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Increased eosinophils in adipose tissue of metabolic syndrome

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ABSTRACT

Aims: Metabolic Syndrome (MetS) is a common global disorder that predisposes to both Type 2 diabetes mellitus (T2DM) and cardiovascular disease (ASCVD). Adipose tissue (AT) contributes significantly to increased inflammation and insulin resistance (IR) in MetS which appear to be the crucial underpinnings of MetS. Compared to macrophages and lymphocytes in human subcutaneous AT (SAT), there is sparse data on the role of other immune cells, especially eosinophils (EOS). In this study, we investigated the abundance of EOS in the SAT of 19 patients with MetS without diabetes, ASCVD, smoking or any inflammatory condition, and matched controls.
Methods: SAT EOS were quantified by immunohistochemistry.
Results: Both circulating and SAT EOS were significantly increased 2-fold in MetS and correlated with each other. Circulating EOS correlated significantly with triglycerides (TG), high-sensitivity CRP, leptin, and IL-6. SAT EOS correlated significantly with plasma glucose, TG, FFA, adipose-IR, leptin, IL-6, endotoxin, chemerin and inversely with adiponectin. They also correlated with SAT markers of fibrosis: collagen and Sirius red staining of SAT.
Conclusion: We make the novel and seminal observation that eosinophils are increased in SAT of MetS patients, and are associated with the pro-inflammatory state. Hence, in humans, they appear to contribute to the dysregulation of SAT biology in MetS.

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1. Introduction

Metabolic Syndrome (MetS) is a cluster of cardio-metabolic risk factors that predisposes the global population to both Type 2 diabetes mellitus (T2DM) and atherosclerotic cardiovascular disease (ASCVD).[1] While the pathophysiology has not yet been elucidated, both insulin resistance and increased inflammation appear to be important participants. In particular, monocyte/macrophages and adipose tissue (AT) have emerged as major contributors of metabolic dysregulation. Although many studies illustrate the role of macrophages and lymphocytes in human SAT, there is sparse data on other immune cells.[2] Previously, we cogently documented increased mast cells in subcutaneous AT (SAT) of patients with MetS without the confounding factors of diabetes, ASCVD and smoking. These mast cells were found to correlate with insulin resistance, increased inflammation and SAT fibrosis.[3]

The predominant role of eosinophils is in allergic reactions and mounting an immune response to parasitic infections. In 2011, the role of eosinophils in AT biology was reported. Studies on eosinophil-deficient mice on a high-fat diet showed AT eosinophils contributed to decreased body weight, improved insulin sensitivity, and activation of alternatively activated (M2) macrophages, promoting an anti-inflammatory function in AT.[4] Other groups have also confirmed in animal models that eosinophils have a role in glucose tolerance and energy expenditure.[5,6] Recently, a study in obese mice showed that increasing AT eosinophils had no significant benefit on improving AT inflammation, glucose tolerance, weight gain or insulin sensitivity.[7] Since there is a real paucity of data in human metabolic disorders, we investigated the status and role of eosinophils in SAT in patients with MetS without the confounding of T2DM, ASCVD or smoking.

2. Methods

Matched controls ($n = 15$) and MetS ($n = 19$) subjects (aged 24–72 yrs.) were recruited from Sacramento county, California using fliers and newspaper advertisements, and selected based on the Adult Treatment Panel III (ATP III) criteria for MetS as detailed previously.[1,3,8–10]

MetS patients fulfilled 3 or more of the ATP III criteria. Both control and MetS patients were tested for normal renal function using serum BUN and creatinine and albuminuria <30 mg/g creatinine. Exclusion criteria that were determined using a screening questionnaire and

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baseline chemistries included the following: diabetes defined by a fasting blood glucose level >125 mg/dL and HbA1c >6.4%, albuminuria >30 mg/g creatinine, clinical atherosclerosis including: coronary artery disease, peripheral vascular disease, and cerebrovascular disease; history of smoking, thyroid disorders including hyper- and hypothyroidism, inflammatory bowel diseases, use of anticoagulants, steroids, anti-inflammatory drugs, statins and other lipid lowering agents, hypoglycemic agents, angiotensin-2 receptor blockers, oral contraceptives, antioxidant supplements within the last 6 months, estrogen replacement therapy in postmenopausal women, >1 oz./day of alcohol; pregnancy, abnormal complete blood count, consumption of N-3 polyunsaturated fatty acids >1.0 g/d, recent surgery, inflammatory or malignant disease, leukocytosis, and human serum C-reactive protein (hsCRP) >10 mg/L.

