Diet induced changes in the adipose tissue of experimental animals

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Abstract:

Several studies have shown a relationship between the distribution of fat mass around the organism, metabolic disorders, and an increased risk of morbidity and mortality. It has been demonstrated that in obese animals there is a big rise in the white fat deposits due to hyperplasia and hypertrophy of the adipocytes. Studies related to weight and health have been more popular regarding obesity rather than extreme caquexia or calorico-proteic deficiencies, but these states are interesting from the point of view of the preferential atrophy of certain organs that may help us in the understanding of undernourishment. Moreover, the discovery of beige adipose tissue have instigated thoughts around the roles played by the different cells in the adipose tissue as well as its adaptability in pathological states.

In our study we carried out morphometric, morphologic, and quantitative measurements of the adipose tissue in an animal model based on a 40-50% diet restriction in comparison to control animals.

We have found differences in the studied parameters that can be considered as possible transformations between the types of adipose tissues, and that could be caused by an adaptive phenomenon to the undernourished state.

Key words: Browning; Undernourishment; adipose tissue

INTRODUCTION

The adipose tissue has always been considered a diffuse organ with a great metabolic activity, but more importantly as an energetic reservoir. However, nowadays it is also considered as a secretory organ, since it secretes adipokines that regulate in an autocrine and paracrine manner functions within itself and other tissues such as muscle, liver, brain or pancreas. It has been described to be involved in regulating processes such as homeostasis of body weight (leptin, CRP30/adipoQ), immune system (TNFα, IL-1, IL-6), or the development of insulin resistance (resistin), (Bełtowski y cols. 2003; Castro et al., 2011; Lomonaco et al., 2011; Guerra et al., 2019).

Three types of adipose tissues are accepted, A) white adipose tissue (WAT) formed by white adipocytes that structurally are unilocular with peripheral nucleus and very little number of mitochondria and its main function is to store lipids in order to generate energy when needed and to protect us from hyperglycaemia. Under normal circumstances, it sends hunger and satiety signals to several brain areas (Brandan et al, 2008). During fasting situations, white adipose tissue cells gradually secrete the stored lipids. B) brown adipose tissue (BAT) (Koh y cols. 2007; Valenzuela y Sanhueza, 2009), is formed by multilocular brown adipocytes being formed by multiple cytoplasmic triglyceride droplets varying in size. It is also highly vascularized and was given such a name due to the colour it presents caused by the numerous mitochondria it counts with. The BAT is able to carry out what is known as adaptative or facultative thermogenesis, thanks to the mitochondrial protein UCP1 to uncouple respiration and dissipate chemical

energy as heat (Park y cols. 2014; Chechi et al., 2013, 2014. Wu et al., 2012; Bruun y cols. 2004, 2005. C) Beige adipose tissue (Gómez-Hernández y cols. 2013, Chechi K y cols. 2013, Park y cols. 2014), formed by cells with intermediate fat drops but higher number of mitochondria, they present a different genetic expression to that of white and brown adipocytes lacking UCP1 expression in basal conditions (Wu et al. 2012, Chechi K et al. 2013, Park et al. 2014).

At least three types of precursors give rise to white, beige, and brown adipose cells separately (Symonds,2013). Precursors of brown adipocytes developmentally originate from dermomyotome and express Pax7 and Myf5, while those for white adipocytes lack such gene expression (Fig. 1). Moreover, while PPARγ is essential for adipogenesis of all fat cells, PRDM16 plays an important role in regulating only brown and beige cells (Wu J et al., 2013), and other transcriptional components, such as growth differentiation factor 5 (GDF5), play distinct roles in the development, commitment, and differentiation of white, beige, and brown fat(Zhang et al., 2019).

However, certain transformations in between distinct adipose tissues can take place under certain stimuli. Age on one hand, leads to a physiological transformation of BAT into WAT (Valenzuela y Sanhueza, 2009; Enerbäck, 2010), which is known as whitening (Bartelt y Heeren, 2014). On the other hand, stimuli such as low temperatures or β3 adrenergic activators, can transform WAT adipocytes into cells with similar characteristics to those of the Brown adipocytes in number of mitochondria with multiple fat droplets and UCP1 upregulation (Park et al. 2014), this process is known as browning (Paulo and Wang, 2019).

