Title: Perivascular adipose tissue – an immune cell metropolis

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SS, AMH and SW all were involved in the Conception or design of the work
SS. AMH and SW contributed to the interpretation of data for the work
SS and SW were involved with drafting of the work or revising it critically for important intellectual content

Abstract:

Perivascular adipose tissue is a heterogenous tissue which surrounds most blood vessels in the body. This review focuses on the contribution of eosinophils located within the adipose tissue to vascular contractility. High fat diet reduces the number of these immune cells with perivascular adipose tissue, and this loss is linked with an increase in vascular contractility and hypertension. We explored the mechanisms by which eosinophils contribute to this function using genetically modified mice, ex vivo assessment of contractility and pharmacological tools. We found that eosinophils contribute to adrenergic signalling, nitric oxide and adiponectin dependent mechanisms in perivascular adipose tissue. Exploring whether manipulation of these pathways in obesity can alleviate cardiovascular complications is now important to determine whether eosinophils are a valid target for obesity-related disease.

This is an Accepted Article that has been peer-reviewed and approved for publication in Experimental Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an Accepted Article; doi: 10.1113/EP087872.

New Findings

- **What is the topic of this review?**

  The review discusses how eosinophils can contribute to the function of perivascular adipose tissue and explores the mechanisms involved.

- **What advances does it highlight?**

  Understanding the communication between the cell populations which constitute perivascular adipose tissue function is important for exploring therapeutic options in the treatment of obesity-related cardiovascular complications. This article highlights that eosinophils are able to directly contribute to healthy perivascular adipose tissue function. These immune cells contribute to adrenergic signalling, nitric oxide and adiponectin dependent mechanisms in perivascular adipose tissue.

Perivascular adipose tissue:

Perivascular adipose tissue (PVAT) refers to the layer of adipose tissue which is common to almost all vessels, excluding those which contribute to the pulmonary and cerebral vasculature (Verlohren et al., 2004). Traditionally this tissue was considered to play a structural role, however it is now well accepted that perivascular adipose tissue plays a crucial role in regulating vascular tone through the secretion of signalling molecules, known as adipokines (Soltis & Cassis, 1991; Szasz & Webb, 2012).

Perivascular adipose tissue is heterogenous in cellular composition and in function, much of this is dependent on its location and reflects the physiological role pertinent to the tissue which the vasculature perfuses (Bulloch & Daly, 2014; Gil-Ortega, Somoza, Huang, Gollasch, & Fernandez-Alfonso, 2015). Adipose tissue can exist in different forms and these are distributed differently throughout the body. Adipose is classically defined as white or brown adipose tissue, the colour reflecting the number of mitochondria within the cell, and further non-classical categories of beige and pink, representing tissue which has undergone a phenotypic change from white to brown, or transformed into lobulo-alveolar glandular structures which produce milk, respectively. This has been reviewed recently in (Saxton, Clark, Withers, Eringa, & Heagerty, 2019). What is common to these different depots is twofold; first, these different types of adipose contain a diverse panel of immune cells, pre-adipocytes, sympathetic nerves and microvasculature (Lynes & Tseng, 2018). Second, these depots release an array of adipokines which elicit physiological effects throughout the body (Ahima & Flier, 2000).

For this brief article, we will focus on perivascular adipose tissue which surrounds mesenteric resistance arteries, i.e. those which are smaller than 200µm and play a key role in blood pressure regulation (Furness & Marshall, 1974; Heagerty, Aalkjaer, Bund, Korsgaard, & Mulvany, 1993). This adipose depot is classically white and all immune cells are represented in the depot, but numbers

and phenotype are known to be influenced by dietary intake. The Heagerty group demonstrated that this depot becomes dysfunctional in obese patients (Greenstein et al., 2009). In subcutaneous gluteal biopsies taken from healthy volunteers, vessels which were left with their surrounding adipose intact, showed a reduction in contractility in response to increasing of noradrenaline compared with a vessel without adipose tissue. Our comprehensive *ex vivo* studies, as well as those by others, identified that this depot elicited an anticontractile, or relaxant effect (Bussey et al., 2018; Greenstein et al., 2009; Lynch et al., 2013; Saxton et al., 2018). When the same experiments were performed in obese patients, there was no difference in contractility of vessels with or without perivascular adipose tissue present. This suggested that the relaxant effect was lost (figure 1). Yudkin and colleagues proposed that this loss of relaxant effect contributed to increased vascular resistance and subsequent impaired glucose delivery and insulin sensitivity (Yudkin, Eringa, & Stehouwer, 2005). The importance of perivascular adipose tissue in regulating blood pressure is further highlighted by the loss of its anticontractile function in the spontaneously hypertensive rat in the absence of any weight gain (Torok, Zemancikova, & Kocianova, 2016).

