UNVEILING THE THERAPEUTIC POTENTIAL OF PERIVASCULAR ADIPOSE TISSUE (PVAT): A SOURCE OF VASOACTIVE COMPOUNDS

By

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ABSTRACT

The interplay between perivascular adipose tissue (PVAT), “the obesity triad” (hypoxia, inflammation, oxidative stress), and the resulting atherosclerosis is gaining a large body of knowledge, but many questions remain to be answered. The state of the adipose tissue surrounding vessels has a significant role in the health of the vascular tissue and cardiovascular function. PVAT is not simply a structure that holds vasculature in place, but rather an active endocrine tissue with many functions including a role in vascular tone and atherogenesis. Recently, there has been growing consensus with evidence to support that high cholesterol levels are not necessarily where the focus should be when addressing atherosclerosis and cardiovascular disease. Recent evidence suggests that an increase in the quantity of adipose tissue (vis-a-vis obesity) negatively affects the composition of PVAT and leads to increases in oxidative stress, decreased blood flow, and increases in immunological activity, increasing inflammatory mediators. These inflammatory mediators are likely more important in forming atherosclerotic plaques than cholesterol. More data-gathering is indicated to find more of those molecules and to understand more deeply how obesity degrades PVAT.

Keywords: Perivascular adipose tissue, white adipose tissue, brown adipose tissue, atherosclerosis, PCSK-9, atherogenesis, obesity, monoclonal antibody, inflammation

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INTRODUCTION

Perivascular adipose tissue (PVAT) has an essential role in the function of cardiovascular health as an active paracrine and endocrine organ, not simply a framework through which blood vessels course (Liang et al., 2020; Sena et al., 2017). PVAT is composed primarily of adipocytes, some of whose precursors are linked to vascular tissue and can differentiate to brown adipose tissue (BAT), which can convert to white adipose tissue (WAT) (Guimarães-Camboa and Evans, 2017; Liang et al., 2020; Qi et al., 2018). For example, one of several paracrine functions of PVAT is the reduction of contractility of blood vessels by the uptake of norepinephrine, in the physiologic state (Ahmad et al., 2019; Liang et al., 2020; Sena et al. 2017). On the other hand, dysfunctional PVAT and activated-associated inflammatory processes are known sequelae of obesity, diabetes, hypertension, and atherogenesis (Greenstein et al., 2009; Liang et al., 2020).

The composition of PVAT, i.e. the predominant adipose tissue type, is a prominent aspect of its potential to contribute to cardiovascular disease (CVD). In the presence of obesity or a high quantity of adipose tissue, BAT adipocytes, especially in visceral locations, differentiate into WAT, which in high quantities is harmful as it is associated with increased immune cell infiltration and, therefore, inflammation (Diamond et al., 2022; Koenen et al., 2021; Manrique et al., 2013; Piche et al. 2020,). Evidence shows that the browning of PVAT reduces hypertension in mice and rats (Kong et al., 2018; Persson et al., 2023). In addition, it is well known that BAT, which contains the adipocytes with thermogenic properties, is associated with less inflammation around vasculature (Becher et al., 2021; Cohade et al. 2003; Cypess et al., 2009; Koenen et al., 2021).

Inflammation precedes several diseases and dysfunctions, being an utmost important factor in the development and progression of atherosclerosis (Abifadel et al. 2003, Patriki et al., 2022). There are a myriad of mediators known to induce inflammation in vascular tissue (Abifadel et al., 2003; Patriki et al., 2022). One mechanism by which inflammation increases is through mediation by proprotein convertase subtilisin/Kexin 9 (PCSK-9) (Fan et al., 2022; Nakashima et al., 2002; Yanan et al., 2020). Research completed in 2003 indicated that a mutation in the PCSK-9 gene

is responsible for familial hypercholesterolemia. **Figure 1** describes the physiological role of PCSK-9 (Abifadel et al. 2003, Roth et al., 2014, Roth et al., 2018). The mechanism by which circulating PCSK-9 induces hypercholesterolemia is by reducing the LDL receptors in the liver. (Abifadel et al., 2003; Peterson et al., 2008). The important aspect is that this discovery spurred research into the role of PCSK-9 as a mediator of inflammation. A potential link between PCSK-9 and local vascular atherogenesis and is, due to reduced re-uptake of LDL, greater levels of LDL oxidation lead to immune response (Roth et al., 2014; Roth et al., 2018; Yanan et al., 2020). Atherogenesis is likely caused by a combination of vascular tissue and atherogenic lipoproteins and an inflammatory process mediated by the immune system and metabolic products (Fan et al., 2022; Nakashima et al. 2002). A body of evidence suggests that high circulating levels of PCSK-9 and inflammation have a strong impact on atherogenesis (Lamb et al., 2021; Patriki et al., 2022; Roth et al., 2014; Roth et al., 2018; Yanan et al., 2020).

With the advent of statins and its chief biomarker used in testing, low-density lipoprotein cholesterol (LDL-C), it was believed that LDL-C could cause atherogenesis and other vascular dysfunction (Diamond et al., 2022; Roth et al., 2014; Roth et al., 2018). However, recent evidence and systematic reviews demonstrate that LDL-C levels are a poor predictor of CVD and a diet lower in carbohydrates and lower calorie intake rather than lower LDL-C is a far better predictor, suggesting that inflammation and obesity are to blame (Diamond et al., 2022; Harcombe et al. 2015; Koenen et al., 2021; Persson et al., 2023; Roth et al., 2014; Roth et al., 2018). A new drug, inclisiran, was approved for use in the U.K. in 2020 in response to the successful use of monoclonal antibody drugs as an adjunct, or total replacement for statins in contraindicated patients, to treatment of atherosclerosis by explicitly targeting the PCSK-9 gene (Ali et al., 2021; Lamb et al., 2021). Inclisiran is an mRNA inhibitor (mRNAi) that reduces circulating levels of PCSK-9 by targeting the gene responsible for PCSK-9 rather than the protein itself (Lamb et al., 2021).

