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CELLULAR CHARACTERIZATION OF ADIPOSE TISSUE IN A MURINE MODEL OF DIET-INDUCED OBESITY

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In memory of our Master Doctor Daniel Andrés Durán Sandoval.

Abstract: Obesity is the main public health problem, globally and particularly in our country, Chile. Therefore, all studies that allow us to understand the biological mechanisms related to the etiology and development of obesity are of clinical and economic interest. In this study, we show some of the morphological characteristics and markers in adipose tissue under obese conditions, these characteristics are constant and help us to establish in vivo, a murine model of diet-induced obesity.
Keywords: Obesity, inflammation, murine model.

INTRODUCTION

The term "obesity epidemic" has been commonly used to refer to the worldwide increase in the rate of this pathology, which began in the 1980s in the United States and continues to this day throughout the world. [1]. In 2014, the World Health Organization (WHO) reported that approximately 500 million adults are obese, showing an increase of 100% compared to a couple of previous years.[2]. In addition, the latest data provided by the National Health and Nutrition Surveys (NHANES) in the USA are much more alarming, indicating an increase of more than 30% in adults who are obese and that around 15% of children and adolescents are overweight [1].

In Chile in recent decades, efforts have been made to strengthen the legislature, programs and strategies to deal with malnutrition, with favorable results when dealing with malnutrition due to deficit, but in the case of malnutrition due to excess, they have been a failure, mainly affecting the child population[3].

Obesity is a relevant issue in public health worldwide, therefore it must be addressed by developed and developing countries, as a priority National Health Strategy. The data provided by the Ministry of Health, in Chile, which correspond to the period 2016-2017, indicates that obesity has had an increase in the entire Chilean population, but mainly women and children, reflecting that Chile is in a situation of high prevalence of obesity. risk factors for noncommunicable diseases and low protective factors.[4].

We know that obesity occurs as a consequence of a deregulation between the intake, expenditure and storage of energy, which may be influenced by different biological, behavioral and environmental factors, and in this sense, adipose tissue (AT) is a fundamental metabolic organ in the energetic homeostasis of the whole body.[5].

In response to changes in nutritional status, the AT undergoes dynamic remodeling, including quantitative and qualitative alterations of the resident cell components[9]. These changes evident in obesity would be closely related to the function of the AT, which include changes in the size and number of adipocytes in the tissue [13]. This would affect the microenvironment of the fatty tissue, which would be accompanied by alterations in the secretion of cytokines, hormones, adipocyte death, hypoxia and increased secretion of free fatty acids[4].

The complex regulation of body weight as a possible indicator of obesity presents us with a challenge to understand the etiology of this pathology in order to develop tools to combat it and finally treat and prevent it. It is in this context that this work presents a mouse model of diet-induced obesity, and the parameters that validate it, in order to obtain a tool to be used in research and expand our knowledge of this pandemic.

MATERIALS AND METHODS

TA isolated from mice of the C57BL6 strain that were kept in an animal facility at the Faculty of Chemistry and Pharmacy

of the University of Concepción, set at a temperature of 25ºC +-1 with 12/12 light/dark cycles with free access, was used. water and fed ad libitum in the following groups.

Group 1:5 female mice fed ad libitum a high-fat diet (HFD); diet D12451, 45% fat. Open source diets, researchs diet, ic. Nww Brunswich, NJ USA)

Group 2: 5 mice fed ad libitum low-fat diet (LFD); 10% calories. Fat derivatives.

After 4 months, the animals in group 1 obtained an obese phenotype evidenced by a significant increase in weight of over 40% compared to baseline, associated with a significant increase in fat mass.

FABRIC SAMPLES

Once the treatment period was completed, the animals were sacrificed, after fasting for 12 hours the animals were put to sleep in an isofluorane hood.

Euthanasia was performed by cervical dislocation. Visceral adipose tissue samples were fixed in 4% paraformaldehyde in PBS (MERCK) for 48 hours, then processed in a Citadel 1000 tissue processor for 13 hours, under standardized protocols by the Histopathology Laboratory of the Faculty of Veterinary Sciences, of the University of Concepcion. Samples were embedded in paraffin and sliced at 4 um for analysis under a Carl Zeiss Axioscop 40 image processor, using Axiovision release 4.6 software.

ANTIBODIES

Antibodies were used for the detection of cytoplasmic cytokines and phenotypic markers by immunohistochemistry: Anti IL-6 (polyclonal rabbit Thermofisher), Anti TNF-a (monoclonal mouse, invitrogen), Anti cd68 (monoclonal mouse santa cruz), Anti iNOS (monoclonal mouse Thermofisher).

IMMUNOHISTOCHEMISTRY

The samples at 4 um were placed on loaded slides, to be deparaffinized, hydrated and blocked from endogenous peroxidase, then they were incubated overnight with each of the antibodies (1:100) separately, the next day the samples were incubated with universal labeled polymer (Immpress universal reactive, Vector) and revealed with DAB chromogenic substrate (Immpact Dab, Vactor).

RESULTS

The results obtained indicate a clear increase in body weight and adipose tissue deposition (TAV) in HFD mice. At the same time, the morphometric analysis determined a larger area of the adipocytes and a significant increase in the number of cells. A higher CD68+ macrophage cell count was determined in HFD mice compared to LFD. (Figure 1)

HFD LFD

(Figure 2).

