A preliminary study of subject factors associated with poor differentiation capacity of visceral and subcutaneous adipose tissue in human obesity

Bhattacharya, Swati
BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

A PRELIMINARY STUDY OF SUBJECT FACTORS ASSOCIATED WITH
POOR DIFFERENTIATION CAPACITY OF VISCERAL AND
SUBCUTANEOUS ADIPOSE TISSUE IN HUMAN OBESITY

by

SWATI BHATTACHARYA

B.Sc., University of Pune, 2001
M.Sc., University of Mumbai, India, 2003

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Approved by

First Reader

Susan K. Fried, Ph.D.
Professor of Medicine and Biochemistry

Second Reader

Kalypso Karastergiou, M.D., Ph.D.
Post Doctoral Research Associate

Third Reader

Janice Weinberg, Sc.D.
Professor of Biostatistics
Director, MS in Clinical Investigation
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ABSTRACT

Background: Fat is stored in adipose tissue. In obesity, differentiation of preadipocytes to
new adipocytes (fat cells) is required for energy storage. Otherwise fat accumulation in
non-adipocytes contributes to fatty liver and diabetes.

Our goal was to assess subject characteristics associated with poor in-vitro differentiation
capacity of preadipocytes from omental (OM) and abdominal subcutaneous (SC) fat.

Approach: A convenience sample of, 4 males and 20 females, age 39±2 (range 20–56)
years, BMI 42 ± 2 (23–63) kg/m² (i.e. from lean to obese), 7 Caucasian, 8 Hispanic, 1
other and 8 African Americans) undergoing elective surgery was studied. Fat samples
collected during surgery were used for histology and preadipocyte isolation. Fat cell
diameters and their distribution (normal or bimodal) were analyzed from histology.

Preadipocyte differentiation capacity was measured in vitro.

Results: In the OM depot, no effect of ethnicity, sex or HbA1c was found. Unexpectedly,
subjects with preadipocytes with poor differentiation capacity tended to be younger (poor
differentiation group 36 ± 2 years versus high 43 ± 3 years, p=0.09) and to have lower
fasting glucose (poor 97 ± 3.65 mg/dl versus high 111 ± 7.08 mg/dl, p=0.06).

In SC, no differences were noted. Fat cell size was not associated with differentiation
capacity in either depot. Bimodal distribution, which may show formation of new
adipocytes, was seen mostly in Caucasian subjects (5 out of 7) compared to Hispanic (3 out of 8) and African Americans (2 out of 8).

Conclusion: It is important to investigate the associations between age/ethnicity and OM preadipocyte differentiation/cell distribution in adequately powered cross-sectional studies.
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LIST OF ABBREVIATIONS

AA.................................................African American
AT...............................................Adipose tissue
BMC............................................Boston Medical Center
BMI............................................Body Mass Index
DC...............................................Differentiation Capacity
FCV.............................................Fat Cell Volume
HbA1C..........................................Hemoglobin A1C
IRB.............................................Institutional Review Board
OM.............................................Omental
SC...............................................Subcutaneous
VAT............................................Visceral Adipose Tissue
  BAT.........................................Brown Adipose Tissue
  WAT.........................................White Adipose Tissue
INTRODUCTION

1. Definition of Obesity:

Overweight and obesity are defined as excessive fat accumulation, which occurs by enlargement of adipose tissue to store excess energy intake. Body Mass Index (BMI) is defined as a person’s weight in kilograms divided by the square of his height in meters (kg/m$^2$). BMI is a key index of weight for height related to both percentage body fat & total body fat and is commonly used to classify underweight, overweight & obesity in adults.\textsuperscript{1} Obesity is a complex, multifactorial metabolic disorder involving genetic, environmental (social and cultural), behavioral, psychological and physiological factors. These risk factors are commonly associated with cardiovascular disease, Type II diabetes mellitus, hypertension, stroke, dyslipidemia, gallbladder disease, hepatic steatosis, sleep apnea, endometrial disorder, and cancer.\textsuperscript{2,3} Here the term "metabolic" refers to the biochemical processes involved in the body's normal functioning. Risk factors are traits, conditions, or habits that increase your chance of developing a disease. The METABOLIC SYNDROME is a complex disorder unifying dyslipidemia, insulin resistance, and hypertension. It is a primary risk factor for diabetes and cardiovascular disease. (Goodpaster et al. 2005)

2. Epidemiology of Obesity:

The most current information from the Center for Disease Control and Prevention (CDC) states that more than one-third (34.9\%) of adults in US population were obese in 2011–2012 (most recent data, Figure 1). More than 78 million adults were obese in that specific
period. In 2011–2012, the prevalence of obesity was higher among middle-aged adults (39.5%) than among younger (30.3%) or older (35.4%) adults.

