A CYTOMORPHOMETRIC ANALYSIS OF ADIPOCYTES FROM THE OMENTAL AND ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE

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INTRODUCTION

Obesity is associated with chronic illness such as hypertension, type 2 diabetes mellitus and cardiovascular disease. Excess body fat has been frequently correlated with dyslipidemic states and alterations in risk factors for cardiovascular disease. A series of prospective studies have shown that obesity is an important predictor of cardiovascular mortality, although this association is smaller in magnitude than the relationship of cardiovascular mortality with well-known risk factors such as smoking, hypertension and dyslipidemia. It is widely accepted that obesity is a metabolically heterogenous condition. Gyonid obesity, encountered especially in women, has not been linked with the known complications of obesity. Prospective studies that made use of the hip-to-waist ratio have confirmed the fact that android obesity (at the present time, the desired nomenclature being abdominal obesity) is much more strongly associated with metabolic complications, such as dyslipidemia, hyperinsulinemia and greater risk of type 2 diabetes mellitus and cardiovascular disease than overall body fat excess.
Cell size is, alongside sex and depot of origin, the key determining factor of adipocyte function. Adipocytes possess a maximum volume, beyond which expansion cannot take place, which is specific to every adipose depot, known as critical cell size. The expansion of white adipose tissue takes place by means of hypertrophy, hyperplasia or through a combination of both mechanisms. Reaching critical cell size halts expansion by hypertrophy, and stimulates hyperplasia instead. Certain adipose depots grow in size during the course of development, in obesity, or after partial lipectomy, especially through increases in the volume of pre-existing adipocytes. Others grow both through an increase in adipocyte volume, as well as through the differentiation of preadipocytes into new mature fat cells. Subcutaneous adipose tissue, which functions as a long-term depot, tends to grow mainly through increasing its cell number, while cell sizes approximate the most thermodynamically stable sizes of triolein droplets. Visceral adipose tissue, with its short term storage function, modifies its size especially through increases and decreases in cell size, rather than modifications in the number of existing cells. Following the removal of adipose tissue, fat redistributes itself to the remaining depots. After liposuction surgery, it was found that women’s breasts grow in size. Subcutaneous fat expands when visceral fat is experimentally removed in animals, and vice versa. These observations raise serious questions about the potential harmful effects of liposuction on visceral fat, and the very same process probably contributes to the enlargement of visceral adipose tissue associated with subcutaneous fat tissue loss in lipodystrophies and aging. Not all fat depots grow in the same manner and amount after removing fat from another region, and this asymmetry was attributed to differences in regional adrenergic receptor distribution. Fat storage is greater in depots with a high density or affinity of β-adrenergic receptors, as compared with α-adrenergic receptors. This difference in regional density could be the consequence of differences in the distribution of sympathetic fibers or in the intrinsic properties of adipocytes. The discovery that adipocytes synthesize and secrete a wide array of hormones, cytokines and growth factors has radically impacted the classic notion of the adipose tissue being a more or less inert energy storage. The endocrine functions of adipocytes reflect their metabolic status and send information about it to numerous other organs and tissues, as well as to the central nervous system. Moreover, adipocytes express a great variety of receptors that allow them to respond to various stimuli. Thus, the adipose tissue plays a key role in regulating metabolic homeostasis. Any defects affecting this dynamic relationship predispose to fat accumulation and are implicated in the appearance of obesity and its related complications. The adipose tissue has a characteristic adaptative capacity of growing in size. Marked weight gain induces substantial remodeling of the adipose tissue, greatly altering local cellularity. Obesity is characterized by growth in number (hyperplasia), as well as size (hypertrophy) of the fat cells, attributed to preadipocytes being stimulated to proliferate and differentiate (adipogenesis) and to the filling with lipid droplets of smaller adipocytes (lipogenesis), respectively. Initially, adipocyte hypertrophy precedes adipogenesis and reaches a plateau when the maximum size is reached for each adipocyte. Conversely, hyperplasia-adipogenesis continues to progress proportionally with weight gain and, consequently, the number of large adipocytes in fat depots will increase as BMI increases. Enlarged adipocytes tend to be more insulin resistant and more lipolytic compared to small adipocytes, and their overall secretory function promotes metabolic dysregulation. Hypertrophic and hyperplastic growth of adipose tissue is also accompanied by an increase in local vascularization and a proliferation of stromal cells. Mononuclear cells migrate from the blood stream into the expanding adipose tissue, increasing the number of resident macrophages.

Adipokine expression and secretion is markedly altered in obesity and correlates with the manifestations of metabolic syndrome. Most adipokines that have deleterious metabolic effects are more expressed in the visceral adipose tissue. This means a greater adipokine secretion at the visceral level than at the subcutaneous level in obesity, supporting the association between visceral adipose tissue accumulation and a higher risk of metabolic complications.

**MATERIALS AND METHODS**

The study aims to investigate differences in size and number between omental and abdominal subcutaneous adipocytes. Paired samples were obtained from a group of 15 surgical patients (11 women and 4 men).
hospitalised for various interventions in the surgical clinics of the “Floreasca” Clinical Emergency Hospital and the “Dr Ioan Cantacuzino” Clinical Hospital in Bucharest. Permanent histological mounts were obtained and observed through optic microscopy (Olympus BX41) and analyzed through computerized cytomorphometry (Cell^B).

