

Defining Eosinophil Function in Adiposity and Weight Loss

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Despite promising early work into the role of immune cells such as eosinophils in adipose tissue (AT) homeostasis, recent findings revealed that elevating the number of eosinophils in AT alone is insufficient for improving metabolic impairments in obese mice. Eosinophils are primarily recognized for their role in allergic immunity and defence against parasitic worms. They have also been detected in AT and appear to contribute to adipose homeostasis and drive energy expenditure, but the underlying mechanisms remain elusive. It has long been recognized that immune cells such as macrophages respond to external signals to regulate adipose homeostasis and energy balance, however, less is known about the relevance of eosinophil activity in AT. As the authors propose in this review, given recent debate over the relative importance of their tissue-specific abundance, the stage is now set for exploring the functionality and activation states of AT eosinophils.

1. Introduction: The Importance of Immune Cells in Metabolism and Adipose Tissue

Obesity and its co-morbidities are increasing in prevalence worldwide, representing a considerable public health burden.^[1] Intensive research efforts have focused on the crosstalk between novel genes, molecules, pathways, and cell types controlling metabolism and energy homeostasis, in order to develop improved therapeutics.^[2,3] One such focus has been hematopoietic cells of the immune system, known for their diverse roles and activities. Cells of the immune system are highly responsive to external stimuli, receiving signals from the environment, reacting, and sending their own signals. Evolution has produced a diverse array of immune cell types that occupy all known tissue niches within the body. Their numbers and activities change in response to local or systemic cues. Adipose tissue (AT), the body's primary energy storage site, is one important niche in which immune cells, including macrophages, eosinophils, innate lymphoid cells (ILCs), and T cells reside (Figure 1).^[4] AT macrophages are the most well

characterized of these resident immune cells; two decades of research demonstrate their high plasticity and ability to influence AT homeostasis in different ways depending on their functional phenotype, or activation state.^[5] In contrast, less is known about other resident immune cells, besides the facts that their abundance changes in normal and disease states, and that they are also involved in secreting signaling molecules.

AT resident eosinophils have recently attracted attention since they have been reported to promote glucose homeostasis and energy expenditure.^[6] Obese mice have fewer eosinophils in AT than lean mice, and interestingly, a genetic mouse model lacking eosinophils displays exaggerated weight gain and glucose intolerance when fed a high calorie diet.^[6,7] On

the other hand, mice genetically predisposed to high eosinophil levels exhibit protection from diet-induced obesity and show improved glucose tolerance.^[6,8] But despite these thought-provoking advances, our current understanding of the role of eosinophils in AT remains in its infancy. The purpose of this piece is to synthesize recent findings linking eosinophils to AT function, with an emphasis on exploring normal and disease states. A greater understanding of eosinophil number and function in AT may reveal whether these cells represent druggable targets for the treatment of obesity and other metabolic disorders.

2. Eosinophils Have Been Proposed to Influence Adipose Homeostasis

When Wu et al.^[6] reported that eosinophils were the primary source of interleukin-4 (IL-4) in AT in 2011, their result catapulted a new cell type into the spotlight in the area of AT homeostasis. IL-4 was known to be the main driver of alternative macrophage activation, which is commonly associated with healthy AT, while classical macrophage activation is associated with inflammation and obesity. By utilizing an IL-4 reporter mouse,^[9] the group showed that the majority of IL-4-expressing cells in AT were eosinophils. They also provided the first functional evidence linking eosinophils to the regulation of adiposity by showing that mice lacking eosinophils due to the deletion of a GATA binding site in the GATA-1 promoter

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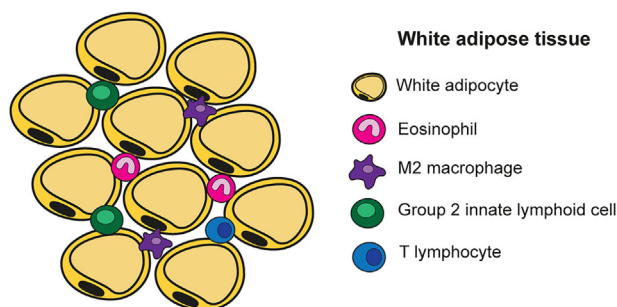


Figure 1. Immune cells reside within adipose tissue. Fat is a more dynamic organ than once believed, hosting a diverse array of resident innate and adaptive immune cells within its stromal vascular fraction. Major cell types include regulatory and helper T cells, M2 (alternatively activated) macrophages, eosinophils, and innate lymphoid cells. These cells are able to move in and out of adipose tissue in response to local and environmental signals, helping to regulate energy homeostasis, tissue repair, and immune surveillance.

(Δ dblGATA mice) had increased AT mass and reduced glucose tolerance compared to wild type mice fed a high-fat diet.