Fig.1: Prevalence of Obesity Among Adults: United States, 2011–2012 (NCHS Data Brief, No. 131, October 2013)


The prevalence of obesity was higher among non-Hispanic black (47.8%), Hispanic (42.5%), and non-Hispanic white (32.6%) adults than among non-Hispanic Asian adults (10.8%) (Figure 2). The overall prevalence of obesity did not differ between men and women in 2011–2012 (Figure 1). Among non-Hispanic black adults, however, 56.6% of women were obese compared with 37.1% of men. The most recent national data from 2011–2012 on obesity prevalence among U.S. adults show that there was no significant change since 2009–2010. Given the focus of public health efforts on obesity, surveillance of trends in obesity remains important. Overall disparities in obesity prevalence continue to exist. Newly available data show a lower prevalence of obesity among non-Hispanic Asian adults than among non-Hispanic white, non-Hispanic black, and Hispanic adults.
Furthermore, obesity prevalence in 2014 varies across states and territories (Figure 3).

1. No state had a prevalence of obesity less than 20%.
2. 5 states and the District of Columbia had a prevalence of obesity between 20% and <25%.
3. 23 states, Guam and Puerto Rico had a prevalence of obesity between 25% and <30%.
4. 19 states had a prevalence of obesity between 30% and <35%.
5. 3 states (Arkansas, Mississippi and West Virginia) had a prevalence of obesity of 35% or greater.
6. The Midwest had the highest prevalence of obesity (30.7%), followed by the South (30.6%), the Northeast (27.3%), and the West (25.7%).
Health Consequences:

Obesity increases the risk of a host of obesity related diseases that can afflict nearly every organ system in the body.\textsuperscript{6} It has been directly linked to the world’s leading cause of death – cardiovascular disease\textsuperscript{7} and to one of the greatest public health threats of the 21st century – diabetes\textsuperscript{1} (Figure 4). According to the National Institutes of Health, epidemiological data show a 50% to 100% higher all-cause mortality rate for obese patients (BMI of $\geq 30$ kg/m$^2$) than for patients with BMIs of 20 to 25 kg/m$^2$.\textsuperscript{6}
At a given body mass index (BMI) level, body fat may vary by age, sex, and racial and ethnic group (Flegal KM et al.). In particular, at a given BMI, Asian adults may have more body fat than white adults. Morbidity and mortality risk may be influenced by factors—such as body composition and fat distribution—that are not completely captured by BMI (Ogden CL et al.).

**Economic Consequences:**

- Overweight and obesity and their associated health problems have a significant economic impact on the U.S. health care system. Medical costs associated with overweight and obesity may involve direct and indirect costs. Direct medical costs may include preventive, diagnostic, and treatment services related to obesity.
• Indirect costs relate to morbidity and mortality costs. Morbidity costs are defined as the value of income lost from decreased productivity, restricted activity, absenteeism, and bed days. Mortality costs are the value of future income lost by premature death.

• National Estimated Cost of Obesity

The medical care costs of obesity in the United States are staggering. In 2008 dollars, these costs totaled about $147 billion.

3. Anatomy of adipose tissue (Fat Depots)

Adipose is a loose connective tissue that fills up space between organs and tissues and provides structural and metabolic support. Adipose tissue / fatty tissue, consists mainly of fat cells (adipose cells) and is specialized to synthesize. It contains large globules of fat, within a structural network of fibers. Mammals have two different types of adipose: white adipose tissue (WAT) and brown adipose tissue (BAT). Here, I will only describe white adipose tissue. White adipose, the most common type, provides insulation, serves as an energy store for times of starvation or great exertion, and forms pads between organs. When muscles and other tissues need energy, substances known as hormones bind to adipose cells and trigger the release of energy-rich fatty acids and glycerol. In humans, adipose tissue is found in specific locations, referred as adipose / fat depots. Whole body adipose tissue has generally been subdivided in two main components: subcutaneous (SC) and internal, visceral adipose tissue (Figure 5). Subcutaneous adipose tissue is usually defined as the layer found between the skin and the aponeuroses and
fasciae of the muscles; SC adipose tissue depots store ~ 80–90% of the total body fat, mainly in the abdominal & thigh area.

Figure 5: Anatomical localization of the main abdominal adipose tissue depots.

Intraperitoneal adipose tissue or so-called visceral adipose tissues (VAT) are associated with digestive organs, and include the omental mesenteric is composed of two major compartments: the omentum (hangs off the stomach) and mesenteric (associated with the intestine). Intraperitoneal means within or the peritoneal membrane that lines the walls of the abdominal cavity and contains/encloses the abdominal organs. The main white adipose tissues (WATs) are abdominal subcutaneous adipose tissue (SAT, and visceral adipose tissue (VAT). VAT surrounds the inner organs and can be divided in omental, mesenteric, retroperitoneal: surrounding the kidney, gonadal and pericardial. The omental depot stars near the stomach and spleen and can expand into the ventral abdomen, while the deeper mesenteric depot is attached in a webform to the intestine. The gluteofemoral adipose tissue is the SAT located to the lower body parts and is measured by hip, thigh, and leg circumference. WAT can also be found intramuscularly.
Adipose tissues located in and around the abdominal cavity can help deciphering the human body fat distribution patterns as well as the pathophysiological association of visceral obesity and other metabolic diseases.