Each participant signed an informed consent form and the study was approved by the Institutional Review Board at University of California (UC) Davis. Fasting blood and SAT biopsies was undertaken between 730 am and 930 am from each patient after taking a history and performing a physical examination. Blood was assayed by standard laboratory techniques for a complete blood count (CBC), plasma lipid and lipoprotein profile, urea nitrogen, creatinine, aspartate and alanine aminotransferases, glucose, thyroid-stimulating hormone (TSH) and high-sensitivity C-reactive protein (hsCRP). Insulin levels were measured using ELISA (Linco Biosystems) and homeostatic model of assessment-insulin resistance (HOMA-IR) was calculated from glucose and insulin levels and adipokines, cytokines, chemokines were analyzed by ELISA as described previously.[3,8–10] AT insulin resistance (Adipo-IR) was calculated as previously reported as the product of FFA and fasting insulin.[3]

SAT biopsy was performed on all subjects as detailed previously,[8] fixed in 10% buffered formalin after cleaning and processed for immunohistochemistry. As reported previously,[10] angiogenesis (CD31, VEGF) and fibrosis (collagen and Sirius red staining) in SAT were quantified by immunocytochemistry. The Combined Eosinophil-Mast cell (hematologic stain, ab150665, ABCAM) is intended for the simultaneous visualization of eosinophils and mast cells. Sections were deparaffinized and then stained with Vital New Red solution for 30 min, rinsed well in 2 changes of distilled water, counterstained with Hematoxylin, dehydrated, cleared and mounted with synthetic mounting media. The eosinophils stain bright red and were quantitated using manual counting and NIH Image Pro. Circulating eosinophils were quantified using the standard automated coulter counter, with at least 5 high power fields (hpf) were viewed for each patient.[9]

Results are expressed as mean and standard deviation (SD) for normally distributed data or as median and interquartile range for skewed variables. Log or square root transformations were applied to variables with skewed distributions prior to parametric analyses. Comparisons between the control and MetS groups were made with Wilcoxon Rank Sum test or 2-sample *t*-tests. Combining the control and metabolic syndrome groups, Spearman rank correlation coefficients were computed to assess the association between AT eosinophils and metabolic variables. Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC). Significance was defined as a p value <0.05.

3. Results

There were no significant differences in the age or gender distribution of the two groups. (Table 1). However, significant differences were found in the waist circumference (WC), plasma glucose, triglycerides (TG), HDL-cholesterol, FFA, HOMA-IR, Adipo-IR and levels of high-sensitivity (hs) CRP. Eosinophil counts were significantly increased in whole blood of MetS patients compared to controls ($p = 0.0225$). As portrayed in Fig. 1A, eosinophil abundance in SAT was significantly increased 2-fold in MetS patients compared to controls ($p = 0.005$). In Fig. 1B is representative staining of eosinophils. In a subgroup analyses of females only both circulating ($p = 0.04$) and SAT eosinophils ($p = 0.02$) were significantly increased.

Both circulating and SAT eosinophils were correlated with each other ($r = 0.40$, $p = 0.03$). Circulating eosinophils correlate positively and significantly with TG ($r = 0.36$, $p = 0.04$), hsCRP ($r = 0.46$, $p = 0.009$) leptin ($r = 0.51$, $p = 0.004$) and IL-6 ($r = 0.61$, $p = 0.001$).

As shown in Table 2, SAT eosinophils correlated positively with cardio-metabolic features including fasting glucose ($r = 0.42$, $p = 0.02$), TG ($r = 0.39$, $p = 0.04$), FFA ($r = 0.81$, $p = 0.0002$), Adipo-IR ($r = 0.64$, $p = 0.003$). The increase in biomarkers of inflammation and AT dysregulation have been reported previously in this cohort.[3,8–10] SAT eosinophils correlated positively with circulating levels of IL-6 ($r = 0.44$, $p = 0.04$), endotoxin ($r = 0.56$, $p = 0.03$), chemerin ($r = 0.57$, $p = 0.01$) leptin ($r = 0.40$, $p = 0.03$) and negatively with adiponectin ($r = -0.39$, $p = 0.048$). Furthermore, SAT eosinophils correlated with markers of fibrosis in SAT: collagen ($r = 0.42$, $p = 0.03$) and Sirius Red, ($r = 0.44$, $p = 0.01$).