RESULTS

Selected images from the mounts obtained, as well as their measurement through computerized cytomorphometry are shown below. Statistically significant results were obtain for two of the four parameters investigated. Thus, a significant difference was found between the mean and minimum adipocyte diameters of omental and abdominal subcutaneous adipocytes, as will be further detailed.

Mean adipocyte diameter is significantly smaller in the omental adipose tissue compared with abdominal subcutaneous adipose tissue (omental 155.96 ± 7.23 µm, abdominal subcutaneous 184.9 ± 9.75 µm; p<0.05).

The mean adipocyte diameter is, on average, 15.65% lower for omental adipocytes compared to abdominal subcutaneous adipocytes.

Table 1: Mean adipocyte diameter in the omental and subcutaneous abdominal adipose depots.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>OMENTAL ADIPOSE TISSUE</th>
<th>ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>114.6</td>
<td>164.8</td>
</tr>
<tr>
<td>2.</td>
<td>109.2</td>
<td>122.6</td>
</tr>
<tr>
<td>3.</td>
<td>155.4</td>
<td>178.8</td>
</tr>
<tr>
<td>4.</td>
<td>179.1</td>
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<tr>
<td>5.</td>
<td>166.2</td>
<td>226.4</td>
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<td>149.1</td>
<td>205.5</td>
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<tr>
<td>7.</td>
<td>145.2</td>
<td>151.6</td>
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<tr>
<td>8.</td>
<td>222.3</td>
<td>251.2</td>
</tr>
<tr>
<td>9.</td>
<td>156.8</td>
<td>142.7</td>
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<tr>
<td>10.</td>
<td>156.2</td>
<td>193.2</td>
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<td>195.6</td>
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<tr>
<td>13.</td>
<td>129.8</td>
<td>183.4</td>
</tr>
<tr>
<td>14.</td>
<td>166.8</td>
<td>248.9</td>
</tr>
<tr>
<td>15.</td>
<td>186.4</td>
<td>203.6</td>
</tr>
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</table>

Average 155.96 184.8933
Standard Deviation 27.98328 37.74789
Number 15 15
Standard Error of the Mean (SEM) 7.22525 9.74646
\( t \)-test \( (2,2) \) 0.02714
\( t \)-test \( (2,3) \) 0.027947
Tabel 2: Maximum adipocyte diameter in the omental and abdominal subcutaneous adipose depots.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>OMENTAL ADIPOSE TISSUE</th>
<th>ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE</th>
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<tr>
<td>2.</td>
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<td>3.</td>
<td>361.7</td>
<td>247.8</td>
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<tr>
<td>4.</td>
<td>253</td>
<td>286.3</td>
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<tr>
<td>5.</td>
<td>252</td>
<td>306.6</td>
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<tr>
<td>6.</td>
<td>222.8</td>
<td>311.9</td>
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<tr>
<td>7.</td>
<td>230.9</td>
<td>233.6</td>
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<tr>
<td>8.</td>
<td>331.5</td>
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<tr>
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<td>290.3</td>
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<td>235.3</td>
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<td>11.</td>
<td>242</td>
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<td>12.</td>
<td>209.4</td>
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<td>13.</td>
<td>187.1</td>
<td>312.6</td>
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<td>14.</td>
<td>237.4</td>
<td>307.6</td>
</tr>
<tr>
<td>15.</td>
<td>264.6</td>
<td>271.7</td>
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Average: 245.9012  272.9267

Standard Deviation: 51.74466  39.67553

Number: 15  15

Standard Error of the Mean (SEM): 13.36041  10.24418

t-test (2,2)  0.124328

t-test (2,3)  0.124904

Table 3: Minimum adipocyte diameter in the omental and abdominal subcutaneous adipose depots.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>OMENTAL ADIPOSE TISSUE</th>
<th>ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE</th>
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<td>58.4</td>
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<td>103.3</td>
</tr>
<tr>
<td>4.</td>
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<td>68.1</td>
</tr>
<tr>
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<td>71.4</td>
<td>128.1</td>
</tr>
<tr>
<td>6.</td>
<td>67.6</td>
<td>88.1</td>
</tr>
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</table>
Table 4: Number of adipocytes per microscopic field in omental and subcutaneous abdominal adipose depots.

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>OMENTAL ADIPOSE TISSUE</th>
<th>ABDOMINAL SUBCUTANEOUS ADIPOSE TISSUE</th>
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<tr>
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<td>4.</td>
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<td>5.</td>
<td>33</td>
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<td>6.</td>
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<td>7.</td>
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<tr>
<td>10.</td>
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<tr>
<td>11.</td>
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<td>38</td>
</tr>
<tr>
<td>12.</td>
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<td>24</td>
</tr>
<tr>
<td>13.</td>
<td>44</td>
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<td>15</td>
</tr>
<tr>
<td>15.</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

Average                      | 43.266666667                   | 33.533333333                |
Standard Deviation           | 20.95050857                    | 16.02171146                 |
Number                        | 15                            | 15                        |
Standard Error of the Mean   | 5.409398054                    | 4.136788111                 |
t-test (2,2)                  | 0.163982728                    |                         |
t-test (2,3)                  | 0.164734967                    |                         |
Fig. 1. Fragments of omental and abdominal subcutaneous adipose tissue, showing greater cellularity at the omental level compared to the subcutaneous one (approx. twice as many adipocytes in the omentum compared with the subcutaneous adipose tissue).