4. Type of Obesity: Central versus Peripheral

Multiple epidemiological studies confirm the detrimental effect of upper-body obesity (android-type / apple shape) or central obesity, and the protective effects of lower-body obesity (gynoid-type / pear-shape) on diabetes, cardiovascular risk and eventually morbidity & mortality. Upper body fat distribution is considered as an additional “new” independent risk factor for coronary heart disease (CHD), whereas increased lower body fat is independently predictive of reduced cardiovascular risk. Body fat distribution varies with sex, genetic background and aging. Women, compared to men, have higher percentages of body fat with relatively more deposits of adipose tissue in the hips and thighs. This ‘female’ fat distribution, independent of total body fat, confers protection against metabolic diseases, such as Type 2 diabetes and atherosclerosis. Adipocytes are smaller in visceral than subcutaneous (SC) depots in women, while they are similar in size in men and extremely obese women.

Why visceral adiposity is bad

The dyslipidemic state frequently observed in patients with visceral obesity is a key feature of the clustering abnormalities of the metabolic syndrome and has been extensively described in the literature. The abnormalities include high levels of triglycerides, low levels of high-density lipoprotein (HDL) cholesterol, relatively normal
total and low-density lipoprotein (LDL) cholesterol levels, but more LDL particles that are smaller and denser than normal. In abdominal obesity, HDL particles are also small in size because of the presence of hypertriglyceridemia. Total and LDL cholesterol levels are generally within the normal range unless unrelated abnormalities are present. In clinical research, hypertriglyceridemia and low HDL cholesterol could be the two major detectable blood abnormalities associated with visceral obesity.

**Type of fat accumulation: Hypertrophy / Hyperplasia**

The primary function of the fat cell (adipocytes) is to control energy balance by storing triacylglycerol during periods of energy excess and mobilizing it during energy deprivation. If the adipose organ is unable to accommodate excess energy, the calories are stored in the liver and muscles with the development of diabetes.

The increased storage of triglycerides in fat cells occurs in two ways: by expanding the size of available adipose (fat) cells (hypertrophy), or by recruiting new fat cells by increasing their number.
The size of each fat compartment / depot results from the integration of the size and number of lipid-filled adipocytes, which represent the main cellular component of adipose tissue. In general, mean adipocyte sizes from all anatomical locations and in both sexes increase along with adiposity level, but reach a plateau in extremely obese individuals. The plateau indirectly suggests that the presence of large adipocytes may trigger the generation of new adipocytes to store excess dietary fat.

With regard to visceral adipocyte size and number, lean to moderately obese men tend to have larger omental adipocytes than women (Fried SK, Kral JG 1987). In lean to moderately obese individuals of both sexes, a strong correlation is observed between abdominal subcutaneous adipocyte size and total body fat mass, suggesting that the contribution of adipocyte hypertrophy to abdominal adipose tissue expansion may be similar in men and women. In adult women, expression of genes involved in preadipocyte differentiation is relatively higher in subcutaneous than in visceral adipose tissue (Drolet R 2008). This finding suggests that in women, expansion of the subcutaneous adipose tissue depot relies more heavily on adipocyte hyperplasia than the visceral adipose tissue compartment, which may be predominantly hypertrophic.

5. Adipogenesis

Obesity is also characterized on the basis of adipose tissue cellularity. Adipogenesis, the process during which fibroblast-like preadipocytes (precursor fat cells) develop into mature adipocytes (fat cells), is a process by which a less specialized cell becomes a
more specialized cell type. In this case, fibroblast like preadipocytes develop into mature adipocytes through (commitment & terminal differentiation). This involves cascade of transcription factors and cell-cycle proteins regulating gene expression and leading to adipocyte development. In order to reach maturity, these cells must go through two vital phases: adipocyte determination and adipocyte differentiation. \(^{17}\)

**Figure 7**: Factors affecting preadipocyte commitment Ref: Cawthorn WP et al. J Lipid Res 2012

Figure 7 describes the relationships between SVCs, ASCs, committed preadipocytes, and mature adipocytes. SVC is the stromal vascular cells (SVCs) as “primary” preadipocytes, which set the stage for extensive characterization of the mechanisms regulating adipogenesis and WAT expansion. Determination phase: This stage results in the conversion of the Adipose stem cell (ASC) to a committed preadipocyte, which cannot be distinguished morphologically from its precursor cell but has lost the potential to differentiate into other cell types. Large number of Adipocyte stromal cells (ASC) or preadipocytes, are isolated with collagenase digestion of Adipocytes. ASCs are multipotent cells (adipogenic, chondrogenic, osteogenic, and myogenic; Cawthorn,
Scheller, & MacDougald, 2012b), are capable of expansion in vitro, and can be cryopreserved for long periods without significant loss in proliferation and differentiation capacity. The differentiated adipocytes display phenotypic characteristics of genuine adipocytes, that is, freshly isolated ones.

**Terminal differentiation phase:** In this stage, the preadipocyte takes on the characteristics of the mature adipocyte. It acquires the machinery that is necessary for lipid transport and synthesis, insulin action and the secretion of adipocyte-specific proteins.