In the years since, other hematopoietic cells have entered the picture. Group 2 innate lymphoid cells (ILC2s) were shown to release IL-5, promoting eosinophil accumulation in AT.^[10] IL-33 was identified as a chief mediator of ILC2 activation, indirectly driving eosinophil expansion, but was also reported to directly increase eosinophil numbers in AT.^[11] Interest in the abundance of eosinophils was further invigorated with their implication in driving thermogenic activation of AT. White AT, where energy is stored in the form of lipids, can be converted to “beige” AT, which uses non-shivering thermogenesis to expend energy, offering a novel therapeutic opportunity for weight loss^[12]

(Figure 2). Exposure to cold temperature, a well-established activator of beige AT, leads to an increase in AT eosinophil abundance and simultaneously causes expansion of alternatively activated macrophages.^[13] Observations of increased eosinophil abundance are now well-documented, but the mechanisms underlying their increase remain ill-defined. Adding to the observation that ILC2-derived IL-5 encourages eosinophil expansion, the chemokine CCL11, also known as eotaxin-1 for its capacity to attract eosinophils, was shown to promote eosinophil recruitment following secretion by adipocytes.^[14] While it was found that CCL11 enhanced type 2 immune signaling through IL-4 expression, further insight into how this chemoattractant might influence genome-wide expression changes within AT eosinophils has yet to be elucidated. Fibroblast growth factor 21 (FGF21) is a metabolic regulator known to stimulate CCL11 secretion, and early clinical studies investigating the effects of an FGF21 analogue called LY2405319 have reported improvements in circulating triglycerides and body weight.^[15] Further to this, a type 2 cytokine signaling network in beige AT activation was uncovered by Qiu et al.^[16] using a combination of genetic knockout and reporter mouse models. This work suggested that eosinophils indirectly act on adipocytes via alternatively activated macrophages, leaving the possibility that eosinophils directly affect AT metabolism unexplored.

3. Increasing AT Eosinophil Numbers Alone Does Not Rescue Metabolic Impairments in Obese Mice

Recently, Bolus et al.^[17] elegantly demonstrated that artificially boosting the number of eosinophils in the AT of obese

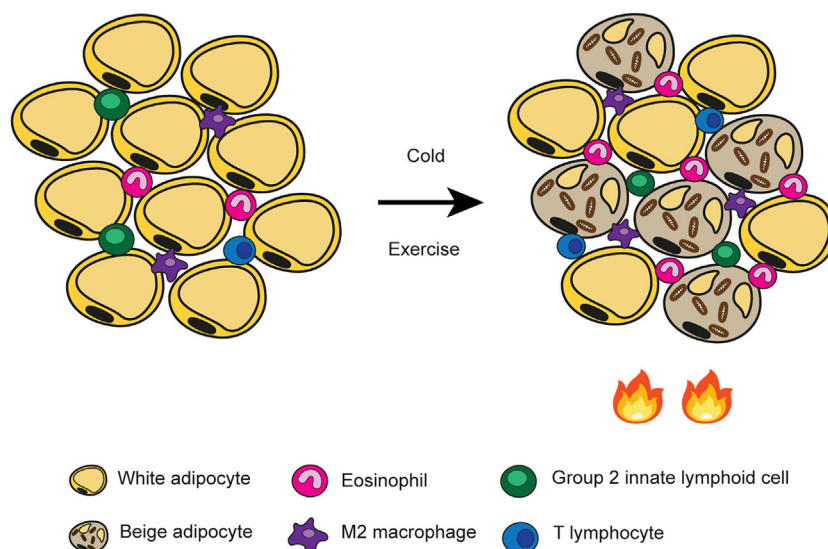


Figure 2. Activation of beige fat is accompanied by a change in adipose immune profile. Cold exposure and exercise are two archetypal instigators of beige fat activation in mammals, including humans. During activation, single large lipid droplets in white adipocytes transition into smaller, multilocular droplets, characteristic of beige adipocytes. This is accompanied by an increase in thermogenesis and mitochondrial biogenesis. Changes in the immune cell profile are also seen during recruitment of beige fat, with increases in the abundance of eosinophils and M2 macrophages, among other cell types. In addition to number, changes in the functional phenotype of these immune cells also appear to be of great importance, however, gaps remain in our current understanding.