Fig. 2. Omental and abdominal subcutaneous tissue, with large white adipocytes. A higher cellularity can be observed at the omental level, roughly 2.5 times greater than in the subcutaneous tissue. Capillary vessels in the omental adipose tissue are better represented.

a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements.
Fig. 3. Omental and abdominal subcutaneous adipose tissue, composed of white adipocytes roughly equal in size and number, with a slightly better visible vessel supply at omental level.

Fig. 4. Smaller and more numerous adipose cells in a given microscopic field for the omental adipose tissue compared to the abdominal subcutaneous one.

a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements.
Fig. 5. Adipocytes roughly equal in size and number, both in the omental and abdominal subcutaneous regions. Capillary vessels of relatively large diameter are visible at both levels.

Fig. 6. Greater cellularity can be observed at the omental level. Numerous vessels, some with relatively large diameters can be observed in the abdominal subcutaneous adipose tissue.

*a: omentum adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements*.
Fig. 7. Adipocytes roughly equal in size are visible in both tissues, however the vessel supply is more abundant at the omental level.

Fig. 8. Large adipocytes and reduced cellularity can be observed in the abdominal subcutaneous adipose tissue.

*a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements.*
Fig. 9. Larger adipocytes in the abdominal subcutaneous tissue, more cells visible in the omental region.

Fig. 10. Greater cellularity can be observed at the omental level, large adipocytes in the abdominal subcutaneous adipose tissue.

a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytormorphometric measurements.
A cytomorphometric analysis of adipocytes

Fig. 11. In this particular case, adipocytes in the omental adipose tissue are larger and fewer compared with those in the abdominal subcutaneous adipose tissue.

Fig. 12. Greater cell number in the omental adipose tissue, larger adipocytes in the subcutaneous abdominal adipose tissue.

*a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements.*
Fig. 13. Greater cell number and smaller cell size in the omental adipose tissue, smaller cell number and greater cell size in the abdominal subcutaneous adipose tissue.

Fig. 14. Numerous vascular elements, adipocytes with an irregular shape, in greater number and smaller size in the omental region.

a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements.
Fig. 15. Vascular elements present. Roughly equal cell number. Greater cell size in the subcutaneous fat. *a: omental adipose tissue, b: abdominal subcutaneous adipose tissue, c-d: cytomorphometric measurements.*

Through cytomorphometric analysis of the mounts, we measured the mean, maximum and minimum cell diameters, as well as the number of cells per microscopic field. We present the results in the tables below.

Fig. 16. A graphical rendering of the differences in mean adipocyte diameter between adipocytes in the omental and abdominal subcutaneous compartments of the adipose organ.
Minimum adipocyte diameter is significantly lower in the omental adipose tissue compared with abdominal subcutaneous adipose tissue (omentaland abdominal subcutaneous 93.76 ± 8.64 µm; p<0.05). Minimum adipocyte diameter is, on average, 26.35% lower for omental adipocytes compared with subcutaneous ones.

Fig. 17. A graphical rendering of the differences in minimum adipocyte diameter between adipocytes in the omental and abdominal subcutaneous compartments of the adipose organ.

The difference between maximum adipocyte diameters between the omental and abdominal subcutaneous adipose depot is not statistically significant, although by and large the maximum diameter in omental adipocytes was found to be somewhat smaller (omentaland abdominal subcutaneous 272.92 ± 10.24 µm; p>0.5).

Fig. 18. A graphical rendering of the differences in maximum adipocyte diameter between adipocytes in the omental and abdominal subcutaneous compartments of the adipose organ. The difference found was not statistically significant.
Similarly, the difference in cell numbers between the two depots was found to be statistically insignificant, although by and large the omental adipose tissue was found to have a slightly greater number of adipocytes than the subcutaneous one (omental $432.67 \pm 56.09$, abdominal subcutaneous $335.33 \pm 41.37$; $p>0.5$).

![Number of Adipocytes per Microscopic Field](image)

**DISCUSSION**

The impact of overfeeding was studied on a group of healthy adults, evaluating the impact on upper body and lower body subcutaneous fat depots and monitoring the difference in adipocyte size through microphotography. Mean abdominal subcutaneous adipocyte size grew in correlation with upper body adipose mass gain. The adipose tissue in the lower body responded to overfeeding through adipocyte hyperplasia. No difference in replication or apoptosis rates were found between the different compartments of the adipose organ, so as to properly explain lower body hyperplasia and upper body abdominal subcutaneous hypertrophy. PPARγ and C/EBPα were higher in abdominal subcutaneous preadipocytes than in femoral ones, consistent with the abdominal subcutaneous adipocytes’ capacity to grow to larger sizes. The intrinsic difference in preadipocyte cell dynamics could be a contributor to each compartment’s distinct response to overfeeding. These data contradict the idea that total adipocyte number is constant in adults. An increase in just 1.6 kg of adipose mass in the lower body leads to the formation of up to 2.6 billion new adipocytes in 8 weeks. The lower body adipocyte progenitors rapidly transform into mature adipocytes in adults as a response to overfeeding, and this response is partly dependant on sex and basal adipocyte size. The number of fat cells in the lower limb is greater in overweight than in lean individuals. There is also proof that massive obesity is associated with marked subcutaneous abdominal hyperplasia. A large and sustained weight gain is necessary before an increase in number of subcutaneous abdominal adipocytes occur, though studies show that certain lean women with relatively large subcutaneous adipocytes can recruit other mature adipocytes. Other groups have reported fixed numbers of abdominal subcutaneous adipocytes.