Moreover, adipose tissue varies according to nutritional status, thus undernourishment causes anatomic alterations in all organs with microscopical atrophy and weight loss (Brasel, 1980). In the same way, maternal undernourishment can cause a programming of diseases in the adulthood, even reducing the number of pancreatic cells in the offspring of undernourished mothers (Steingrimsdottir et al., 1980). Furthermore, in vitro studies show how the adipocytic cultures from muscle and subcutaneous tissue, have an increased lipid accumulation when the cells came from undernourished rats, what suggests that through a still unknown mechanism, the mother rat following external aggressions such as calorico-proteic restrictions, programmes its embryos through biochemical or hormonal means, for them to increase the lipid storage inside adipocytes (Moreno, 2002). This seems to be an energetic strategy through which the mother prepares the offspring to survive in food scarce periods, seeming protective. However, it also increases the probability for them to develop obesity in the adulthood together with metabolic and cardiovascular diseases amongst others (Rodríguez Scull, 2004). These engrossed adipocytes that the pups present might undergo a browning process to render heat through thermogenesis processes as Wu et al. (2012) suggest by the increase of UCP1 expression as a response to cyclic AMP, which is known to be increased in situations such as malnutrition (Serezani et al. 2008; Ineke et al., 2019).

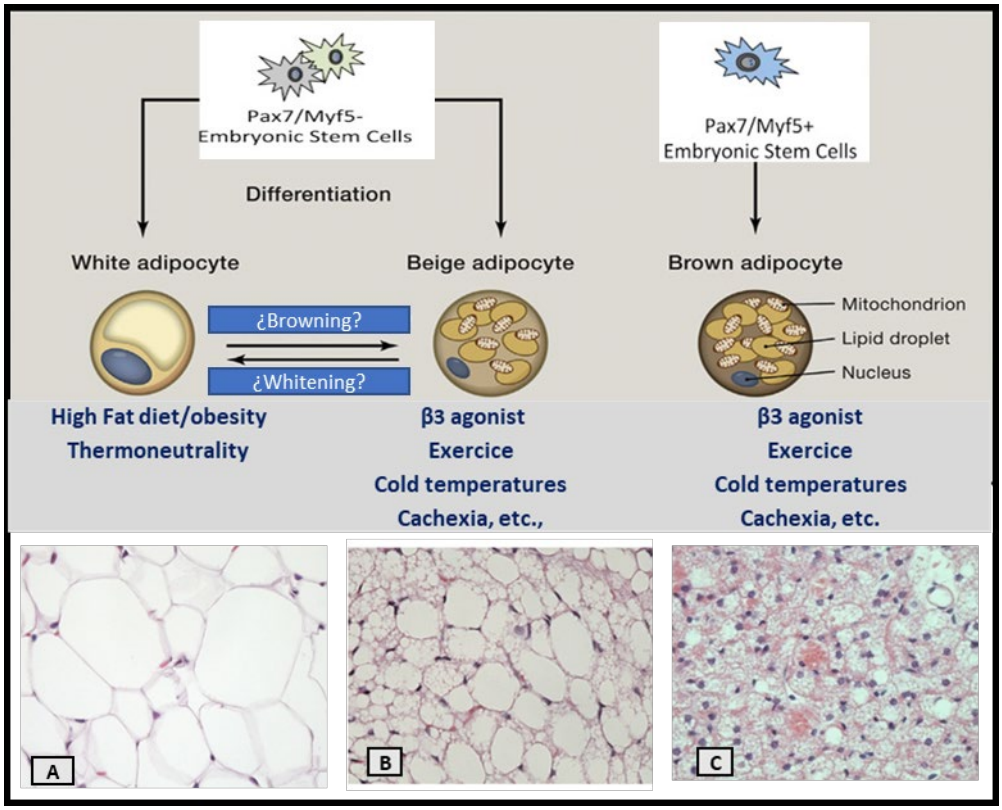

Figure 1. Development and intertransformations of different adipose cells.

