JCI insight

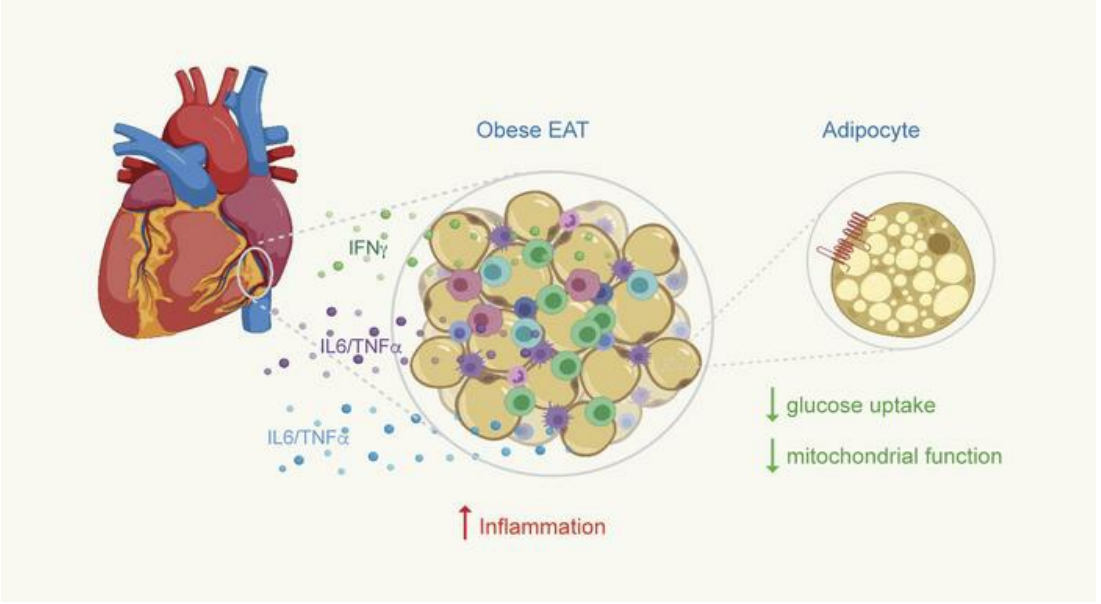
Obesity and diabetes are major risk factors for epicardial adipose tissue inflammation

Vishal Vyas, … , Malcolm C. Finlay, M. Paula Longhi

JCI Insight. 2021. https://doi.org/10.1172/jci.insight.145495.

Clinical Medicine In-Press Preview Cardiology Inflammation

Graphical abstract



Obesity and diabetes are major risk factors for epicardial adipose tissue inflammation

Vishal Vyas[1,2*], Hazel Blythe[1*], Elizabeth G. Wood[1*], Balraj Sandhar[1], Shah-Jalal Sarker[3], Damian Balmforth[1,4], Shirish G. Ambekar[4], John Yap[4], Stephen J. Edmondson[4], Carmelo Di Salvo[4], Kit Wong[4], Neil Roberts[4], Rakesh Uppal[1,4], Ben Adams[4], Alex Shipolini[4], Aung Y. Oo[1,4], David Lawrence[3,4,5], Shyam Kolvekar[3,4], , Kulvinder S. Lall[4], Malcolm C. Finlay[1,2,6], M. Paula Longhi[1,6]

1 William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK.

[2]Department of Cardiology, Barts Heart Centre, St Bartholomew's Hospital, London, UK.

[3]Research Department of Medical Education, UCL Medical School, University College London, London, UK.

[4]Department of Cardiac Surgery, Barts Heart Centre, St Bartholomew's Hospital, London, UK.

[5]University College London Hospitals NHS Foundation Trust, London, UK.

[6] These authors jointly supervised this work

[*]contributed equally

Correspondence:

M. Paula Longhi

Address: Heart Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, EC1M 6BQ

Tel: +44 (0) 207 882 6566

Email: m.longhi@qmul.ac.uk

Conflict of interest

V.V and M.F. received funding from Abbott.

Abstract

Background: Epicardial adipose tissue (EAT) directly overlies the myocardium with changes in its morphology and volume associated with myriad cardiovascular and metabolic diseases. However, EAT's immune structure and cellular characterization remain incompletely described. This study aimed to define the immune phenotype of EAT in humans, and compare such profiles across lean, obese and diabetic patients.

Methods: A total of 152 adult patients undergoing open chest coronary artery bypass grafting (CABG), valve repair/replacement (VR) surgery or combined CABG/valve surgery were recruited to the study. Patients' clinical and biochemical data alongside epicardial adipose tissue (EAT), subcutaneous adipose tissue (SAT) and pre-operative

blood samples were collected. Immune cell profiling was evaluated by flow cytometry and complemented by gene expression studies of immune mediators. Bulk RNA-seq was performed in EAT across different metabolic profiles to assess whole transcriptome changes observed in these groups.

Results: Flow cytometry analysis demonstrated that EAT is highly enriched in adaptive immune (T and B) cells. Whilst overweight/obese and diabetic patients had similar EAT cellular profiles to lean control patients, the EAT exhibited significantly ($P \leq .01$) raised expression of immune mediators including: interleukin1 (IL1), IL6, tumour necrosis factorα (TNFα) and interferonγ (IFNγ). These changes were not observed in either SAT or blood. Neither underlying coronary artery disease nor the presence of hypertension significantly altered the immune profiles observed. Bulk RNA-seq demonstrated significant alterations in metabolic and inflammatory pathways in the EAT of overweight/obese patients compared with lean controls.

Conclusions: Adaptive immune cells are the predominant immune cell constituent in human EAT and SAT. The presence of underlying cardiometabolic conditions, specifically obesity and diabetes, rather than cardiac disease phenotype appears to alter the inflammatory profile of EAT. Obese states markedly alter EAT metabolic and inflammatory signalling genes, underlining the impact of obesity on the EAT transcriptome profile.

Keywords: Inflammation, obesity, epicardial adipose tissue, T cells

Translational Perspective:

Epicardial adipose tissue (EAT) is implicated in the pathophysiology of a spectrum of cardiometabolic disorders including coronary artery disease (CAD) and diabetes. However, the immune cell types and mediators involved in driving changes in EAT are poorly elucidated. Here, we demonstrate that overweight/obesity and type 2 diabetes are independently associated with EAT inflammation regardless of the severity of CAD. Interleukin1, 6, tumour necrosis factorα and interferonγ are the key mediators elevated several-fold in the EAT of overweight/obese and type 2 diabetic patients, while EAT inflammatory and metabolic signalling pathways are markedly perturbed in obese states. Indeed, such pathways and immune mediators be novel targets to reduce the cardiovascular morbidity and mortality associated with these conditions.

Introduction

Epicardial adipose tissue (EAT) is the visceral adipose tissue of the heart, covering 80% of its surface and up to 20% of its weight (1). EAT is in direct contact with the underlying myocardium without fascial interruption, allowing direct interactions between the two tissues. EAT has been implicated in a host of cardiovascular diseases (2) and has been proposed as a transducer (3) of the effects of systemic conditions such as obesity on the heart. Significant associations between EAT thickness and cardiovascular risk factors have been reported, including diabetes (4) and hypertension (5). Although variable (4,6,7) a relationship has been described between EAT size and the severity of coronary artery disease (CAD) (8) as well as cardiovascular events (9,10). However,

EAT size does not necessarily correlate with the degree of inflammation observed (3) and how EAT inflammation correlates with the severity of CAD remains controversial (11)

The pivotal role of inflammation in the pathogenesis of many cardiovascular disorders is increasingly recognised (12,13). The EAT has been identified as a rich local source of vasoactive molecules, pro- and anti-inflammatory adipokines, growth factors and other agents that can exert paracrine and vasocrine effects on the myocardium (1,8). Inflammatory mediators released by EAT may directly contribute to the inflammation of the myocardium and coronary arteries. Indeed, higher levels of the pro-inflammatory mediators interleukin1β (IL1β), IL6, monocyte chemoattractant protein1 (MCP1),tumour necrosis factorα (TNFα) and reduced levels of adiponectin were observed in the EAT of CAD and diabetic patients (14-17). Additionally, increased infiltration of 'pro-inflammatory' $CD11c^+$ vs 'anti-inflammatory' $CD206^+$ macrophages was observed in the EAT of CAD patients (18). Aside from these reports, much of our current understanding of adipose tissue-mediated inflammation is derived from studies of non-cardiac depots, such as abdominal visceral adipose tissue (VAT), where pro-inflammatory macrophages predominate (19). The description of the inflammatory cell population and pathways in EAT in humans remains incomplete, and an understanding of how these may vary between conditions such as severe CAD and valvulopathy is absent.