The capacity of healthy adults to expand their lower body’s adipose reserve through hyperplasia hinders or delays abdominal subcutaneous hypertrophy, by sequestering the excess lipids. Lower body adipose mass is, therefore, a negative predictor of upper body adipocyte size.
Subcutaneous adipocyte hypertrophy is a predictor for the onset of insulin resistance and type 2 diabetes mellitus. This explains why lower body fat depots are said to have a positive health effect. These conclusions are consistent with the overflow hypothesis, according to which the adipose tissue of the lower body is the primary compartment for expansion, and its capacity determines the degree to which secondary compartments (upper body subcutaneous and visceral) expand during weight gain.38

The growth of adipose tissue occurs through volume expansion of preexisting adipocytes (hypertrophy), by generating new adipocytes (hyperplasia) or through both these mechanisms. Although the amount and distribution of adipose tissue are independently correlated with insulin resistance, type 2 diabetes mellitus and other metabolic disorders, adipocyte size is also extremely important in this regard. Increased adipocyte size correlates with insulin serum levels, insulin resistance and increased risk for type 2 diabetes mellitus. Obese subjects with lower numbers of large adipocytes are more glucose intolerant and have higher serum insulin levels than subjects with the same obesity type that have a higher number of small adipocytes. Moreover, adipocyte hypertrophy impairs the adipose tissue’s normal function by inducing local inflammation, mechanical stress and an altered metabolism. There is large variation as far as adipocyte size is concerned between lean and obese individuals. Lean individuals can possess larger adipocytes than obese individuals and vice versa. To the present day, a particular methodology for estimating adipocyte morphology has yet to be developed. Adjusting adipocyte size to BMI through linear regression is insufficient, because the relationship between BMI/adipose tissue mass and adipocyte size is curvilinear.39

The processes responsible for the development of the different forms of adipocyte morphology are largely unknown, although the involvement of differences in adipocyte turn-over is a prime suspect. The rate of adipocyte turn-over is high for all adult ages and body fat levels. Approx. 1/10 of total adipocyte mass is renewed every year through continuous adipogenesis and cellular death.39

Investigations were performed to establish the part played by adipocyte turn-over in the development of different subcutaneous adipose tissue morphologies (the body’s dominant adipose tissue depot). The distribution of hyperplasia and hypertrophy were found to be independent of sex and body weight, but correlated with basal serum insulin and insulin sensitivity, independent of adipocyte size. The total number and morphology of adipocytes were negatively correlated, meaning that the total number of adipocytes was the greatest in cases of pronounced hyperplasia and the lowest in cases of pronounced hypertrophy. The absolute number of new adipocytes generated per year was 70% lower for hypertrophy than for hyperplasia, and the individual values of adipocyte generation and morphology were strongly correlated. The relative rate of cell death (roughly 10% per year) or the median age of adipocytes (approx. 10 years) have not been correlated with cell morphology.39

Therefore, adipose tissue morphology is correlated with insulin levels and is intimately related with the total number of adipocytes independent of sex and body adipose depot levels. Low rates of adipocyte production are correlated with adipose tissue hypertrophy, while high rates are associated with hyperplasia.39

A study conducted on a group of Pima Indians showed that subcutaneous adipocyte enlargement is associated with hyperinsulinemia, insulin resistance and glucose intolerance. After adjusting for age, sex and fat composition %, the mean subcutaneous adipocyte size was 19, respectively 11% larger in subjects with diabetes and IGT, as compared with subjects with normal glucose tolerance. Insulin sensitivity was inversely correlated with abdominal subcutaneous adipocyte size, even after adjusting for fat composition percent. An increase in mean abdominal subcutaneous adipocyte size, but not a high percentage of body fat, was an independent predictive factor for diabetes, as well as lowered insulin sensitivity and acute insulinic secretory response. Modifications of insulin sensitivity are inversely and independently correlated with modifications of median abdominal subcutaneous adipocyte size and body fat percentage. Although transversally, higher median abdominal subcutaneous adipocyte size is associated with insulin resistance, prospectively, both anomalies are independent and additive predictive factors for diabetes mellitus type 2.40

The adipocytes’ metabolic activity and their response to lipolytic agonists differ markedly from one compartment to another, the main
determinant being the difference in adipocyte size. According to recent studies, for most values on the adiposity spectrum, omental adipocytes are approximately 20% smaller than subcutaneous adipocytes in women, a correlation which was also observed in the male sex (in the case of which, however, the maximum diameter is lower)[42]. The adipose tissue morphology and physiological characteristics differ with each adipose depot as well.

In both sexes, subcutaneous and omental adipocytes become larger with obesity, but adipocyte size reaches a plateau in extreme obesity[42,43]. In lean to obese women, omental adipocytes are 20-30% smaller than subcutaneous adipocytes for most values of the adiposity spectrum[42,43]. Omental and subcutaneous adipocytes reach similar sizes only for markedly elevated BMIs in women (>45 kg/m²)[42,43]. In men, omental and subcutaneous adipocytes have similar values for most of the adiposity spectrum. The maximum adipocyte size is lower in men (approx 120 µm) compared to women (approx 140 µm)[42]. Studies that reveal differences between small and large adipocytes in the same adipose depot of an individual show that lipolysis, lipogenesis, glucose uptake, as well as gene expression were strongly influenced by adipocyte size[44-47]. Therefore, adipocyte size is a critical determinant of its function[44] and the differences related to sex, depot and degree of adiposity in this parameter play an important part in the relation between visceral obesity and metabolic alterations that ensue.