4. Discussion

We make the novel observation of increased circulating and SAT eosinophils in patients with MetS without the confounding of diabetes, ASCVD, or smoking. Furthermore, the SAT eosinophils correlated with both cardio-metabolic features and biomediators of inflammation and adipose tissue dysregulation in MetS.[11]

Whilst eosinophils play a major role in allergy and parasitic infections they have also been incriminated in many other disorders.[6,12,13] However, their specific role in these other disorders is far from settled. They are phagocytic cells that possess numerous receptors that can mediate inflammation given their rich arsenal of biomediators including eosinophil peroxidase, major basic protein1/2,eosinphilic cationic protein, IL-6, etc.[12] Interestingly, circulating eosinophils are signaled to migrate to inflamed tissues via leptin secreted by adipocytes.[14] We show a

Table 1
Salient baseline characteristics of patients with metabolic syndrome and controls.

Variable	Controls ($n = 15$)	MetS ($n = 19$)	p-Value Controls vs MetS[a]
Sex, F/M	14/1	15/4	0.35
Age (yrs)	47 ± 14	53 ± 10	0.11
Waist (cm)	94 ± 14	112 ± 16	0.001
BP-systolic (mmHg)	124 ± 12	126 ± 113	0.71
Glucose (mg/dL)	87 ± 8	103 ± 13	0.0002
Total cholesterol (mg/dL)	186 ± 30	186 ± 29	0.69
Triglycerides (mg/dL)	83 (60, 97)	121 (94, 156)	0.001
HDL cholesterol (mg/dL)	51 ± 13	42 ± 13	0.04
HOMA-IR	1.9 (1.1, 2.9)	3.8 (2.4, 5.8)	0.005
hsCRP (mg/L)	2.3 (0.4, 4.0)	4.4 (1.7, 6.0)	0.03
Free fatty acids (mmol/L)	0.44 ± 0.19	0.78 ± 0.13	0.0008
Eosinophils, whole blood (K/mm^3)	0.16 ± 0.08	0.25 ± 0.13	0.0225

Results are presented as Mean ± standard deviation for normally distributed data or Median and (25th percentile, 75th percentile) inter-quartile range for skewed data.
[a] p-Value for sex from Fisher's Exact test, p-values for continuous variable from the Wilcoxon Rank Sum test.