Here, we aimed to describe the human EAT immune infiltrate and provide comparisons with subcutaneous adipose tissue (SAT) and blood inflammatory profiles (adipose

tissue and systemic comparators accessible during cardiac surgery). Significant challenges in accessing human EAT were overcome by limiting this investigation to patients undergoing cardiac surgery for two commonly encountered cardiovascular diseases: severe CAD and valvular heart disease without severe CAD. Further, we hypothesized that the underlying risk factors associated with the EAT, specifically obesity, diabetes and hypertension rather than CAD *per se,* alter the inflammatory profile of EAT. Here, we first outline the immune profile of EAT and how it differs to SAT and blood, demonstrating that T cells and not macrophages are the predominant immune cell population in both EAT and SAT. We then show that CAD and valvular heart disease patients exhibit a similar pattern of inflammation. We go on to illustrate that it is obesity and diabetes that drive changes in immune infiltrates and that these changes are uniquely observed in the EAT, underlining its critical significance in cardiovascular disease pathophysiology.

Results

T cells are the primary immune cell type present in EAT

To gain a more complete understanding of the immune profile of EAT, we investigated the presence of key immune populations by flow cytometry. The gating strategy is outlined in supplemental figure 1. The most striking differences in the immune profile are noted between blood and adipose tissue. In the blood, 60% immune cells are neutrophils and 3% adaptive immune cells (B and T cells) while in the EAT and SAT, adaptive immune cells make up approximately 60% of immune cells and neutrophils

less than 7% cells (Figure 1a). In addition, the immune infiltrate is significantly lower (*P*≤.001) in SAT than EAT. We focused particularly on T cell subsets given adipose tissue is now a well-established reservoir of memory T cells (20,21). We used unsupervised t-distributed Stochastic Neighbor Embedding (t-SNE) plots to investigate the presence of naïve, central memory (TCM), effector memory (TEM) and tissue-resident memory T (TRM) cells in SAT, EAT and blood (Figure 1b). As expected, $CD4^+$ and $CD8^+$ TRM cells made up a sizeable proportion of the T cell repertoire in adipose tissues but was absent in blood. TRM cells in the adipose tissue were $CD103^-$, $CD69^+$ and $PD1^+$ (Supplemental Figure 1) , which was consistent with that observed in murine models (20).

Next we evaluated the phenotype of those T cells by intracellular cytokine staining in a small cohort of patients undergoing CABG (n=17) and VR (n=8) where sufficient adipose tissue was available for additional analyses. We found that IFNγ is the most highly expressed cytokine in both $CD4^+$ and $CD8^+$ T cells, with negligible levels of IL17- and IL22-producing T cells (Supplemental figure 1c). However, there were marked differences in IFNγ production in each individual patient's EAT compared with their blood and SAT highlighting that blood is not necessarily a good gauge of tissue-level inflammation (Fig 1c).

EAT inflammation is independent of CAD severity

We set out to investigate whether the immune profile of EAT from patients with severe CAD (CABG patients) differed from those with non-severe CAD (VR patients). There were marked differences in the groups. As expected, patients were typically in their seventh decade of life, CABG and combined CABG/valve surgery patients had a high proportion of cardiovascular risk factors including diabetes, hypertension, hyperlipidemia and a history of smoking. Medications reflected their underlying cardiac co-morbidities. Notably, baseline inflammatory markers (C-reactive protein and neutrophil:lymphocyte ratio) were similar between the groups (Table 1). In order to first comprehensively characterize the key immune populations present in the blood and adipose tissue of patients with severe vs non-severe CAD, we used the CABG and VR surgery cohorts. Given only 16 patients had combined CABG/valve surgery, the group was too small for inferential statistical analysis following the propensity matching and hence was not included in this analysis. Patients with a history of myocardial infarction were excluded. A total of 48 patients in the CABG and VR surgery groups were thus included (Table 2) following propensity matching.

The numbers of macrophages remained similar between groups as did the proportion of M2-polarized 'anti-inflammatory' $CD206^{+}$ macrophages. Similarly, no significant differences ($P \leq 0.9$) were noted in numbers of key immune cells in both adipose tissue and blood across both groups (Figure 2 & Supplemental Figure 2). Similarly, no differences in expression levels of immune mediators were observed across the two groups (Figure 2c). Reviewing cell numbers in blood and adipose tissue, again no significant changes in T cell subsets were noted nor were there any differences in IFNγ

production across the EAT, SAT or blood (Figure 2d-f). Thus, our data demonstrate that CAD is not associated with alterations in immune cell numbers or inflammatory mediator levels in EAT.

Obesity promotes EAT inflammation

Hypercholesterolemia, overweight/obesity, hypertension and diabetes are known to be key risk factors for the development of CAD. Thus, the next step was to identify whether these conditions are associated with an altered inflammatory picture observed in different patients.

First, we considered the effect of obesity as this is most likely to impact adipose tissue biology directly inducing an inflammatory response. Given that patients with T2D were in general prescribed anti-hyperglycemic medications, they were analyzed as a separate group despite having similar BMIs to the overweight/obese cohort. Table 3 details the demographic and clinical characteristics of the patients utilized for the overweight/obesity and T2D group analysis.

Absolute numbers of key adaptive immune cells were similar across groups (Figure 3a-c & Supplemental Figure 3). However, the immune mediator profile is dramatically altered (Figure 3c) uniquely in the EAT compared to blood and SAT (Figure 3c & Supplemental Figure 4). Overweight/obese and T2D patients were observed to have greatly elevated expression levels of the same pro-inflammatory immune mediators, specifically a 5 to 10-fold increase in IL1, IL6, TNFα and IFNγ. IL6 and TNFα can be

produced by myeloid cells and adipocytes, while IL1 and IFNγ and mainly released by immune cells, e.g. myeloid cells and T cells respectively. All these cytokines are known to contribute to adipose tissue inflammation and insulin resistance. As expected, overweight/obese and T2D patients exhibit increased expression levels of the adipokine leptin. Notably, levels of adiponectin were elevated in obese patients. The proportion of TEM and TRM cells were similar between groups. Yet a clear increase in $CD4^+$ $IFN\gamma^+$ and $CD8^+$ $IFN\gamma^+$ T cells could be observed in both overweight/obese and T2D groups being T cells the main producer of IFNγ. The level of immune mediators in blood were similar, with the exception of TNFα that was elevated in in the overweight/obese cohort. These data indicate that although the cellular immune infiltrate in EAT remains the same, obesity and diabetes induce a change in their phenotype towards a pro-inflammatory state.

Obesity induces extensive EAT remodelling

In orders to further investigate the differences in gene expression in EAT between lean and overweight/obese and T2D, we performed bulk RNA-seq. The thresholds of differentially expressed genes were fold change >2 and adjusted *p* value < 0.05. There were 133 differentially expressed genes in overweight/obese compared to lean EAT patients, while 94 were found to be significantly upregulated in T2D patients (Figure 4A & Supplementay figure 5A). Inflammatory and metabolic genes were differentially regulated in both overweight/obese and T2D samples compared to Lean (Figure 4B & Table S1-2). Upregulation of inflammatory genes indicate activation of myeloid and lymphoid cells including *IL6*, *IL1b, CXCL8, CCL3, NLRP3,ATF3, OSM, CD83,*

GPR183,VCAN, CCR3, CXCL12 and CCL2. Interesting, T2D patients show a lower expression of inflammatory genes in EAT compared to overweight/obese (Figure 4A-B & Supplemental Figure 5B). A similar trend was observed in the RT-PCR and intracellular cytokine production by T cells (Figure 3C, F). Obesity induces a profound metabolic rewiring in EAT, with downregulation of genes associated with glucose metabolism (e.g. *SLC24A, CS, GPT, OGDH, ACO2, GPI, LDHD*) and lipid metabolism (e.g.*GYS2, GPAT3, CRAT, FASN, ACADVL, DGAT1, DGAT2, NAT8L, SCD*) as well as changes in genes related to adipogenesis (*HES1, MXD3, NR4A2, RGS2, PPP1R15B, ADAMTS1, CEBPD, KDM7A)*. The metabolic phenotype was more evident in EAT from diabetic patients (Fig 4B & Supplementary Figure 5C-D). Interestingly, certain genes associated with CAD were dysregulated in obese EAT such as *HBEGF, ADAMTS1 and ADAMTS4* while the novel adipokine spexin (*SPX),* which regulates adipose tissue inflammation and was shown to protect cardiomyocytes from hypoxia-induced metabolic distress, was downregulated in EAT from both obese/diabetic patients. Overall, these data highlights significant metabolic and inflammatory changes in EAT induced by obesity.

EAT inflammation is not associated with hypertension

EAT volume was found to be increased in hypertensive compared to normotensive patients (5). Thus, we investigated if the presence of hypertension alters the EAT inflammatory state in Table 4 and found that it did not ($P \geq .14$). Both hypertensive and non-hypertensive (control) patients demonstrate similar immune cell profiles in the blood, EAT and SAT (Figure 5A-B & Supplemental Figure 6) alongside similar relative

expression levels of key immune mediators (Figure 5C). Looking at specific T cell subsets and their phenotype, again similar absolute numbers of T cell subsets and IFNγ production are seen across both groups and across tissues (Figure 4D-E & Supplemental Figure 6). Hence, we show that hypertension as a cardiovascular risk factor in isolation is not a driver of EAT inflammation.