The response of adipocytes to lipolytic agonists differs between the visceral and subcutaneous compartments[42,43,48-50], adipocyte size being one of the main determinants of this regional difference[42,44]. Large adipocytes from any given adipose tissue have elevated lipid synthesis and lipolysis, as well as an increased flow of fatty acids across their cell membranes[51]. Basal lipolysis can be encountered in the omental depot, compared to the subcutaneous one in women, consistent with the difference between adipocyte sizes observed in these two compartments[42,48,50]. Therefore, in women and perhaps in highly lean men, visceral adipose tissue does not contribute to the pool of circulating free fatty acids under basal conditions[52]. Compared with the subcutaneous depot, lipolysis in the omental fat is more responsive to stimulation with β-adrenergic agonists[43,48,50] and less responsive to suppression by insulin[53,54]. In men, lipolytic activity is higher than in women, without regional differences in isoproterenol-stimulated lipolysis[42,43]. In absolute terms, more free fatty acids are released into the portal bloodstream by the visceral fat in men[52]. In comparison with women, this increases the impact of the omental adipose tissue on hepatic metabolism and the appearance of a metabolic profile that favours the onset of diabetes and atherosclerosis[42].

Regional differences in triglyceride accumulation are closely tied with adipocyte size as well. Certain studies have failed to find differences in lipoprotein lipase activity in the omental and subcutaneous regions[55,56]. Other studies, however, showed that lipoprotein lipase activity in the subcutaneous adipose tissue is higher, especially in women[43,54,57], and lower in the studies that included more men[42,57,58]. The hypothesis was put forth that regional differences in lipoprotein lipase are sex-specific and reflect the propensity of different depots towards lipid accumulation in each sex. Accordingly, triglyceride synthesis in women is reduced in the omental fat compared to the subcutaneous one[48,59], and no differences were reported in men[48].

To study the relative capacity of each abdominal adipose compartment to store excess fat (through hypertrophy and hyperplasia), a group of women were examined, in whom CT measures of abdominal fat areas were performed, and omental and subcutaneous adipose tissue samples were obtained surgically, in order to characterize adipocytes by size and profile of adipogenic gene expression. A marked difference was observed in the regressions of omental and subcutaneous adipocyte size to total body fat mass, as well as in the regression of adipose tissue areas and total body fat mass. It was found that obese women have proportionately larger adipocytes in both these adipose compartments than lean women do. Also, subcutaneous adipose tissue was found to be hyperplastic in obese women. Therefore, in women, according to this study, hyperplasia is predominant in the subcutaneous fat depot, whereas fat cell hypertrophy is observed in both omental and subcutaneous adipose tissues[60]. A greater storage capacity of subcutaneous adipose tissue through hyperplasia (as observed in women) could theoretically reduce the dependency on the visceral depot and other ectopic compartments, exercising
a protective metabolic role in dealing with excess energy.\textsuperscript{41}

The relationship between subcutaneous and omental adipocyte hypertrophy and metabolic alterations independent of body composition and fat distribution in women has been assessed. The mean adipocyte diameters of paired samples of subcutaneous adipose and omental tissues were calculated for women with BMIs ranging from lean to obese. For each adipose depot, the women with larger adipocyte than predicted by linear regression models were considered to have adipocyte hypertrophy, and those with adipocytes smaller than predicted were considered to have adipocyte hyperplasia. Women characterized by omental adipocyte hypertrophy had high plasma VLDL and triglycerides, as well as a higher total cholesterol/HDL cholesterol ration, compared to wome characterized by omental adipocyte hyperplasia. Furthermore, women characterized by subcutaneous hypertrophy or hyperplasia have a similar lipidic profile. A 10% increase of omental adipocyte size raises the risk for hypertriglyceridemia independent of body composition and fat distribution measures. An increase of 10% in the number of visceral adipocytes also increases the risk of hypertriglyceridemia. Thus it is suggested that omental adipocyte hypertrophy, but not subcutaneous hypertrophy, is associated with an altered lipidic profile independent of body composition and body fat distribution in women.\textsuperscript{60}

It has been postulated that, in the course of the growth process, adipocytes can react at first in a physiological manner and then in a pathological one. The view put forth by the Romanian school of diabetology has been that the behaviour of adipocytes can be included in three categories: “quiet” (“silent”) adipocytes, which constitute the majority in lean individuals, “restless” adipocytes, which constitute the majority type in overweight individuals (with BMI in the range of 25 to 30) and, finally, “aggressive” adipocytes, in obese individuals (BMI higher than 30). The latter two categories are characterized by a progressive increase in pathogenic adipokines and a decrease in the protective adipokines such as adiponectin.\textsuperscript{62}

As obesity has risen exponentially in developed countries, it constitutes a major public health concern through raising the risk of metabolic and cardiovascular diseases. Differences in gene expression profiles, as well as metabolic and biochemical properties between omental and subcutaneous adipose tissue have been documented at length in scientific literature.\textsuperscript{63}

Because omental adipose tissue accumulation has been strongly associated with the development of insulin resistance, diabetes mellitus type 2 and cardiovascular disease, proteins differentially expressed between the two depots have been sought out. Through electrophoretic and mass spectrometry, a study has discovered 43 such proteins, part of which have been validated through immunologic analysis. The results have demonstrated the existence of tissue-specific molecular differences in protein content of the two adipose depots, particularly connected with differences in metabolic processes such as glucose metabolism, lipid metabolism, lipid transportation, protein synthesis and packaging, stress response and inflammation. This suggests elevated metabolic activity, as well as raised cellular stress in the omental adipose tissue compared with subcutaneous adipose tissue.\textsuperscript{63}