In morbidly obese men and women, SC and OM adipocyte size increases with BMI. In women, OM fat cells are smaller than SC cells independent of BMI. Severely obese patients with healthy metabolic profiles have significantly smaller omental adipocytes with increased preadipocyte size compared to a metabolically unhealthy group.

According to Anand & Chada, centrally obese people lose the ability to accommodate excess energy by differentiating new adipocytes. A study on obese Pima Indians showed a strong correlation between fat cell size and difficulty in differentiating new adipocytes during the onset of diabetes. Another in vitro study demonstrated that newly differentiated adipocytes is greater in lean than in obese subjects. The majority of studies report that preadipocyte adipogenesis is higher in SC than in OM fat cells. There are no other studies found that examined how the ability of primary preadipocytes from various fat depots (SC & OM) undergoing in vitro differentiation is associated with adipocyte size & visceral obesity.
Data from Dr. Fried’s lab show that in most of the obese subjects, visceral fat cells from different individuals show wide variations in differentiation capacity. However, it is unknown what variables play an important role in triggering these variations. Some visceral preadipocytes are unable to differentiate into new mature adipocytes irrespective of having good number of OM preadipocytes.

In very few (10%) cases, omental preadipocytes produce large numbers of mature adipocytes compared to the remaining 90% cases where the number of adipocytes produced are very small. It is not known what differentiates these two groups of subjects (good vs. bad omental differentiators). SC preadipocytes differentiate well in almost all subjects. But very little is known about the adipocyte size and its relation with differentiation capacity in adult adipocyte cells.

OBJECTIVES

The primary objective of this study is to investigate associations between human adipocyte (fat cell) size and the differentiation capacity of preadipocytes (precursor cells) in visceral & subcutaneous (SC) fat depots. (DC is measured as total triglyceride content in the cell) morbidly obese, obese and lean subgroups will be examined if possible. Secondary objective is to assess whether this association is independent of phenotypic characteristics & metabolic variables in the subject population (BMI, age, sex, ethnicity, Cholesterol, HbA1c & Blood glucose)

Third objective is to find relation between adipocyte size & metabolic health in the study subject population. (The concept of "metabolically healthy obesity" — that is, individuals
with a body mass index (BMI) above 30 who do not have metabolic-syndrome factors that put them at risk for cardiovascular disease. Up to 35% of obese individuals may be metabolically healthy despite their size, the researchers write, but the true prevalence of this phenomenon is difficult to assess due to large disparities in defining metabolic health).\textsuperscript{30}

**Thesis Question:**

1. In Om depot, is there an association between differentiation capacity and the variables listed and the ability of preadipocytes to differentiate (as measured in vitro):
   - Cell size
   - Age
   - Sex
   - Race
   - BMI
   - Metabolic characteristics (HbA1c, Blood glucose, Cholesterol)

2. In SC depot, is there an association between differentiation capacity and:
   - Cell size
   - Age
   - Sex
   - Race
   - BMI
   - Metabolic characteristics (HbA1c, Blood glucose, Cholesterol)
HYPOTHESIS:

1. In the Om depot, the differentiation capacity will be higher in:
   - Subjects with small adipocytes
   - Younger subjects
   - Women
   - Caucasians>Hispanics>AA
   - Obese subjects
   - With normal HbA1c, Cholesterol, FPG

2. Preadipocytes from subjects with large adipocytes associates with low / poor differentiation capacity (DC) in both visceral & SC depots. The above mentioned association is independent of other variables (BMI, age, sex, ethnicity, Cholesterol, HbA1c & Blood glucose) - which could be confounding factors.

People having large adipocyte size are more likely to be metabolically unhealthy; high HgBA1c, high glucose, independent of BMI, age & sex.

STUDY DESIGN: (Cross-sectional)

In order to examine the relationship / associations between one or more independent variables (age, sex, BMI, adipocyte size, HbA1c, cholesterol) and a dependent variable (fat cell Differentiation capacity), as they exist in a convenient population at one particular time, a cross-sectional study will be performed. All study measurements will be made at a single time-point & no follow-up period involved in this study. This primarily involves organizing the subject medical data, calculating mean cell size. Data
analysis by finding correlation between differentiation capacity and phenotypic variables (cell size, age, sex, BMI) and logistic regression will be done on data collected from a population obtained from an investigator initiated clinical study.
METHODS

Subjects: Sample of adipose tissue were obtained from 24 healthy adults: 4 males, 20 females, who were lean (n=2, BMI < 25.0 kg/m\(^2\)), overweight (n=9, BMI: 25.0 kg/m\(^2\) to 30.0 kg/m\(^2\)) or obese (n=13, BMI > 30.0 kg/m\(^2\)) who have undergone elective surgeries & volunteered for studies of adipose tissue in Fried lab in the Boston Medical center. These surgeries included laparoscopic bariatric surgeries: Gastric bypass or Sleeve Gastrectomy for weight loss – (abdominal surgeries for severe obesity), kidney donors. The participation of the subjects end with the donation of blood and fat sample during surgery. Each participant provided written informed consent. The study was approved by the Institutional Review Board of the Boston University School of Medicine (Nutritional Regulation of Leptin Production, IRB Number H-28139). Information on the height and weight of volunteers, medications, medical conditions and serum levels of glucose, HbA1c, blood pressure were obtained from the medical records and recorded in lab records with a code number.

