

# VALIDATION OF ADIPOSOFIT: AN OPEN SOURCE SOFTWARE TO ANALYZE ADIPOSE TISSUE CELLULARITY

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## 1. INTRODUCTION

Obesity is caused by an increased adipose tissue mass, which may be caused by an increase in the number of adipocytes or an enlargement of the adipocytes due to lipid accumulation. Furthermore, the precise depot-location of the enlarged adipocytes is relevant in order to understand the differential response to dietary treatments. Thus, determining the cause and location of the increase of adipose tissue mass in histological sections is essential to study the effects of different diets. Manual measurements are time consuming and prone to error. Therefore, we present and validate Adiposoft, an open source software that analyzes adipose tissue cellularity providing accurate and repetitive quantitative measurements.

## 2. METHODS

*Experimental setup and image acquisition.* Male Wistar rats of approximately 400g were sacrificed and small pieces from retroperitoneal –RP-, mesenteric –MES- and subcutaneous –SC- fat depots were fixed, sectioned at 4 microns, stained with hematoxylin/eosin and imaged at 20X using an Axio Imager.M1 Zeiss microscope. All images were stored in uncompressed 24 bit color TIFF format.

*Image analysis.* Our software was entirely developed using MATLAB v.7.1.0 and the DIPlib v1.6 C libraries under OS Red Hat Linux AS 2.6.18-53.el5. The images were acquired in a region of interest and then segmented and quantified. The user can select a color threshold to binarize the image and the minimum size of objects that will be considered as adipocytes. With these parameters, the software performs a sequence of operations: threshold, erosion, filling holes, removing edge objects and separation of clusters. The results (adipocyte diameters and adipose tissue cellularity) are stored in an xls file. Adipocyte number in each tissue was estimated using the method of Bourgeois et al [1]

*Validation.* For the validation, we studied a well-characterized phenomenon, the increase in adipocyte size in RP. The results of our software were compared with those obtained by manual analysis of the images, and controlled measuring the of total DNA content by picogreen analysis.

## 3. RESULTS

The three types of fat depots studied and analyzed using Adiposoft have different adipocyte size pattern. Thus, retroperitoneal was significantly different to the two other fat depots analyzed. These differences mark the response of white adipocyte to obesity and a future nutritional intervention. The results show sufficient correlation between the measurements obtained from Adiposoft and those obtained using manual analysis of the images or measuring DNA content per gram of tissue study.

[1] Bourgeois, F; Alexiu, A; and Lemonnier D, “Dietary-induced obesity: effect of dietary fats on adipose tissue cellularity in mice”, *Br J Nutr*, **49**, 17-26 (1983).