Micro-RNA (miRNA) are small uncodifying nucleic acids with important regulatory roles in a wide array of biological processes – development, differentiation, apoptosis and metabolism. In mammals, miRNA modulates adipocyte differentiation. A global quantification of miRNA gene expression in different adipose depots of overweight and obese individuals was undertaken, in order to indentify whether it possessed depot specificity in humans and whether it is associated with different parameters of obesity and glucose metabolism. It was found that no miRNA was expressed exclusively in one of the two depots, suggesting a common origin of both adipose depots. 16 miRNA (4 found in normo-glycemic subjects, 12 in diabetic patients) show a depot-specific expression pattern. Significant correlations between the expression of miRNA-17-5p, -132, -99a, -134, 181a, -145, -197, on the one hand, and adipose tissue morphology and parameters such as blood sugar, circulating leptin, adiponectin and IL-6, on the other. It was thus concluded that differences in miRNA expressions could contribute to intrinsic differences between omental and subcutaneous adipose tissue. Moreover, the expression of miRNA in human adipose tissue is correlated with adipocyte phenotype, parameters of obesity and glucose metabolism.\textsuperscript{64}

Different fat depots have differential gene expression and indicate that there are substantial differences between the sexes as far as adipose
gene expression patterns are concerned. A subtractive hybridisation strategy was used in order to assess gene expression differences in subcutaneous and omental adipose tissue of obese males. 44 potentially differentially expressed genes were identified, and 5 genes were confirmed to be differentially expressed in subcutaneous or omental adipose tissue from male or female obese patients. One gene was found exclusively in male, and was discovered to be expressed more prominently in subcutaneous tissue.

The protein levels of eNOS are markedly elevated in omental adipose tissue as compared with subcutaneous adipose tissue in obese subjects. Since basal lipolysis is much lower in omental adipose tissue compared with subcutaneous adipose tissue, it is likely that elevated regional NO production, especially by eNOS, plays a part in the differences in basal lipolysis between adipose depots in obese subjects. iNOS is expressed at significantly lower and barely detectable levels in both subcutaneous and omental regions. Basal lipolysis rate was found to be twice as high in subcutaneous adipose tissue compared with omental adipose tissue.

IL-6 production and its production by adipocytes from different depots of obese subjects and its regulation by glucocorticoids were investigated. It was shown that fragments of omental and abdominal subcutaneous adipose tissue release immunodetectable quantities of IL-6 into the medium during acute incubation. Omental adipose tissue releases 2-3 times more IL-6 compared to subcutaneous adipose tissue. Isolated adipocytes prepared from samples of omental tissue released greater quantities of IL-6 than isolated adipocytes from subcutaneous tissue, as well, but this constitutes only 10% of the total quantity released at tissue level. Adipose tissue cultures exposed to dexamethasone for 7 days have shown a markedly suppressed IL-6 production. This data has shown that IL-6 is released in substantial amounts (up to 75 ng/mL) into the medium, both by the adipocytes alone, as well as the adipose tissue. Although the effects of IL-6 on adipose tissue are insufficiently established, a well-known effect is the down-regulation of the adipose tissue lipoprotein lipase. The regulation of this multifunctional cytokine’s production may modulate regional adipose tissue metabolism and contribute to the correlation between the blood IL-6 levels and the degree of obesity that was recently uncovered.

Plasma and adipose tissue sex steroid levels have been examined in a sample of 28 men aged 24-62 (average BMI of 46.3 ± 12.7 kg/m²). BMI and waist circumference were negatively correlated with plasma testosterone and dihydrotestosterone, and positively correlated with estrone levels. Regional differences in the levels of sex steroid hormones in adipose tissue were observed for dihydrotestosterone, androstenedione and dehydroepiandrosterone, with significantly higher concentrations in the omental adipose tissue compared with the subcutaneous fat. Significant positive associations between the circulating levels and concentrations in the omental and subcutaneous adipose tissues were found for estrone, testosterone and dihydrotestosterone. Positive correlations between dehydroepiandrosterone-sulfate and the omental and subcutaneous dehydroepiandrosterone levels were found. Positive associations were found between adipocyte responsivity to lipolytic stimuli and plasma or omental androgen levels. It was concluded that, although plasma androgen and estrogen levels are strongly correlated with both omental and abdominal subcutaneous steroid levels, a series of regional differences can be observed. Differences in concentration of androgens in omental adipose tissue compared to abdominal subcutaneous adipose tissue suggest a depot-specific impact of these hormones on adipocyte function and metabolism.

TNFα is involved in the relationship between obesity and insulin resistance/diabetes mellitus type 2. To better understand this association, the gene expression patterns of TNF, TNFR1 and TNFR2 were profiled, and the effects of TNF on glucose uptake in isolated adipocytes and adipose tissue explants from omental and subcutaneous depots from individuals with BMIs ranging from lean to obese were investigated. TNF expression correlated with TNFR2 expression, but not with TNFR1 expression, and both of these are elevated in obese individuals. The expression of TNFR1 is higher in omental than in subcutaneous adipocytes. There is no difference in the levels of expression for TNF and the two receptors between the adipocytes of the individuals with central and peripheral obesity. TNF suppresses glucose uptake in the insulin-stimulated subcutaneous adipose tissue, and the aforementioned suppression has only been observed in the lean
TNFα expression was higher in omental adipose tissue than in subcutaneous adipose tissue. Significant positive linear correlations were found between omental TNFα and plasma PAI-1 in obese subjects. Omental TNFα was positively correlated with HOMA-IR, triglycerides and negatively correlated with HDL-cholesterol levels. TNFα expression could play a key part in the development of cardiovascular risk in subjects with central obesity.