Discussion

A number of previous analyses (11,15) studying patients undergoing cardiac surgery with and without significant CAD (CABG vs VR surgery patients) suggest that it is the specific adipose tissue depot, namely EAT, that drives the unique inflammatory changes observed. With EAT's anatomical intimacy with the myocardium and lack of fascial boundaries between them, immune mediators can have a direct and potentially deleterious impact on the heart. This highlights the unique significance of EAT in giving an indication of the local tissue environment.

To date and despite a wealth of data indicating a link between EAT volume and cardiovascular disease, in-depth investigations of EAT inflammation remain sparse. Indeed, we still lack a detailed overview of the immune profile of EAT and how it differs between SAT and blood samples and in different cardiovascular conditions. Here we show a clear enrichment of adaptive immune cells, in particular $CD4^+$ T cells, in the adipose tissue (EAT and SAT) compared with blood, where neutrophils are the dominant cell type. Of note, much of the earlier literature has focused on innate immune cells, such as macrophages within EAT (16,18) yet the immune profiling illustrates these

represent a lower proportion of the immune ($CD45^+$) cell population compared with adaptive immune cells. Embyrologically, both EAT and abdominal adipose tissue are derived from the splanchnopleuric mesoderm (1) and both tissues utilize the same vasculature and lymph drainage as their underlying organs, the intestine and myocardium respectively. However, we have demonstrated EAT is dominated by adaptive immune cells while abdominal adipose tissue by macrophages (23). This would suggest that is the anatomical location of the adipose tissue that determines its immune profile.

The notable finding of tissue-resident memory T (TRM) cells within the EAT is deserving of comment, these have not previously been characterized in EAT. As the name indicates, TRM cells remain local to the tissue, affording long-lasting immune surveillance, and can rapidly re-activate and recruit circulating T cells when required (24). Their presence enables adipose tissue to act as a local source of adaptive immune cells; such organ-specific immunity has been previously described in other organs (25). Notably, TRM cells are high in programmed cell death protein1 (PD1) expression and TRM reactivation has been implicated in numerous cancers but their exact role in the heart remains enigmatic (26).

We have highlighted that blood and SAT may offer a comparative lack of insight into the local tissue environment, given the dominant immune mediator produced from T cells (IFNγ) can vary considerably between tissues in a single individual. This is a critical point as blood is typically assayed for its accessibility in giving an indication of the

patient's condition and inflammatory status, yet blood may bear little correlation with the local tissue inflammation. Indeed, this point was highlighted in one of the earliest descriptions of EAT inflammatory mediators (14). Clinicians should be mindful of the limitations in insights available from blood analysis alone.

A key finding is that once underlying cardiovascular risk factors are balanced, we have demonstrated no differences in the inflammatory profile between severe vs non-severe CAD. Some previous publications (15,27,28) have reported the EAT of patients with significant CAD to have a unique inflammatory profile. However, it is noteworthy that many of these reports failed to control for other important cardiometabolic conditions such as diabetes and overweight/obesity (16-18) or focused exclusively in lean individuals (28), which are not typical of patients undergoing cardiac surgery. A significant strength of our work is that the large patient cohort allowed propensity matching to be performed, reducing bias between our patient groups. Our extensive comparison of a range of immune cells and immune mediators shows the immune profile to be similar in patients with severe (CABG) and non-severe (VR) CAD across blood, EAT and SAT. Indeed, no differences in absolute numbers of immune cells are observed in overweight/obese patients or those with diabetes. However, the immune mediators are greatly elevated in both overweight/obesity and diabetes compared with lean patients. In EAT, TNFα, IFNγ, IL1, IL6 and leptin levels are all elevated several-fold in both of these groups. Inflammation is a key mechanism of cardiovascular disease. Pro-inflammatory cytokines such as IL1 and TNFα can amplify local inflammation, regulate endothelial cell activation, induce ROS production and cardiomyocyte

apoptosis. Bulk RNA-seq transcriptomic analysis further confirmed this finding. Pro-inflammatory cytokines were elevated in overweight/obese EAT and to a lesser extend in EAT from T2D pateints. Pathway enrichment analysis further indicates upregulation of pathways associated with adaptive immune responses in addition to monocyte/macrophage activation. A reduced inflammatory response in T2D patients could be attributed to the anti-diabetic drug metformin, which is considered to have an anti-inflammatory effect (29). The overwhelming majority of T2D patients in our cohort were on metformin, given it is a first-line indicated drug for T2D patients. The study lacked statistical power to evaluate the effect of metformin in EAT inflammation. Cytokine-targeting therapies have emerged as possible non-invasive treatment for heart disease. Considering that adipose tissue is the primary organ affected by overnutrition and given the anatomical proximity between EAT, coronary arteries and myocardium it is possible to envisage EAT as the primary source of heart inflammation in obesity.

T cells were found to be the main producers of IFNγ. Certain mediators have previously been described to be elevated in the EAT (15,27) but not attributed to specific co-morbidities through a robust propensity-matched analysis. Similarly, a host of studies have illustrated increased EAT size in patients with increased BMI (4,6) and diabetes (15,27) but a detailed understanding of changes in immune cell types and inflammatory mediators has been lacking. Significantly, we have demonstrated that adiponectin was uniquely elevated in the overweight/obese group. Reduced adiponectin in EAT was found to be associated with atherosclerotic plaque development but this association is not consistent between studies (16,18,30) with reports not accounting for potential

confounding variables such as BMI (16) and with patients being entirely or almost exclusively male (16,31). Adiposity and diabetes appear to alter specific inflammatory mediators rather than having a more holistic impact on the immune phenotype. This is crucial for future studies analyzing immune differences of adipose tissue between patients with varying metabolic risk profiles.

Infiltration of inflammatory cells in expanding adipose tissue can result in adipocyte dysfunction and metabolic dysregulation. Several human studies mainly in subcutaneous fat, have linked obesity to reduced mitochondrial oxidative metabolism and biogenesis as well as to impaired glucose and lipid metabolism in adipose tissue (32,33). The insulin-regulated glucose receptor GLUT4 is downregulated in obese adipose tissue but not skeletal muscle, however, deletion of GLUT4 selectively in adipose tissue is sufficient to induce insulin resistance (34). Downregulation of GLUT4 results in reduced adipocyte glucose uptake and *de novo* fatty acid synthesis (35), which in turn contributes to systemic metabolic perturbations in obesity. Our findings in EAT are in agreement with previous studies showing decreased glucose metabolism and lipid synthesis in obese adipose tissue from other sites (abdominal and subcutaneous).

Adipose tissue is one of the main sites of mitochondrial branched-chain amino acids (leucine, valine and isoleucine) catabolism (36). Impaired BCAA metabolism correlates with insulin resistance, altered cardiac metabolism and greater risk of cardiovascular disease (33,37,38). Genes associated with BCAA catabolism, including *ACAD8,ALDH6A1,ALDH9A1,BCKDHA,BCKDHB* and *HADH*, were downregulated in

EAT from overweight/obese and T2D patients, which may contribute to cardiac dysfunction. Overall, our data suggest that the metabolic perturbation observed in obese adipose tissue from other sites is similarly observed in EAT.

Finally, EAT size has been associated with the presence of hypertension (5,39). However, hypertension does not appear to specifically impact the EAT immune phenotype. First, this highlights a point alluded to earlier that EAT size does not necessarily correlate with the degree of inflammation. Second, when considering the studies assessing hypertension and EAT, it is noteworthy that hypertensive patients often tend to be co-morbid with overweight/obesity and T2D, which when adjusted for, reduces the significance of the association with EAT size and hypertension (7,39). This is consistent with the changes observed uniquely in the overweight/obese and T2D groups in our study, when co-morbidities have been adjusted for.

There are a number of limitations to our study that are important to recognise. The number of patients per group is relatively small compared to other observational studies, but is the largest detailed immunophenotype analysis in EAT. In addition, the immune mediator alterations observed in the EAT of overweight/obese and diabetic patients are independent associations. A causal relationship between these conditions and EAT inflammation cannot be established given the inability to perform a randomized-controlled trial with the prolonged time period required for conditions such as obesity and diabetes to exert an impact on EAT. Moreover, EAT can only be ethically sampled during cardiac surgery (and not in healthy volunteers for instance). Due to the

challenges in safely harvesting EAT, we were not able to perform multi-site EAT sampling to assess for whether regional differences in the EAT inflammatory profile could be observed from the same patient. We utilised the CABG vs VR surgery groups to compare severe vs non-severe CAD. While we recognise these are not ideal comparisons groups, as alluded to above, healthy controls could of course not be recruited. The decision for CABG was based on a joint cardiology-cardiac surgery multidisciplinary team discussion concluding severe CAD was present warranting CABG. VR alone was performed with a similar multidisciplinary team discussion concluding there was not severe CAD to require concomitant CABG and VR. Thus, VR was used as a control group accepting the limitations as outlined above. In the context of these limitations, we surmised that a propensity-matched analysis using fresh human tissue samples was the best approach to study the risk factor-EAT inflammation relationship.