mice to normal physiological levels did not improve metabolic parameters such as energy expenditure and insulin signaling. They administered recombinant IL-5 to mice on a normal chow diet or a high-fat diet twice weekly for 8 weeks. The effect of this treatment was restoration of eosinophils to physiologically normal levels in the AT of mice fed a high-fat diet, which would otherwise have had greatly reduced numbers of these cells. As increased eosinophil abundance has been inversely correlated with body weight and a host of metabolic impairments,^[6] the simple approach of restoring their numbers in AT was expected to ameliorate the glucose intolerance and weight gain associated with obesity. Surprisingly, elevating eosinophils in obese mice failed to restore any metabolic parameters to levels seen in normal healthy mice. A near-exhaustive suite of local and systemic metabolic assays were performed to elucidate how increased eosinophils might affect metabolic fitness. However, no differences were seen between vehicle- and IL-5-treated mice with respect to body mass composition, insulin signaling, glucose uptake, energy expenditure, and beige AT activation, among other parameters. These findings are particularly perplexing given that mice genetically engineered to overexpress IL-5 (IL-5tg mice) have elevated numbers of eosinophils and are known to exhibit protection from obesity, reduced AT mass, and improved glucose tolerance on a high-fat diet.^[6] The metabolic benefits seen in IL-5tg mice suggest that positive outcomes may only be manifested over a longer duration than an 8-week period of IL-5 administration, or may result from factors earlier in development. It should be noted however, that IL-5tg mice display substantially higher supraphysiological numbers of eosinophils compared to mice administered with IL-5, which may also explain differences observed in the metabolic phenotype.

In reporting these unexpected findings, the authors concluded that while their results do not invalidate a role for eosinophils in improving AT homeostasis, they do bring into question the simple assumption that increasing the number of eosinophils in AT will improve metabolic functions. The work challenges our early hypotheses about eosinophils in AT, suggesting that eosinophil abundance is not necessarily sufficient for conveying metabolic benefits. Instead, we are encouraged to consider both the number and the nature of AT eosinophils – their expression profiles, signaling networks, and secretomes.

4. The Importance of Number and Nature of AT Resident Eosinophils in Adipose Homeostasis

Is it time to re-evaluate our understanding of eosinophils in AT homeostasis? Significant progress has been made in terms of explaining how AT macrophages and lymphoid cells, like T helper cells and ILC2s, are activated in response to different biochemical signals. Despite several exciting findings, the literature on eosinophil function and activation is disproportionately lacking, providing a timely opportunity for researchers in the field to fill this void. It would be premature to rule out metabolic benefits stemming from

increased eosinophil abundance, but has the field's preoccupation with number prevented it from developing a greater appreciation for the complex crosstalk between resident eosinophils and their AT niche? We propose that while keeping number in consideration, we should expand our attention toward understanding the nature and activities of AT eosinophils. What external cues influence eosinophil activation in AT? How do eosinophils adapt their gene expression profile accordingly and how does this translate into changes to signaling networks and secreted factors for communicating with other cells in the tissue microenvironment? Do eosinophils in obese AT have a different molecular signature to eosinophils in healthy AT? These are just several lines of enquiry that will be pivotal to our understanding of these cells in regulating AT biology.

The ultimate goal of improving our understanding of immune cells in AT is to develop tailored therapeutic approaches for addressing obesity and other metabolic disorders. Rather than simply boosting or reducing numbers of specific immune cells in the AT, targeting their activity may prove more successful both in terms of minimizing side effects and in achieving the intended physiological outcomes – enhanced energy expenditure and weight loss.

5. Targeting Eosinophil Function and Activity for Metabolic Therapeutics

We have seen that administering IL-5 increases eosinophil numbers in AT, but without any measurable amelioration of metabolic impairments in the short term.^[17] Additionally, boosting eosinophil number may bring about unwanted side effects associated with eosinophilia pathologies.^[18] Given this, how can we target the activity of immune cells like eosinophils in AT to produce intended outcomes like weight loss, while simultaneously minimizing harmful side effects? Infection with parasitic worms, or helminths, has yielded some positive results for metabolic improvement. As opposed to simply elevating eosinophil abundance, helminth infection promotes more widespread type 2 immunity in AT, which confers metabolic benefits such as improved insulin signaling and reduced AT mass.^[6,19] These effects persist for at least 6 weeks following clearance of the worm infection in mice, suggesting that resident immune reprogramming is sustainable. It is interesting that as the developed world has seen a gradual decrease in helminth infections, a concomitant increase in allergic, autoimmune, and metabolic diseases has been witnessed^[20] – of course, there are numerous other societal factors that may be responsible for this increase too, such as dietary and lifestyle changes – but the co-evolution of humans and parasitic worms may have provided a stable metabolic environment that in the absence of worms results in increases in obesity and allergies. A concerted research and clinical effort is seeking to utilize helminth-derived factors as biologic therapeutics to treat metabolic and inflammatory diseases,^[20,21] and if the key components can be identified without resorting to live worms, the unwanted side effects and inevitable public apprehension over worm-derived treatments may be overcome.