It has been found that resistin is synthesized and released in much higher quantities by the omental adipose tissue than by subcutaneous adipose tissue. Further research is necessary to determine whether this can be attributed directly to adipocytes or to the non-adipocytic cells of the human adipose tissue.

The activity of various insulin signaling molecules in the adipose tissue in vivo and compared reaction for visceral and subcutaneous adipose tissues. Paired omental and subcutaneous biopsies were obtained from non-obese subjects with normal insulin sensitivity, under basal conditions, at 6 and 30 minutes after administering intravenous insulin. Insulin receptor phosphorylation were more rapid and intense and the insulin receptor protein content was greater in omental adipose tissue compared with subcutaneous adipose tissue. The insulin-induced phosphorylation of Akt was greater and earlier in the omental depot than in the subcutaneous one, without modifications with respect to the Akt content. Thus, the phosphorylation of glycogen synthase kinase-3, the substrate of Akt, was more responsive to insulin stimulation in the omental depot. The content in extracellular signal-regulated kinase (ERK)-1/2 was three times higher in the omental depot than in the subcutaneous one, and ERK phosphorylation reached an early peak at 6 minutes in the omental fat, compared with the gradual increase observed in the subcutaneous fat. Therefore, the insulin signaling system of the omental adipose mass shows responses to insulin that are faster and of a higher magnitude, than that of the subcutaneous abdominal adipocytes.

The finding that African American women lose less weight and at a lower rate than Caucasian women under the same conditions was attributed to a decreased mobilization of fat, possibly involving differences in the responsiveness of fat tissue to sympathetic stimulation. A study was undertaken to determine the differences in number and affinity for β-adrenergic receptors in omental and abdominal subcutaneous adipose tissues in obese African American and Caucasian women. The total number of receptors, both in the omental and subcutaneous adipose tissue, is greater in African American than in Caucasian women. The densities of β1, β2 and β3 in African American women are higher in omental adipose tissue, but not different in the subcutaneous adipose tissue. No racial differences in kd values were found for adrenergic agents, agonists and antagonists, with regard to β1, β2 and β3 receptors in both omental and subcutaneous adipose tissues. The protein mass of β1 and β2 receptors is significantly greater in omental preparations, but not subcutaneous ones, in African American women. In vitro data that show an increase in β receptors in the omental adipose tissue of obese African American women suggest that the potential for lipolysis is greater in these women. Future studies are required to assess the biological significance of these distribution differences for β-adrenergic receptors in vivo.

Screening a subtracted cDNA library was performed so as to identify genes that are differentially expressed in omental adipose tissue of patients with type 2 diabetes mellitus. One clone showed a marked decrease in the omental adipose tissue of patients with type 2 diabetes mellitus. The respective clone was shown to have been, in fact, the gene coding the adipocyte-specific secreted protein gene apM1. apM1 mRNA was expressed by human adipocytes in culture, but not by preadipocytes, in a like manner with its orthologue in mice. It was confirmed that apM1 mRNA levels were markedly reduced in the omental adipose tissue of obese patients with type 2 diabetes mellitus, compared with normoglycemic subjects, be they lean or obese. apM1 mRNA levels are reduced in the subcutaneous adipose tissue of diabetics, though this reduction is less pronounced. Although the biological function of apM1 is not yet known, its cellular-specific expression, structural similarities with TNFα and deregulated expression in obese and diabetic patients suggest that it might play a role in the development of cardiovascular risk in these patients.
part in the pathogenic processes that lead to insulin resistance and diabetes.\textsuperscript{34}

While the most widely used measurement of body fat distribution is the waist-to-hip ratio, it does not distinguish the amount of visceral adipose tissue from the subcutaneous adipose tissue. Imaging techniques such as CT and MRI have made quantifying the amount of adipose tissue in various body compartments much more accessible. Beginning with the late 1980s a series of studies undertook the task of evaluating the correlates of abdominal-visceral obesity in various populations and physiological conditions. It was found that, for any given body fat mass value, men have significantly more visceral adipose tissue than women of fertile age.\textsuperscript{75} Whether this difference could account for the documented difference in cardiovascular risk factors between the two sexes has been examined in further studies, comparing subgroups of men and women with matching levels of visceral adipose tissue. It was found that this procedure eliminated to a large degree the majority of differences in glucose tolerance and plasma lipoprotein levels (apoB, triglycerides). This study showed that plasma HDL-C levels remain higher in women than men, even after controlling for sex differences in the amount of visceral adipose tissue.\textsuperscript{76} This has been attributed to the fact that androgens and estrogens modulate hepatic lipase activity levels directly.\textsuperscript{77}