The present work aims to carry out a quantitative and morphological study of the different adipose tissues in the experimental animals in order to compare control and undernourished animals to further comprehend the variations in the types of tissues that may take place implying the thermoregulatory and metabolic needs and lacks that the undernourished animals may experience.

MATERIALS AND METHODS.

Experimental design

All studies were conducted under an approved animal protocol by Ethics Committee for Animal Experimentation of the Universidad Complutense, Madrid (EC 280790000085).

Wistar rats, 90-200 g, were housed under a 12-h light/12-h dark cycle in a temperature-controlled room (22 ºC) and were fed ad libitum with a commercial standard laboratory diet containing by weight 19 % protein, 56 % carbohydrate (starch and sucrose) 3.5 % lipid, 4.5 % cellulose, vitamin and mineral mix, and 12 % water. Females were caged with males, and mating was confirmed by the presence of spermatozoa in vaginal smears. Each dam was housed individually from the 14th day of pregnancy.

Control received standard laboratory diet and restricted rats received 10 g of the standard food daily from 16th day of gestation until delivery, which represents 40-50 % of that ingested by controls and prevents the 20 % increase of body weight observed in these rats during this period (Escrivá et al. 1992). The number of pups was evened out to 8 in all litters.

The restricted mothers received 40 % of the food consumed by controls, that is: 15, 20 and 25 g daily of food during the 1st, 2nd and 3rd week of lactation, respectively. We selected the females of each litter for this study, and during the period between weaning and until their sacrifice (day 70), control group (n=5) received standard diet and undernourished rats (n=5) received 35 % of the diet daily consumed by controls (Lizarraga et al. 2013). Water was given ad libitum and animals were observed and weighed before their sacrifice

Tissular processing and analysis

Tissue samples of WAT (Inguinal zone) and BAT (interscapular zone) were fixed in Paraformaldehyde 4%, dehydrated with ethanol and subsequently embedded in paraffin. Thin serial discontinuous sections (4 µm) were obtained with a vertical rotation microtome (Leica RM 2125RT), mounted on glass-slides and stained with hematoxylin/eosin (Thermo Scientific, Spain). After staining, sections were dehydrated in ethanol and xylene, mounted with DPX. For the morphological, quantitative and morphometric studies samples were observed by using an ECLIPSE 50i-NIKON microscope, equipped with a video-camera (DS-5M) and a NIS-ELEMENTS image analysis software.

Morphometric and quantitative and study

A morphometric analysis of the area (µm²) of white adipocytes was carried out (Fig.3A). Up to 200 adipocytes from each animal were analyzed, both of controls and undernourished rats.

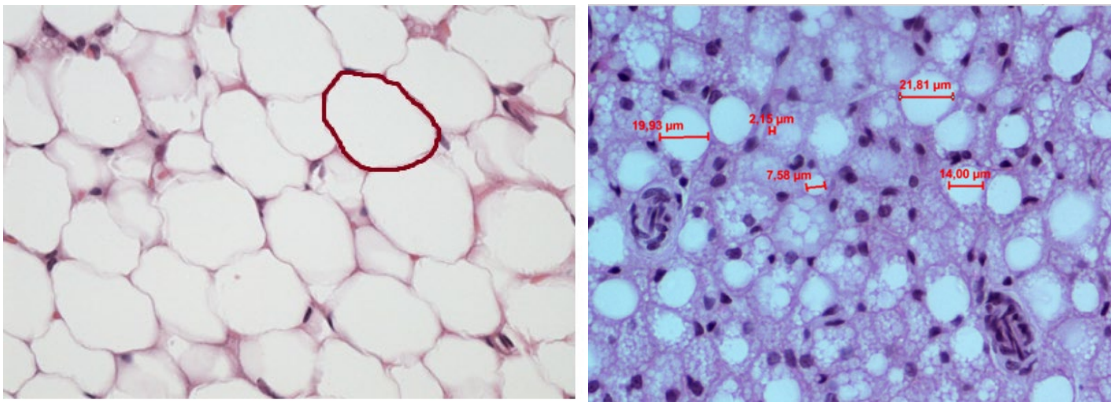

Figure 3: Morphological and morphometric analysis. A) Areas (µm²) of white adipocytes B) Different diameters ((µm) of lipid droplets in the brown adipose tissue. 40X (H-E)