In summary, we have performed a detailed immune analysis of EAT, SAT and blood in a substantial cohort of clinically well-phenotyped patients undergoing cardiac surgery. We have demonstrated the overall immune profile is dominated by adaptive immune cells in EAT and SAT compared with neutrophils in blood, and that the blood is not necessarily an accurate gauge of tissue-level inflammation. Finally, we have shown that key cardiometabolic conditions, namely overweight/obesity and T2D, are independently associated with significant changes in the EAT inflammatory picture rather than just the presence or absence of severe CAD.

Methods

Study population and sample collection

Adult patients (≥18 years) undergoing open chest coronary artery bypass grafting (CABG), valve repair/replacement (VR) or combined CABG/valve surgery were recruited from Barts Heart Centre, St Bartholomew's Hospital (London, UK) from 2017 to 2020 (n=152). Exclusion criteria included patients with underlying congenital heart disease, those with co-existing systemic inflammatory/neoplastic disorders and those on immunomodulatory agents. Fasting blood samples were collected pre-operatively in the anesthetic room. Approximately 0.8-1g of adipose tissue samples were collected in ice-cold phosphate-buffered saline with 2% fetal bovine serum. SAT was collected immediately following the median sternotomy incision and EAT was obtained following opening up of the pericardial sac with tissue typically collected over the body of the right ventricle. Following tissue collection, samples were transferred to the William Harvey Research Institute, Queen Mary University of London (London, UK) for further processing (supplemental figure 7).

The protocols of the studies complied with the Declaration of Helsinki, and all patients provided informed written consent. The demographic characteristics are presented in table 1 to 4.

Sample processing

Fasting blood samples were collected pre-operatively to include: 6.5mls of peripheral blood divided into 2.5mls collected in a PAXgene® (PreAnalytiX) tube for RNA isolation and the remaining 4mls in an ethylenediaminetetraacetic acid (EDTA) tube (BD). Peripheral blood mononuclear cells (PBMCs) isolated using the Ficoll-Paque ™ PLUS (GE Healthcare) as per manufacturer's instructions. PBMCs were then stained using antibodies for flow cytometry analysis (Table S3). The gating strategy is depicted in supplementary figure 1.

Following adipose tissue sample collection, samples were divided into a portion for flow cytometry (~0.1-0.4g) analysis, a portion for subsequent RNA extraction (~0.1-0.2g) which was snap frozen and a sample fixed in 4% formaldehyde solution (Sigma-Aldrich) for future immunohistochemical analysis (~0.05-0.2g). The sample of adipose tissue aliquoted for flow cytometry analysis was first mechanically minced using microscopy scissors and then digested enzymatically using 5668 IU collagenase II (Sigma-Aldrich) and 55.5 IU DNase (Sigma-Aldrich) per gram of adipose tissue for 30 minutes. Immune cells present in the stromal vascular fraction were obtained following centrifugation and lysed for red blood cells prior to antibody staining.

T cell stimulation assays

In a proportion of patients where sufficient adipose tissue samples were available for further analyses, tissue was aliquoted for T cell stimulation assays. Briefly, phorbol 12-myristate 13-acetate (PMA)/ionomycin with the addition of brefeldin A was used to

stimulate the immune cell fraction of the digested adipose tissue for 4 hours followed by interleukin17 (IL17), IL22 and interferon (IFNγ) intracellular staining as shown in supplemental figure 1.

Real-time Polymerase Chain Reaction Analysis

Total RNA was extracted from the adipose tissue using QIAzol (QIAGEN) and the RNeasy Lipid Tissue Mini Kits (QIAGEN) following the manufacturer's instructions. Total RNA was extracted from whole blood samples stored in PAXgene® (PreAnalytiX) tubes using the PAXgene® blood RNA kit (QIAGEN). RNA was quantified using the NanoDrop spectrophotometer (ThermoFisher). Reverse transcription to complementary deoxyribonucleic acid (cDNA) was performed using High-Capacity RNA-to-cDNA kits (Applied Biosystems) and stored at -80°C. The relevant primer sequences can be found in table S4 and were purchased from Invitrogen. Gene expression was performed using SYBR Green Supermix (Bio-Rad), as per manufacturer's instructions and analysed using the Light Cycler System (Roche). Relative gene expression values were determined using the ΔΔCT method and normalized to a stable reference housekeeping gene control (GAPDH). The control values were set at a 1. Given the ΔΔCT methods is not normally distributed, the geometric mean was used for the representation of the data (40). Illumina sequencing was carried out at Novogene (Novogene Bioinformatics Technology Ltd).Data was deposited in Gene Expression Omnibus (GEO) GSE179455.

Propensity Matching of Groups

To account for differences in baseline clinical variables between groups, a propensity matching algorithm was applied. A 1:1 propensity score matching (PSM) optimal algorithm was utilized using dedicated propensity matching software (XLSTAT, Addisoft). The confidence interval was set at 95% and a caliper width at 0.2. For the initial analysis comparing differences in severe vs non-severe CAD, CABG and VR surgery were used as the dependent variables and age, gender, body mass index (BMI), hypertension, hyperlipidaemia and diabetes as co-variates. For the PSM of subsequent group analyses, BMI and diabetes were used as dependent variables and age, gender, hypertension and hyperlipidaemia as co-variates. While for the hypertension group analysis, the variables to be matched for included age, gender, BMI, hyperlipidaemia, and diabetes status.

Role of co-morbidities and cardiovascular risk factors: subgroup assessment.
We evaluated cardiovascular risk factors which are known to impact adipose tissue specifically, namely obesity/overweight (4,6), diabetes (15,27) and hypertension (5,39) to determine the extent to which they alter the inflammatory profile in different groups of patients. All diabetic patients included suffered from type 2 diabetes (T2D).

Flow cytometry analysis, real-time polymerase chain reaction (RT-PCR) analysis and T cell stimulation assays (as per the above methodology) were undertaken to identify the key immune cells and mediators that differ between the groups. Comprehensive

absolute changes in numbers of all immune cells available in the supplemental data figures 3 to 5.

Statistics

SPower calculations were based an effect size of 1.16, power of 0.95 and α error of 0.05. Statistical significance was determined for continuous variables where three groups were assessed using the ANOVA (or Kruskal-Wallis for non-parametric data) test with Dunnett's T3 (or Dunn's for non-parametric data) multiple comparisons post-test applied. The student's *t*-test was used for two groups of continuous data where the data was parametric and the Mann-Whitney test for non-parametric data. The chi-squared test or Fisher's exact test was utilized for categorical data. Data were analyzed on GraphPad Prism version 8 (GraphPad Software LLC). Normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk test. Where parametric data are represented, the mean and standard deviation values are reported and for non-parametric data, median and inter-quartile ranges are reported. A *p*-value of less than .05 was considered statistically significant.

Study approval

The study was approved institutionally via the ethical framework of the Barts Bioresource (research ethics committee reference: 14/EE/0007) and written informed consent was obtained from each patient. Written consent was obtained prior to sample collection and patients were identified with an arbitrary Barts Bioresource number.

Authors contribution. M.P.L. and M.F. conceived the study; V.V., H.B., E.G.W and M.P.L. designed experiments; V.V., H.B., E.G.W. and B.S. performed and analysed experiments. S.S provided statistical analysis; D.B., S.G.A, J.Y.,S.J.E., C.D.S., K.W., N.R.; R.U., B.A., A.S., A.Y.O., D.L., S.K. and K.S.L provided clinical assessment and tissue samples. V.V and M.P.L. wrote the manuscript.

Amongst the co-first authors, V.V. was assigned first given he wrote the manuscript with M.P.L. followed by H.B. and then E.G.W. based on their relative contribution to performing and analysing experiments.

Acknowledgments. This work was supported by Barts Charity MGU0413 (V.V. and M.P.L.), Abbott (V.V. and M.F), Medical Research Council MR/T008059/1 (V.V), British Heart Foundation FS/13/49/30421 (M.P.L., H.B.), PG/16/79/32419 (E.G.W.), BHF 4Y PhD programme (B.S). This work forms part of the research areas contributing to the translational research portfolio of the Biomedical Research Centre at Barts which is supported and funded by the National Institute for Health Research.

We are indebted to all members of the Barts Heart Centre, Professor Steffen Petersen, Ms Maudrian Burton and the Barts Bioresource staff who assisted with consent and access to medical records, and all the patients that kindly agreed to participate in this research. We are grateful to members of the Marelli-Berg laboratory at QMUL for their technical support and scientific discussions.

