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Inflammatory Characteristics of Adipose Tissue Collected by Surgical Excision vs. Needle Aspiration

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Abstract

Subcutaneous adipose tissue can be obtained for research during an elective, clinically indicated operation by standard surgical excision approaches and by needle aspiration in pure research settings. Whether measurements of inflammatory markers and cells made in tissue collected these two different ways are comparable is debatable. We sought to determine whether these two techniques yield systematically different results for measurements of inflammation, cellular senescence, and adipose tissue composition. Twelve subjects undergoing surgery participated. At the time of surgery abdominal subcutaneous adipose tissue from adjacent sites was removed by excision and needle aspiration. Stromovascular cell composition (flow cytometry), the number of senescent cells (senescence-associated- β -galactosidase staining), and IL-6, IL-1, TNF- α , MCP1 mRNA (RT-PCR) were measured in each sample. We found no statistically significant differences between the two sample collection approaches for any of the parameters measured. We conclude that these two methods of obtaining adipose tissue do not systematically differ in the results of cytokine mRNA content, cellular senescence, or stromovascular cell composition.

Keywords

adipose tissue biopsy techniques; flow cytometry; mRNA; cellular senescence; adipose needle aspiration

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Introduction

White adipose tissue functions include fat storage and release, endocrine signaling and effects on physiological homeostasis through metabolic pathways and inflammation. Adipose tissue inflammation may be an important factor in the pathophysiology of obesity-associated comorbidities, including cardiovascular disease and diabetes (1). Although normal adipose tissue contains a variety of immune cells, including macrophages, T cells, B cells, plasma cells, and mast cells, the accumulation of excess inflammatory cells in obesity may contribute to local tissue dysfunction and systemic inflammation (2,3). Another potential contributor to adipose tissue inflammation is the accumulation of senescent preadipocytes. Inflammatory cytokines can induce preadipocyte cellular senescence (4). Senescent cells may propagate the inflammatory adipose tissue environment through further increases in pro-inflammatory cytokine expression.

Many studies of local inflammation, adipose tissue composition, and cellular senescence in humans have used adipose tissue excised at the time of surgery (5-11). However, if the study of adipose inflammation is restricted to surgically-obtained samples, this will constrain the experimental questions that may be asked, as well as the types of participants that can be studied. Subcutaneous adipose tissue samples can easily be obtained by needle aspiration in outpatient clinical research settings, allowing greater protocol flexibility. Previous comparisons of adipose tissue obtained by needle aspiration and surgical excision were samples from different participants (16-18). Our goal was to assess adipose tissue characteristics obtained by these two techniques from the same individuals. We tested whether markers of inflammation, cellular composition, and senescence are systematically different if the tissue is obtained by needle aspiration or surgical excision.

Methods

General Protocol

Twelve surgery patients provided informed, written consent to participate in this study. Because our goal was to test whether the adipose tissue collection method affects the assay results using paired samples with a wide range of values, we recruited volunteers from patients scheduled for a variety of procedures. There were no other inclusion or exclusion criteria. Near the completion of the operation, 2-4 g of subcutaneous abdominal adipose tissue was collected by surgical excision and needle aspiration from adjacent sites. Surgically excised tissue was removed by cauterization. For needle aspiration, 0.9% saline was first injected subcutaneously to mimic the research approach we employ and adipose tissue was aspirated using a 12 gauge, monoeye cannula (Mayo Clinic Department of Engineering, Rochester, MN) with negative pressure generated by a 10 mL syringe. The monoeye is 1 cm in length and comprises ~ 50% of the diameter of the cannula, such that tissue fragments of ~ 2 mm in diameter are readily aspirated. Tissue was immediately aliquoted for flow cytometry and cellular senescence analysis or flash frozen for mRNA analysis. Aliquots were immediately transported to our laboratory (5 min travel time) for further processing. The protocol was approved by the Institutional Review Board of Mayo Clinic.

mRNA

RNA was extracted using Qiagen's RNeasy lipid tissue mini kit. Applied Biosystem's High Capacity cDNA Archive kit was used to make the cDNA library. An ABI 900 was used to perform the quantitative RT-PCR using primer and probe sets from Applied Biosystems. The housekeeping gene that was used was cyclophilin A (12).

