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FRACTAL CHARACTERISTICS OF ADIPOCYTE DYNAMICS IN MICE

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Abstract
The functional status of adypocites is reflected in their morphology and is directly related to the metabolic state of the individual. Here, we investigate if fractal analysis is useful in highlighting the impact on adipocytes of progressive exposure to a hypercaloric diet. A total of 18 NMRI mice were assigned to 3 groups: control group (C), obesity induced by hypercaloric diet at one (M1) and two months (M2). Samples from mesenteric, omental, perirenal and inguinal subcutaneous adipose tissue samples were collected from each subject and analyzed through fractal dimension (FD) method. Within the performance range of current medical tests, FD showed differences between M1 and C (area below ROC curve > 0.9), as well as between M2 and C. Data collected from the inguinal subcutaneous site provided a statistical distinction between M2 and M1 (area below ROC curve 0.714). Thus, we concluded that FD represents a reliable method for identifying the smallest changes in the adipose tissue morphology.

Key words: *adipocytes, obesity, fractal dimension, fractal analysis, mouse.*

INTRODUCTION

Understanding obesity may be crucial for future developments in clinical therapy and diagnosis. The mechanisms by which excess energy is managed, underlies the entire metabolic process. Both diet and genetic background of a species can influence the expansion and contraction dynamics of the adipose tissue. In our current view there are two main growth mechanisms. One of these mechanisms is represented by hyperplasia (a cell number increase) and the other by hypertrophy (cell size expansion) (Drolet et al., 2008; Spalding et al., 2008; Faust et al., 1978). Unlike other essential organs (brain, lung, heart, kidney), the adipose tissue has the widest range of expansion due to his storage function. Adipocyte hypertrophy can block the proper function of body fat by inducing mechanical stress and local inflammation, thus, initiating hyperplasia. When obesity is experimentally induced, various parameters of adipocytes can be quantified, such as diameter, perimeter, area, and other non-classical parameters. One of these non-classical parameters is represented by cell membrane contours of neighborhood adipocytes. We considered fractal analysis as the main method for quantifying such a relationship (Mandelbrot,1967; Mandelbrot, 1983). The computed fractal dimension of the microscopic picture may reveal existing relationships between hyperplasia and hypertrophy of adipocytes in different fat regions (Arner & Spalding, 2010; Britton & Fox, 2011; Smith et al., 2008; Spalding et al., 2008). Here, adipocytes from four distinct anatomical regions have been considered, namely the omental, mesenteric, perirenal, and subcutaneous inguinal region. The parallel analysis of these four regions has been the basis in elucidating different relationships between mice white adipocytes. The main objective consisted in capturing the dynamics/evolution of the adipose tissue expansion in all four regions. The secondary objective was to evaluate the

method itself, to see if it is suitable for such a task.

MATERIALS AND METHODS

A total of 18 NMRI mice were assigned to 3 groups, each consisting of 6 individuals: control group (C), obesity induced by hypercaloric diet at one (M1) and two months (M2). Samples from mesenteric, omental, perirenal, and inguinal subcutaneous adipose tissue were collected from each subject (Figure 1).

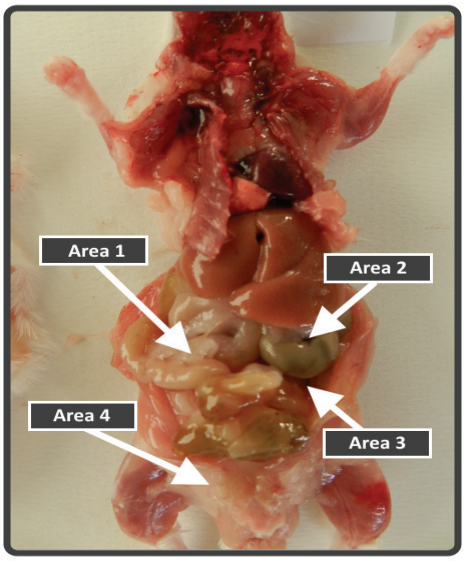

Figure 1. Adipose tissue samples. The samples were collected from mesenteric area (Area 1), omental area (Area 2), perirenal area (Area 3), and inguinal subcutaneous area (Area 4).

The adipose tissue samples were fixed immediately after harvesting in buffered 10% formalin solution for 24 h. Processing of the samples and paraffin embedding were made automatically by the tissue processor 120-3 Thermo Scientific STP. Onward, blocks were sectioned at 3 μm using Leica microtome RM 125RTS. All slides were stained with hematoxylin eosin using Thermo Scientific Microm HMS 70. Thus, 360 photomicrographs 400x have been made using an Olympus BX 41 microscope equipped with Olympus SP350 video camera (Figure 2A). The photomicrographs were digitally processed and their fractal dimension (FD) was calculated (Figure 2).