References

1. Sacks HS, Fain JN. Human epicardial adipose tissue: A review. *Am Heart J*. 2007;153(6):907-917.
2. Ansaldo AM, et al. Epicardial adipose tissue and cardiovascular diseases. *Int J Cardiol*. 2019;278:254-260.
3. Packer M. Epicardial Adipose Tissue May Mediate Deleterious Effects of Obesity and Inflammation on the Myocardium. *J Am Coll Cardiol*. 2018;71(20):2360-2372.
4. Song DK, et al. Increased epicardial adipose tissue thickness in type 2 diabetes mellitus and Obesity. *Diabetes Metab J*. 2015;39(5):405-413.
5. Austys D, et al. Epicardial adipose tissue accumulation and essential hypertension in non-obese adults. *Med*. 2019;55(8):1-11.
6. Yorgun H, et al. Increased epicardial fat tissue is a marker of metabolic syndrome in adult patients. *Int J Cardiol*. 2013;165(2):308-313.
7. Graeff DB, et al. Epicardial fat thickness: distribution and association with diabetes mellitus, hypertension and the metabolic syndrome in the ELSA-Brasil study. *Int J Cardiovasc Imaging*. 2016;32(4):563-572.
8. Iacobellis G. Local and systemic effects of the multifaceted epicardial adipose tissue depot. *Nat Rev Endocrinol*. 2015;11(6):363-371.
9. Mahabadi AA, et al. Association of epicardial fat with cardiovascular risk factors and incident myocardial infarction in the general population: The Heinz Nixdorf recall study. *J Am Coll Cardiol*. 2013;61(13):1388-1395.
10. Mahabadi AA, et al. Noncoronary Measures Enhance the Predictive Value of Cardiac CT Above Traditional Risk Factors and CAC Score in the General Population. *JACC Cardiovasc Imaging*. 2016;9(10):1177-1185.
11. Fitzgibbons TP, et al. Coronary disease is not associated with robust alterations in inflammatory gene expression in human epicardial fat. *JCI Insight*. 2019;4(20).
12. Bäck M, et al. *Nat Rev Cardiol*. 2019;16(7):389-406. doi:10.1038/s41569-019-0169-2
13. Adamo L, et al. Reappraising the role of inflammation in heart failure. *Nat Rev Cardiol*. 2020;17(5):269-285.
14. Mazurek T, et al. Human Epicardial Adipose Tissue Is a Source of Inflammatory Mediators. *Circulation*. 2003;108(20):2460-2466.
15. Cheng KH, et al. Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. *Int J Obes*.
16. Bambace C, et al. Inflammatory profile in subcutaneous and epicardial adipose tissue in men with and without diabetes. *Heart Vessels*. 2014;29(1):42-48.
17. McAninch EA, et al. Epicardial adipose tissue has a unique transcriptome modified in severe coronary artery disease. *Obesity*. 2015;23(6):1267-1278.

18. Hirata Y, et al. Coronary atherosclerosis is associated with macrophage polarization in epicardial adipose tissue. *J Am Coll Cardiol*. 2011;58(3):248-255.
19. Guzik TJ, et al. The role of infiltrating immune cells in dysfunctional adipose tissue. *Cardiovasc Res*. 2017;113(9):1009-1023.
20. Han SJ, et al. White Adipose Tissue Is a Reservoir for Memory T Cells and Promotes Protective Memory Responses to Infection. *Immunity*. 2017;47(6):1154-1168.e6.
21. O'Sullivan D, et al. Memory CD8+ T Cells Use Cell-Intrinsic Lipolysis to Support the Metabolic Programming Necessary for Development. *Immunity*. 2014;41(1):75-88.
22. Drolet R, et al. Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women. *Obesity*. 2009;17(3):424-430.
23. Jaitin DA, et al. Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. *Cell*. 2019;178(3):686-698.e14.
24. Mueller SN, Mackay LK. Tissue-resident memory T cells: Local specialists in immune defence. *Nat Rev Immunol*. 2016;16(2):79-89.
25. Zhang N, Bevan MJ. Transforming growth factor-β signaling controls the formation and maintenance of gut-resident memory T cells by regulating migration and retention. *Immunity*. 2013;39(4):687-696.
26. Dumauthioz N, et al. Tumor resident memory T cells: New players in immune surveillance and therapy. *Front Immunol*. 2018;9(SEP):1-6.
27. Gruzdeva OV, et al. Adipokine and Cytokine Profiles of Epicardial and Subcutaneous Adipose Tissue in Patients with Coronary Heart Disease. *Bull Exp Biol Med*. 2017;163(5):608-611.
28. Wang QC et al. Exploring the Role of Epicardial Adipose Tissue in Coronary Artery Disease From the Difference of Gene Expression. Front Physiol. 2021 Mar 30;12:605811.
29. Saisho Y. Metformin and Inflammation: Its Potential Beyond Glucose-lowering Effect. Endocr Metab Immune Disord Drug Targets. 2015;15(3):196-205.
30. Woodward L, et al. Unravelling the adiponectin paradox: novel roles of adiponectin in the regulation of cardiovascular disease. *Br J Pharmacol*. 2017;174(22):4007-4020.
31. Iacobellis G, et al. Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease. *Cytokine*. 2005;29(6):251-255.
32. Kusminski CM, Scherer PE. Mitochondrial dysfunction in white adipose tissue. Trends Endocrinol Metab. 2012 Sep;23(9):435-43.
33. Pietiläinen KH, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. PLoS Med. 2008 Mar 11;5(3):e51.
34. Abel ED, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001 Feb 8;409(6821):729-33.
35. Ortega FJ, et al. The gene expression of the main lipogenic enzymes is downregulated in visceral adipose tissue of obese subjects. Obesity (Silver Spring). 2010 Jan;18(1):13-20.

36. Herman MA, et al. Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. J Biol Chem. 2010 Apr 9;285(15):11348-56.
37. Li T, et al. Defective Branched-Chain Amino Acid Catabolism Disrupts Glucose Metabolism and Sensitizes the Heart to Ischemia-Reperfusion Injury. Cell Metab. 2017 Feb 7;25(2):374-385.
38. Tobias DK, et al. Circulating Branched-Chain Amino Acids and Incident Cardiovascular Disease in a Prospective Cohort of US Women. Circ Genom Precis Med. 2018 Apr;11(4):e002157.
39. Dicker D, et al. Increased Epicardial Adipose Tissue Thickness as a Predictor for Hypertension: A Cross-Sectional Observational Study. *J Clin Hypertens*. 2013;15(12):893-898.
40. Macdougall CE, et al. Visceral Adipose Tissue Immune Homeostasis Is Regulated by the Crosstalk between Adipocytes and Dendritic Cell Subsets. *Cell Metab*. 2018;27(3):588-601.e4.

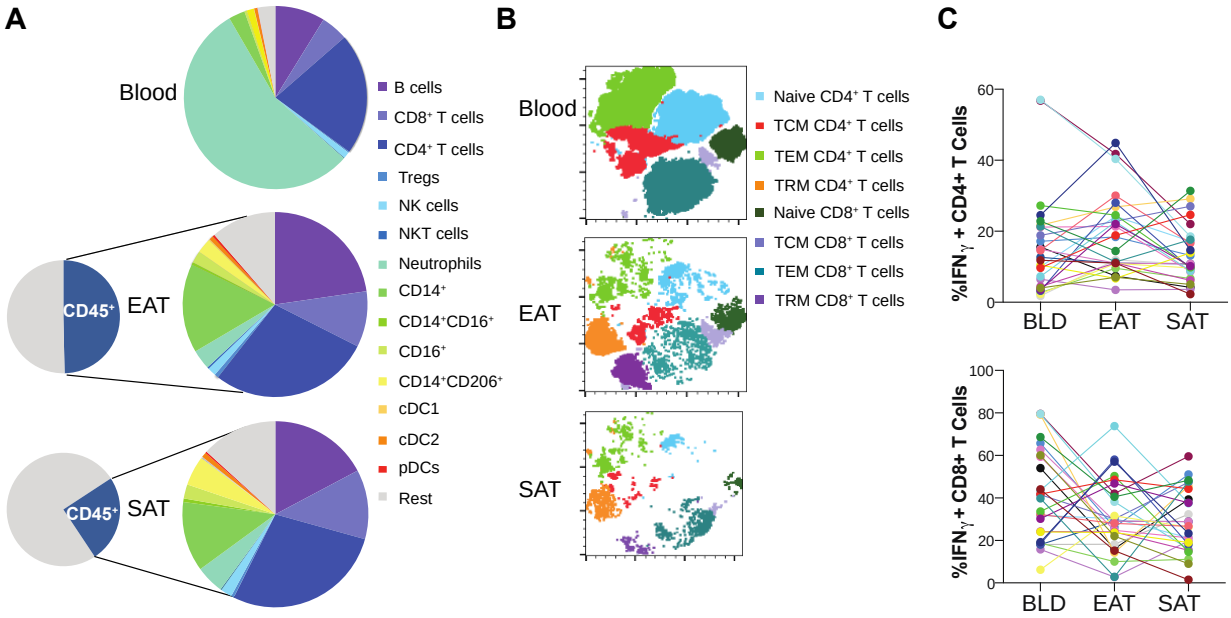


Figure 1. CD4⁺T cells are the dominant immune population in EAT and SAT. (A) Pie chart illustrating relative proportions CD45⁺ cells from the stromal-vascular fraction across tissues (n=152). (B) Representative t-SNE plot to cluster different T cell subsets into a 2-dimensional plot across blood, EAT and SAT. (C) IFNγ levels produced by T cells in blood, EAT and SAT (n=25). Each color line represents the same patient.

