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Original Article

Epicardial adipocyte size does not correlate with body mass index

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ABSTRACT

Background: Epicardial adipose tissue (EAT) deposition has a strong association with aspects of metabolic dysfunction, including obesity. The size of the EAT adipocytes in relation to obesity, however, has rarely been researched. Therefore, to contextualise EAT within the broader framework of pathophysiological adipocyte size changes in obesity, we aimed to determine whether EAT adipocyte size is associated with body mass index (BMI).
Methods: During routine post-mortem examination, adipose tissue biopsies were obtained from four depots of 43 cases, including EAT, as well as pericardial (PAT), appendix mesenteric (AAT), and clavicular subcutaneous (SAT) adipose tissues. Tissues were fixed, sectioned, and stained using haematoxylin and eosin. The size (measured as area) of each adipocyte imaged from the depots was analysed in relation to BMI.
Results: Mean size of EAT adipocytes was significantly smaller than that from SAT and AAT depots, while not differing from PAT adipocytes. BMI positively correlated with the size of adipocytes isolated from SAT (r=0.5893, P<.0001), PAT (r=0.5854, P<.0001), and AAT (r=0.5829, P<.0001) depots, but not from EAT (r=0.1242, P=.4274), even after multivariate adjustment for age and sex.
Conclusions: EAT adipocyte size is not associated with increased BMI despite significant associations within adipocytes from other adipose depots.

1. Introduction

Epicardial adipose tissue (EAT) is the fat depot located between the surface of the myocardium and the visceral pericardium. Clinical interest in EAT has piqued in the past 20 years due to associations of macroscopic EAT deposition, whether measured as thickness or volume, with cardiovascular disease and anthropometric indices of obesity, including body-mass index (BMI) [1–3]. Moreover, as a fat depot, EAT shares unique contiguity with the underlying tissue that is not constrained by a fascial boundary. Consequently, EAT and the myocardium share a microcirculation and, therefore, a direct paracrine and vasocrine signalling relationship [4,5]. Despite the clinical interest in EAT, basic research from a morphological aspect has lagged behind. In particular, the understanding of EAT adipocyte size, both in health and in metabolic dysfunction, is poor, especially when compared to other visceral and subcutaneous adipose depots.

Microscopic adipose tissue morphology, which encompasses adipocyte size and number, has been comprehensively researched in various subcutaneous and visceral adipose tissue depots (recently reviewed by Tandon et al. [6]). Although distinct regionality exists between adipose depots, the remodelling associated with an increase in obesity-indices is conserved. Studies of human subcutaneous adipose tissue (SAT) adipocytes, most commonly from an abdominal origin, have consistently shown that adipocyte size (or hypertrophy) positively correlates with BMI or body fat percentage [7–12]. Moreover, a similar correlation exists with adipocytes isolated from other visceral adipose tissue (VAT) depots, including from omental, retroperitoneal, and mesenteric origins [12,13]. SAT and VAT adipocyte hypertrophy has been established as a risk factor for metabolic derangements, including insulin

Abbreviations: EAT, epicardial adipose tissue; SAT, subcutaneous adipose tissue; AAT, appendix mesenteric adipose tissue; PAT, pericardial adipose tissue; VAT, visceral adipose tissue; BMI, body mass index.

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resistance and dyslipidaemia, and with abnormalities in the circulating levels of adipocyte-derived inflammatory and fibrotic markers [7,14–16]. Therefore, because macroscopic EAT deposition is emerging as an important clinical marker for metabolic dysfunction, it is pertinent to determine whether concurrent alterations occur within the microscopic morphology of EAT.

Previous investigations of changes in EAT adipocyte size with obesity have yielded divergent results [17]. Sons and Hoffmann found that with an increase in body weight, adipocytes derived from the EAT of post-mortem hearts were slightly larger when from obese cases relative to lean cases [13], whereas others either found no correlation of EAT adipocyte size with BMI or the results were inconclusive [9,10].

We aimed to clarify the discrepancy that currently exists in the understanding of the EAT adipocyte morphology and obesity relationship. To this end, we have measured the size of adipocytes isolated from the EAT of post-mortem hearts from 43 cases with a range of BMIs, which is the largest sample size used for such a study. Further, we have analysed paired adipocytes from other SAT and VAT depots to serve as positive controls.