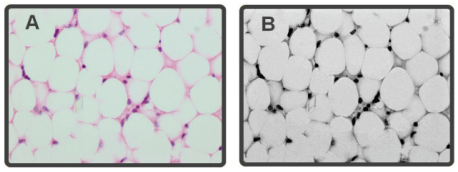

Figure 2. Examples of photomicrographs used for fractal analysis. (**A**) Original image of the microscopic field, (**B**) black and white image used for fractal analysis.

Prior to the image analysis, the digital processing of photomicrographs consisted of transforming each pixel color from the original image to the corresponding gray palette (Gagniuc et al., 2013). The fractal dimension for each processed picture was then calculated using FracLab 2.05 software (INRIA Saclay Research Center designed - Ile-de-France). FracLab 2.05 uses the box method (which provides a very good approximation of the Hausdorff dimension). A given set of points (pixels on the image – Figure 2B) is broken down into ε-sized boxes and the number N of boxes which include elements of the set is counted. For various ε a respective N value is determined. The dependence $N(\varepsilon)$ is plotted on double logarithmic coordinates, where the slope of the dependence corresponds to the value of the fractal dimension. The fractal dimension (D_H) formula is defined below:

$$D_H = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log \frac{1}{\varepsilon}}$$

where ε is the size of the box, and N represents the number of positive boxes. Hence, the dependence is shown by $N(\varepsilon) \sim 1/\varepsilon^D$. Thus, when the differences between means (medians) of D_H for each group/anatomic site were found significant ($P<0.02$), the performance of a differentiation-test based on FD was evaluated by ROC (Receptor Operating

Curve) plot analysis (Gaiță et al., 2013). The statistical analysis used StatsDirect v. 3.0.

RESULTS AND DISCUSSIONS

The fractal dimension remains a useful measure of the complexity of a bidimensional contour. Therefore, fractal analysis has already found widespread applications in biology, medicine, and their related fields (Losa, 2012). Here, digital image analysis has been adapted to measure the fractal dimension of adipocyte profiles (Gagniuc et al., 2013; Losa, 2009; Condrut et al., 2015). Specifically, the aim was to determine whether the complexity of the branching pattern between adipocyte cell membranes reflects their function in regard to the state of induced obesity in mice. Within the performance range of current medical tests, FD showed differences between M1 group and C group (ROC curve >0.9), as well as between M2 and C groups (Figure 3A-F). Data collected from the samples taken from the inguinal subcutaneous site provided a statistical distinction between M2 group and M1 group (area below the ROC curve 0.714).