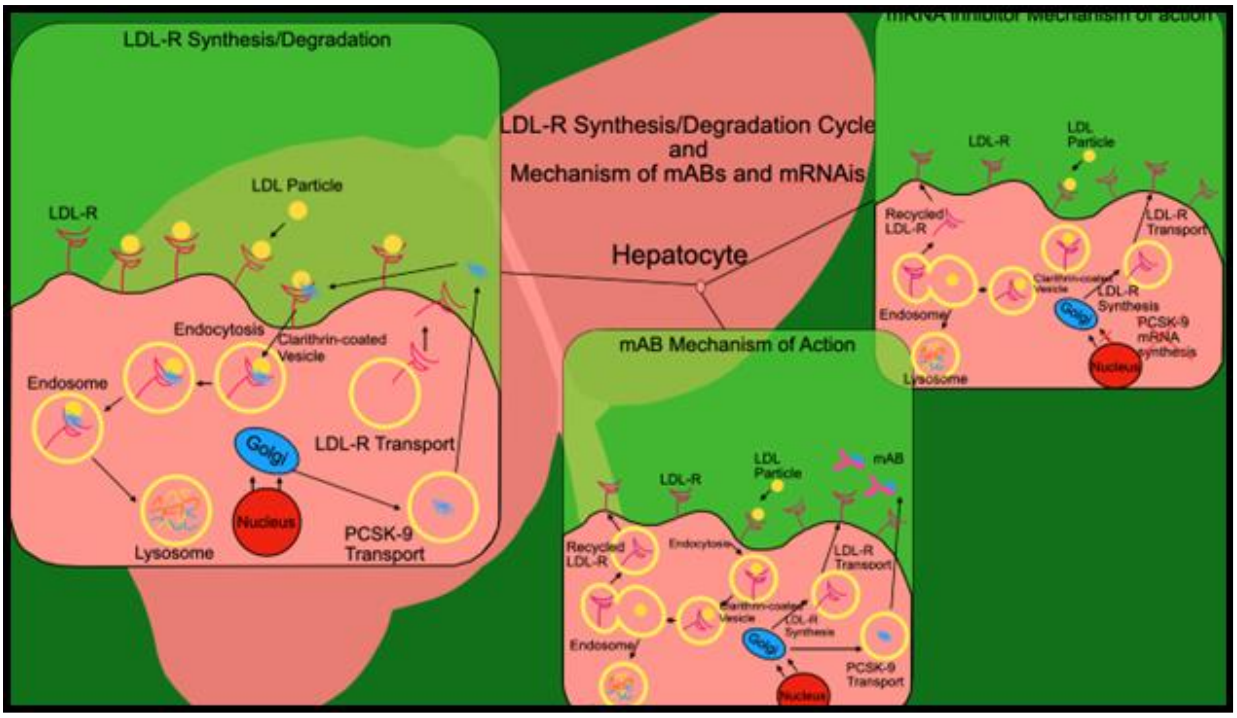

Figure 1: Physiological role of PCSK-9 and Mechanisms of mABs and mRNA inhibitors. PCSK-9 is an enzyme that plays a role in the degradation of LDL receptors. In the presence of LDL cholesterol and PCSK-9, the receptor is endocytosed and metabolized via lysosome action. In a physiological state, the enzyme helps to balance the need for cholesterol and the need to excrete it. In patients with gain-of-function PCSK-9 gene mutations, familial hypercholesterolemia results, while in patients with loss-of-function mutations, hypocholesterolemia is the outcome. When an LDL-R is not combined with PCSK-9 and an LDL-C particle binds it, the receptor is recycled. Monoclonal antibody drugs bind the PCSK-9 protein and it is phagocytose and excreted, resulting in the savage of the receptor. mRNA inhibitors block the synthesis of PCSK-9 and negate the downstream effects and the receptor is not compromised by the protein. (Adapted from Roth et al., 2014 and Roth et al., 2018)

Given the previously mentioned evidence and review literature, there is sufficient reason to suspect that the interplay of oxidative stress, and the downstream effects that cause PVAT dysfunction, are important players in forming atherosclerotic plaques. Dysfunctional PVAT may play a critical role in the induction of oxidative stress (in addition to the oxidative stress caused by immune activity in dysfunctional PVAT) and may add to our understanding of atherogenesis. Additionally, the origin and function of important molecules like PCSK-9 that have great influence on atherogenesis and need further investigation. There are gaps in the understanding of how mRNAi drugs, statins,

and MAB drugs reduce PVAT oxidation that may suggest the focus should shift from dyslipidemia and cholesterol to enhancing the treatment of inflammation. Finally, an important gap in the literature that needs to be addressed is the origin of the various types of tissue in PVAT.

This review aims to cover the above deliverables by identifying what is known and specifically where more data and research is needed. Initially, we present studies approaching the role of PVAT in CVD and atherosclerosis and its physiological role in vascular homeostasis. Subsequently, we bring data discussing PVAT dysfunction and obesity and the processes related with the whitening of BAT in PVAT and the downstream implications on endocrine and immune function. Finally, we discuss the current knowledge about PCSK-9 and its role in atherogenesis, as well as established and novel therapies.