2. Methods

2.1. Post-mortem cases

Adipose tissue biopsies were collected from four anatomically distinct sites during consecutive routine coronial post-mortem examinations as part of a 6-month prospective study at Auckland City Hospital, Auckland, New Zealand. Cases excluded from analysis were all paediatrics, those with suspicious or homicidal cause of death, or those with potential medico-legal implications. Further exclusion was applied to cases with advanced decomposition or trauma that resulted in loss of suitable tissue. No cases were severely malnourished and/or cachectic. Tissue sampling and processing for histological analysis formed part of the post-mortem examination and all post-mortem examinations were authorised by the chief coroner. No tissue was retained following processing. Information available for each case was limited to the age, sex, cause of death, as well as body weight and height, which was used to calculate BMI (body weight (kg)/height $(m)^2$). No other potentially identifying information could be deduced from the adipose images used for analysis.

2.2. Adipose tissue procurement

The EAT biopsy was taken as part of routine right coronary artery examination from the atrioventricular groove, between the right coronary artery orifice and the right lateral ventricular wall. For comparison to EAT, a SAT sample from the clavicular fat was examined as part of biopsy for skin and muscle histology. Additionally, adipose tissue samples were obtained from routine appendix and pericardial wall examinations. The appendix mesenteric (AAT) and pericardial (PAT) adipose tissues were utilised as VAT controls. PAT was defined as the adipose tissue external to the parietal pericardium.

2.3. Adipose tissue processing

Adipose biopsies were immediately transferred to a histology cassette and immersed in 10% formalin for fixation. The tissues were fixed for 24–48 h and transferred to an accredited histology processing laboratory (Department of Anatomical Pathology, LabPLUS, Aukland City Hospital). The biopsies were processed using the Leica Peloris Tissue Processor (Leica, Germany). This involves a stepwise infusion of formalin, alcohol, xylene and finally paraffin wax. After tissue processing, the biopsies were embedded into paraffin wax and sectioned at 4 μm using a Thermo scientific HM325 microtome (ThermoFisher Scientific, USA). Sectioned tissues were then stained using Haematoxylin and Eosin using an automated Leica Multistainer model ST5020 (Leica Biosystems, Germany). The histological slides were viewed using an Olympus BX53 microscope (Olympus, Japan) using an UPlanFL N 20× objective. One digital image was taken using an Olympus UC50 digital camera (Olympus, Japan) and analysed for each adipose depot from each case.

2.4. Measuring adipocyte area

The measurement of adipocyte area was performed using Aperio ImageScope software (Leica Biosystems Pathology Imaging, Germany). The researcher was blinded to the origin of the adipose tissue image as well as the BMI of the case. To measure the adipocyte area, the Positive Pen tool of the ImageScope software was used to trace the border of every adipocyte within the field of view. Adipocytes with ambiguous or broken cell membranes, as well as adipocytes cut-off by the image edge, were not traced for analysis. The adipocyte area (contained within each adipocyte tracing) was calculated based on the pixel size per μm^2, which at the 20× objective used for imaging equated to 0.289 μm^2 per pixel.

2.5. Data analysis

Researcher blinding was maintained during data analysis. Results are presented as mean ± standard deviation (SD) or standard error (SEM) as appropriate. Case BMI was categorised into the standardised ranges, with <25 kg/m^2 allocated into the lean group, ≥25<30 kg/m^2 into the overweight group, and ≥30 kg/m^2 into the obese group [18]. Adipocyte size frequency distributions were plotted from every adipocyte measured from each adipose depot. Differences of mean adipocyte size between adipose depots were determined using one-way ANOVA followed by post-hoc Tukey's multiple comparisons test. Univariate (Pearson) correlations were used to assess the correlation between adipocyte size and BMI. Follow-up multiple linear regressions were used to adjust for age and sex in relation to each adipocyte depot. Scatter plots present the average adipocyte size from each fat depot of one case. Differences determined from all analyses were considered statistically significant if $P<.05$. All statistical analysis was performed using GraphPad Prism 8 software (GraphPad Software, Inc. CA, USA).