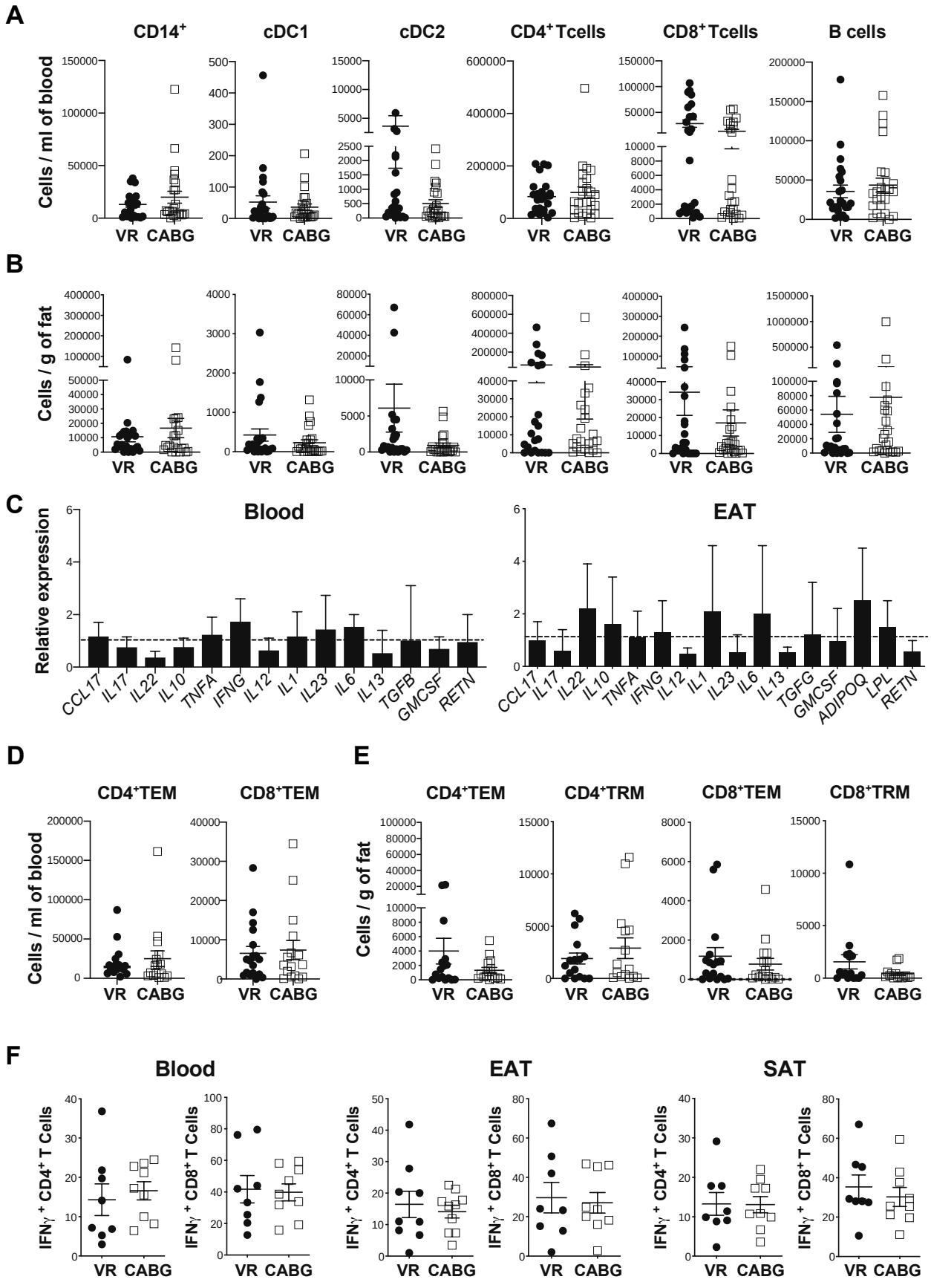

A
CD14+
cDC1
cDC2
CD4+ Tcells
CD8+ Tcells
B cells
Cells / ml of blood
VR CABG
B
Cells / g of fat
C
Blood
EAT
Relative expression
CCL17 IL17 IL22 IL10 TNFA IFNG IL12 IL1 IL23 IL6 IL13 TGFB GMCSF RETN
CCL17 IL17 IL22 IL10 TNFA IFNG IL12 IL1 IL23 IL6 IL13 TGFG GMCSF ADIPOQ LPL RETN
D
CD4+TEM
CD8+TEM
Cells / ml of blood
E
CD4+TEM
CD4+TRM
CD8+TEM
CD8+TRM
Cells / g of fat
F
Blood
EAT
SAT
IFNγ+ CD4+ T Cells
IFNγ+ CD8+ T Cells

Figure 2. Comparison of EAT immune profiling between CAD patients and controls. (A-B) Absolute number of immune cells in CABG and VR patients across blood (A) and EAT (B) (n=24 patients/ group). (C) Relative expression levels of immune mediators in blood and EAT respectively (n=24 patients/ group). Expression levels were normalized to GAPDH expression. Bars represent expression in CABG patients compared to VR surgery, which was set at 1 and indicated with doted lines. Error bars show the geometric mean. (D-E) Graphs showing T cell subsets in blood (D), EAT (E). (F) IFNγ production amongst live $CD4^+$ and $CD8^+$ T cells in blood, EAT and SAT respectively (n=7-8 patients/ group). Statistical significance was determined by Mann-Whitney test, denoted as $*P < 0.05$ and data represented as median.

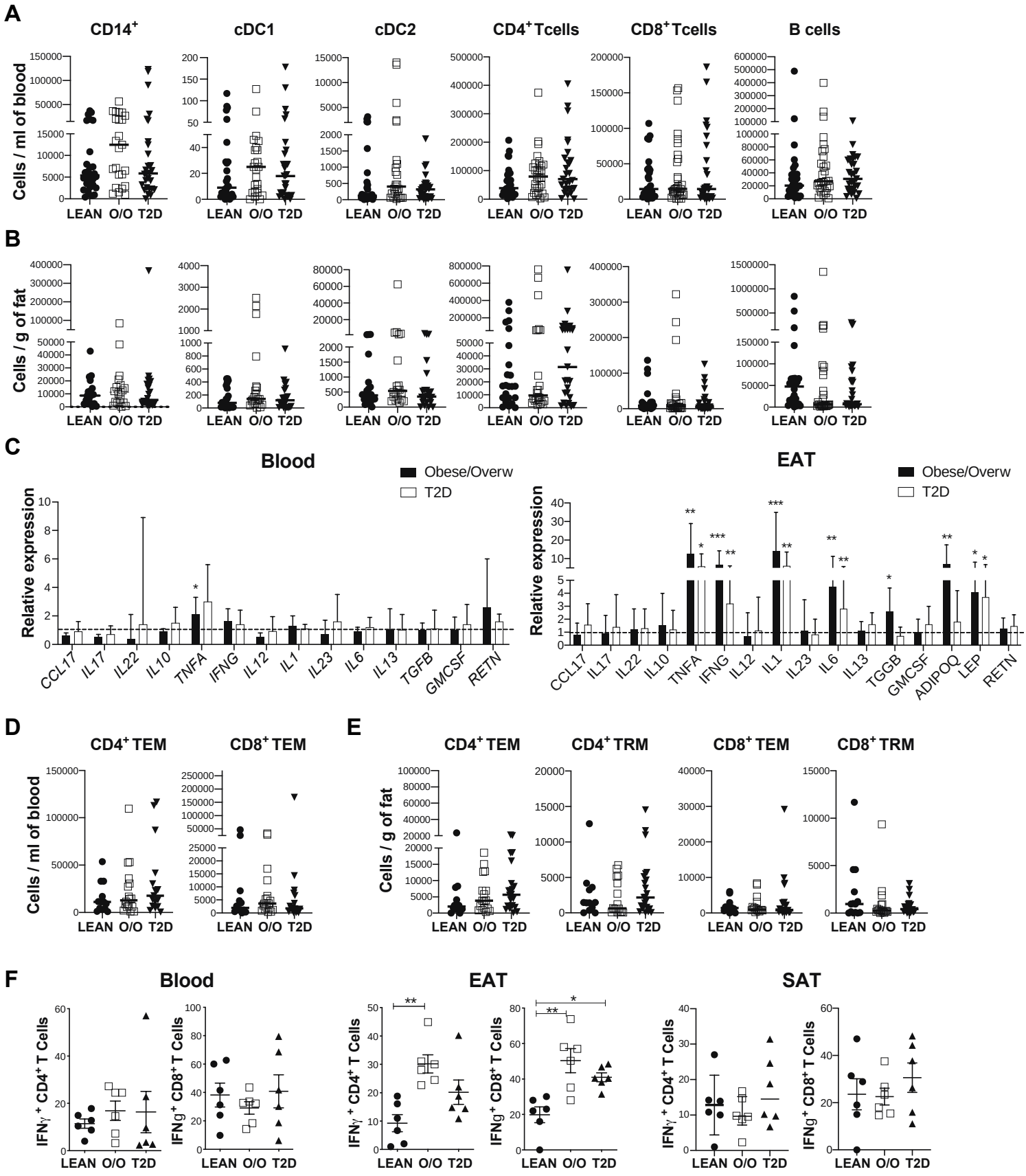


Figure 3. Comparison of EAT immune profiling between obese/overweight and T2D

patients and controls. (A-B) Absolute number of immune cells in overweight/obesity (O/O) and T2D patients across blood (A) and EAT (B) (n=30 patients/ group). (C) Relative expression of immune mediators in overweight/obesity and T2D patients compared with lean non-diabetic patients across blood and EAT(n=30 patients/ group). Gene expression was normalised to GAPDH and control set as 1, indicated with doted lines. Error bars show the geometric mean. (D-E) Graphs showing T cell subsets in blood (D) and EAT. (F) IFNγ production amongst live $CD4^+$ and $CD8^+$ T cells in blood, EAT and SAT respectively (n=6 patients/ group). Statistical significance was determined by the Kruskall-Wallis Tests with Dunn's multiple comparisons post-test correction applied. Significance denoted as *p<0.05; **p<0.005; ***p<0.0005 and data represented as median.