METHODS

The articles used in this review were found in PubMed and Google Scholar. The literature review was conducted from August to September 2023. PVAT dysfunction-related literature were gathered using the following keywords: “Perivascular adipose tissue”, “white adipose tissue”, “brown adipose tissue”, “atherosclerosis”, “PCSK-9”, “atherogenesis”, “obesity”, “LDL cholesterol”, “HDL cholesterol”, “statin”, “inclisiran”, “diabetes”, “mRNAi”, “monoclonal anti-body”, “inflammation”, “hypertension”, and combinations thereof. Material was also gathered by reading titles cited in reviews found by searches. Inclusion criteria included selecting reviews, systematic reviews, clinical trials, cohort studies and cross-sectional studies. Additionally, all articles had to attempt to describe PVAT dysfunction and related sequelae or factors contributing to PVAT dysfunction. Exclusion criteria included journal reviews published before 2020, articles not written in English, non-peer review articles, and articles unrelated to PVAT and cardiovascular disease. Older studies were included as source material that supported findings in the various reviews.

PRIMARY LITERATURE SCREENING

During the initial search, no duplicate data entries were identified in either database (Google Scholar and PubMed). Review literature was the focus of the primary search. Articles with content related to PVAT, inflammation, atherosclerosis and obesity were retained; all others were discarded. Remaining articles and articles included as references were reviewed a second time to ensure that they addressed the main research question. No records were removed in the second screening process.

SECONDARY LITERATURE SCREENING

In order to identify primary source material, reference sections of the initial reviews included were screened. Those references identified underwent the same screening process as the primary literature search.

RESULTS

ANALYSIS OF PRIMARY LITERATURE SCREENING

On PubMed, the first literature search yielded a total of 99 articles using the phrase “inflammation perivascular adipose tissue” and filtering for “review” and “publication date 5 years.” Of those articles, 30 were screened for relevance to the research question. Using the same database, the phrase “perivascular adipose tissue statins” (with “review” and a “publication date 5 years” filters) yielded four total results. All four were screened for relevance to the research question. Both terms were entered into the Google Scholar database and a total of 6,410 records were identified, with 15 screened for relevance.

A total of 15 reviews were retained from the primary screening. Of those 15 reviews identified in the initial search, seven were used directly in the introduction (Becher et al., 2021; Diamond et al., 2022; Koenen et al., 2021; Liang et al., 2020; Patriki et al., 2022; Qi et al., 2018; Yanan et al., 2020). The remaining eight were used later, or as a source mine for primary references (Ali et al., 2021; Ding et al., 2022; Fan et al., 2022; Li et al., 2021; Marie-Eve et al., 2020; Mu et al., 2021; Rhoades et al., 2018; Tran et al., 2018).

ANALYSIS OF SECONDARY LITERATURE SCREENING

A total of 66 articles were screened for inclusion on PubMed from the reference sections of the reviews identified in the primary search. The remaining 44 references were screened using similar criteria as the primary search. A total of 59 articles were selected as references for this review.

RATIONALE TO INCLUDE PRISMA

While this is a narrative review and does not follow the process of using protocols for statistical analyses, we applied the following rigorous criteria to avoid internal bias:

1. Quality of the included articles was based on being published in peer-reviewed journals.
2. Inclusion and exclusion criteria (discussed above) were developed prior to the search for literature.
3. The research question was used as guidance in searching for literature, thereby making the selection analysis qualitative.
4. Keywords and key phrases were chosen with the research question used as guidance.
5. The preliminary literature search was done using PubMed and Google Scholar.

Figure 2 on the following page, is a chart resembling the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow diagram to help the reader understand how we screened articles for inclusion. It is a visual representation on the search strategy used for information gathering in this review.