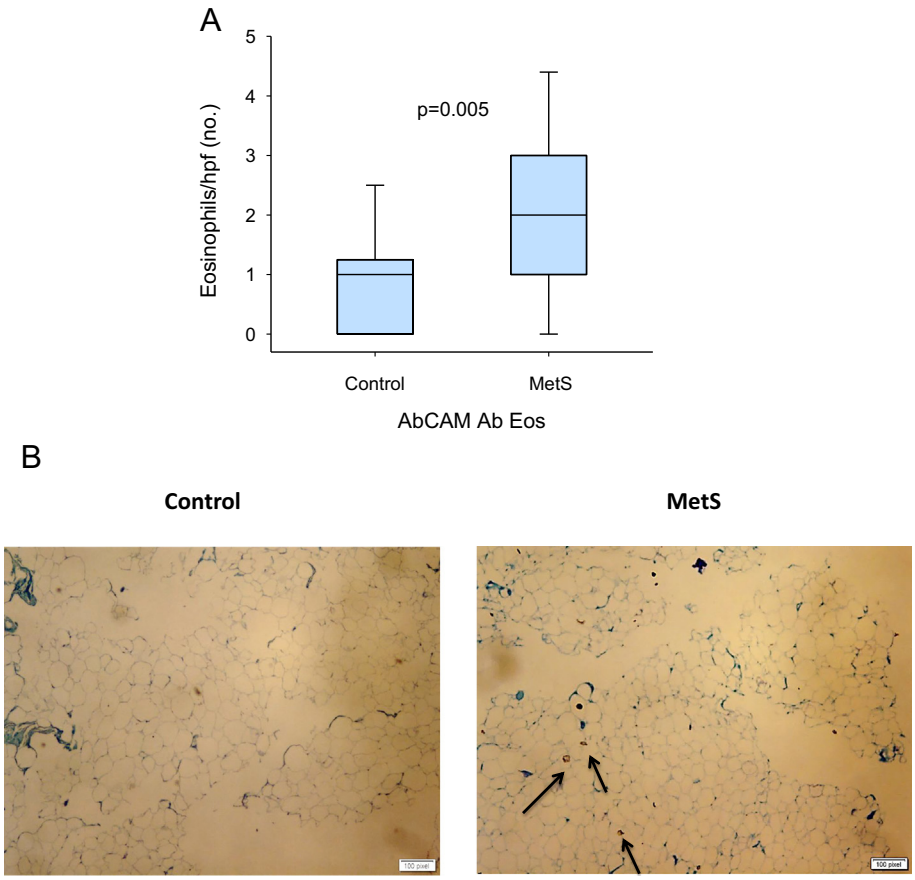

Fig. 1. A This figure depicts the density of eosinophils in SAT tagged with AbCAM antibody in MetS patients compared to controls. The lower and upper limits of the box indicate the 25th and 75th percentiles, the line within the box depicts the median, and the whiskers (error bars) below and above the box indicate the 10th and 90th percentiles. B In this figure is shown representative staining for eosinophils in SAT. Most of the eosinophils are shown by arrows.

significant correlation between leptin and both circulating and SAT eosinophils in this communication.

In 2011, Wu et al. showed an increase in AT inflammation, increased adiposity and impaired glucose tolerance in genetically eosinophil-deficient mice fed a high fat diet.[4] Inducing eosinophilia through parasitic infection, they showed that the increased eosinophils ameliorated the metabolic disturbances. Many groups have similar findings and the prevailing consensus is that eosinophils promote polarization of the alternatively-activated macrophages (AAM) in animal models.[4–6,15,16] These macrophages (AAMs/M2), in concert with group 2 innate lymphoid cells (ILC2s) and eosinophils, improve glucose tolerance, insulin sensitivity, promote beiging of fat to reduce obesity and its complications. While the role of eosinophil deficiency in mice is relatively consistent, the role of increasing eosinophils is far from clear, even in animal studies, as critically appraised recently.[16] Bolus et al. showed that restoration of AT eosinophils to the levels in lean mice using recombinant IL-5 failed to improve AT inflammation, glucose tolerance, lipid tolerance, mixed meal tolerance, energy substrate utilization and expenditure, insulin signaling, and beiging capacity of fat, in adult mice with normal development.[7] Furthermore, Brestoff et al. showed that IL-33 augmentation of ILC2s induced beiging that did not require eosinophils or IL-4.[17]

Gomes et al. identified IL-6 as a marker of fibrosis as it is secreted by both eosinophils and eosinophil-mediated fibroblasts[18] and we show a significant correlation between blood and SAT Eosinophils and IL-6. Hart et al. showed, in their model of non-alcoholic fatty liver disease (NAFLD) using interferon-gamma deficient mice fed a high fat diet, that there was successful induction of non-alcoholic steatohepatitis (NASH) with fibrosis.[19] Interestingly in their model there was an

Table 2
Spearman's correlation between SAT Eosinophil (ABCAM-Vital New Red stain) and relevant biomarkers.

	Glucose	TG	HOMA- IR	Adipo-IR	Leptin	Adiponectin	IL-6	Endotoxin
Spearman correlation coefficient	0.42	0.39	0.29	0.64	0.40	−0.39	0.44	0.56
Prob > |r| under H_0, Rho = 0	0.02*	0.04*	0.17	0.0033**	0.03*	0.048	0.04*	0.03*
N	29	29	24	19	28	26	23	15

	Free fatty acids	Chemerin	Collagen	Sirius Red
Spearman correlation coefficient	0.81	0.57	0.42	0.44
Prob > |r| under H_0, Rho = 0	0.0002***	0.01*	0.02*	0.02*
N	15	18	29	29