Study subjects were enrolled by the clinical coordinators, PI on the basis of the elective surgery they were undergoing at BMC and therefore women are no more vulnerable than the other participants. Pregnant women are not enrolled. No children (< 18 years) were enrolled for this study. Potential subjects were given the time to think about participation in the study and were encouraged to ask questions of the investigators or their own doctors. If potential volunteers were contacted on the day of their surgery, and were willing to discuss participation in our study, we proceed to describe the purpose and procedures involved in the study, and risks, and ask them to sign an informed
consent. Subjects provided written informed consent before their inclusion in the study and copies of the signed consent from were placed in: 1) the patient's research chart, 2) the investigator's locked cabinet.

Inclusion criteria:

Participants were required to be healthy, or type 2 diabetic, 18 to 80 years of age, having BMI > 19 kg/m², scheduled for elective surgery, free of major organ disease, physical impairment limiting normal activity, current cancer, unstable cardiac disease, other unstable system disorders that are not being treated, untreated Endocrine disorders, such as hypo- or hyperthyroidism, pituitary disorders, etc., except Type 2 Diabetes, polycystic ovarian syndrome and Cushing’s syndrome.

Ethical Statement: The sample size mentioned in BMC IRB application is 243, which is for a far more complex & big study & it’s still ongoing. My study is a part of that approved study for which I have selected 24 subjects having data available with differentiation capacity. To understand how fat cell function changes in obesity, and in particular how the fat cells regulates the production of leptin, we will study these processes in samples of human adipose tissue obtained from patients undergoing elective surgery. We sample fat from different places in the body (called depots), because we know that they act differently. Once we obtain a fat sample, all tests will be conducted in test tubes.

The study (IRB # H-28139) is approved by the ethics committee of our institution & all 24 subjects (selected from a large pool of participants) gave written informed consent before the beginning of the study. Subjects were included in the study only once they met
the inclusion / exclusion criteria. There is a check off in Informed consent form so the subject can agree to be re-contacted for future studies. There is also a check off to determine whether subjects will agree to bank their tissue & DNA for genetic testing.

**Adipose tissue sampling, adipocyte isolation, and cell size measurement**

We obtained blood and subcutaneous -omental adipose tissue samples from the 24 subjects. Four of the 24 patients are diagnosed with pre-diabetic borderline to type 2 diabetes (DM2). Approximately 1–3 gms of omental adipose tissue, or 1 gms of abdominal subcutaneous adipose tissue, was obtained at the time of bariatric & other types of surgery. Subcutaneous adipose tissue samples were collected at site of the surgical incision (lower abdomen) while omental samples were removed from the distal part of the greater omentum. A piece of this tissue was immediately fixed in Z-fix, prior to paraffin mounting and preparation of H&E slides. The remaining tissue was placed in M-199 transported to the lab, and processed within 1 hour and used for adipocyte isolation.

**Blood Sampling:** Blood samples were taken on the day of the surgery in the overnight fasting state. Serum was separated with centrifugation for 15 min at 2500 rpm and 4°C and stored at -80°C for future analyses.
Fat cell size and number calculation:

The fixed tissue was sent to laboratory of Tufts University Department of Histology core for slicing, and H&E staining. Cells were sized after digital photography using imaging software (Image J, NIH). Two hundred fifty cell size as adipocyte area (µm²) and diameters per depot (µm) were calculated by Photoshop & Image J software. Average volume of adipocytes were measured by the weighted average volume method.

The demographic and metabolic characteristics (Age, sex, BMI, Cholesterol level, HbA1c) of the participating subjects were recorded from the hospital medical chart via BMC logician access. These variables will be studied and compared to find correlation with fat cell differentiation capacity.

Statistical Analysis: Data are presented as mean and their standard errors, unless otherwise indicated. A paired t-test (two-tailed distribution) was carried out to compare the mean adipocyte size in Subcutaneous (SC) & visceral (OM) depots in the selected subject population. A non-paired t-test was carried out to compare age, BMI, HbA1c, FPG, cholesterol between good & poor differentiation groups. Differences among race categories were analyzed by 1-way ANOVA, followed by Tukey’s post-hoc test. A P value of <0.05 was considered as statistically significant. Statistical analyses were performed with the Graph pad Prism 5 (Graph Pad, a Jolla, CA) software.
RESULTS

1. Subject Characteristics

Samples from twenty-four subjects were available for the preliminary analyses planned. Subject characteristics are shown in Table 1. 83% were females (n=20) and 17% (n=4) males. Their age varied from 20–56 years with overall mean age of 39 ± 1.96 years. Their mean BMI was 42 ± 2.10 kg/m² (range 23–63 kg/m²).