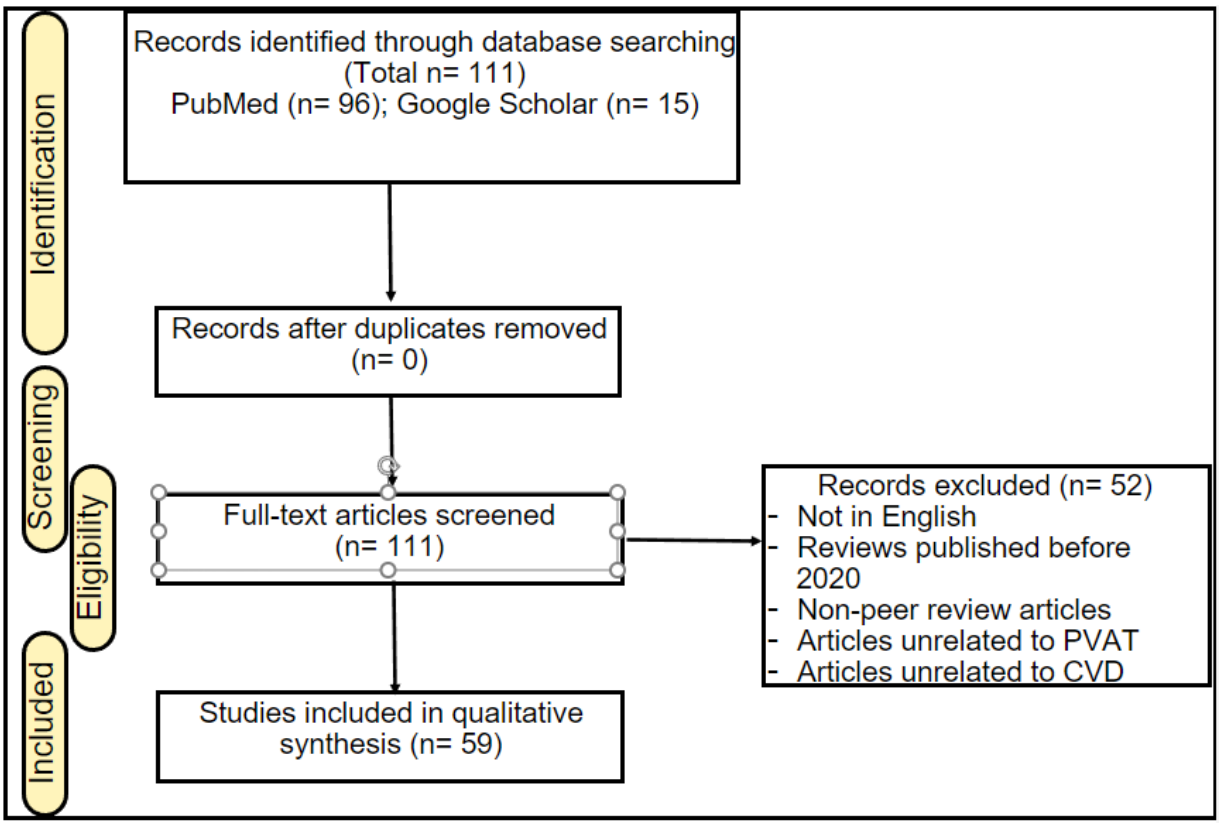

Figure 2: Database search strategy. Initial search strategy using 2020 PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines for the primary analysis. Our primary question that led to this investigation is, “What changes does PVAT undergo in pathological states that lead to the factors that contribute to atherosclerosis, hypertension and CVD overall?”

DISCUSSION

Though there is a large body of data and research addressing the function, mechanisms of vasoactive effects, and composition of PVAT, it is clear that there is a long way to go. More questions are generated with every query into the literature than answered. For instance, there is little consensus on the origin of PVAT; some authors claim it's progenitors lie in the vascular intima while others believe it originates in adipose tissue and still others believe individual adipocytes in the PVAT matrix are produced in different tissues (Camboa et al., 2017; Cattaneo et al., 2020; Guimaraes-Merrick et al., 2019; Li et al., 2021; Rodeheffer et al., 2008). There is even debate over whether PVAT in different anatomical locations have consistent progenitors (Hepler et al., 2017; Li et al., 2021; Tran et al., 2018). Knowing the origin of these adipocytes may be a potential avenue of treatment in the future.

Additionally, the "humors", such as PCSK-9, that contribute to PVAT dysfunction are not well known. It is a relatively new concept that inflammation (partially from the *oxidation* of LDL-C) and obesity, not LDL-C, is responsible for chronic CVD (Diamond et al., 2022; Harcombe et al. 2015; Koenen et al., 2021; Persson et al., 2023; Roth et al., 2014; Roth et al., 2018). Although the mechanism of PCSK-9 has been identified, it is not well know if it has local effects on the PVAT itself or if it is solely responsible for downregulation of LDL-C in the liver (Roth et al., 2014; Roth et al., 2018). Surely, there is great potential for other unknown mediators of inflammation in dysfunctional PVAT (outside of PCSK-9 and immune factors) where another path to therapy may lie.

One novel treatment for atherosclerosis targets the gene expression of PCSK-9 by mRNA inhibition (Lamb, 2021). This prevents the downstream effects of oxidation that leads to PVAT dysfunction (Roth et al., 2014; Roth et al. 2018, Lamb et al., 2021). In a 2021 study, patients were administered the new drug inclisiran and saw a reduction in LDL-C of 45.8% compared to the placebo group that only saw a 4.0% reduction (Lamb, 2021). This is a great example of the value of understanding details of a problem and should encourage further research to clarify the different unknown aspects of PVAT and atherogenesis.

STRENGTHS AND LIMITATIONS

This review was conducted under the auspices of a faculty-mentor (CBAR) and student (AW) relationship. The primary author, AW, worked in close collaboration with CBAR who has expertise in writing narrative reviews and has many publications in peer reviewed journals. CBAR subjected the work conducted by AW to rigorous scrutiny and applied high standards in the creation of this paper. While the inexperience of AW could be a liability, it was offset by CBAR.

One area where this paper may have benefitted is in the amount of articles screened. With only one author conducting the research, there was limited time to complete the review. However, when inclusion criteria was applied to articles, the amount of literature on the topics covered in this review is limited. That may be a limitation, but that is also one of the realities this review attempts to address and spur more research.

THE ROLE OF PERIVASCULAR ADIPOSE TISSUE (PVAT) IN CARDIOVASCULAR DISEASE RISK FACTORS

Whether the PVAT surrounding a given artery is BAT or WAT lends much predictive value to the course of disease that may arise due to two principal contributors to CVD; inflammation (and subsequent atherosclerosis) and hypertension (Cypess et al., 2009; Galvez et al., 2008; Nosalski et al., 2017). Dysfunctional PVAT with attenuated anti-contractile properties and/or more local oxidative stress can be the result of many pathological processes, including genetic abnormalities, tobacco use, obesity, metabolic syndrome, diabetes, hypoxia, aging, etc. (Diamond et al. 2022; Soltis and Cassis 1991; Galvez et al. 2006; Koenen et al. 2021; Nosalski et al. 2017). The main focus is on the modifiable risk factors of obesity and associated sequelae of obesity (hypertension, hyperlipidemia, metabolic syndrome, atherosclerosis, etc.).

The understanding of atherogenesis and associated hypertension and thrombogenesis, has shifted from LDL-C as the implicated culprit to inflammation (Galvez et al., 2008; Nosalski et al. 2017; Roth et al., 2014; Roth et al., 2018). The interplay between dysfunctional PVAT, hypoxic PVAT and the immune system is key to understanding atherogenesis and hypertension (Galvez et al., 2008; Koenen et al. 2021; Nosalski et al., 2017; Patriki et al., 2022). Key pro-inflammatory markers include IL-1, TNF alpha, NFKB and IL-6 which are activated when tissue necroses (often in the presence of hypoxia), LDL-C oxidizes, or the composition of PVAT changes (Hansson et al., 2011; Nosalski et al., 2017; Rhoades et al., 2018). The inflammatory mechanism leading to atherosclerosis is described in **Figure 3** on the following page.