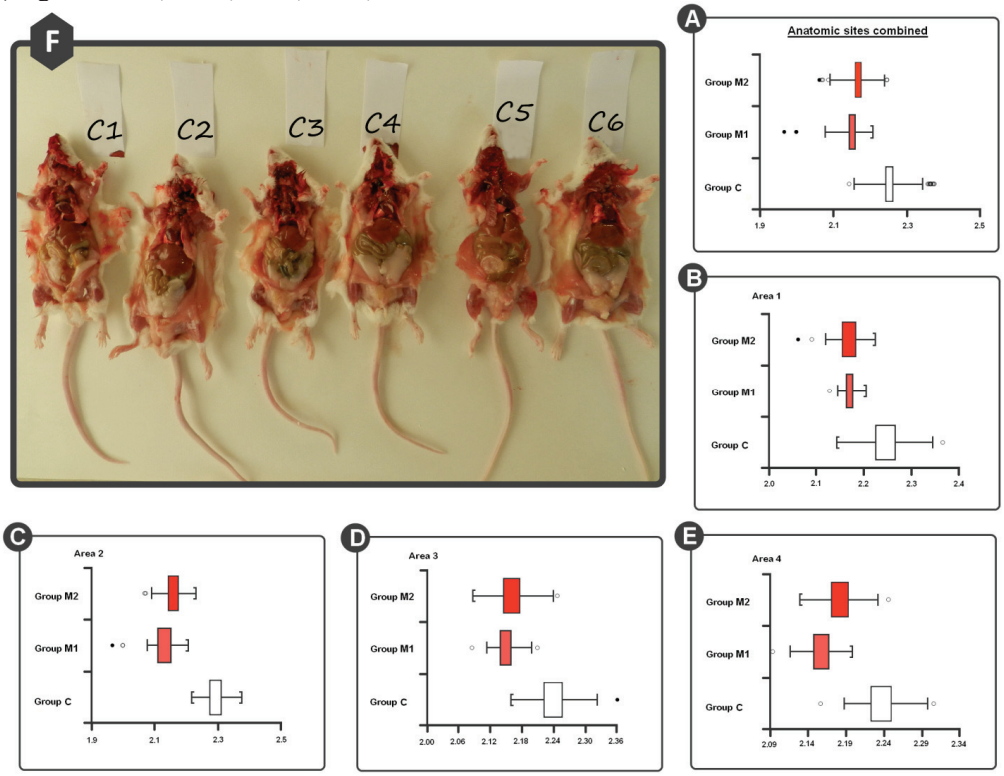

Figure 3. Box-and-whisker plottings for fractal dimension. (**A**) Fractal dimension of adipocytes for Group M1, Group M2, Group C (control) in all the regions (mean values for the four regions). Fractal dimension of adipocytes for Group M1, Group M2, Group C (control) in regions (**B**) mesenteric area (Area1), (**C**) omental area (Area 2), (**D**) perirenal area (Area 3), (**E**) inguinal subcutaneous area (Area 4), (**F**) individuals from group C.

Thus, these observations suggest that FD represents a reliable method for identifying the smallest changes in the adipose tissue morphology (Figure 4A-D). Although previous reports had hinted at the omental region, the inguinal subcutaneous site was

found to provide the most sensitive samples to both emergence and dynamics of changes in the adipose tissue status induced by exposure to hypercaloric diet (Figure 4C).

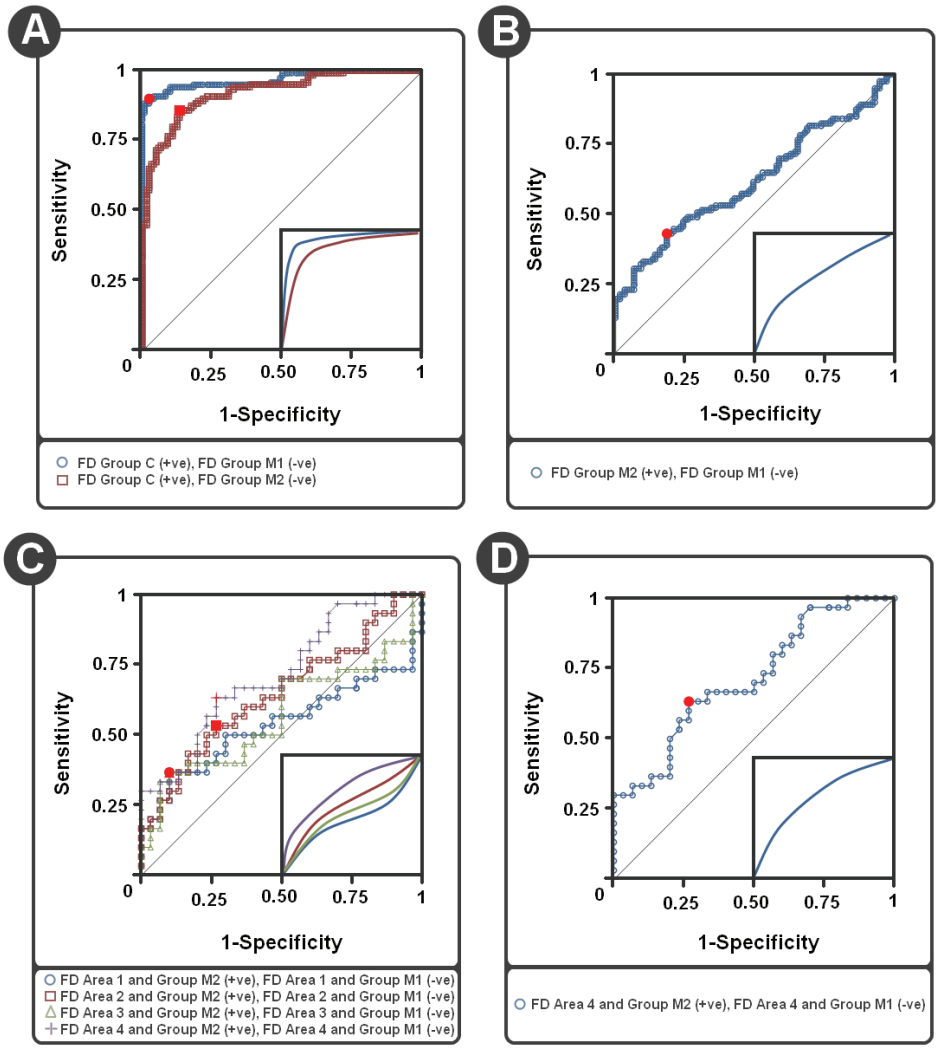

Figure 4. ROC distributions. (**A**) Group C/Group M1 (in all areas) and Group C/Group M1 (in all areas), (**B**) Group M1/Group M2 in all areas, (**C**) the entire group analyzed in all areas, (**D**) Group M2/Group M1. Cutoff value was the threshold for which the analysis was performed (red dots).