The study included 8 (F7 M1) African Americans, 7 (F7) Caucasians, and 8 (F6, M2) Hispanics (Table 1). The participants underwent laparoscopic surgery: 17 subjects had gastric bypass, 5 subjects had sleeve gastrectomy, and 2 had donor nephrectomy and their characteristics are described in Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Overall (n= 24)</th>
<th>Men (n= 4)</th>
<th>Women (n= 20)</th>
<th>Caucasians (n=7)</th>
<th>AA (n=8)</th>
<th>Hispanics (n=8)</th>
<th>p value between races</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39 ± 1.96 (20–56)</td>
<td>30 ± 3.63 (20–37)</td>
<td>41 ± 2.04 (20–56)</td>
<td>45.3 ± 3.0 (35–56)</td>
<td>39.1 ± 2.8 (24–48)</td>
<td>36.3 ± 3.3 (20–48)</td>
<td>0.1457</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>42 ± 2.10 (23–63)</td>
<td>55 ± 2.72 (50–63)</td>
<td>39 ± 1.99 (23–54)</td>
<td>40 ± 0.78 (38–44)</td>
<td>45 ± 3.35 (25–55)</td>
<td>40 ± 3.51 (23–63)</td>
<td>0.4809</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>198 ± 7.72 (157–280)</td>
<td>179.3 ± 6.0 (162–190)</td>
<td>203.1 ± 9.4 (157–280)</td>
<td>197.3 ± 14.93 (172–280)</td>
<td>207.3 ± 14.57 (157–257)</td>
<td>193.5 ± 7.3 (183–215)</td>
<td>0.7957</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6 ± 0.098 (5.1–6.8)</td>
<td>6 ± 0.25 (5.5–6.6)</td>
<td>6 ± 0.10 (5.1–6.8)</td>
<td>6.1 ± 0.14 (5.7–6.8)</td>
<td>5.9 ± 0.22 (5.1–6.6)</td>
<td>5.9 ± 0.14 (5.7–6.3)</td>
<td>0.6576</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>102 ± 3.76 (77–139)</td>
<td>106 ± 12.7 (77–139)</td>
<td>101 ± 3.79 (84–131)</td>
<td>106 ± 7.24 (84–131)</td>
<td>101 ± 4.99 (88–124)</td>
<td>99 ± 9.1 (77–139)</td>
<td>0.7844</td>
</tr>
</tbody>
</table>

Table 1: Subjects characteristics with metabolic variables

BMI: Body mass Index (weight in kilograms divided by the square of the height in meters)  AA: African Americans  Data shown as Mean ± SEM

The women in this sample were older (p = 0.0386) and less obese (lower BMI) (p=0.0023) as compared to men.
2. Differences in cell volume between OM and SC

In the total population, SC adipocytes were larger than omental adipocytes: 309.1 ± 29.44 vs 480.7 ± 35.73 pl, \( p = 0.006 \), as previously reported in the literature.

**Figure 8:** Mean of Fat cell volume vs. SC-OM depot.

Photos of histology sections from one representative subject are shown in (**Figure 9**). The sections are stained with H&E stains. The outlines of the cells were traced and perimeters were measured from which the cell diameter was calculated.

**Figure 9:** Cell images showing larger adipocytes in SC (right) compared to OM (left)
In some subjects, a bimodal distribution was found: i.e. the volumes of the cells did not follow a normal distribution, but two populations of small and large adipocytes were found (Figure 10).
The subjects (48-OM, 143-OM, 218-OM-SC, 220-SC and 74 SC) that showed bimodal distribution were all women, Caucasian, age 36 and 56 years old, with BMI range of 38–40. Subject 14-OM is the only AA male with BMI 55.
Then, OM and SC cell volume were compared in subgroups (paired t test- it is a within person paired comparison).

**a. Men and women**

In men (n=4), OM cell volume was $388.8 \pm 24.72$ pl, which was smaller (not statistically significant) than SC cell volume $458.5 \pm 60.69$ pl (N=4, p= 0.3281).

In women (n=20), OM cell volume was $293.2 \pm 34.05$ pl, which was smaller than SC cell volume $485.1 \pm 41.62$ (N=20, p= 0.0010***).

**b. AA and Hispanics**

In AA subjects, OM cell volume was $301.4 \pm 57.04$ pl, which was smaller than SC cell volume $484.8 \pm 42.41$ pl, (n= 8, p= 0.0218*).

In Hispanic subjects, OM cell volume was $255.3 \pm 42.90$ pl, which was tended to be smaller than SC cell volume $356.5 \pm 28.62$ pl, (n=8, p= 0.0698).