Notes.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

enrichment of eosinophils in the liver of the mice with fibrosis. They also confirmed this increase in eosinophils and markers of fibrosis in an amylin liver NASH model (AMLN). More importantly, in human liver biopsies, they showed the presence of eosinophils, speculating that they could be a biomarker of advancing NASH with fibrosis. However they did not correlate eosinophil infiltration with disease severity in their NASH samples nor did the compare with a non-NASH model of liver fibrosis. In their recent scholarly review, Bolus and Hasty provide us with an excellent update on the role of eosinophils in the dysregulation of AT biology and adiposity.[16] They state that animal models of eosinophil deficiency support a role for AT eosinopenia impairing glucose tolerance. However, they emphasize the caveats of increasing eosinophils in AT since most published models used IL-5tg mice, parasitic infections, the honey bee extract, propolis; these inducers have other effects that can account for the benefits.

There is a paucity of data on AT eosinophils in humans with metabolic disorders.[11] Hence, our novel findings of increased eosinophils in SAT of metabolic syndrome patients adds another wrinkle to the initial hypothesis that augmenting eosinophils is beneficial. In fact, in our carefully selected patients, we show that the SAT eosinophils correlate positively with circulating glucose, TGs, hsCRP ($r = 0.35$, $p = 0.066$), leptin, FFA, IL-6, endotoxin, chemerin, and Adipo-IR. Interestingly, our data showed an inverse relationship between AT eosinophils and adiponectin in MetS further support against an anti-inflammatory role in humans. Whilst correlations do not imply causality, we posit that, in the perturbed milieu of MetS, SAT eosinophils in concert with other immune cells such as mast cells, could be a contributor to MetS genesis and not be a protector as shown in mice studies. Furthermore, we showed a correlation with 2 measures of SAT fibrosis[10]; picrosirius red staining and collagen density arguing that they could also contribute to fibrosis in SAT, as with our previous report with the mast cells in SAT.[3] In fact, there was a significant correlation between mast cells and eosinophils in SAT ($r = 0.60$, $p = 0.0005$). Also our findings with fibrosis support that of Hart et al. in their model of NASH although they did not provide any correlations.[19] A study by Qin et al. demonstrated that priming eosinophils with oxidized-LDL resulted in a shift from M2 macrophages to M1 macrophages, suggesting that eosinophils are capable of being anti-inflammatory or pro-inflammatory depending on environmental stimuli and cues.[20,21] A possible explanation for our discrepant findings in humans compared to the studies in mice, is that the majority of our patients were females whilst in the mice studies in which gender is reported only male mice were studied. Hence the role of eosinophils in human metabolic disorders needs to be addressed in a serious and urgent fashion since the noxious meta-inflammatory milieu of MetS and diabetes could provide stimuli that can convert them to a pro-inflammatory phenotype as proposed recently supporting their plasticity.[15,20,21]

The increase in circulating eosinophils we report has been supported by some groups.[22–25] We showed significant correlations with TG, hsCRP, leptin and IL-6 suggesting they are also pro-inflammatory. Others have reported increased eosinophils in MetS with morbid obesity that correlated with impaired lung function[22] and also in obesity and MetS.[23,24] Furthermore, Fukui et al.[25] argued eosinophils may contribute to progression of diabetic retinopathy and diabetic nephropathy in men with T2DM, however Zhu et al.'s study on Chinese adults with T2DM revealed an inverse association between eosinophils and type 2 diabetes and insulin resistance.[26] The major focus of this report is the role of the increased eosinophils in SAT of MetS since our sample size does not allow us to speculate too much on the increased circulating eosinophils.

In conclusion, our study clearly advances the field with respect to the role of immune cells in AT of Metabolic Syndrome. We make the seminal observation in a human model that eosinophilia in SAT correlates with increased inflammation and do not appear to be anti-inflammatory as suggested in animal studies. However, we cannot comment on eosinophils in other adipose tissue beds such as visceral and omental AT since our study was confined to SAT. Further studies are urgently required in human models to confirm our novel contribution with respect to immune cells in adipose tissue dysregulation in human studies.

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All authors contributed to this submission (IJ and SD undertook the study and performed analyses and guided the manuscript to submission, BH undertook the statistical analyses, K M and PG researched the field, developed drafts and were mentored in writing this manuscript to final submission).

None of the authors have any conflict of interests.