The fat droplets within the brown adipocytes were classified into 5 groups according to their diameter: 0-5 µm; 5-10 µm; 10-15 µm; >15 µm diameters. Subsequently, the percentage of adipocytes containing the different droplet sizes was quantified.

Statistical analysis

To carry out the statistical analysis PRISM 6 (GraphPad software, San Diego, CA, USA.) was used. Mean (X) and Standard Error of the Mean (SEM) were obtained for each analyzed value. The comparative study of the area of the adipocytes of the white adipose tissue from control group and undernourished group was done by using T Student test. The comparative study according to the droplet size and the percentage of adipocytes containing each type of sized droplets was carried out through a 2-ANOVA. Statistical significance was considered with a P value < 0.05.

RESULTS

Animal weight

Dietary restricted animals showed a significant decrease in weights (99,40 ± 2,75) compared to control ones (188±3,59). (Fig. 4).

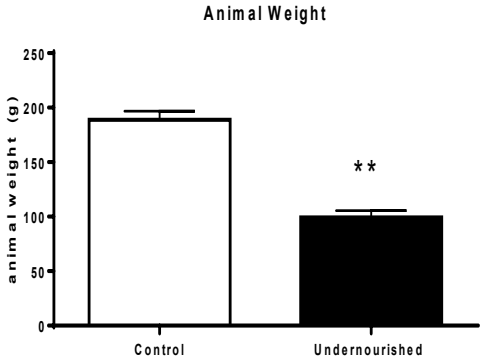

Figure 4: Weight of the control and undernourished rats. ** p< 0,001 vs control.

Morphological and morphometric study of the White Adipose Tissue

White adipose tissue in both groups had a normal morphology resembling a mesh, cells had their nucleus to the bounds and a great central fat droplet. In both groups different sizes and shapes could be found as well as the presence of some multilocular cells among white adipocytes undernourished rats (Fig 5).

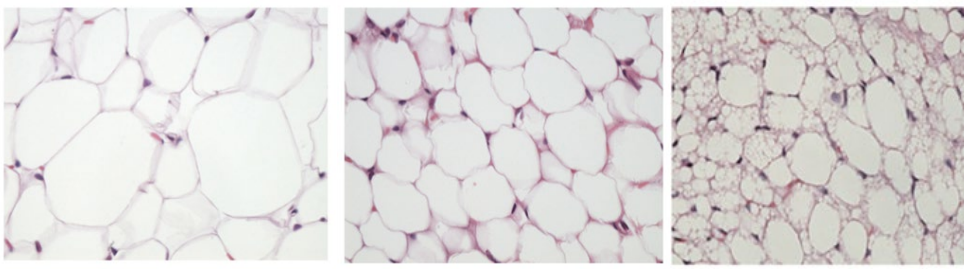

Figure 5: Morphological analysis of the White adipose tissue of control (A) and undernourished rats (B) 40x. (HE)

The morphometric study, revealed a significant decrease in size of the adipocytes coming from the undernourished rats (1280 ±27.94) compared to that of control rats (1807± 22.57) (X±SEM) (Fig.6)

