue (cm ²)	83.3 (18.8-268.8)	248.5 (54.6-443.7)	541.1 (104.5-990.1)	<.001
Serum tryptase (ng/mL)	3.1 (0.7-6.7)	3.5 (0.9-15.0)	2.7 (0.7-15.0)	.068

*Defined as a BMI of greater than or equal to the 5th percentile but less than the 85th percentile.

†Defined as a BMI of greater than or equal to the 85th percentile but less than the 95th percentile.

‡Defined as a BMI of greater than or equal to the 95th percentile.

§Significance for differences among groups. The χ^2 test was used for qualitative traits, and the Kruskal-Wallis test was used for quantitative traits.

TABLE E2. Clinical and biological parameters of patients by BMI

Parameter	Lean* (mean [range])	Overweight† (mean [range])	Obese‡ (mean [range])	P value§
No. of subjects	58	48	78	
Age (y)	13.1 (8.7-17.8)	13.9 (10.8-17.7)	14.9 (10.2-18.3)	<.001
Sex (female/male)	35/23	21/27	33/45	.087
Race (white/others)	30/28	21/27	36/42	.691
Glucose tolerance (normal/impaired/type 2 diabetes mellitus)	56/0/2	20/11/17	39/12/27	<.001
BMI (kg/m ²)	21.3 (13.9-25.6)	29.0 (26.1-31.8)	38.6 (32.0-50.7)	<.001
BMI percentile	71.5 (0.3-97.0)	96.9 (90.7-99.0)	99.2 (97.0-99.9)	<.001
Height (cm)	157.0 (133-182)	164.1 (135-193)	168.3 (152-191)	<.001
Weight (kg)	53.1 (29-76)	78.6 (56-118)	109.7 (81-151)	<.001
Fat mass (kg)	14.0 (2.7-29.9)	29.6 (12.2-45.6)	46.6 (26.1-62.8)	<.001
Body fat (%)	25.4 (6.1-40.5)	38.5 (16.3-49.5)	44.5 (24.9-53.9)	<.001
Waist circumference (cm)	73.6 (54.0-89.0)	92.5 (76.0-110.0)	113.1 (88.0-142.5)	<.001
Hip circumference (cm)	87.1 (68.0-106.0)	102.2 (86.0-115.0)	121.7 (96.0-146.0)	<.001
Waist/hip ratio	0.8 (0.75-0.99)	0.9 (0.76-1.10)	0.9 (0.74-1.08)	<.001
Visceral adipose tissue (cm ²)	23.9 (5.6-87.0)	53.4 (14.7-117.1)	86.4 (26.3-242.4)	<.001
Visceral adipose tissue (%)	18.5 (6.3-62.5)	13.0 (5.2-22.8)	13.6 (4.6-50.0)	<.001
Subcutaneous adipose tissue (cm ²)	137.0 (7.0-413.2)	357.7 (84.2-482.7)	580.4 (291.6-875.8)	<.001
Subcutaneous adipose tissue (%)	81.5 (37.5-93.7)	87.0 (77.2-94.8)	86.4 (50.0-94.3)	<.001
Total adipose tissue (cm ²)	159.0 (18.8-443.7)	441.2 (104.5-584.2)	668.3 (372.1-990.1)	<.001
Serum tryptase (ng/mL)	3.4 (0.7-15.0)	3.3 (1.0-13.0)	3.0 (0.7-15.0)	.449

*Defined as a BMI of less than 26 kg/m².†Defined as a BMI of greater than or equal to 26 kg/m² but less than 32 kg/m².‡Defined as a BMI of greater than or equal to 32 kg/m².§Significance for differences among groups. The χ^2 test was used for qualitative traits, and 1-way ANOVA was used for quantitative traits.