3. Results

Post-mortem adipose tissue biopsies (SAT, AAT, PAT, EAT) were procured from 43 post-mortem cases. As shown in Table 1, these post-mortem cases included males ($N=27$) and females ($N=16$), with a mean age of 53±16 years (range 22–77 years), and a mean BMI of 26.8±4.3 kg/m^2 (range 19.0–35.5 kg/m^2) for both sexes. In 17 of the cases, the cause of death was of a cardiovascular disease (sudden cardiac death, ischaemic heart disease, valvular heart disease, hypertensive heart disease), while the remaining 26 were of a non-cardiovascular cause.

Table 1
Post-mortem case characteristics ($N=43$)*

Variable	Mean ± SD	Range
Age (years)	53±16	22–77
Sex (Male/Female)	27/16	
BMI (kg/m^2)	26.8±4.3	19.0–35.5

* *Footnote*: BMI = body mass index. SD = standard deviation.

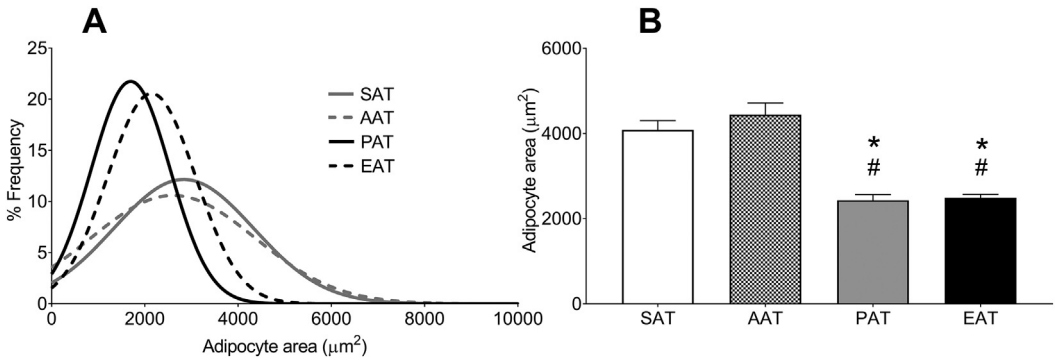

Fig. 1. Adipocyte size frequency distributions and mean adipocyte sizes from subcutaneous (SAT), appendix (AAT), pericardial (PAT), and epicardial (EAT) adipose tissues. **A**, Unimodal frequency distribution of adipocyte size presented as % frequency. Frequencies calculated from every adipocyte analysed from each respective depot. SAT *N*=2577, AAT *N*=2522, PAT *N*=4428, EAT *N*=3524. **B**, Mean adipocyte area SAT, AAT, PAT, and EAT depots. Values are unadjusted for BMI (average body mass index=26.8±4.3 kg/m^2). **P*<.0001 vs SAT, #*P*<.0001 vs AAT. Results are presented as means ± SEM. Statistical difference determined using one-way ANOVA with post-hoc Tukey's multiple comparisons test. *N*=43 for each depot.

3.1. Mean adipocyte sizes from different adipose depots

Frequency distributions of size revealed that adipocytes from each depot have a unimodal distribution profile (Fig. 1A). Adipocytes from different fat origins have different sizes [12]. As shown in Fig. 1B, adipocytes isolated from SAT (4088.9±209.1 μm^2) and AAT depots (4443.6±266.6 μm^2) were significantly larger in mean when area when compared to adipocytes from PAT (2431.0±131.3 μm^2; *P*<.0001 vs SAT or AAT) and EAT (2487.3±83 μm^2, *P*<.0001 vs SAT or AAT). Mean adipocyte area was not significantly different between cells from SAT and AAT depots (*P*=.55). Interestingly, relative to PAT mean adipocyte area, EAT adipocyte area was not different (*P*=1.0).

3.2. Adipocyte size relationship with body mass index

As shown in Fig. 2, when stratified into lean, overweight, and obese BMI categories, a significant greater mean adipocyte area was found between lean and overweight categories, and lean and obese categories in cells isolated from SAT, AAT, and PAT depots (Fig. 3A–C). However, there was no difference in the size of EAT adipocytes measured from cases in the lean, overweight, and obese categories (Fig. 2D). To circumvent the arbitrary categorisation of BMI grouping, we performed univariate correlation analyses of adipocyte size and BMI (as a continuous variable). A significant positive correlation was found between BMI and adipocyte area of