In addition to inflammation, anti-inflammatory cytokines are down regulated when PVAT is dysfunctional, including adiponectin and IL-17 (Alambrouk et al., 2018; Fernandez et al., 2013; Greenstein et al., 2009). In the next section, the mechanism of PVAT dysfunction will be discussed at length, especially in the context of obesity and poor diet high in carbohydrates and fat (Fuster et al., 2016; Mu et al., 2021; Nosalski et al., 2017). PVAT converts from adipocytes that resemble BAT to WAT (Fernandez et al., 2013; Mu 2021). BAT cells are more dense, more metabolically active and thermogenic and smaller than their WAT counterparts (Liang et al., 2020; Guimarães-Camboa and Evans, 2017; Qi et al., 2018,). An obese body habitus sets the conditions

for accumulation of more visceral and perivascular WAT, as well as insulin insensitivity that leads to diabetes mellitus type two, which have many deleterious vascular implications (Aghamohammadzadeh et al., 2013; et al., Li et al., 2022,).

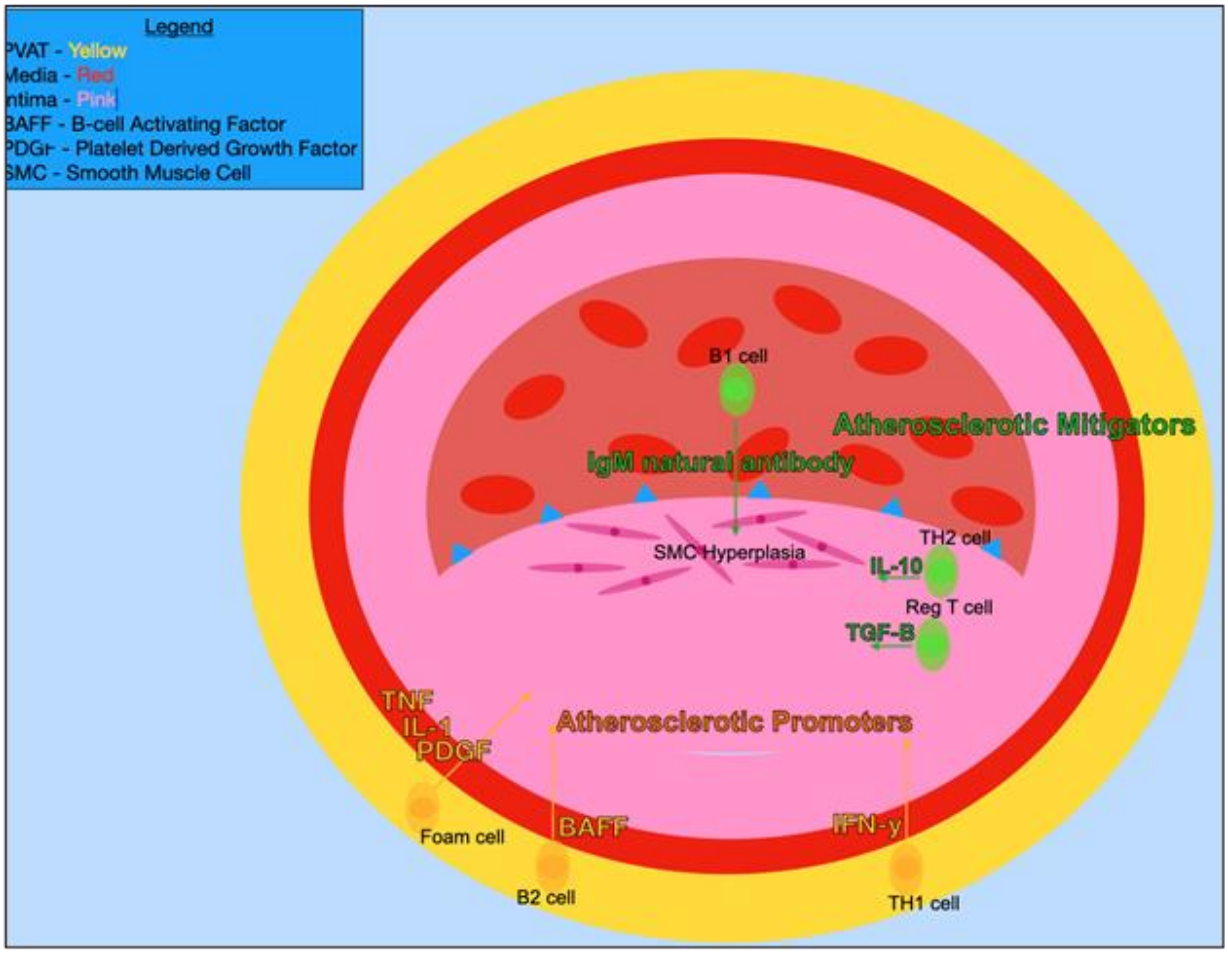

Figure 3: Key mechanisms of Atherogenesis. The intima is the innermost layer of the artery, which has a thin layer of epithelium in contact with the bloodstream. These endothelial cells express adhesion molecules that attract their complementary ligands and promote inflammation by chemoattraction of TH1 and B2 cells that in turn migrate to the intima through the epithelium and release pro-inflammatory cytokines. In addition, macrophages that phagocytose lipids and become foam cells. Smooth muscle cells proliferate because of immune action, which expands the intima and occludes the luminal side of the artery. Note that this is not an exhaustive list of factors. (Adapted from Libby et al., 2021).