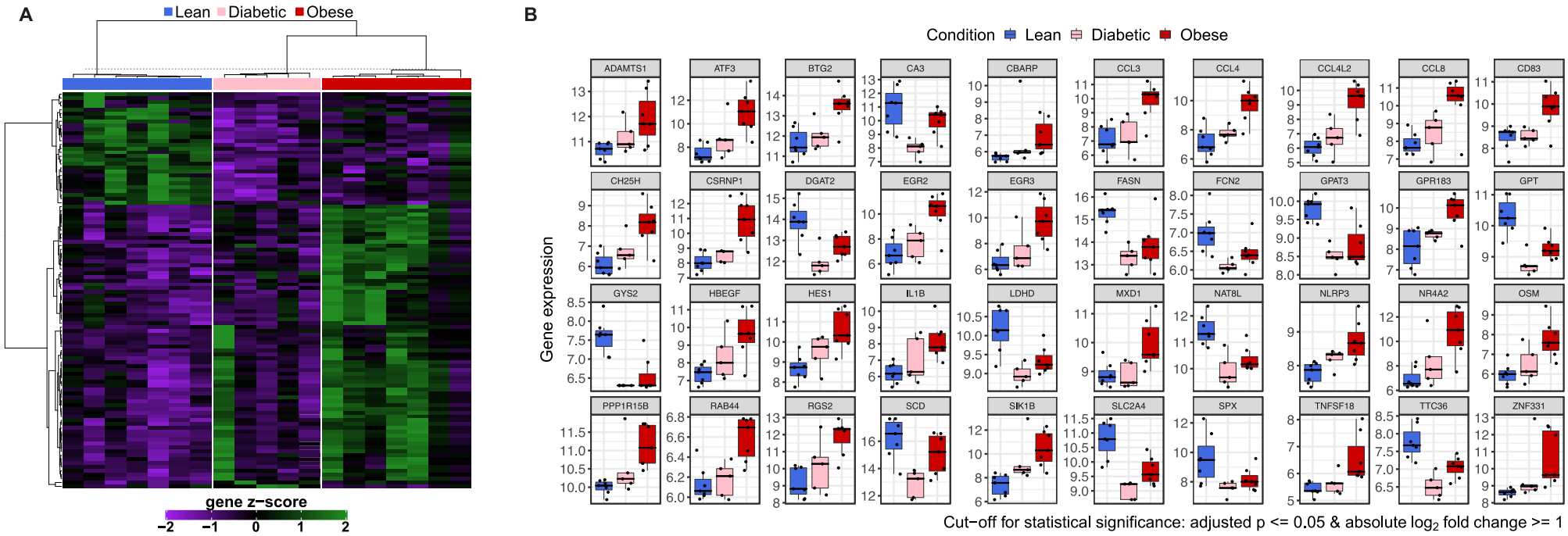


Figure 4. Differentially expressed genes in EAT from obese/overweight and T2D patients compared to lean. (A) Heatmap of differentially expressing genes in EAT, cut-off: adjusted *p*<0.05; Log2FC>1. (B) Bars represents gene expression value for the top 40 differentially expressed genes in all 3 groups.

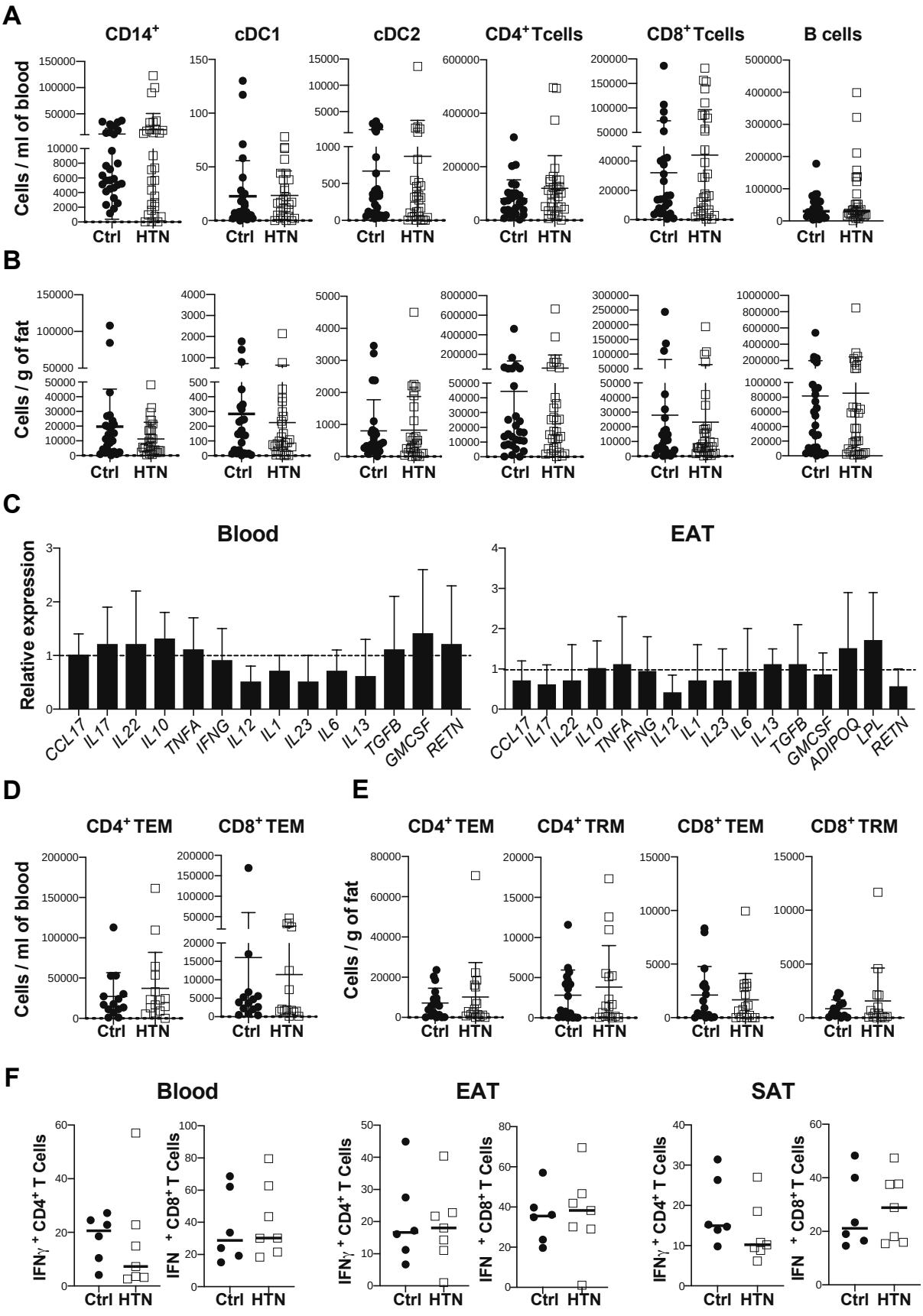


Figure 5. Comparison of EAT immune profiling between hypertensive patients and controls. (A-B) Absolute number of immune cells in hypertensive and control patients

across blood (A) and EAT (B) (n=32 patients/ group). (C) Relative expression of immune mediators in hypertensive vs control patients across blood and EAT(n=32 patients/ group). Gene expression was normalised to GAPDH and control set as 1, indicated with doted lines. Error bars show the geometric mean. (D-E) Graphs showing T cell subsets in blood (D) and EAT (E). (F) Graph represent IFNγ production amongst live $CD4^+$ and $CD8^+$ T cells in blood, EAT and SAT respectively (n=6 patients/ group). Statistical significance was determined by Mann-Whitney test, and data represented as median.