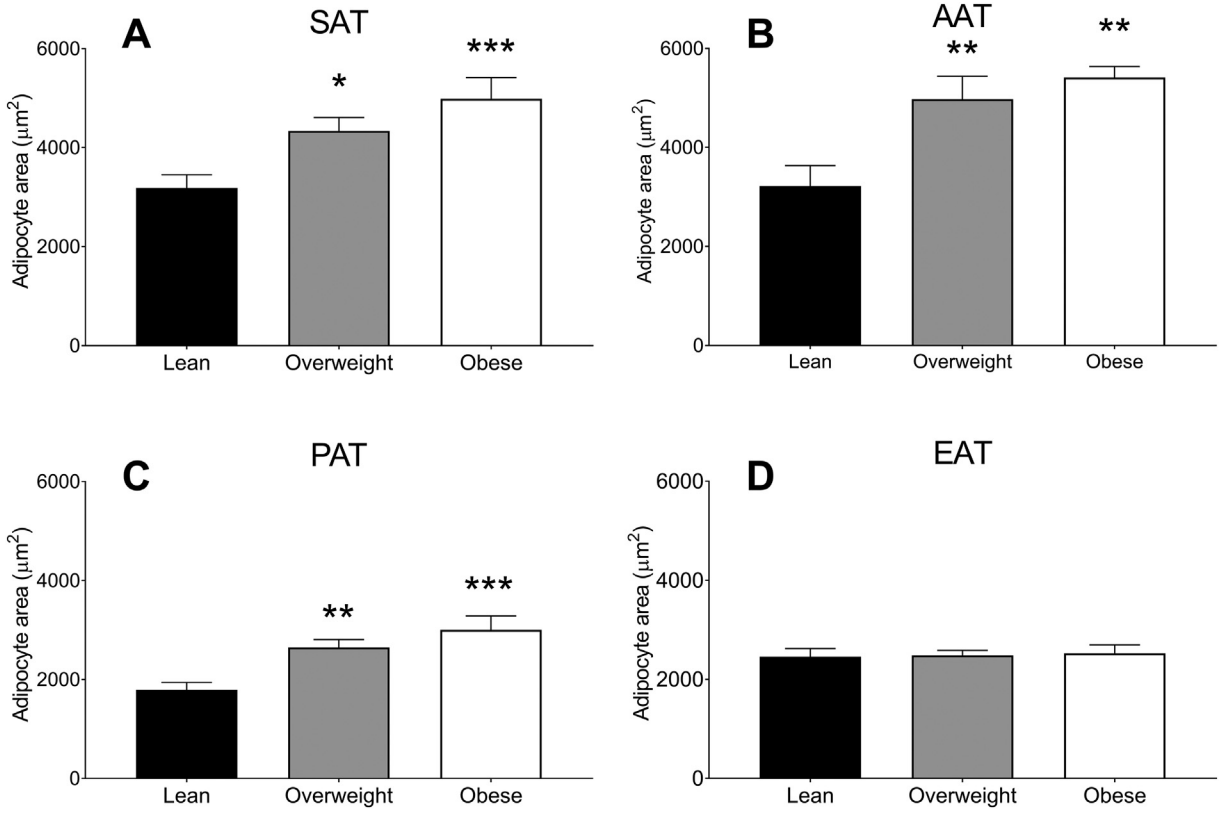

Fig. 2. Mean adipocyte sizes in lean, overweight, and obese groups. Subcutaneous (SAT, **A**), appendix (AAT, **B**), pericardial (PAT, **C**), and epicardial (EAT, **D**) depots. **P*<.05 vs lean group, ***P*<.01 vs lean group, ****P*<.001 vs lean group. Data are presented as means ± SEM. Statistical difference determined using one-way ANOVA with post-hoc Tukey multiple comparisons test. *N*=43 for each depot.