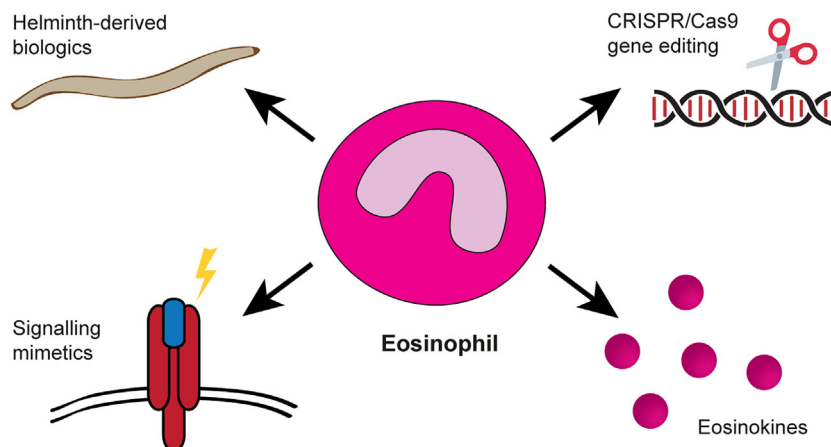


Figure 3. Eosinophils are potential therapeutic targets for metabolic disorders. Eosinophils present an attractive option for therapeutic intervention to promote energy expenditure and weight loss. Helminths are known to influence eosinophil abundance and activity, and trials using helminth-derived biologics are in progress. Advances in the versatility and efficacy of gene editing technologies like CRISPR/Cas9 present an alternative avenue for manipulating gene expression. Boosting levels of beneficial factors by gene therapy provides one possible strategy for promoting weight loss. Discovery and characterization of eosinokines, molecules derived from eosinophils, will open up new options for clinical translation. Modulating their secretion and activity, or mimicking their signaling through surface receptors may prove to be worthwhile therapeutic approaches.

It may also be possible to specifically target intrinsic eosinophil activity – to modulate their gene expression and secretion of beneficial factors. IL-4, produced by eosinophils in AT, is a well-characterized promoter of alternative macrophage activation and beige AT biogenesis. Intraperitoneal administration of IL-4 activates beige AT and increases oxygen consumption in cold-exposed mice;^[16] unfortunately however, IL-4 treatment unexpectedly promotes neural inflammation and can exacerbate weight gain on a high-fat diet.^[22] Direct infusion of recombinant signaling factors, despite some promise, has experienced drawbacks, such as reduced pharmacokinetics compared to endogenous counterparts, off-target effects, and suboptimal post-translational modification. The discovery that CD300f, a transmembrane surface receptor on AT eosinophils, negatively regulates IL-4 production^[23] may present a viable initial target for modulating eosinophil activity, for instance by administering a CD300f antagonist to boost IL-4 secretion. In addition to IL-4, eosinophils have also been proposed to secrete adiponectin,^[24] an archetypal insulin-sensitizing molecule. Identification and characterization of additional factors secreted by eosinophils, which we have termed “eosinokines,” may provide new avenues for the development of tailored therapeutics that target AT eosinophil activity directly. The discovery of eosinokines that specifically activate beige AT and regulate AT homeostasis will provide attractive avenues for therapeutic manipulation, whether via small molecule mimetics or targeting receptors of these eosinokines.

A recently pioneered unconventional approach involves micro-needle-mediated delivery of pharmacological agents directly into AT in mice, and is thus a potential method for delivery of eosinokines or bioengineered analogues into AT.^[25] Rapid developments in the field of CRISPR/Cas9 gene editing also present potential options for modulating eosinophil activity, owing to the fact that blood cells can be manipulated *ex vivo* and re-infused into hosts with relative ease compared to most tissue types.

6. Conclusions and Prospects

The exciting discovery that eosinophils play a role in driving AT homeostasis and energy expenditure opened up new approaches to therapeutic intervention for people suffering from metabolic diseases (summarized in **Figure 3**). A recent paper demonstrating that boosting eosinophil numbers in AT was insufficient for ameliorating metabolic impairments, has provoked reflection upon our current understanding of these cells in AT biology. In this piece we have highlighted the importance of considering both the *number* and *nature* of eosinophils – that is, building on our current knowledge to better define their functional activity and phenotype, spatial niche, gene expression signatures, and secretome profile under normal and obese conditions. Identification of novel eosinokines and characterization of their *in vivo* signaling networks and receptors will provide opportunities for more targeted manipulation of eosinophils in the hope of developing improved therapeutic strategies to address obesity and other metabolic sequelae.

Abbreviations

AT, adipose tissue; IL, interleukin; ILC, innate lymphoid cell.

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Conflict of Interest

The authors declare no conflict of interest.

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