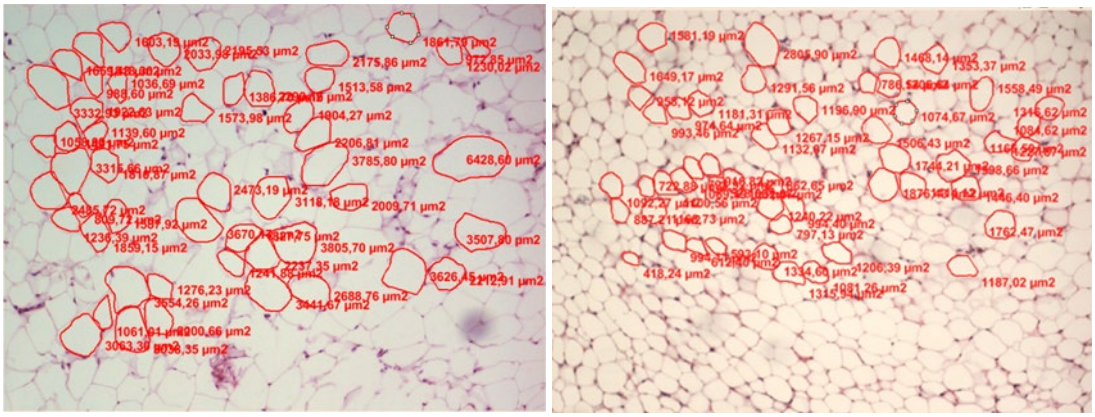

Fig 6. Morphometric analysis of the areas of the white adipocytes of control (A) and undernourished rats. (H-E). NIS-ELEMENTS image analysis software (10x).

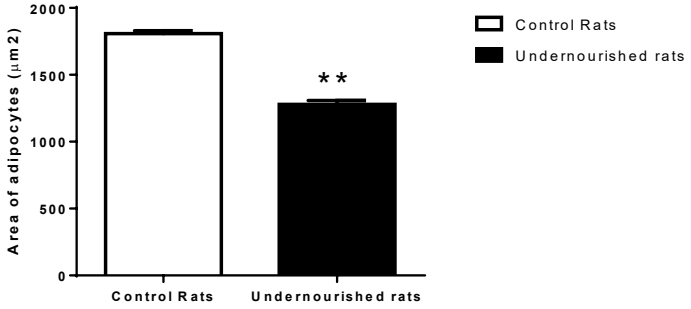

Figure 7: Comparative analysis of the areas of the White adipocytes of Control and Undernourished rats (X±SEM) (** p< 0,001 vs control)

Morphologic morphometric and quantitative study of the Brown Adipose Tissue

Brown adipose tissue in both treatment groups showed a normal morphology showed several lipid droplets, The diameter of the lipid droplets was measured for each group and adipocyte cells and quantifies the percentage of brown adipocytes grouped by the diameter of their lipid droplets (0-5 ;5-10; 10-15 and >15 µm). Table 1, and Fig 8.

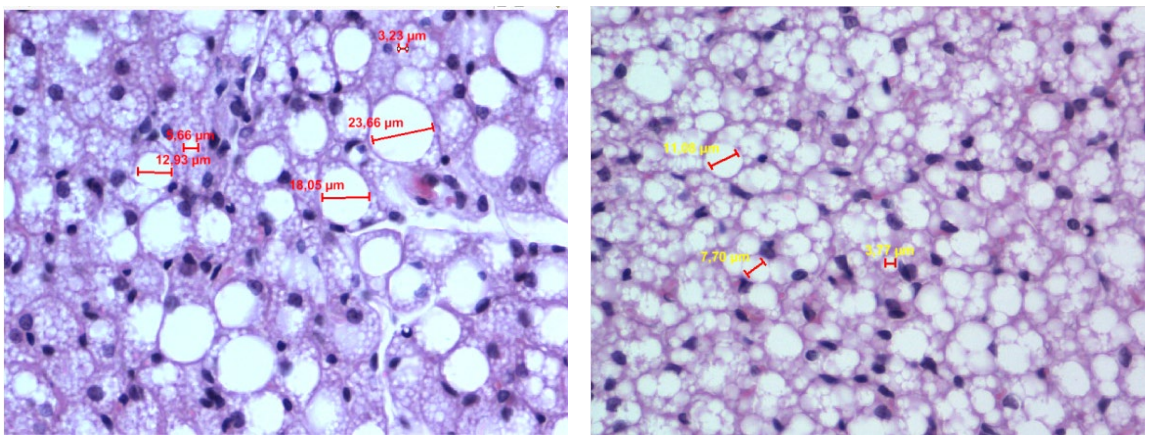

Figure 8: Morphometric analysis of the diameters of the brown adipocytes of control (A) and undernourished rats. (H-E), NIS-ELEMENTS image analysis software (40x)