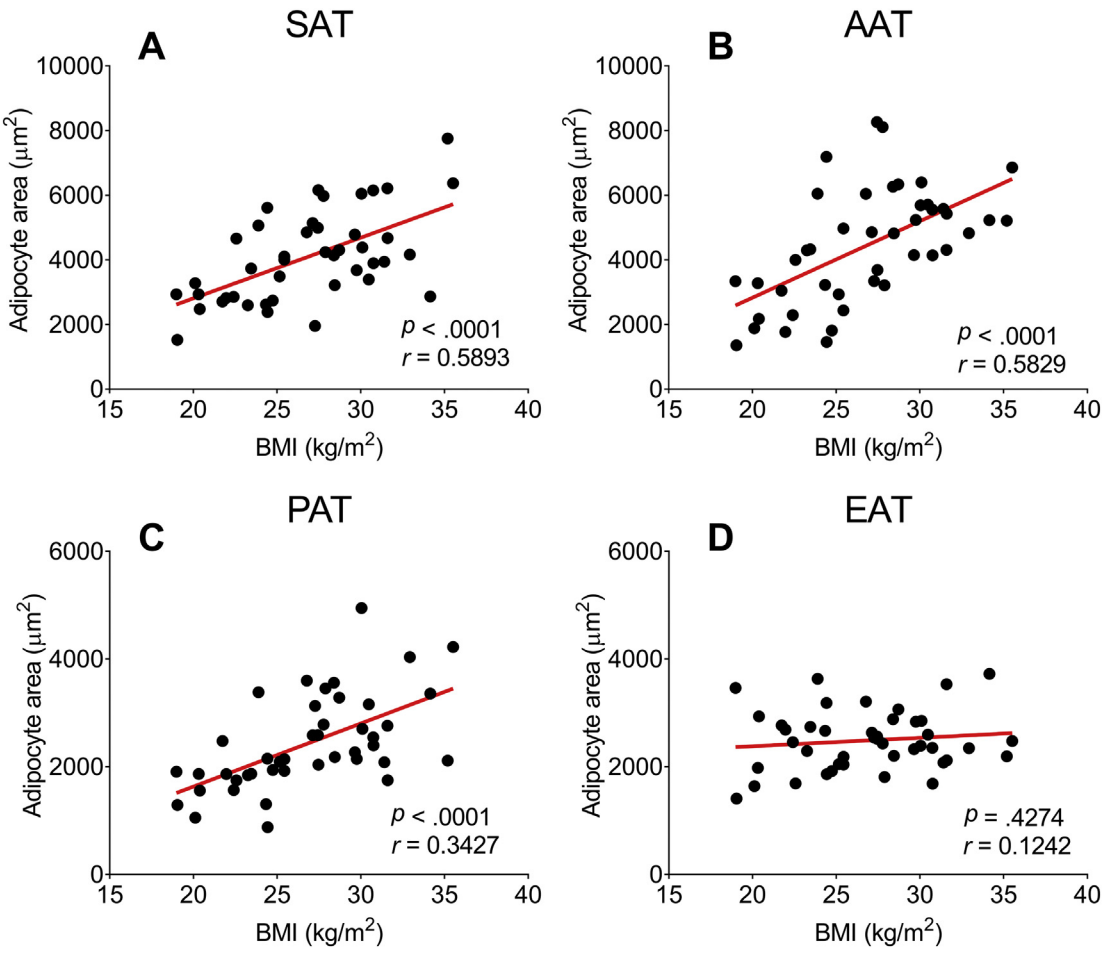

Fig. 3. Univariate correlation analyses of mean adipocyte area vs BMI of cells from subcutaneous (SAT, **A**), appendix (AAT, **B**), pericardial (PAT, **C**), and epicardial (EAT, **D**) depots. Note that *P* values presented within the Figures are unadjusted. BMI = body mass index. *N*=43.

cells isolated from SAT (r=0.5893, 95% confidence interval [CI]: 0.35, 0.76, P<.0001), PAT (r=0.5854, 95% CI: 0.35, 0.75, P<.0001), and AAT (r=0.5829, 95% CI: 0.34, 0.75, P<.0001) depots (Fig. 3A–C). These associations remained after adjustment for age and sex (not shown). No such correlation could be found between mean EAT adipocyte area and BMI, either with or without adjustment for age and sex (unadjusted: r=0.1242, 95% CI: −0.18, 0.41, P=.4; adjusted: R^2=0.1182, P=.6) (Fig. 3D).

4. Discussion

We aimed to determine the relationship between EAT adipocyte size and obesity. EAT adipocyte size, we expected, would be analogous to adipocytes from other well-researched SAT and VAT depots and would correlate positively with increasing BMI, an anthropometric proxy for obesity. Our main findings showed that an increase in BMI was not associated with hypertrophy of EAT adipocytes, despite this association being observed for adipocytes isolated from SAT, AAT, and PAT depots. This suggests that EAT adipocytes do not conform to the obesity-driven morphological size changes attributable to subcutaneous and visceral adipose depots.