Flow Cytometry

Aspirated and excised adipose tissue were analyzed by flow cytometry according to the protocol by Brake & Smith (13). Any cauterized material was removed from the surgically excised tissue prior to processing. The tissue was weighed then digested with 1 g/mL collagenase (Sigma Type II C-6885, St. Louis, MO) in Krebs Henseleit Buffer (280 U/mL) at 37°C for 40 min. The cells were filtered and centrifuged to separate the adipocytes from stromo-vascular fraction (SVF). The supernatant was removed and the SVF was re-suspended in Krebs Henseleit Buffer, centrifuged, and isolated twice more. The SVF was then resuspended in 100 μ L of PBS with 0.2% BSA. Following re-suspension, the SVF was incubated with the following conjugated-monoclonal antibodies: anti-CD14-PE, anti-CD34-FITC, or anti-CD14-APC. Multicolor flow cytometry was performed with a 4 color FACS Canto (BD Biosciences, San Jose, CA).

Cellular Senescence

Cells were fixed in 0.5% glutaraldehyde, washed with PBS and incubated at 37°C for 16 h with senescence-associated- β -galactosidase (SA- β -gal) activity solution (14). After a water wash, Hoechst 33342 dye was used to stain nuclei. The tissue was then placed between two mounting slides and examined with a fluorescent microscope (Nikon). The number of cells with SA- β -gal activity was counted and expressed as a function of number of nuclei counted using NIS software (Nikon, Champigny-sur-Marne, France).

Statistical Analysis

Paired t-tests were used to compare measurements made in aspirated and excised adipose tissue. Spearman's rank correlation and Bland Altman tests were used to examine the agreement and bias between measurements. We found no paired data from similar experiments allowing us to perform robust power calculations. Thus, we used the sample size of 12 based on feasibility, precision gains about the mean and variance, and consideration of regulations (15). SPSS for Mac (version; SPSS Inc, Chicago, IL) was used for statistical analysis. Data are presented as mean \pm SEM. Differences were defined as statistically significant at a p value <0.05 .

Results

Subject Characteristics

Of the 12 participants, 7 were female. Participants were 51 ± 4 years of age and weighed 112.3 ± 9.0 kg with an average BMI of 39 ± 3 kg/m². Six patients were undergoing bariatric surgery, 3 were undergoing revisions of bariatric procedures, 2 were undergoing hernia

repair and 1 had a cholecystectomy. Nine cases were laparoscopic procedures and 3 were open incision procedures.

mRNA

There were no statistically significant differences between excised and aspirated adipose tissue in mRNA of inflammatory cytokines (IL-6, IL-1, TNF, MCP1) or PAI-1 (Table 1). The Bland Altman plots and correlation coefficients indicated good agreement in mRNA measured in aspirated and excised adipose tissue. There was no bias in the measurements: neither method of collection provided a consistently greater or lesser expression of mRNA.

Flow Cytometry

Flow cytometry analysis was conducted in the SVF of ASP and EX collected adipose tissue. CD45⁺ cells were considered hematopoietic cells, including hemolymphoid cells. CD34⁺ cells were considered endothelial and hematopoietic progenitor cells. CD14⁺ cells were considered monocyte/macrophages. As seen in Table 1, there were no significant differences between the proportion of cells in the SVF of tissue collected by ASP or EX that were CD45⁺, CD34⁺, or CD14⁺. The Bland Altman plots and correlation coefficients further showed that there was no bias between methods of collection.

Cellular Senescence

There were no statistically significant differences in the percent of senescent cells between adipose tissue collected by ASP or EX (Table 1). The Bland Altman plots and correlation coefficients indicated that there was good agreement and no bias between methods.

Discussion

To our knowledge, this is the first study examining whether adipose tissue characteristics differs between samples collected using surgical excision *vs.* needle aspiration from the same depot at the same time. We found that the cytokine mRNA values, cellular senescence content, SVF cell composition were not systematic or statistically significant different between the two approaches. Cytokine mRNA, cellular senescence and flow cytometry determined cellular composition were compared because these endpoints have been previously shown to be markers of adipose tissue inflammation.