Groups	**0-5 µm**	**5-10 µm**	**10-15 µm**	**> 15 µm**
Control	54,13 ± 3,014	23,88 ± 1,29	21,00 ± 2,00	3,75 ± 0,83
Undernourished	43,88 ± 2,29	39,63 ± 2,18	14,63 ± 1,32	1,87 ± 0,49

Table 1: Percentage of Brown adipocytes grouped by the diameter of their lipid droplets (0-5 ;5-10; 10-15 and >15 µm) (X±SEM). (N =200)

The statistical analysis showed significant differences in the percentages of adipocytes in each group, showing a bigger number of adipocytes with droplets sized 5-10 µm in undernourished animals as well as a smaller percentage in those sized 10-15 µm when compared to control animals.

Moreover, a decrease in the number of adipocytes with big droplets (>15 µm) in undernourished rats was observed in comparison to that of control ones, almost in the signification limit (p=0.08) (Fig. 9).

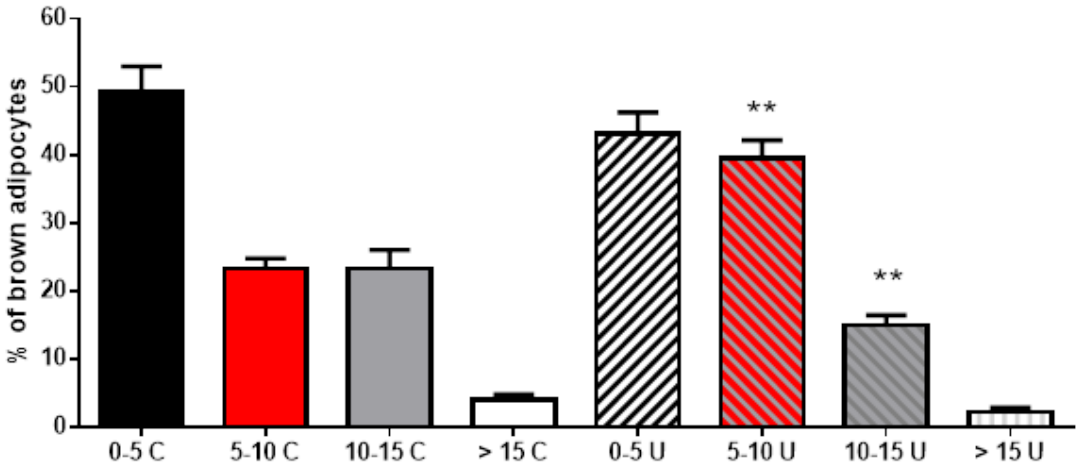

Figure 9. Percentage of adipocytes according to the size of their lipid droplets and statistical analysis between control (C) and undernourished (U) rats. ** p < 0.001 vs control. (n= 200)

DISCUSSION

The use of animal models of caloric-proteic restrictions has been up to date a key point in the study of the mechanisms related to several metabolic syndromes (Moreno, 2002; Rodriguez Scull, 2004).

Classical adipose tissue, (white and brown adipose tissues), not only show differences in their colour but also in their morphology, distribution, genes and function. Moreover, it is already known that the quantitative balance of both can be altered as a response to several factors such as age, temperature, obesity, undernourishment amongst others (Moreno et al 2002, Spaldin et al.2008, Van Market et al. 2009).