4.1. Lack of epicardial adipocyte size and body mass index correlation

Previous studies have found conflicting results when determining the relationship of EAT adipocyte size to BMI. Measuring EAT adipocytes from 10 sampling sites of 34 post-mortem adult hearts, Sons and Hoffman reported a robust positive correlation between EAT cell size and body weight [13]. Moreover, at the sampling site most similar to that used in our study (beside the aortic ostium at the arteria coronaria dextra above the wall of the right ventricle), 21% and 47% increases in mean EAT cell size were reported in groups with average BMIs of 30.1 and 34.7 kg/m^2, respectively, when compared to the group with a 22.2 kg/m^2 average [13]. Conversely, and more recently, as part of their assessment of EAT morphology in coronary artery disease patients, Eiras et al found that no correlation existed between EAT adipocyte size and BMI, despite finding a positive correlation with adipocytes from SAT depots [10]. Our study has found a similar adipocyte size to those reported by Sons and Hoffman and Eiras et al (Fig. 1B), but extended and directly clarified with a larger sample size that no association exists between mean EAT adipocyte size and BMI (Fig. 3D). While EAT adipocyte size did not correlate with BMI, adipocytes from the SAT, AAT, and PAT depots did (Fig. 3A–C). Moreover, this adipocyte hypertrophy was dependent on the category of BMI, with mean adipocyte size increasing significantly in the overweight and obese groups relative to the lean group (Fig. 2A–C). Hypertrophic growth of adipocytes from various SAT and VAT depots with increasing BMI is well-established in the field. Whether measured as cell lipid content [11,19], diameter [9,12], or area [10], adipocytes isolated from SAT and VAT biopsies have been shown to positively correlate with BMI. This includes adipocytes with a subcutaneous manubrium sternal origin [9], which has anatomical proximity to the clavicular adipose depot sampled in

our study, as well as cells of mesenteric or omental origin that share anatomical proximity to the appendix adipose used in our study [12,13]. Our study, therefore, corroborates previous reports of adipocyte-BMI associations from other SAT and VAT depots, while directly measuring the association of EAT and BMI with a greater sample size and paired comparison with 3 other adipose depots.

This absence of EAT adipocyte hypertrophy, therefore, is not concordant to adipocytes from other depots. Increased BMI has not only been correlated with an increase in SAT adipocyte size, but concurrently with an increase in the expression and activity of key lipid-handling enzymes, including lipoprotein lipase and hormone-sensitive lipase [12,20]. As an energy storage and release organ, increased lipoprotein lipase and hormone-sensitive lipase activity, coupled with increased lipolysis rates, results in enhanced lipid and fatty acid flux across the adipocyte membrane, thereby facilitating excess energy storage and release as required [6]. We were unable to assess EAT adipocyte fatty acid metabolism as part of this study because it is not part of routine post-mortem examination; however, previous studies using EAT isolated from an array of other mammalian species have found that EAT has a uniquely active role in fatty acid uptake, lipogenesis, and lipolysis [21,22]. Relative to other VAT depots, including pericardial, popliteal, and perirenal, maximal lipogenic and lipolytic capacities of EAT are more than 2-fold greater [22]. Moreover, fatty acid incorporation is approximately 1.7-fold greater in EAT compared to pericardial, and more than 3-fold higher than all other VAT depots [21]. Furthermore, EAT fatty acid incorporation has been found to significantly increase in guinea-pigs fed a high-fat diet [21]. Collectively, this creates a curious contradiction. EAT fatty acid handling has been found to increase with an obesity-inducing diet, but this is not accompanied by the adipocyte hypertrophy that is characteristic of other adipose depots. As part of their early work, Sons and Hoffman also noted that in a very small number of post-mortem cases matched for sex and height but with different body weights, there was near-identical EAT cell sizes. In these particular cases, EAT deposition was found to be increased, thereby suggesting that an alternative, hyperplastic expansion of EAT adipocytes is a possible mechanism of EAT remodelling [13]. Therefore, based on the hypothesis that EAT functions as a buffer against localised lipotoxicity at the myocardium, hyperplastic remodelling of EAT adipocytes may predominate over hypertrophy to maintain adequate lipid handling [4,22].