The study of adipose tissue cellular characteristics and inflammatory properties has led to important advances in understanding the pathophysiology of obesity. Many such studies use samples collected by the excision method during surgery (5-11), mostly from upper body subcutaneous and visceral fat. Advantages of this approach are the ability to collect relatively large samples and to access visceral fat, whereas limitations include restrictions on the populations that can be studied (healthy adults seldom undergo abdominal surgery), difficulty collecting repeated samples and, usually, lack of access to leg fat. In contrast, needle aspiration of subcutaneous adipose tissue is an outpatient procedure that can be employed to study more varied populations, as well as can be repeated in the same individual to assess the effects of an intervention.

A few investigators have compared characteristics of adipose tissue obtained by needle aspiration and surgical excision (16-18). However, the samples were obtained from different individuals whereas we compared samples obtained from adjacent sites from the same individual. One report indicated a number of gene expression and histology differences between aspirated and excised subcutaneous adipose tissue (17), but used a different size and type of needle to collect tissue and studied different subjects. Consistent with our data, neither Eto *et al.* (16) nor Wosnitza *et al.* (18) found differences between the two techniques in the proportion of adipose tissue CD34⁺ cells. However, whereas we found no statistically significant differences in CD45⁺ cells, Eto *et al.* (16) observed that the percentage of CD45⁺ cells was 4 times greater in aspirated than excised adipose tissue. The greater proportion of CD45⁺ cells (16) could indicate infiltration of blood-derived cells during the aspiration procedure. However, our data suggest that the differences between excised and aspirated adipose tissue they reported (16) are due to collection of samples from different individuals. We found no studies that compared markers of local inflammation or cellular senescence in adipose tissue collected using aspiration vs. excision.

Although we found no statistically significant differences in the measured outcome variables, our sample size was only 12 paired observations. The Bland Altman analysis showed the average difference between the means was small. Based upon our results, future studies designed to detect 50% differences with 80% power would need to include 17, 45, and 48 subjects for TNF, CD34, and cellular senescence, respectively. However, in the context of the tenfold or greater range of observed values for most of the variables we measured, we had acceptable statistical power to detect meaningful differences between the two sampling methods. Another limitation is that the excised and aspirated tissue could have been exposed to different environments (air, instruments) during the operation. Although we used tissue distant from the wound to minimize this problem, this could account for some of the poor correlations we observed. Finally, larger numbers of mRNA markers and cell types could have been attempted, but by focusing on the most commonly assessed cells/genes we reduced the type 2 statistical error risk.

In summary, we found no significant differences in measures of cellular composition, inflammatory cytokine markers, and cellular senescence in adipose tissue collected by surgical excision vs. needle aspiration. This suggests that these two commonly employed adipose collection techniques provide comparable data, which broadens the range of observational and interventional studies that are feasible to gain insight into the adipose tissue microenvironment and function.

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Table 1
Inflammatory cytokine mRNA, flow cytometry cell characterization, and cellular senescence in adipose tissue

	Aspirate Tissue	Excised Tissue	Correlation Coefficient (ρ)	Bland Altman (mean difference of means)
<i>mRNA (relative to calibrator)</i>				
IL-6	0.51±0.27	0.48±0.12	0.50	0.03±0.03
IL-1	1.02±0.19	1.35±0.34	0.58	-0.33±0.34
TNF	1.56±0.31	1.54±0.39	0.72	0.02±0.35
MCP1	0.58±0.10	1.25±0.61	-0.06	-0.67±0.60
PAI1	0.83±0.19	1.40±0.82	0.71	-0.57±0.84
<i>Flow Cytometry (% SVF cells)</i>				
CD14	3.9±1.0	2.6±1.2	0.46	1.4±1.2
CD45	6.9±1.9	5.4±2.2	0.74	1.5±2.2
CD34	11.6±3.0	14.7±3.9	0.75	-3.0±2.6
<i>Cellular Senescence (%)</i>				
SA- β -gal	0.02±0.01	0.04±0.01	0.30	-0.01±0.01

ASP represents tissue collected by needle aspiration and EX represents tissue collected by surgical excision. Mean \pm SEM