**c. Correlations between OM-SC cell volume and age, BMI, cholesterol, Blood glucose level**

**d.** The results are shown below (Table 2). There was a close to significant correlation between fat cell volume and BMI in the OM depot ($r = 0.3775$, p = 0.0689), but not in the SC depot (Figure 11).
Table 2: Correlations between FCV (OM-SC) & metabolic parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>OM cell volume</th>
<th>SC cell volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Age (Y)</td>
<td>0.0449</td>
<td>0.8348 (ns)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.3775</td>
<td>0.0689 (ns)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>-0.0619</td>
<td>0.8073 (ns)</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>0.208</td>
<td>0.3655 (ns)</td>
</tr>
</tbody>
</table>

Figure 11: Correlation of FCV (OM-SC) with BMI

3. Comparison between the Poor & Good Differentiation groups in OM depot

Preadipocytes from the OM depot differentiated poorly in 14 subjects and average in 10 subjects. The preadipocyte differentiation was done by another group of people and the data were available in the lab. Their characteristics are shown in Table 3.
Table 3: describes the relationship between subject characteristics & differentiation capacity of OM.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean Differentiation ± SEM (OM)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium (n=10)</td>
<td>Poor (n=14)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43 ± 2.87 (31–56)</td>
<td>36 ± 2.49 (20–48)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>40 ± 3.45</td>
<td>43 ± 2.7</td>
</tr>
<tr>
<td>Sex</td>
<td>M= 1 (10%) F=9 (90%)</td>
<td>M= 3 (21.4%) F=11 (78.5%)</td>
</tr>
<tr>
<td>Race</td>
<td>C=5 (50%), AA=2 (20%), H=3 (30%)</td>
<td>C=2 (14.3%), AA= 6 (42.9%), H=5 (35.7%)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>205 ± 12.29</td>
<td>192 ± 10</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6 ± 0.08</td>
<td>6 ± 0.16</td>
</tr>
<tr>
<td>Blood Glucose (mg/dl)</td>
<td>111 ± 7.08</td>
<td>97 ± 3.65</td>
</tr>
<tr>
<td>Hx of Diabetes</td>
<td>Y=2</td>
<td>Y=2</td>
</tr>
</tbody>
</table>

BMI: Body mass Index, AA: African American, C: Caucasian, H: Hispanic
Data shown as Mean ± SEM

Subjects with medium differentiation capacity did not differ from those with poor differentiation capacity in age, BMI and metabolic variables. These differences may not be statistically significant due to small sample size but some are close to being statistically significant: subjects with OM preadipocytes with medium differentiation tended to be older (43 ± 2.87 years) compared to subjects with preadipocytes poor differentiation capacity (36 ± 2.49 years) (p value 0.095). Also subjects with poor DC tended have lower blood glucose level (97 ± 3.65 mg/dl) compared to subjects with medium DC having higher blood glucose level (111 ± 7.08 mg/dl) with a p value of 0.057.
Next the cell volume in OM adipose tissue was calculated from histology sections. No difference was noted between Poor and Medium differentiators (Table 4).

**Table 4: Comparison between the Poor & Good Differentiation groups in OM depot**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean Differentiator ± SEM (OM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Medium (n=10)</td>
</tr>
<tr>
<td>Cell size-vol (pl)</td>
<td>333.9 ± 47.7</td>
</tr>
</tbody>
</table>

4. Comparison between the Poor & Good Differentiation groups in SC depot

**Table 5** describes the relationship between subject characteristics & differentiation capacity of SC.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean Differentiation ± SEM (SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (n=16)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>41.44 ± 2.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>40.75 ±2.68</td>
</tr>
<tr>
<td>Sex</td>
<td>M=1 (6.25%) F=15 (93.7%)</td>
</tr>
<tr>
<td>Race</td>
<td>AA=3 (18.7%), C=7 (43.75%), H=6 (37.5%)</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>197.9 ± 9.6</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6 ± 0.1</td>
</tr>
<tr>
<td>Blood Glucose (mg/dl)</td>
<td>103.2 ± 5.3</td>
</tr>
<tr>
<td>Hx of Diabetes</td>
<td>y=3</td>
</tr>
</tbody>
</table>
Subjects with high differentiation capacity did not differ from those with medium differentiation capacity in age, BMI and other metabolic variables. No statistical significant associations were found & this could be due to small sample size.

The cell volume in SC adipose tissue was calculated from histology sections. No significant difference was noted between high and medium differentiators (Table 6).

**Table 6: Comparison between the Poor & Good Differentiation groups in SC depot**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean Differentiator ± SEM (SC)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High (n=16)</td>
<td>Med (n=8)</td>
<td>p value</td>
</tr>
<tr>
<td>Cell size-vol (pl)</td>
<td>502.4 ± 49.37</td>
<td>437.3 ± 41.67</td>
<td>0.4023</td>
</tr>
</tbody>
</table>
DISCUSSION

It was hypothesized that preadipocytes from subjects with large adipocytes associates with low / poor differentiation capacity (DC) in both visceral & SC depots. We have examined whether the differentiation of preadipocytes is influenced by any metabolic factors. The findings from our lab (Dept. of Medicine, Section of Endocrinology, Diabetes and Nutrition) does not support the hypothesis as we didn’t find any significant association of adipocyte size and difference in differentiation capacity in SC & OM depots. Our data indicate that, under a defined differentiation program, abdominal SC and OM preadipocytes from lean to obese men and women differentiate similarly in primary culture. No significant differences were found in fat cell size and clinical characteristics with good & poor differentiator in SC-OM depot. Due to the lack of associations found, no further statistical tests were performed to check whether any confounding factors were involved in the study. It’s also found that people with poor DC in OM depot have lower blood glucose level compared to the SC depot. But the opposite is not true.