Finally, our finding that PAT and EAT have similar mean adipocyte sizes but different BMI associations highlights a further difference between PAT and EAT depots. Although often conflated, PAT and EAT arise from differential origins and are, consequently, anatomically distinct and are thought to have different roles in adipose physiology [4,5]. Importantly, PAT does not have contiguity with the myocardium. This means that PAT and the myocardium have different circulations and no physical contact, thereby preventing direct paracrine and vasocrine signalling between the tissues [5]. Therefore, not only is it apparent that PAT and EAT have divergent macroscopic associations with the underlying cardiac muscle, our results show clearly that the response of each fat depot to increased adiposity is also different.

4.2. Smaller epicardial adipocyte size

We also obtained adipose samples from three other fat depots, one of SAT origin, and two from non-EAT VAT depots. Regional distinction in adipocyte morphology has been well-established. With regard to adipocyte size, those from SAT depots have consistently been shown to be larger, whether measured as cell diameter, volume, lipid content, or area than those from numerous VAT depots. Studies of patient cohorts have found that without adjustment for BMI abdominal SAT adipocytes are 12−30% larger than adipocytes from omental, retroperitoneal, and mesenteric VAT depots [12,20,23−25]. Moreover, adipocytes isolated from abdominal, thoracic, and sternal SAT depots and other VAT depots, including omental, peritoneal, and pericardial fat, have consistently been reported as larger than EAT adipocytes, typically by ~20% and ~10%, respectively [9,10,13,22]. Our study has corroborated this well-established regional adipocyte size distinction; however, the size differences were greater than previous studies as the clavicular SAT adipocytes we isolated were ~40% greater than both the PAT and EAT adipocytes (Fig. 1B). We did not, however, find a size difference between adipocytes of PAT and EAT origins, which has previously been reported in other mammalian species, but not in humans [21]. As shown, PAT adipocyte size positively correlates with BMI while EAT adipocytes are not (Fig. 3). Therefore, the adiposity of the mammalian species utilised for this previous work would influence size variation between the depots and could explain the discrepancy between our studies.

4.3. Limitations

Our study was limited in the information that could be obtained from each adipose case. Made available to us was the age, sex, and the BMI for each case; however, other measures such as body fat percentage, blood glucose levels and HbA1c, and macroscopic EAT thickness were not available. Blood glucose, and by extension insulin resistance, have been well documented as metabolic derangements that are correlated with altered adipocyte morphology [7]. Because these measures were not available for this study, we cannot exclude the possible effect these measures have on EAT adipocyte morphology. EAT thickness correlates strongly with BMI [1−3], therefore future work will be required to directly determine how macroscopic EAT thickness and EAT adipocyte size correlate. Additionally, the maximal case BMI used for this study was 35.5 kg/m^2. Adipocyte hypertrophy is finite in the extent to which the remodelling can occur and it is hypothesised that fat cells increase in size initially, but when a critical size is reached an alternative hyperplastic remodelling occurs, which ultimately impacts the mean adipocyte size [23]. Therefore, because cases with BMI >40 kg/m^2 were unavailable for our study, we could not assess whether EAT adipocyte morphology is altered in extreme adiposity. Finally, the method of cell size analysis used for this study generated a mean adipocyte size distribution with a unimodal profile (Fig. 1A). Other methods, such as particle counters, generate bimodal distributions [23,26]. This bimodality, in turn, allows the determination of a ‘nadir’ in the distribution and the categorisation of ‘small’ and ‘large’ adipocytes. These categories can be used as proxy markers for pre-adipocytes and mature adipocytes, respectively [26]. Without a bimodal size distribution, we could not differentiate small adipocytes from large and, therefore, we could not determine whether hyperplastic EAT adipocyte remodelling could account for the results of the study.

5. Conclusion

We have shown that the size of EAT adipocytes isolated from routine post-mortem cases does not correlate with BMI. Further, we found this lack of association despite corroborating previous findings of adipocytes from SAT and other VAT depots having a positive correlation with BMI. These results extend our understanding of EAT as a fat depot, and begin to contextualise EAT adipocytes within the framework of well-established adipocyte morphology. Finally, they provide the rationale for future investigations of the pathophysiological consequences of EAT adipocyte remodelling on the function of EAT, as an adipose tissue, and on the health of the myocardium.

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