One of the proposed mechanisms by which obesity contributes to the loss of perivascular adipose tissue function is the hypertrophic remodelling of adipocytes. Chronic dietary excess results in expansion of adipose tissue, mainly through adipocyte hypertrophy as opposed to hyperplasia. It has been proposed, by our group and others, that the increased in adipocyte size exceeds the oxygen diffusion coefficient. As this is coupled with a shift towards secretion of anti-angiogenic factors from adipocytes, pockets of hypoxia may form which therefore drive a low-grade, chronic, inflammatory response. This is reflected by a shift in adipose tissue immune cells from an anti- to pro-inflammatory panel. There has been much work focussed on macrophages, perhaps due to their relative abundance compared with other immune cell populations. Studies have shown polarisation of M2 macrophages to the M1 phenotype following high fat diet, and increased recruitment and proliferation of macrophages due to high calorie diet is associated with insulin resistance. However, macrophages are considered as 'late' immune responders and their regulation is controlled by other immune cells.

Based on the observation by Wu and colleagues that high fat-fed mice develop insulin resistance and impaired glucose tolerance in the absence of eosinophils which is restored upon helminth-induced adipose eosinophilia, we questioned whether eosinophils played a role in mediating the anticontractile effect of perivascular adipose tissue. Our study published in 2017, a collaboration with Cruikshank, Else and Svensson-Frej (Withers et al., 2017), brought together expertise and animal models from immunology and cardiovascular physiology to dissect the role of eosinophils in elucidating the anticontractile effect of perivascular adipose tissue.

We found that a mouse model which was deficient in eosinophils mimicked the obese phenotype, specifically perivascular adipose tissue had no anticontractile effect and eosinophil deficient mice had increased blood pressure compared with healthy controls. These effects were independent of changes in the size of dendritic cells, neutrophil and monocyte populations and there were no changes in body weight. We were able to rescue this phenotype by reconstituting eosinophils from isolated spleens or bone marrow; the anticontractile effect of perivascular adipose tissue mimicked the normal, healthy response and blood pressure was restored to normal levels. Our observations add to the growing field of research which identifies roles of eosinophils beyond simply an 'anti-

parasitic' immune cell (Jacobsen, Helmers, Lee, & Lee, 2012), yet how these cells could function in this way was somewhat surprising.

Previous publications from the Heagerty group have identified adiponectin and NO (Bussey et al., 2018; Greenstein et al., 2009; Lynch et al., 2013; Saxton et al., 2018) as key signalling molecules in mediating the anticontractile effect of perivascular adipose tissue following adrenergic stimulation. Adiponectin is negatively correlated with body mass and we and others have demonstrated that its absence or inhibition diminishes the capacity of perivascular adipose tissue to evoke vascular relaxation (Aghamohammadzadeh et al., 2013; Almabrouk et al., 2018; Lynch et al., 2013). There is a wealth of literature which demonstrate insulin sensitising, anti-inflammatory and fatty acid oxidation properties of adiponectin, and these effects have been attributed to the activation of AMP-activated protein kinase (AMPK) and increased NO bioavailability (Shearer et al., 2004; Wong et al., 2011). Studies in experimentally hypertensive mice have also demonstrated that the AMPK activator, metformin can reduce hypertension via vascular relaxation. β_3-adrenergic receptor activation induces adiponectin exocytosis/secretion in cultured adipocytes (Komai et al., 2016) and our *ex vivo* data support the sympathetic driven release of adiponectin in mediating the anticontractile effect of perivascular adipose tissue (Saxton et al., 2018). We therefore explored whether eosinophils could contribute to any of these effects.

Reconstitution of eosinophils isolated from adiponectin and iNOS knockout mice into the eosinophil deficient mouse, implicated adiponectin in eosinophil mediated effects in perivascular adipose tissue. Some studies have shown that adiponectin can influence eosinophil recruitment (Amarsaikhan, Tsoggerel, Hug, & Templeton, 2019), and some immune cells are known to express adiponectin receptors (Luo & Liu, 2016), however we have yet to determine the precise relationship between adiponectin and eosinophils in our system. The use of wire myography and pharmacological tools further demonstrated that eosinophils elicited a NO dependent effect but were not a source of NO in perivascular adipose tissue themselves. This was somewhat surprising as eosinophils have been shown to generate NO-derived oxidants both *in vitro* and *in vivo* (MacPherson et al., 2001). Whether this is linked to the type of knockout we used, or whether this was because eosinophil reconstitution was performed in an otherwise healthy animal is unknown.