PVAT DYSFUNCTION DEPENDENCY ON OBESITY AND DIET

Foundational to the notion that obesity and poor diet are responsible for PVAT dysfunction and atherogenesis was investigated by Aghamohammadzadeh et al. in 2013, they conducted a comparative study of healthy, lean adults vs. bariatric surgery candidates, where they harvested PVAT from the obese group prior to and 6 months after surgery, and compared in vitro function with wire myography (Aghamohammadzadeh et al., 2013). Fascinatingly, they found three benefits to reducing obesity; first, bariatric surgery and the resulting reduction in adipose tissue reduces the damage to PVAT and restores anti-contractile properties, second, that those improvements to the composition of PVAT are independent of the endothelium of the artery, and third, that local adiponectin and NO bioavailability are increased (Aghamohammadzadeh et al., 2013). They also found that PVAT adipocytes were reduced in size (Aghamohammadzadeh et al., 2013). This is a contentious aspect of the literature in that, there is no clear evidence supporting the nature of the progenitor cells of PVAT; the only evidence is that PVAT may have origins in vascular tissue and that it resembles WAT or BAT (Camboa et al. 2017; Hu et al. 2004; Koenen et al. 2021; Manrique et al. 2013; Mu et al. 2021; Qi et al. 2018). What is known is that PVAT is an admixture of adipocytes, progenitors, nerves and mast cells (Hu et al. 2004).

Regardless of origin, when PVAT resembles WAT more than BAT, the literature agrees that it becomes dysfunctional. In an obese state, the human body habitus has less neovascularization due to physical inactivity, experiences more oxidative stress due to caloric intake and more oxidation of LDL-C, and increased immuno-activity due to an increase in WAT or analogous PVAT (again, the literature cannot definitively identify the origin of PVAT) and the resulting necrosis from hypoxia and immuno-permeability of large adipocytes *(*Camboa et al., 2017; Aghamohammadzadeh et al., 2013; Qi et al., 2018; Koenen et al., 2021; Manrique et al., 2013; Mu et al. 2021*).* This all lends credibility to the argument that obesity and PVAT dysfunction leads to atherogenesis and CVD.

There is evidence that PVAT dysfunction is dependent on an increase in visceral fat (specifically WAT) and a diet rich in carbohydrates and fat (Diamond et al., 2022; Greenstein et al., 2009; Liang et al., 2020; Patriki et al., 2022; Persson et al., 2023;

Sena et al., 2017). One inflammatory marker associated with LDL-C, increase in WAT and immuno-activity is PCSK-9 (Abifadel et al. 2003; Camboa et al. 2017; Yanan et al. 2020).

PERIVASCULAR ADIPOSE TISSUE (PVAT) COMPOSITION, WHITENING OF BROWN ADIPOSE TISSUE (BAT), EFFECTS ON ENDOCRINE FUNCTION, AND IMMUNE CELL LOCAL ENVIRONMENT IN THE PRESENCE OF CHRONIC OBESITY

BAT has been positively correlated with vaso-protection, reduced inflammation and immune involvement, and functional PVAT in many larger vessels including the aorta and those in the supraclavicular region and is negatively correlated with higher body mass index (BMI) (Cypess et al., 2010; Koenen et al., 2021; Patriki et al., 2022). Soltis and Cassis generated evidence in 1991 that PVAT is vaso-dilatory if functional, and since then a good deal of evidence, describes some of the factors that functional PVAT yields to maintain vascular health, especially those that reduce atherosclerosis and vasodilation (Fernandez-Alfonso et al. 2013; Soltis and Cassis 1991). One aspect of PVAT dysfunction that has been under scrutiny, is the conversion of BAT to WAT, which happens significantly in established diet-induced obesity (Fernandez-Alfonzo et al. 2013). In 2013, Schroeter et al. demonstrated with mice fed a high-fat diet, that in early obesity, PVAT retains its vasoprotective properties, but that later, hyperleptinemia leads to leptin insensitivity and reduces vaso-dilation (Fernandez-Alfonzo 2013; Schroeter et al. 2013). In the next section, the “obesity triad,” a term coined by Xia et al. in 2016, will be discussed in depth. For now, it is sufficient to state that obesity leads to a greater quantity of WAT in PVAT (Cypess et al., 2009; Koenen et al., 2021; Patriki et al., 2022; Xia et al., 2016).

Endocrine and paracrine functions are compromised in the presence of chronic obesity and the PVAT local immune environment (Xia et al., 2016; Kumar et al., 2020; Kumar et al., 2021; Soltis and Cassis, 1991). This leads to increased stiffness of larger vessels, vasoconstriction of mesenteric and peripheral vessels, and atherosclerosis (Kumar et al., 2021; Libby et al., 2021; Soltis and Cassis, 1991; Xia et al., 2016). In their 2021 study, Kumar et al. conducted an experiment on several groups of Dahl salt-sensitive rats (Kumar et al., 2021). They demonstrated that the density of immune cells increases and the physical composition of the cells change, especially macrophages (Kumar et al. 2021). These changes in long-term obesity account for poor cardiovascular outcomes.

Additionally, much more research is needed in the area of identification of PVAT's origin. In their 2021 review, Li et al. outlined some of the known and challenged pathways proposed and studied. Without going into the minutiae of specific biomarkers used to identify cellular lineage (methods include genetic assays, surface protein identification and the cluster of differentiation identification) there is some consensus, but more debate about which cells serve as progenitors (Camboa et al., 2017; Cattaneo et al., 2020; Guimaraes-Merrick et al., 2019; Li et al., 2021; Rodeheffer et al., 2008). Where there is consensus, there is still debate about whether there is parallel or distinct differentiation and that is dependent on anatomical location (Hepler et al., 2017; Li et al., 2021; Tran et al., 2018)! What is important is that the identification of these progenitors may provide insight into drug development or future treatments.