Table 1. Clinical characteristics of all study participants

Variable	Valve Repair/Replacement (n=54)	CABG (n=82)	Combined CABG/Valve Surgery (n=16)	P value
Age (years)	67.4 ± 11	64.9 ± 9.9	70.4 ± 6.8	0.08
Body Mass Index (kg/m^2)	26.6 (22.9-29.9)	29.2 (25.4-31.6)	28.9 (26.5-31.1)	0.008**
Male Gender (%)	34 (63.0)	67 (81.7)	14 (87.5)	0.02*
Female Gender (%)	20 (37.0)	15 (18.3)	2 (12.5)	
Hypertension (%)	28 (51.8)	61 (74.4)	12 (75)	0.02*
Type 2 Diabetes	8 (14.8)	24 (29.3)	8 (50)	0.004**
Hyperlipidemia (%)	26 (48.1)	58 (70.3)	8 (50)	0.02*
Smoking history (%)	20 (37.0)	54 (65.8)	9 (56.2)	0.004**
Prior myocardial infarction (%)	10 (18.5)	36 (43.9)	0 (0)	0.0003****
Prior Atrial Fibrillation (%)	20 (37.0)	7 (8.5)	3 (18.8)	0.0002****
Post-operative atrial fibrillation (%)	7 (13.0)	18 (21.9)	8 (50)	0.04*
Left ventricular ejection fraction (%)	59 (55-63)	53 (46-60)	51 (40-60)	0.001***
Pre-operative use of beta blockers (%)	22 (40.7)	64 (78.0)	9 (56.3)	0.001***
Pre-operative use of ACE inhibitors/ARBs (%)	24 (44.4)	59 (71.9)	8 (50)	0.004**
Pre-operative use of Aspirin (%)	12 (22.2)	63 (76.8)	12 (75)	0.0001****
Pre-operative use of Statins (%)	30 (55.5)	68 (82.9)	13 (81.2)	0.001**
Pre-operative use of Metformin (%)	4 (7.4)	17 (20.7)	3 (18.7)	0.04*
CRP (mg/L)	3 (1-5.5)	2 (1-5)	1 (1-8.2)	0.36
Neutrophil: lymphocyte ratio	2.1 (1.7-3.1)	1.9 (1.4-3.2)	2.6 (1.7-3.4)	0.2

ANOVA/Kruskall-Wallis for continuous data with Dunnett's T3 (or Dunn's for non-parametric data) multiple comparisons post-test applied. Chi-squared/Fisher's exact test for categorical data was used where appropriate.

Table 2. Propensity-matched patients for valve repair/replacement vs coronary artery bypass surgery comparison.

Variable	Valve repair/replacement surgery (n=24)	CABG surgery (n=24)	P value
Age (years)	66.8± 12.4	65 ± 7.9	0.54
Body Mass Index (kg/m^2)	26.4 (22.1-29.9)	27.8 (25.2-30.1)	0.23
Male Gender (%)	14 (58.3)	19(79.2)	0.22
Female Gender (%)	10 (41.7)	5 (20.8)	
Hypertension (%)	13 (54.2)	15 (62.5)	0.77
Type 2 Diabetes	3 (12.5)	6 (25)	0.46
Hyperlipidemia (%)	15 (62.5)	19 (79.1)	0.34
Smoking history (%)	10 (41.7)	16 (66.7)	0.15
Prior myocardial infarction (%)	0 (0)	0 (0)	0.99
Prior Atrial Fibrillation (%)	6 (25)	5 (20.8)	0.73
Post-operative atrial fibrillation (%)	7 (29.2)	4 (16.7)	0.30
Left ventricular ejection fraction (%)	60 (60-63)	58 (55-60)	0.07
Pre-operative use of beta blockers (%)	12 (50)	18 (75)	0.13
Pre-operative use of ACE inhibitors/ARBs (%)	10 (41.7)	11 (45.8)	0.99
Pre-operative use of Aspirin (%)	5 (20.8)	12 (50)	0.07
Pre-operative use of Statins (%)	13 (54.2)	17(70.8)	0.37
Pre-operative use of Metformin (%)	2 (14.3)	5 (20.8)	0.99
CRP (mg/L)	3.8 (1.7-5.2)	2.7 (1.3-3.8)	0.13
Neutrophil: lymphocyte ratio	2.5 (1.6-2.9)	2.2 (1.5-2.4)	0.27

Student's t-test/Mann-Whitney test for continuous data or Chi-squared/Fisher's exact test for categorical data was used where appropriate.

Table 3. Propensity-matched patients for overweight/obese, diabetic and control patients

Variable	Lean non-diabetic (BMI<25) (n=30)	Overweight /obese non-diabetic (BMI≥25) (n=30)	Overweight /obese (BMI≥25) Type 2 diabetic (n=30)	P value
Age (years)	66.1 ± 12.2	65.7 ± 8.8	66.1 ± 8.4	0.98
Body Mass Index (kg/m^2)	22.8 ± 1.5	30.3 ± 2.5	30.5 ± 3.6	N/A
Male Gender (%)	20 (66.6)	23 (76.7)	22 (73.3)	0.51
Female Gender (%)	10 (33.3)	7 (23.3)	8 (26.7)	
Hypertension (%)	17 (56.7)	17 (56.7)	21 (70)	0.47
Hyperlipidemia (%)	17 (56.7)	15 (50)	20 (66.7)	0.42
Smoking history (%)	14 (46.7)	20 (66.7)	15 (50)	0.24
Prior myocardial infarction (%)	7 (23.3)	6 (20)	11 (36.7)	0.27
Prior Atrial Fibrillation (%)	7 (23.3)	3 (10)	6 (20)	0.37
Post-operative atrial fibrillation (%)	8 (26.7)	4 (13.3)	6 (20)	0.43
Left ventricular ejection fraction (%)	54.7 (49-60)	55.8 (52-60)	53.7 (50-57)	0.35
Pre-operative use of beta blockers (%)	15 (50)	14 (46.7)	21 (70)	0.08
Pre-operative use of ACE inhibitors/ARBs (%)	12 (40)	19 (63.3)	20 (66.7)	0.25
Pre-operative use of Aspirin (%)	14 (46.7)	16 (53.3)	21 (70)	0.06
Pre-operative use of Statins (%)	17 (56.7)	21 (70)	28 (93.3)	0.005**
CRP (mg/L)	2.1 (1.4-3.3)	3.6 (2.5 -5.1)	2.5 (1.7-3.8)	0.1
Neutrophil: lymphocyte ratio	2 (1.6-3.2)	2.4 (1.9-3.1)	1.8 (1.4-4.1)	0.7
Valve repair/replacement surgery (%)	15 (50)	13 (43.3)	9 (30)	0.27
CABG surgery (%)	13 (43.3)	15 (50)	18 (60)	0.43
Combined CABG/ Valve surgery (%)	2 (6.7)	2 (6.7)	3 (10)	0.85

ANOVA/Kruskall-Wallis for continuous data with Dunnett's T3 (or Dunn's for non-parametric data) multiple comparisons post-test applied. Chi-squared/Fisher's exact test for categorical data was used where appropriate.

Table 4. Propensity-matched patients for hypertensive and control patients

Variable	Hypertensive group (n=32)	Control non-hypertensive group (n=32)	P value
Age (years)	67.4 ± 7.9	65.8± 13	0.56
BMI (kg/m^2)	28.1(23.2-29.9)	27.5 (21.1-29.8)	0.87
Male Gender (%)	25 (78.1)	24 (75)	0.99
Female Gender (%)	7 (21.9)	8 (25)	
Hyperlipidemia (%)	17 (53.1)	17 (53.1)	0.99
Smoking history (%)	15 (46.9)	20 (62.5)	0.31
Prior myocardial infarction (%)	8 (25)	8 (25)	0.99
Prior Atrial Fibrillation (%)	6 (18.7)	5 (15.6)	0.74
Post-operative atrial fibrillation (%)	8 (25)	6 (18.7)	0.54
Left ventricular ejection fraction (%)	55 (48-60)	60 (55-60)	0.53
Pre-operative use of beta blockers (%)	16 (50)	21 (65.6)	0.31
Pre-operative use of ACE inhibitors/ARBs (%)	22 (68.7)	16 (50)	0.2
Pre-operative use of Aspirin (%)	24 (75)	21 (65.6)	0.58
Pre-operative use of Statins (%)	3 (9.4)	4 (12.5)	0.99
Pre-operative use of Metformin (%)	2 (1-6)	3 (2-9)	0.14
CRP (mg/L)	1.9 (1.4-3.9)	2.3 (1.8-2.8)	0.7
Neutrophil: lymphocyte ratio	7 (21.9)	6 (18.7)	0.99
CABG surgery (%)	14 (43.8)	15 (46.9)	0.99
Valve repair/replacement surgery (%)	15 (46.9)	15 (46.9)	0.99
Combined CABG/Valve surgery (%)	3 (9.4)	2 (6.3)	0.99

Student's t-test/Mann-Whitney test for continuous data or Chi-squared/Fisher's exact test for categorical data was used where appropriate.