The wide range of the upper and lower quartile observed in M1 and M2 groups, shows that adipocytes of different anatomical areas respond differently to induced obesity in mice (Figure 3A-F). Some histopathological aspects of the adipose tissue collected from group M1 (HE stain, x400), are shown in Figure 5. An important observation was that hyperplasia and hypertrophy of adipocytes show radically different outlines (in all four anatomical regions) which are therefore interpreted differently through FD.

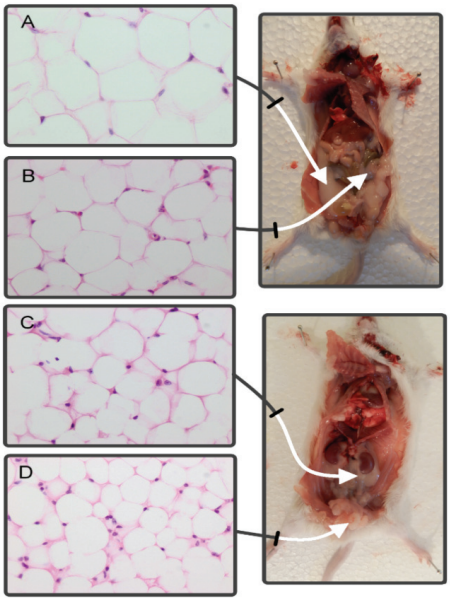

Figure 5. Histopathological aspects of the adipose tissue in mice. (**A**) omental area, (**B**) mesenteric area, (**C**) perirenal area, (**D**) subcutaneous inguinal area.

Even adipocytes from different anatomical sites clearly differ from each other in the context of membrane contours (Figure 5A-D).

Our initial question was if FD may or may not be used as a deterministic method on obesity-related pathology. In obese mice we observed significant structural changes in the adipose tissue compared to non-obese mice (Figure 6A-C).

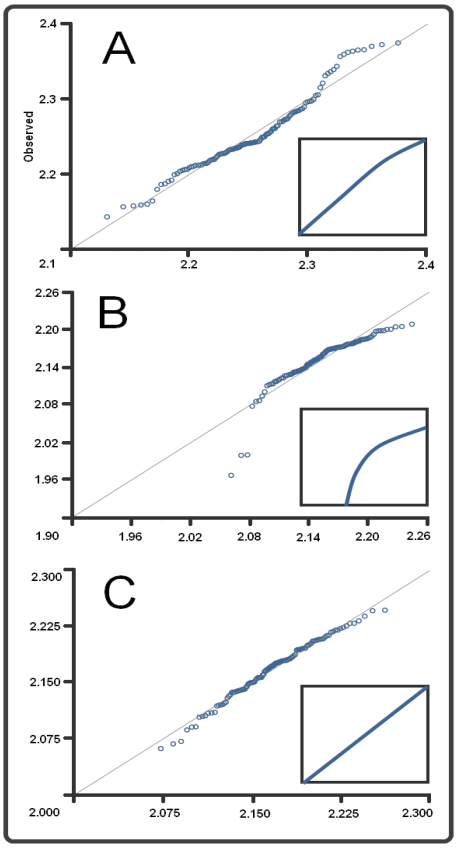

Figure 6. Distribution of FD values. (**A**) group M1, (**B**) group M2, (**C**) group C.

Thus, FD may be an effective histo-pathological parameter for the early detection of changes in adipocytes. However, it is difficult to predict whether this parameter can enter the diagnosis industry as a stand-alone methodology.

CONCLUSIONS

In this study, fractal analysis has proven to be effective for an early detection of changes in fat cells. Our results suggest that adipocytes from subcutaneous inguinal area show the most sensitive changes when mice are exposed to a high calorie diet. To make specific use of the fractal dimension data as a deterministic tool, a more complex approach is required in the future. By correlating different clinical and/or biochemical data with observations made through the fractal dimension method, new

diagnostic and/or prognostic models may be on the horizon.

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