Our previous work identified β3 stimulation as an important mediator of the anticontractile capacity of perivascular adipose tissue (Bussey et al., 2018; Saxton et al., 2018). Although studies have indicated that some immune cells have the capacity to synthesise, store and release catecholamines (reviewed (Flierl, Rittirsch, Huber-Lang, Sarma, & Ward, 2008)), we examined whether eosinophils had this capacity. We identified that eosinophils express tyrosine hydroxylase, a rate-limiting enzyme involved in catecholamine synthesis and therefore, could act as a source of catecholamines. This observation was confirmed using ELISA and a pharmacological inhibitor of tyrosine hydroxylase, Alpha-methyl-p-tyrosine (Vaughan, Zarebidaki, Ehlen, & Bartness, 2014), which showed impaired eosinophil-induced relaxation. Using established inhibitors of adrenergic receptors, we determined that β3-adrenoceptors were key to the relaxation elicited by eosinophils. Although this is a novel observation, the interplay between eosinophils and adrenergic signalling is not unrecognised (Humphreys & Raab, 1950; Tachibana et al., 2002). Furthermore, eosinophils have been shown to release granule protein (EPO) which interacts with β2-adrenoceptors in the lung reinforcing the capacity for eosinophils to affect adrenergic signalling pathways (Motojima, Fukuda, & Makino, 1992).

Concluding remarks:

Our work has defined an important role for eosinophils in mediating the effects of perivascular adipose in health (Withers et al., 2017). The use of knockout animals and pharmacological tools has allowed us to identify, at least in part, the mechanism by which they function. However, understanding whether eosinophils can rescue the pathophysiological changes to perivascular adipose tissue following high fat feeding, is essential if we are to exploit any therapeutic potential of these immune cells. All the animals we used were fed normal diet. It is likely that under the challenge of high fat diet we will see other immune populations change and eosinophils may not be able to restore function. However, our unpublished, preliminary studies show that manipulation of eosinophil numbers in obese animals shows promise as novel targets for the development of therapies for obesity and obesity-related cardiovascular complications.

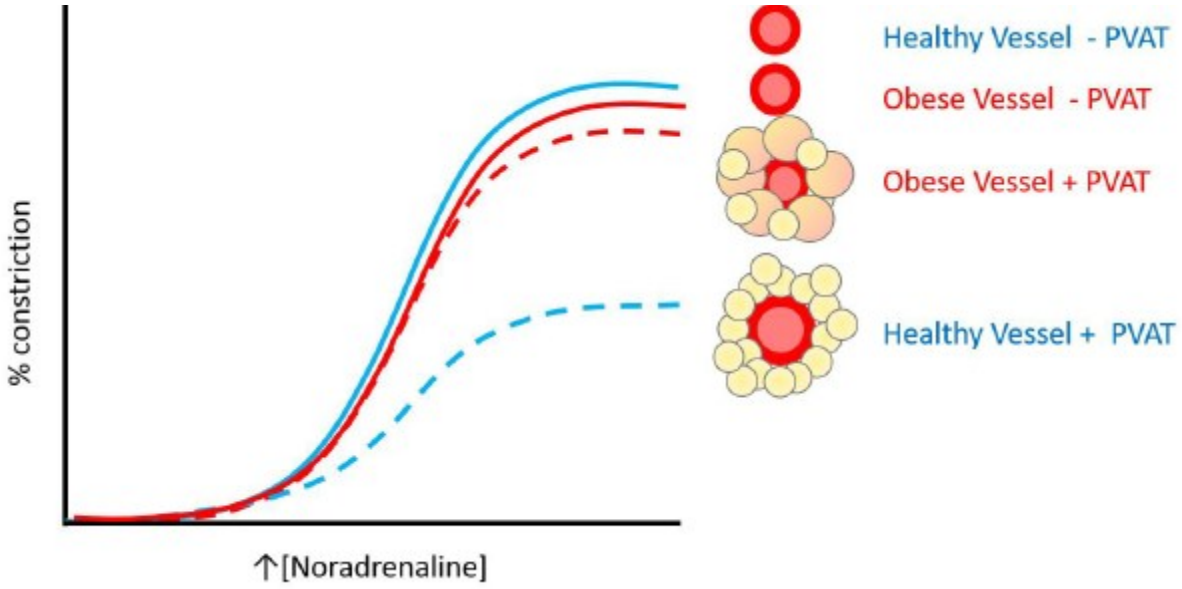

Figure 1. A schematic of the effect of perivascular adipose tissue (PVAT) on vessel contractility in response to noradrenaline. In health (blue), the presence of healthy PVAT (blue dashed line) has an anticontractile, or relaxant effect in response to increasing concentrations of noradrenaline compared with a healthy vessel without PVAT (blue solid line). This relaxant effect is lost in obese patients (red dashed line), and vessels contract to comparable level as their no PVAT counterparts (red solid line).

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