A slight trend of cell size increase with BMI is seen by using both the methods that’s been used for cell size calculation. This result supports the established fact of hypertrophy that occurs in both fat compartments as SC & OM adipocyte sizes increases with BMI in men & women.11

The lack of statistical significance is likely due to the small number of cases; however this finding should not be completely dismissed because it has been observed in other studies. This is a possible trend that warrants further investigation in future studies.
Depot-specific adipogenic differences, specifically those between SC and OM preadipocytes, have been investigated previously in other studies, but only to a limited extent. Our finding of SC depot having higher DC (qualitative) compared to OM depot is in agreement with a report based on 14 women (obese and non-obese) showed that abdominal SC preadipocytes have a 4.5-fold greater differentiation capacity than those of the intra-abdominal OM region.

An additional observation was a trend showing correlation between BMI and omentum cell size but not of subcutaneous cells. A dependence of adipocyte size on age & BMI has been shown previously. However, a novel finding may be that this relation might not be true for the omental region. This observation could be important as studies in humans suggest that aging & BMI is associated with a preferential growth of the omental adipose tissue in both men and women. This underlines the role of regional differences in adipose tissue growth.

There were no major gender differences with regard to preadipocyte differentiation and proliferation in this study. These correlations were not found in the study group, probably because this group was too small to perform any valid linear regression analysis.

**Limitations:** There are several limitations to this project. The first limitation is the sample size of the study population. This could have potentially affected the precision and accuracy of the data reported as some of the results are quite close to statistical significant. A larger sample would have been preferable because it may have resulted in a more stable estimate of the population parameters. As a result, the conclusions of this
study may be based off of a varied data set and caution should be used when interpreting these findings.

Secondly, the DC is data is based on the qualitative value. Overall, the induction of differentiation was satisfactory, as indicated qualitatively by the morphological extent of differentiation. Quantitative data on DC, which were unavailable during the course of this thesis, would have been more helpful to interpret the results in more accurate way.

Thirdly, the ratio of male & female population is not balanced.

In fourth, no measures of body fat and fat distribution were assessed. Adipocytes numbers in each depot can be calculated to compare with the in vitro results. Finally, the results of this study can only be generalized to populations similar to those treated at Boston Medical Center. While that was the original intent of the study, it also becomes a limitation if an attempt is made to compare the data to other heterogeneous populations, which should also be studied before one is able to make solid statements surrounding the prevalence of obese patients in general population.

**Future Directions:** Further studies powered for race, age and sex are needed to address the proposed question. Since most of the study population was severely obese, it’s important to study non-obese male & female. A subject population with more men need to be studied to understand if there is a sex difference and a race difference (match for BMI, AGE).

In conclusion, the results of this study indicate that there may exist regional differences in adipose tissue growth with special reference to proliferation capacity, whereas no
substantial difference in differentiation capacity between subcutaneous and omental preadipocytes was observed. A clinically interesting aspect was that adipocyte cell size increased with BMI only in the omental but not the subcutaneous depot, which could contribute to the preferential expansion of the SC adipose tissue depots with BMI.
## LIST OF JOURNAL ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tr>
<td>Am J Clin Nutr</td>
<td>American Journal of Clinical Nutrition</td>
</tr>
<tr>
<td>Cell Metab</td>
<td>Cell Metabolism</td>
</tr>
<tr>
<td>Int J Obes</td>
<td>International Journal of Obesity</td>
</tr>
<tr>
<td>Int J Obes Relat Metab Disord</td>
<td>International Journal of Obesity and Related Metabolic Disorders</td>
</tr>
<tr>
<td>JAMA</td>
<td>JAMA: The Journal of the American Medical Association</td>
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<tr>
<td>J Clin Endocrinol Metab</td>
<td>Journal of Clinical Endocrinology and Metabolism</td>
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<td>J Lipid Res</td>
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<td>Physiol Rev</td>
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</table>
REFERENCES


VITA

Swati Bhattacharya, MS
85 East Concord Street, Department of Gastroenterology, Suite 7725,
Boston Medical Center, Boston MA 02218.
Phone (617)638-8293. Email: swatimbc@bu.edu

YEAR OF BIRTH: 1979
PLACE OF BIRTH: West Bengal, India

EDUCATION:

MS in Clinical Investigation
Boston University School of Medicine September 2012 – Jan 2016

Certificate in Biotechnology: Biomedical Laboratory & Clinical Sciences
(GPA 3.85/4.00) Boston University September 2008 – June 2009

MS in Biotechnology (Average grade: A)
Mumbai University, Mumbai, India July 2001 – April 2003

BS in Microbiology (Average grade: A)
Pune University, Pune, India July 1998 – April 2001

PROFESSIONAL EXPERIENCE

Feb 2004 – April 2005: Research Assistant, Molecular Biology Research laboratory,
USV Limited, Mumbai, India

Dec 2013 – April 2015: Clinical Research Assistant, Boston Medical Center, Dept. of
Medicine (Section: Endocrinology, Diabetes, Nutrition)

May 2015 – Present: Sr. Clinical Research Assistant, Boston Medical Center, Dept. of
Gastroenterology.