PCSK-9: A MULTIFACTORIAL MEDIATOR OF ARTERIAL INFLAMMATION AND PERIVASCULAR ADIPOSE TISSUE (PVAT) DYSFUNCTION

Information is lacking on the development of the theory of the mechanism of PVAT dysfunction and subsequent inflammatory protein for the last decade. Verhagen et al. published data in 2010 that contributed to the “outside-in” theory of PVAT inflammation that leads to atherosclerotic plaques (Verhagen et al. 2010). In fact, an experiment conducted in 1989 demonstrated that infiltration of leukocytes from the adventitial side of the artery led to atherosclerotic plaques in rats that had endotoxin-soaked cotton placed on the luminal side of the artery (Prescott et al., 1989; Verhagen et al., 2010). Much of what has developed in the research since that first experiment (and subsequent similar experiments on pigs) has been seeking out the various proteins that mediate the immune response. The Prescott experiment found that the particular leukocytes that invaded into the adventitia were neutrophils and mononuclear cells (Prescott et al., 1989). As stated previously, IL-1, TNF alpha, NFKB and IL-6 are key pro-inflammatory mediators that stimulate this response *(*Hansson et al., 2011; Nosalski et al., 2017; Rhoades et al., 2018).

Currently, the idea of ‘the obesity triad,’ as described in **Figure 4**, of hypoxia, inflammation and oxidative stress, is supported by a broad body of experiments and research (Fuster et al., 2016; Greenstein et al., 2009; Pasarica et al. 2009; Xia et al., 2017). The chart on the following page summarizes the ways in which an increase in adipocyte size and downregulation of angiogenesis leads to hypoxia, inflammation of PVAT and finally oxidation by the immune system (Xia et al. 2017).

One area that needs further elucidation is the role that the oxidation of LDL-C plays in the contribution to atherosclerosis (Ding et al., 2022; Ferretti et al. 2018; Ruuth et al. 2018; Tsimikas et al., 2005). Little is known about exactly which aspect of the overall molecule fundamentally changes due to oxidation that triggers stress on the intima and adventitia and there is even debate over which apolipoprotein is most responsible, however, LDL-C is implicated in many experiments and studies (Ding et al., 2022; Ferretti et al. 2018; Ruuth et al. 2018; Tsimikas et al., 2005).

However, what is known is that PCSK-9 has a significant role in both circulating LDL-C and in PVAT oxidative stress and dysfunction (Abifadel et al., 2003; Ali et al.,

2021; Lamb et al., 2021; Yanan et. al., 2020). PCSK-9 increases LDL-C in serum by reducing LDL receptors (LDL-R) in the liver, thereby preventing uptake of LDL-C (Poirier et al., 2009; Shimada et al., 2015). The focus here will be on unregulated PCSK-9 production in the livers of obese patients and the resulting increase in LDL-C, which leads to more oxidative stress and PVAT dysfunction.