A series of studies focused on the relationship between visceral fat accumulation and the gender differences in LDL particle size, the fact that men tend to have smaller LDL particles than women being well established.\textsuperscript{78} A comparison between subgroups of both sexes with elevated triglyceride concentrations of similar value and roughly equal amounts of visceral adipose tissue, revealed that LDL particle size remains significantly lower in men than in women, suggesting that, while plasma triglyceride levels and visceral adipose tissue are strong predictors of the size of LDL particles, they are insufficient in explaining entirely the differences between sexes in LDL size.\textsuperscript{79} It has been shown that a strong genetic component underlies the small and dense LDL phenotype, very likely masking the contribution of visceral adipose tissue to sex differences in LDL size.\textsuperscript{80,81} Thus, visceral adipose tissue can be said to be a significant contributor to sex differences in a series of metabolic parameters, alongside other hormonal and hereditary factors.\textsuperscript{41}

In a recent study, a number of patients with type 2 diabetes mellitus underwent abdominal CT with the purpose of evaluating the visceral and subcutaneous adipose tissue and, at the same time, recording their anthropometric data (BMI, waist and hip circumference). After adjusting for age, sex, anti-diabetic therapy, duration of disease, smoking, statin use and A1C levels, a positive correlation has been found between visceral adipose tissue and the number of VLDL and LDL particles, and a negative correlation between LDL and HDL size. Therefore, in diabetic patients, higher visceral adipose tissue independent of BMI is associated with higher VLDL and LDL particle number, larger VLDL particles and smaller LDL and HDL particles.\textsuperscript{82}

A series of studies have reported the existence of a subgroup of obese individuals with a normal metabolic profile. It remains unclear as to what factors are responsible for this phenomenon. It has been hypothesized that adipocyte size could be a key protection factor for the metabolically healthy obese (MHO), as they are known. A group of patients subjected to bariatric surgery were classified either as metabolically healthy obese (MHO) or metabolically unhealthy obese (MUO), according to thresholds established by the IDF definition of the metabolic syndrome. A moderate correlation between omental adipose size and subcutaneous adipose size was found. The MHO group had a mean omental adipocyte size significantly lower than that in the MUO group. Mean subcutaneous adipocyte size was similar in the two groups. Omental adipocyte size, but not subcutaneous adipocyte size, correlated with the degree of insulin resistance measured by HOMA-IR, as with other metabolic parameters, including the triglyceride/HDL-cholesterol ratio and HbA1c. Of all the patients subjected to hepatic biopsy, 46% had steatosis and fibrosis, and 50% (including all patients with MHO) only had steatosis. Both omental adipocyte size and subcutaneous adipocyte size correlated significantly with the degree of steatosis, but only omental adipocyte size was an independent predicting factor for the presence of fibrosis or absence thereof. It was therefore concluded that omental adipocyte size is strongly correlated with the degree of metabolic health and an important predicting factor of hepatic steatosis to hepatic fibrosis.\textsuperscript{83}

The current study has demonstrated the presence of differences between white adipose
tissue originating in the omental and subcutaneous abdominal compartments of the adipose organ. It was demonstrated that mean diameter and minimum diameter are larger in subcutaneous adipocytes and smaller in omental adipocytes. This is indicative of the fact that critical cell size is higher in the subcutaneous compartment, and smaller in the case of the omental compartment. The differences in size found between omental and abdominal subcutaneous adipocytes (approx 15% for mean diameter, approx 26% for minimum diameter) are compatible with the data found in scientific literature\textsuperscript{11}, that reveal a difference in size of approximately 20-30% between the adipocytes of the two depots.

Although we failed to find statistically significant correlation between the number of adipocytes per microscopic field and maximum diameter on the one hand, and compartment of origin on the other, the overall trend suggests a similar distribution (maximum diameters slightly larger for abdominal subcutaneous adipose tissue, slightly greater cell number in the omental fat). These correlations need be further clarified by extending the study group, thus increasing the degree of statistical significance.

Individual cases that constituted exceptions from the general rule were the following:

- patients 4, 9, 11 had a larger mean adipocyte diameter in the omental adipose tissue compared to the abdominal subcutaneous adipose tissue;

- patients 9, 15 had a larger minimum adipocyte diameter in the omental adipose tissue compared to the abdominal subcutaneous adipose tissue;

- patients 3, 9, 11 had a larger maximum adipocyte diameter in the omental adipose tissue compared to the abdominal subcutaneous adipose tissue;

- patients 9, 11 had lower cellularity at the omental level compared to the abdominal subcutaneous adipose tissue, and patient 12 had an equal number of cells per microscopic fields for both compartments.

A thorough clinical and paraclinical investigation of these cases is in order, so as to formulate working hypotheses for the existence of these discrepancies. Patients 9 and 11 are especially interesting in this regard.

The correlation that we found are consistent with the existent data in scientific literature, that point out the presence of significant differences in gene expression, protein content and metabolic activity of these two compartments and, consequently, to their different impact on the pathogeny of the complications of obesity, type 2 diabetes mellitus and cardiovascular disease.

**CONCLUSIONS**

Omental and subcutaneous adipose tissue in the abdominal region show significant differences in adipocyte size. The mean adipocyte diameter is significantly lower in the omental region than it is in the subcutaneous fat, as is the minimum adipocyte diameter. The other parameters we measured (maximum adipocyte size and cell number) were not found to differ significantly between the two compartments. Critical cell size can be said to be lower in the omental adipose tissue and higher in the subcutaneous abdominal adipose tissue. The contribution of cell hypertrophy and hyperplasia as mechanisms of adipose expansion can be inferred to be different between the two compartments.

**REFERENCES**

A cytomorphometric analysis of adipocytes


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