DISCUSSION

Given the relevance of adipose tissue in the development of metabolic manifestations in obesity, this work aims to provide a tool that allows us to better study the processes that influence these pathologies. The factors that determine the fat mass in an individual are not completely clear yet, however it is evident according to our results that the rate of adipose tissue can expand at the cost of an increase in the volume and/or the number of adipocytes, there are reports that have found a positive correlation between the amount of fat mass and the volume of fat cells, both in subcutaneous patches, which represent about 80% of all body fat, and in visceral fat patches, which have a strong link with metabolic complications in obesity [8].

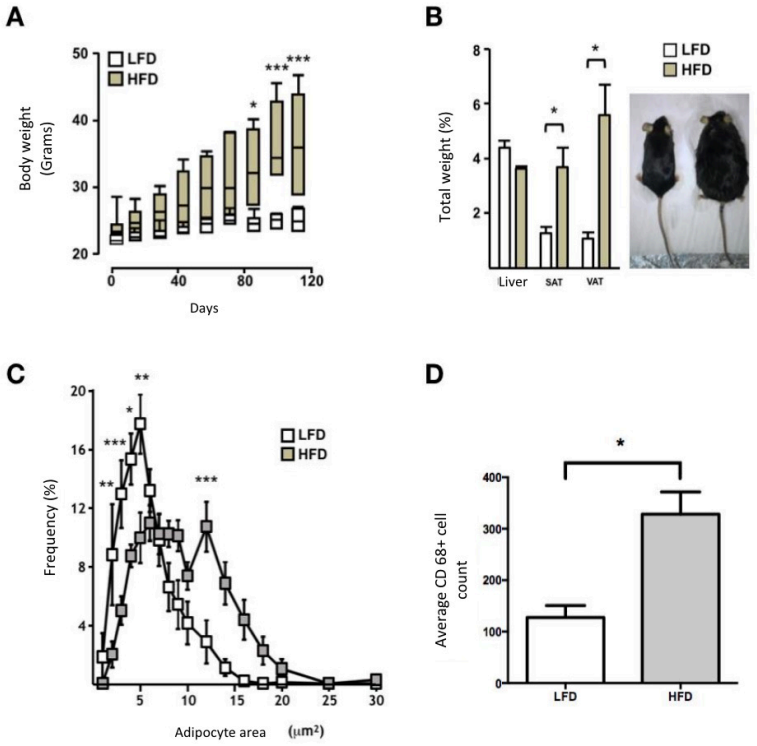

Figure 1. In vivo characterization of the mouse model of obesity induced by HFD. A. Determination of body weight of mice fed LFD and HFD (*p<0.05, ***p<0.001.). B. Percentage representation of liver weight, SAT(subcutaneous adipose tissue) and VAT(visceral adipose tissue)(*, p<0.05). C. Morphometric analysis of VAT adipocytes in relation to their area and frequency (*p<0.005, **p<0.01, ***p<0.001.). D. CD68+ macrophage count in VAT (*, p<0.05).

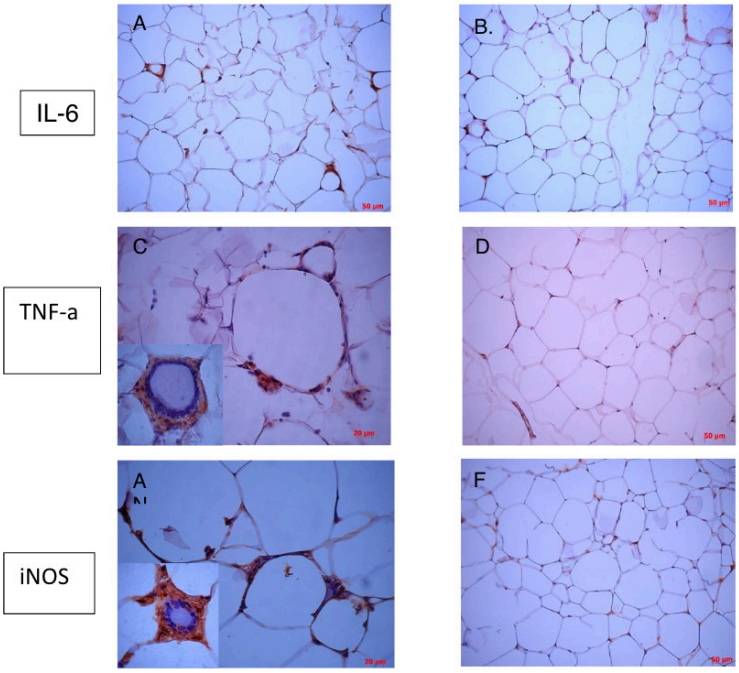

Figure 2. In vivo characterization of the mouse model of obesity induced by HFD and LFD. Determination of the expression of inflammatory cytokinesIL-6, TNF-a and iNOS (macrophage marker M1). Thumbnails at higher magnification in C and E correspond to adipocytes in necrosis, surrounded by macrophages with expression of inflammatory markers, TNF-a and iNOS (M1) correspondingly.

The cellular changes observed in this model would be related to a low-grade chronic inflammatory process [10], which include changes in the number and size of adipocytes[8]. Under conditions of overnutrition, TA increases the number of immune cells, particularly macrophages[5]

The results obtained would allow us to conclude that obesity induced with a HFD diet would induce an inflammatory process characterized by mononuclear recruitment in response to the metabolic adaptation of AT generated by overfeeding a diet rich in fat.

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