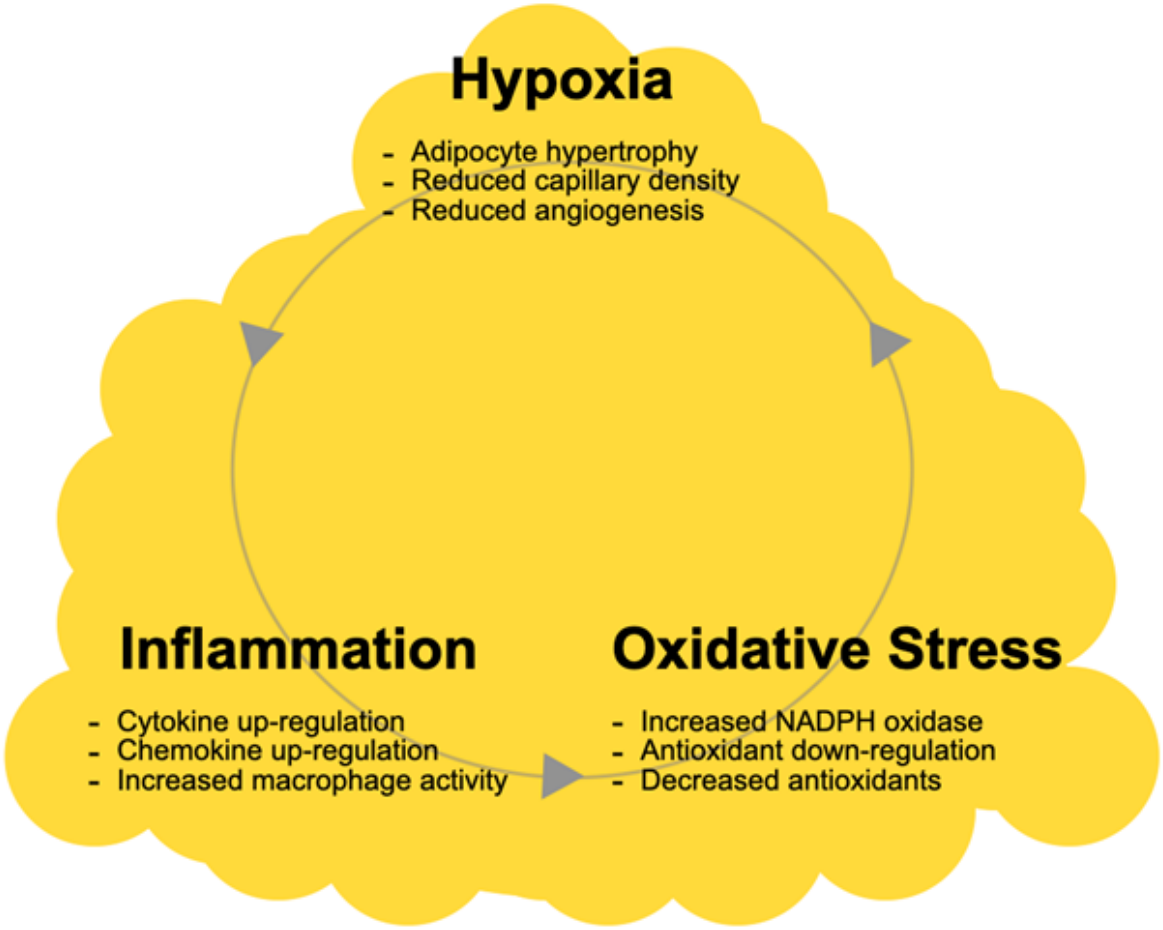

Figure 4: "Obesity triad" Effects on PVAT. Hypoxia ensues with the accumulation of excess adipose tissue, especially in visceral and abdominal zones. Reduced angiogenesis and capillary density are the result of adipocyte hypertrophy, which tends to occur with with the whitening of adipose tissue in obesity. This hypoxia leads to necrosis of PVAT which initiates an immune response to phagocytose dead cells. Inflammation results as cytokines and chemokines are up-regulated. Increased macrophage activity and subsequent change to foam cells, as they phagocytose adipocytes, lead to oxidative stress. Increased NADPH oxidase and antioxidant down-regulation (which decreases antioxidant density) leads to increased oxidative stress. This PVAT dysfunction then causes atherosclerotic plaques to form, as described in **Figure 3**. (Adapted from Xia et al., 2017).

ONE-DIMENSIONAL DRUG EFFECTS ON ATHEROSCLEROSIS

Using of monoclonal antibody drugs to bind and reduce circulating PCSK-9 is a promising adjunct therapy to statins or even as a replacement for statins when contraindicated in patients (Moriarty et al., 2015; Ridker et al., 2017). Odyssey Investigators studied Alirocumab as a treatment for reducing PCSK-9 with monoclonal antibodies in patients that were intolerant to statins (Moriarty et al., 2015). They found a 45% reduction in PCSK-9 compared to a trial using ezetimibe, a mAB drug (Moriarty et al., 2015).

This and other clinical studies in animals led investigators to develop a drug that was approved in the UK in 2021 and the US in July of 2023 that inhibits the mRNA that codes for PCSK-9 (Ali et al., 2021; Lamb et al., 2021). Inclisiran is the first drug developed to specifically block the production of PCSK-9 downstream and the results of the Lamb study are promising (Lamb et al. 2021). The inclusion criteria included patients with a history of familial hyperlipidemia, diabetes type 2, atherosclerotic cardiovascular disease (ASCVD), or a greater than or equal to 20% 10-year risk of a cardiovascular event on the Framingham CVD risk score (Lamb et al. 2021). They were on max dosage of statin therapy with or without other treatment for hyperlipidemia (Lamb et al. 2021). The patients in the study experienced a 45.8% reduction in LDL-C compared to the placebo group which only experienced a 4.0% reduction (Lamb et al. 2021).

This method of lowering circulating LDL-C, thereby reducing oxidative stress on PVAT and mitigating dysfunction, as in the study, can be used as an adjunct in patients who are at their pharmacological limit of reducing lipid levels and can be used as a stand-alone therapy (like monoclonal antibodies) to reduce hyperlipidemia in patients contraindicated (due to, for instance, kidney disease) or intolerant of other therapies like statins (Moriarty et al., 2015; Lamb et al. 2021). More data is needed to parse exactly which part of the LDL-C molecule causes the most oxidative stress and what other adipokines in PVAT may be implicated in inflammatory processes in the presence of obesity. However, due to the success of PCSK-9 inhibitors, stopping pathology upstream by gene silencing seems an excellent direction to go (see **Figure 1** for a summary of PCSK-9 synthesis and drugs that address excess PCSK-9).

CONCLUSION

In conclusion, perivascular adipose tissue has transitioned from a passive anatomical feature to a dynamic regulator of vascular function. Recognizing its role as a source of vasoactive compounds has ignited interest in its therapeutic potential. As researchers delve deeper into the complexities of PVAT biology, there is growing optimism that targeting this adipose depot may offer innovative strategies for managing cardiovascular and cardiometabolic diseases. The journey from bench to bedside in harnessing the therapeutic promise of perivascular adipose tissue is an exciting and evolving frontier in medical research.

PVAT is a complex and mysterious endocrine and paracrine organ. Though the identification of PVAT as a major player in 1989 has led to a strong interest in research and experimentation, more investigation is warranted. Parsing the differences in PVAT composition and PVAT type in different physiologic sectors of the body (mesenteric vs. larger vessels like the aorta, peripheral vessels, etc.) could lead to discovery of differences in the composition of the vascular tissue. The interesting discoveries found through experimentation demonstrate different responses of vascular tissue to dysfunctional PVAT depending on location.

Additionally, more research is needed both to find more factors that induce atherosclerosis and most effective and appropriate treatments to reduce their effects. Important mediators of atherosclerosis like PCSK-9 now have an available treatment to halt the synthesis of the protein in the first place. Gene-silencing and monoclonal antibody drugs are fairly new in the clinical world and should be explored further and studied with large populations. On the other hand, another solution could be to address the problem of dysfunctional PVAT directly and to reduce the quantity of WAT and other components that create dysfunction. Regardless, these tools are only a temporary solution for better patient outcomes until a successful solution is reached for the real issue - diet-induced obesity and diabetes.

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