In this sense, our restricted animals show a significant decrease in the area of the white adipocytes compared to that of control animals. This decrease could be explained by a use of these fat deposits as

a way of energy obtention, or by a browning phenomenon, or a transformation into brown adipose tissue, passing through a more beige one, in order to favor thermogenesis (Palou et al 2014, Liu et al. 2009). This need of heat production had already been postulated by some authors like Park and coworkers who described how a moderate restriction during gestation causes a reduction in the ability of the offspring´s brown adipose tissue to have a normal thermogenic function, leading to an increase such tissue both to produce more heat as well as to insulate better (Park et al. 2014). Still pending would be to figure out if this transformation can induce the production of new organelles such as mitochondria which are necessary for this purpose. Therefore, the determination of UCP-1 expression in this tissue would be essential, as well as of FGF21 that Alemán and coworkers have described is increased by low protein diets, and which can induce the browning of white adipose tissue in order to increase thermogenesis by enhancing the expression of UCP1 (Alemán et al., 2019).

Moreover, and pointing in this direction, our results show how brown adipose tissue seems to become browner, similarly to the well-known browning phenomenon described by many authors. Undernourishment seems to cause a decrease in the middle sized fat droplets that become smaller ones, changing into a more homeostatic energy phenotype (Chen et al., 2016), probably in an apparent attempt of using its fat droplets in order to obtain heat (thermogenesis). process that has already been described in humans by naming this kind of brown adipose tissue “active adipose tissue” making reference to the process of heat production by changing chemical energy stored as triglycerides into thermal one (Martin et al., 2013).

This finding is consistent with the findings of Rojas and coworkers that describe how a breeding rat undergoing external aggressions such as caloric-proteic restriction, programs its offspring, through biochemical or hormonal signals, to make them accumulate more lipids in their adipose tissue as part of an energetic strategy to help them survive in food- scarce periods (Rojas et al. 2009). Moreover, the size of brown adipocytes seems to be bigger in undernourished animals than in control ones suggesting a hypertrophy of the tissue similar to that of white adipose tissue in obesity (Owens, 2014). These effects seem to be opposite to that found in the analysis of the white adipose tissue, as if a whitening process of the brown adipose tissue were taking place, again suggesting the formation of a beige adipose tissue able to carry out both, energetic supply and thermogenesis.

The undernourished animals in our study present a normal morphology and infiltration of inflammatory cells in adipose tissue. Contrarily, other authors have described how undernourishment leads to a decrease in the secretion of leptin to T lymphocytes, monocytes and dendritic cells increasing therefore the risk of infection (Palacios et al. 2002).

Altogether, these results make evident interchanges taking place in between white and brown adipose tissues, and the different types of this last one, under calorico-proteic modifications suggesting the existence of browning phenomena, or activation one. Bearing this in mind, and taking into account the uprising prevalence of obesity that has made the research on new therapeutic approaches essential, a good therapeutic approach seems targetting a possible transformation of white adipose tissue into brown adipose cells through a “Browning” phenomenon (Bartelt and Herren, 2014; Vargas-Castillo et al., 2017), or activation of brown adipose tissue as other authors suggest (Otero-Diaz et al., 2018), hence increasing

energy expenditure and reducing fat storage, thus Activation of brown and/or brite adipocytes reduces metabolic diseases, at least in murine models of obesity (Chen et al., 2016).

CONCLUSIONS

The significant decrease in the size of white adipose cells in the undernourished animals reinforces the thought that under certain circumstances they would use their fat droplets as fuel as a mean to obtain needed energy and the glucose homeostasis. Furthermore, the increase in the Brown adipose cells containing medium sized droplets coming from the reduction of those with big droplets could be thought as being a process targeted to a bigger production of heat that should also happen together with the cellular transformations such as an increase in mitochondria. The calórico-proteic restriction during gestation causes changes in adipogenesis probably due to hormonal variations.

The formation of brown-like adipocytes called beige adipocytes, within white adipose tissue (WAT), has attracted much attention as a therapeutic target due to its inducible features when stimulated, resulting in the dissipation of extra energy as heat. There are various dietary agents that are capable of modulating the beige-development process by interacting with critical molecular signaling cascades, leading to the enhancement of thermogenesis. Although challenges still remain regarding the origin of the beige adipocytes (Wang et al., 2019), all of our findings point in a promising direction in such approach but should be further studied to reconfirm our hypothesis.

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