Jialal is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
2. Singer K, Lumeng CN. The initiation of metabolic inflammation in childhood obesity. J Clin Invest 2017;127:65-73.
3. Gurung P, Moussa K, Huet B, Devaraj S, Jialal I. Increased mast cell abundance in adipose tissue of nascent metabolic syndrome: relevance to the pro-inflammatory state and increased adipose tissue fibrosis. American Journal of Physiology-Endocrinology and Metabolism 2019;316:E504-9.
4. Wu D, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 2011;332:243-7.
5. Moloksky AB, Nussbaum JC, Liang HE, et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J Experimental Medicine 2013;210:535-49.
6. Marichal T, Mesnil C, Bureau F. Homeostatic eosinophils: characteristics and functions. Front Med 2017;4:101-6.
7. Bolus WR, et al. Elevating adipose eosinophils in obese mice to physiologically normal levels does not rescue metabolic impairments. Mol Metab 2017;8:86-95.
8. Bremer AA, Devaraj S, Afify A, Jialal I. Adipose tissue dysregulation in patients with metabolic syndrome. J Clin Endocrinol Metabol 2011;96:E1782-8.
9. Jialal I, Huet BA, Kaur H, Chien A, Devaraj S. Increased toll-like receptor activity in patients with metabolic syndrome. Diabetes Care 2012;35:900-4.
10. Jialal I, Adams-Huet B, Major A, Devaraj S. Increased fibrosis and angiogenesis in subcutaneous gluteal adipose tissue in nascent metabolic syndrome. Diabetes and Metabolism 2017;43:364-7.
11. Jialal I, Devaraj S. Subcutaneous adipose tissue biology in metabolic syndrome. Horm Mol Biol Clin Invest 2018;33, 29353263.
12. Klion A. Recent advances in understanding eosinophil biology. F1000Research 2017:1084.
13. Chusid M. Eosinophils: friends or foe. J Allergy Clin Immunol Pract 2018;6:1439-44.
14. Wong CK, Cheung P-FY, Lam CWK. Leptin-mediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation. Eur J Immunol 2007;37:2337-48.
15. Knights AJ, Vohralik E, Hoehn K, Crossley M, Quinlan KGR. Defining eosinophil function in adiposity and weight loss. Bioessays 2018, https://doi.org/10.1002/bies.20180009830132936.
16. Bolus WR, Hasty AH. Contributions of innate type 2 inflammation to adipose function. J Lipid Res 2018, jlr.R085993, https://doi.org/10.1194/jlr.R085993.
17. Jr B, Bs K, Saenz S, et al. Group2 innate lymphoid cells promote beiging of adipose and limit obesity. Nature 2015;519:242-6.
18. Gomes I, et al. Eosinophil-fibroblast interactions induce fibroblast IL-6 secretion and extracellular matrix gene expression: implications in fibrogenesis. J Allergy Clin Immunol 2005;116:796-804.
19. Hart KM, Fabre T, Sciurba J, et al. Type 2 immunity is protective in metabolic disease but exacerbates NAFLD collaboratively with TGF-Beta. Sci Transl Med 2017:eaal3694.
20. Qin M, Wang L, Li F, et al. Oxidized LDL activates eosinophils polarize macrophages phenotype from M2 to M1 via CD 36 receptor. Atherosclerosis 2017;263:82-91.
21. Bolus WR. Diversity of adipose tissue immune cells: are all eosinophils created equal? Bioessay 2018, https://doi.org/10.1002/bies.20180015030216490.
22. Huisstede A, Castro-Cabezas M, Birnie E, et al. Systemic inflammation and lung function impairment in morbidly obese subjects with metabolic syndrome. Journal of Obesity 2013, 131349, https://doi.org/10.1155/2013/131349.
23. Kim DJ, Noh J, Lee B, et al. The association of total and differential WBCs with obesity, hypertension, dyslipidemia and glucose intolerance. J Korean Med Sci 2008;23, 1930198.
24. Ws S, Kim E, Kang, et al. The association of total and differential white cell count with metabolic syndrome in diabetes. Diabetes Res and Clin Pract 2006;73:284-91.
25. Fukai M, et al. Eosinophil count is positively correlated with albumin excretion in T2DM. CJASN 2009;4:1761-5.
26. Zhu L, et al. Eosinophil inversely associates with type 2 diabetes and insulin resistance in Chinese adults. PLoS One 2013;8, e67613.